



**EDUVET**  
**INTERNATIONAL VETERINARY SCIENCES**  
**CONGRESS**  
**25-27 JUNE 2021**

Sharing Experiences About Veterinary Medicine in a Friendly  
Scientific Atmosphere



**PROCEEDINGS BOOK**  
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***HAYATTA EN HAKİKİ MÜRŞİT İLİMDİR***  
***OUR TRUE MENTOR IN LIFE IS SCIENCE***  
***MUSTAFA KEMAL ATATÜRK***

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*We would like to thank HIPRA VETERINER MUSTAHZARLARI TIC. LTD. STI. for their kind support to the Congress.*

*Organizing Committee*

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# EDUVET INTERNATIONAL VETERINARY SCIENCE CONGRESS

## SCIENTIFIC PROGRAM

**(TIME IS LOCAL ISTANBUL TIME)**

DAY 1	
FRIDAY, 25.06.2021	
09.30-10.00	WELCOME AND OPENING CEREMONY <i>ASSOC. PROF. DR. HANDAN HİLAL ARSLAN YAVUZ</i>
<b>SESSION I: INVITED SPEAKERS</b> <b>CHAIR: PROF. DR. M. ERMAN OR</b>	
10.00-10.45	IMPORTANCE OF MONITORING CALVING TO DECREASE STILLBIRTH RATE IN DAIRY FARMS <i>PROF. DR. OTTO SZENCI</i>
10.50-11.35	METABOLIC PROFILES IN COWS <i>PROF. DR. JOSÉ RAMIRO GONZÁLEZ-MONTAÑA</i>
11.40-12.40	REST AND TREADMILL ENDOSCOPIC FINDINGS IN RACE HORSES PRESENTED FOR POOR PERFORMANCE <i>ASSOC. PROF. DR. FRANCESCO FERRUCCI</i>
<b>12.40-13.30 LUNCH</b>	
<b>SESSION II: INVITED SPEAKERS</b> <b>CHAIR: PROF. DR. M. ERMAN OR</b>	
13.30-14.15	SURGICAL APPROACH TO END STAGE OF OTITIS IN DOG AND CATS <i>ASSOC. PROF. DR. STEFANO ROMUSSI</i>
14.20-15.05	THE ROLE OF ENTREPRENEURSHIP EDUCATION IN VETERINARY MEDICINE <i>INSTRUCTOR HIROKO KAWAMORITA</i>
15.10-15.20	<b>BREAK</b>
<b>SESSION III: ORAL PRESENTATIONS (IN ENGLISH)</b> <b>CHAIR: PROF.DR. OGUZHAN YAVUZ</b>	
15.20-15.35	CAUSES AND REMEDIES FOR NON-INFECTIOUS INFERTILITY IN SHEEP AND GOATS <i>PROF. DR. ÖMER UÇAR</i>

15.35-15.50	SEX DETERMINATION IN GIANT ANTEATER ( <i>MYRMECOPHAGA TRIDACTYLA</i> ) USING PCR AMPLIFICATION <i>ASSOC. PROF. SENA ARDIÇLI</i>
15.50-16.05	MANAGEMENT OF RECTAL PROLAPSE IN ANIMALS <i>PROF. DR. ÖZLEM NİSBET</i>
16.05-16.20	CAN WE USE PROBIOTICS IN CANINE PARVOVIRUS INFECTION AND DOES IT WORK? <i>ASSOC. PROF. HANDAN HİLAL ARSLAN</i>
<b>16.20-16.30</b>	<b>BREAK</b>
<b>SESSION IV: ORAL PRESENTATIONS (IN ENGLISH)</b>	
<b>CHAIR: PROF. DR. ÖZLEM NİSBET</b>	
16.30-16.45	INVESTIGATION OF PERSISTENT ORGANIC POLLUTANTS IN HAIR SAMPLES COLLECTED FROM DOMESTIC CATS AND DOGS <i>PROF. DR. OĞUZHAN YAVUZ</i>
16.45-17.00	ISOLATION OF CANINE PARVOVIRUS FROM RECTAL SWABS OF SUSPECTED DOGS FROM VARIOUS REGIONS IN NORTHERN INDIA <i>PARIKSHIT SINGH, DVM</i>
17.00-17.15	EVALUATION OF PULMONARY ARTERY STIFFNESS IN ASTHMA AFFECTED HORSES <i>ELENA ALBERTI, DVM</i>
17.15-17.30	THE FIRST TIME ISOLATION AND CHARACTERIZATION OF INDIGENOUS BOVINE RESPIRATORY SYNCYTIAL VIRUS STRAINS OF TURKEY FROM RESPIRATORY DISORDERS CAUSING DEATHS IN CATTLE <i>AHMED EISA ELHAG IBRAHIM, DVM</i>
17.30-17.45	GREEN SYNTHESIS OF SILVER NANOPARTICLES USING COMMON POPPY ( <i>PAPAVER RHOEAS</i> ) AND EVALUATION OF THEIR POTENTIAL ANTIBACTERIAL ACTIVITY <i>ÇIGDEM DIKBAS</i>
<b>17.45-18.00</b>	<b>BREAK</b>
<b>SESSION V: ORAL PRESENTATIONS (IN TURKISH)</b>	
<b>CHAIR: PROF. DR. GÜL FATMA YARIM</b>	
18.00-18.15	ESTIMATION OF MILK YIELD LOSSES FROM SUBCLINICAL MASTITIS IN DAIRY COWS <i>ASSIST. PROF. MURAT TANDOĞAN</i>

18.15-18.30	EFFECT OF DIFFERENT AGE AND PLUMAGE COLOR ON EGG QUALITY WITH CHOLESTEROL LEVEL IN JAPANESE QUAIL EGGS <i>RES.ASSIST. EMRE ARSLAN</i>
18.30-18.45	INVESTIGATION OF MYCOPLASMA AGALACTIAE FROM GOATS WITH MASTITIS BY BACTERIOLOGICAL AND MOLECULAR METHODS <i>RES.ASSIST. HAFİZE TUĞBA YÜKSEL DOLGUN</i>
18.45-19.00	BIOSENSORS IN DIAGNOSIS OF BACTERIAL DISEASES IN VETERINARY MEDICINE <i>RES.ASSIST. SONGÜL ÖTKÜN</i>
19.00-19.15	INVESTIGATION OF THE MICROBIOLOGICAL EFFECT OF CHITOSAN BASED PEANUT PINE RESIN ADDED HYDROGEL <i>MERVE YİĞİTER</i>
19.15-19.30	PLANTS GROWING IN TURKEY, WITH REPELLENT AND ACARICIDAL EFFECTS ON TICKS <i>ÖZGE MARANGOZ</i>
19.30-19.45	DEVELOPMENT OF ELISA KIT FOR SEROLOGICAL DIAGNOSIS OF Q FEVER IN ANATOLIAN BUFFALOES <i>RES.ASSIST. MERVE GİZEM SEZENER</i>
<b>DAY 2</b>	
<b>SATURDAY, 26.06.2021</b>	
<b>SESSION VI: ORAL PRESENTATIONS (IN TURKISH)</b>	
<b>CHAIR: ASSIST.PROF.DR. SİNAN KANDIR</b>	
09.00-09.15	EVALUATION OF ROOSTER SEMEN FROZEN WITH SHILAJIT CONTAINING EXTENDER <i>ASSOC.PROF. DR. MURAT SELÇUK</i>
09.15-09.30	THE IMPORTANCE OF VOLATILE COMPOUNDS IN DETECTING HEAT IN COWS <i>ASSIST.PROF.DR. ESER AKAL</i>
09.30-09.45	EFFECT OF SODIUM SELENITE (Na <sub>2</sub> SeO <sub>3</sub> ) ON THE MOTILITY PARAMETERS OF CRYOPRESERVED DOG SPERMATOZOA <i>RES.ASSIST. BURCU ESİN</i>
<b>SESSION VII: INVITED SPEAKERS</b>	
<b>CHAIR: ASSOC.PROF. ÖZLEM BÜYÜKTANIR YAŞ</b>	
10.00-10.45	EPIDEMIOLOGY OF BRUCELLOSIS IN RUMINANTS AND CURRENT CONTROL STRATEGIES USED IN ALBANIA <i>PROF. DR. XHELİL KOLECI</i>

10.50-11.50	CHF DIAGNOSIS AND MANAGEMENT; EQUINE ARRHYTHMIA AND THERAPEUTICS <i>ASSOC. PROF. DR. ENRICA ZUCCA</i>
11.55-12.40	EQUINE MYOPATHIES: CURRENT CLASSIFICATION, DIAGNOSIS AND THERAPEUTIC APPROACH <i>DR. GIOVANNI STANCARI</i>
<b>12.45-13.30 LUNCH</b>	
<b>SESSION VIII: INVITED SPEAKERS</b>	
<b>CHAIR: PROF. DR. OĞUZHAN YAVUZ</b>	
13.30-14.15	MOST COMMON CARDIAC DISEASES, ECHOCARDIOGRAPHY IN CATS AND DOGS <i>DR. MARA BAGARDI</i>
14.20-15.05	<i>WILDLIFE IN TURKEY (TÜRKİYE'DE YABAN HAYATI) (IN TURKISH)</i> <i>PROF. DR. ERDOĞAN UZLU</i>
<b>15.10-15.20</b>	<b>BREAK</b>
<b>SESSION IX: ORAL PRESENTATIONS (IN TURKISH)</b>	
<b>CHAIR: PROF. DR. ÖZNUR ASLAN</b>	
15.20-15.35	TREATMENT PRINCIPLES IN CALF DIARRHEA <i>PROF. DR. SÜLEYMAN KOZLU</i>
15.35-15.50	THE TREATMENT OF A CALF WITH CHRONIC PNEUMONIA DUE TO <i>ACINETOBACTER BAUMANNII</i> COMPLEX AND IDENTIFICATION OF THE PATHOGEN BY MALDI-TOF-MS <i>ZEYNEP YERLİKAYA</i>
15.50-16.05	DETERMINATION OF THE EFFECT OF FILGRASTIM USE ON HEMATOLOGICAL PARAMETERS IN CATS AND DOGS WITH LEUKOPENIA AND NEUTROPENIA: A PRELIMINARY STUDY <i>MEHMET AKİF RIHTİM</i>
16.05-16.20	A CASE OF CONGENITAL ATRIAL SEPTAL DEFECT IN A THREE- DAYS OLD CALF <i>KENAN ÇAĞRI TÜMER</i>
16.20-16.35	DETERMINATION OF THE RELATIONSHIP BETWEEN PLASMA B-TYPE NATRIURETIC PEPTIDE LEVELS WITH THE HEMOGRAM AND SOME BIOCHEMICAL PARAMETERS IN DIARRHEIC AND HEALTHY CALVES <i>EMRE TÜFEKÇİ</i>
<b>16.35-16.50</b>	<b>BREAK</b>

**SESSION X: ORAL PRESENTATIONS (IN TURKISH)****CHAIR: ASSIST.PROF. ESER AKAL**

16.50-17.05	CAN SURGICAL ORTHOPEDIC INTERVENTION BE MADE WITHOUT GENERAL ANESTHESIA IN NEW-BORN CALVES? <i>ASSIST.PROF.DR. MÜMİN GÖKHAN ŞENOCAK</i>
17.05-17.20	PREANESTHETIC EVALUATION OF RESPIRATORY SYSTEM IN CATS AND DOGS <i>EYLEM BEKTAŞ BİLGİÇ</i>
17.20-17.35	ASSESSMENT OF INTRAOCULAR PRESSURE IN ROMANOV SHEEP OF DIFFERENT AGE GROUPS USING REBOUND TONOMETRY <i>SELVİNİZ YAKAN</i>
17.35-17.50	THE EFFECT OF MELOXIKAM ON INTRAOCULAR PRESSURE IN HORSES <i>BÜŞRA KIRLANGIÇ</i>
17.50-18.05	HIGH RESOLUTION IMAGE ACQUISITION WITH 3 TESLA MRI IN SHEEP BRAIN FIXED WITH FORMALDEHYDE: A METHODOLOGICAL STUDY. <i>SEDAT AYDOĞDU</i>
<b>18.05-18.15</b>	<b>BREAK</b>

**SESSION XI: ORAL PRESENTATIONS (IN TURKISH)****CHAIR: ASSOC.PROF. MURAT SELÇUK**

18.15-18.30	EFFECT OF LOW AND HIGH DOSE LEVOTHYROXINE ON P-GLYCOPROTEIN EXPRESSION, PROTEIN LEVEL AND FUNCTION IN MICE <i>HATİCE ESER FAKI</i>
18.30-18.45	PREVALENCE OF EIMERIA SPECIES IN CALVES IN BURDUR REGION OF TURKEY <i>ONUR KÖSE</i>
18.45-19.00	INVESTIGATION OF PREVALENCE OF HYDATID CYST IN CATTLE IN SIVAS PROVINCE <i>OSMAN FURKAN URHAN</i>
19.00-19.15	EFFICIENCY OF CONDENSED TANNIN IN RUMINANT NUTRITION <i>ZEKERİYA SAFA İNANÇ</i>
19.15-19.30	EXPRESSION OF THE KISS1 GENE AND RECEPTOR REGION (KISS1R) IN FRESH AND FROZEN RAM SEMEN <i>MELİH AKAR</i>



19.30-19.45	ISOLATION OF EXTRACELLULAR VESICLE (EXOSOME) FROM CANINE SEMINAL PLASMA USING AQUEOUS TWO-PHASE SYSTEM (ATPS)  <i>MERVE DENİZ GENÇ</i>
<b>DAY 3</b> <b>SUNDAY, 27.06.2021</b>	
<b>SESSION XII: INVITED SPEAKERS</b>	
<b>CHAIR: ASSOC.PROF.DR. HANDAN HİLAL ARSLAN</b>	
10.00-10.45	MORPHOLOGICAL EVALUATION OF EXPERIMENTAL AUTOLOGOUSRECTUS FASCIA SHEATH VASCULAR GRAFTS USED FOR ARTERIALREPLACEMENT IN A DOG MODEL <i>PROF. DR. TIBOR NÉMETH</i>
10.50-11.35	PROSPECTIVE EVALUATION OF CORRELATIONS BETWEEN CERTAINCLINICAL FINDINGS OF PERINEAL HERNIA IN 47 DOGS <i>PROF. DR. TIBOR NÉMETH</i>
11.40-12.25	INFERTILITY OF HORSES- STALLION PERSPECTIVE <i>PROF. DR. NIKICA PRVANOVIC BABIC</i>
<b>12.30-13.30 LUNCH</b>	
<b>SESSION XIII: INVITED SPEAKERS</b>	
<b>CHAIR: ASSOC.PROF.DR. ENRICA ZUCCA</b>	
13.30-14.15	ADRENAL GLAND DISEASES IN DOGS AND CATS <i>ASSOC. PROF. IVANA KIŠ</i>
14.20-15.05	TOOTH PRESERVATION WITH ENDODONTIC THERAPIES IN EQUINE DENTISTRY <i>DR. TIBOR AKOS HEVESI</i>
15.10-15.55	NEW DIAGNOSTIC APPROACHES IN ZONOTIC DISEASES <i>ASSOC. PROF. DR. ÖZLEM BÜYÜKTANIR</i>
<b>16.00-16.10</b>	<b>BREAK</b>
<b>SESSION XIV: ORAL PRESENTATIONS (IN TURKISH)</b>	
<b>CHAIR: PROF.DR. MUSTAFA ACICI</b>	
16.10-16.25	FIRST RECORD OF IXODES RICINUS (ACARI; IXODIDAE) IN EUROPEAN GLASS LIZARD (PSEUDOPUS APODUS; ANGUIDAE) AND STUDIES ON ECTOPARASITE IN REPTILES FROM PAST TO PRESENT IN TURKEY  <i>GÖKHAN EREN</i>
16.25-16.40	DISTRIBUTION OF HELIGMOSOMUM COSTELLATUM IN MICROTUS SPECIES IN TURKEY  <i>ÖYKÜ BARILI</i>
16.40-16.55	THE PREVALENCE AND MOLECULAR CHARACTERIZATION OF FASCIOLA SPECIES IN INTERMEDIATE HOSTS İN SAMSUN REGION: FIRST RESULTS  <i>ELİF BURCU GENÇAY TOPÇU</i>

16.55-17.10	NEOSPOROSIS IN TURKEY <i>RÜBEYDA DİNÇ</i>
17.10-17.25	PUERPERAL HEMOGLOBINUREA CASE IN A COW <i>EMRE TÜFEKÇİ</i>
17.25-17.35	<b>BREAK</b>
<b>SESSION XV: ORAL PRESENTATIONS (<u>IN TURKISH</u>)</b>	
<b>CHAIR: ASSOC.PROF.DR. HANDAN HILAL (ARSLAN) YAVUZ</b>	
17.35-17.50	EFFECTS OF FEEDING AT DIFFERENT METABOLIC ENERGY LEVELS ON DIGESTIBILITY BODY WEIGHT BODY CONDITION SCORE AND STOOL QUALITY IN DOGS <i>OĞUZHAN KAHRAMAN</i>
17.50-18.05	THE EFFECTS OF ADDING WASTE SESAME SEEDS TO DIETS OF MALE AND FEMALE KARAYAKA LAMBS ON SOME BLOOD PARAMETERS <i>BORA BÖLÜKBAŞ</i>
18.05-18.20	RAW PROTEIN, RAW OIL AND ASH CONTENT OF BLACK SEA ORIGINATED FISH FLOURS <i>SONGÜL ÖZDEN</i>
18.20-18.35	THE BIOCHEMICAL EFFECTS OF STRESS CAUSED BY THE TRANSFER OF LIVE RAINBOW TROUT ( <i>ONCORHYNCHUS MYKISS</i> ) <i>UTKU DURAN</i>
18.35-18.50	THE EFFECT OF MERCURY, COPPER AND ZINC ON PARAOXONASE (PON) ENZYME ACTIVITY IN BONITO ( <i>SARDA SARDA</i> ) FISH <i>BÜŞRA ŞAHİN</i>
18.50-19.00	<b>BREAK</b>
<b>SESSION XVI: ORAL PRESENTATIONS (<u>IN TURKISH</u>)</b>	
<b>CHAIR: ASSOC.PROF.DR. HANDAN HILAL (ARSLAN) YAVUZ</b>	
19.00-19.15	POSTER SESSION Q&A

*The ethical, scientific and legal responsibility of the articles belongs to the authors.*



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## **Importance of Monitoring Calving To Decrease Stillbirth Rate in Dairy Farms**

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### **Summary**

Successful genetic selection for higher milk production has caused a dramatic decline in the reproductive performance of dairy cows all over the world. Achievement of optimum herd reproductive performance requires concentrated management activities especially during calving and during the first 100 DIM. The following management activities are needed to pursue during the early postpartum period to reach or approach the optimal reproductive performance such as careful surveillance and assistance at calving, prevention of post parturient metabolic diseases, early diagnosis and treatment of post parturient uterine diseases, accurate detection of oestrus, correct timing of insemination, reducing the effect of heat stress and early pregnancy diagnosis. Among these main activities only careful surveillance and assistance at calving and their effects on reproductive performance as well as on newborn calves are discussed, because this is one of the most important management activities in a dairy farm whereas its absence could lead to a dramatic increase in the number of stillbirths and vulvovaginal lacerations, as well as the number of stress-induced retained foetal membranes and consequent clinical metritis/endometritis. Since the cause of stillbirth with a non-infectious aetiology is likely to be multifactorial and difficult calving may explain only about half of them therefore it is very important to examine the risk factors of stillbirth especially in large-scale dairy farms. While it is not possible to eliminate dystocia, adequate management of growing heifers and close observation during calving are essential for reducing the stillbirth rate. Since in many cases there are no visible clinical signs of the onset of calving, therefore especially in a large dairy farm it is difficult to recognize it.

Many protocols have been suggested to predict the exact time of calving by measuring hormonal changes, or/and evaluating clinical signs (relaxation of pelvic ligaments, decrease in body temperature), recording feeding and rumination behaviour before calving, and determining the electrolyte concentrations in mammary gland secretion. Although these methods may help predict the exact time of calving, inaccuracy and practical limitations of some methods may limit their use in the field. On-farm devices like inclinometers and accelerometers detecting tail raising and behavioural changes, abdominal belts monitoring uterine contractions, intravaginal thermometers detecting a drop in body temperature and/or the expulsion of the allantochorion, and devices fixed in the vagina or at the vulvar lips signalling calf expulsion via SMS are currently marketed for automated calving detection. There is a paucity of information regarding the performance of these devices on commercial

dairy farms, therefore we have tested the effectiveness of an intra-vaginal thermometer in predicting calving in the field as well as determined its impact on the health of dams and newborn calves. By using a vaginal thermometer (Vel'Phone, Medria, Châteaugiron, France) from about Day 5 before the expected calving stillbirth rate, delayed calving assistance and its consequences can be decreased by alarming via SMS about the onset of calving. It seems that the target prevalence of stillbirth (<3%) can be achieved in the field by using such a device.

**Keywords:** Dairy cow, monitoring calving, stillbirth

## **Metabolic Profiles in Ruminants**

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### **1. Introduction**

Animal production, as we understand it today, is supported by five aspects, which are: zootechnical management, nutrition, health, reproduction and genetics. All of them must be aimed towards obtaining efficient and profitable animals in any production system.

The Pathology of Nutrition deals with those morbid processes that have their origin in alterations in animal nutrition, understood as the function of the organism that consists, on the one hand, in incorporating the exogenous products (nutrients) that it needs for the maintenance of body structures, and on the other hand, the necessary energy to perform the functions entrusted to it.

Today its great importance lies on the one hand, in the strong specialization of current livestock and its close relationship with modern animal production techniques and, on the other hand, in livestock, whether they are extensive livestock, with the close dependence It is established between the animal and the characteristics of the place where it grazes, or in intensive livestock farms, where the increase in production prevails.

Intensive livestock farming has imposed severe efforts on the metabolism of the animals to obtain the maximum production at a minimum cost. For this, new feeding methods have been incorporated into livestock farming, and sometimes unconventional handling procedures. Under these conditions, the problem of production diseases is very serious.

### **2. What are metabolic profiles?**

Metabolic profiles (PM) are tools that "allow us to determine elements related to the normal functioning of animal metabolism, directly or indirectly expressing their circulating level in blood, plasma or serum".

The term metabolic profile was proposed by Payne et al (1970) when referring to the study of different blood parameters carried out in dairy cattle in order to evaluate, diagnose and prevent metabolic disorders. Of course this has been taken to other animal species, and it can be spoken about metabolic profiles in sheep, goats and even in companion animals (dogs and cats).

The metabolic profile can be useful to study the nutritional balance of herds, since, in some situations, it can lead to nutritional imbalances in the concentrations of some metabolites, both in blood and in

other biological fluids, such as milk, urine or saliva (Contreras, 2000). However, the metabolic profile is not a nutritional test, since the metabolites are not indicators of the nutritional condition of individuals, but rather indicate when the condition of homeostasis has been altered, being, therefore, indicators of the metabolic balance. of animals (Wittwer, 2000). This information, which was initially aimed at ruminant farms, and especially dairy cows, can be aimed at any other type of livestock farm.

Clinical profiles are a valuable diagnostic tool that can be used to evaluate various body systems. When used in conjunction with medical history, physical examination and other laboratory tests (e.g. complete blood count, urinalysis, etc.) can be helpful in establishing baseline parameters for a patient (or group of patients), the formulation of a problem or an exclusion list, which helps confirming a diagnosis, determining the prognosis and the planning of therapeutic options and response to treatment.

We must also know that the results of the metabolic profiles have no value by themselves, but we must assess all those circumstances that encompass animals such as food analysis, their use, the general state of the animals, behaviour, the handling, its production etc. Although these tests may have a low specificity, they will serve as a first warning sign of a possible problem so that, in case of detecting a change, the pertinent diagnoses can be made and thus correct the situation.

One of the greatest difficulties in using this tool is its interpretation, due in many cases to the lack of adequate reference values. We cannot forget that the different metabolites present variations in the reference values between racial groups, times of the year, sex, different ages, production and management phases, etc., although normally they are not of sufficient magnitude to prevent their use as comparison values.

### **3. What are they used for?**

The routine use of metabolic profiles is a reality in developed countries and should be considered whenever it is intended to increase the productive efficiency of livestock. These analyses serve as a first warning sign in the face of a problem, they allow to reach the opportune diagnoses and, above all, they help to correct or take measures in the face of a possible problem.

In ruminants, and therefore in cattle and sheep, they can be useful not only to check the productive (and even reproductive) potential of the females (including also of the rams), but it is also useful to detect multiple diseases. In collaboration with the clinical signs, we can diagnose ketosis, gestational toxemia, white muscle disease, hypoglycemia of calves or lambs, milk fever (puerperal or not), hypomagnesemia, enzootic ataxia, etc. to name a few of them. And what is more important: the subclinical manifestations of these deficiencies, either before these signs appear or even when they are absent. They also serve us to detect possible intoxications or diseases with primary aetiologies of parasitic or infectious origin.

Basic goals of a metabolic profile:

- evaluate the nutritional metabolic condition of a group of animals,
- diagnose the existence of possible metabolic disorders in a herd,
- maintain a record of the metabolic balance and the health condition of the herd,
- evaluate the response to the measures proposed in the face of a certain problem,
- serve as a metabolic assessment tool in some trials or research.

As we have already commented, a metabolic profile does not constitute a nutritional test, but it does help us to indicate an alteration in the homeostasis capacity, therefore, a metabolic profile is a complement of indications for the nutritionist and serves to guide the veterinary doctor in their decisions. Metabolic profiles are faster and more specific than other data (milk production, body condition score, reproductive rates, fertility, etc.) to evaluate a feed. In addition, they are used to assess the influence of food on certain pathological processes, such as bovine ketosis, ovine pregnancy toxemia, mastitis, infertility, etc.

When the indicators studied in the metabolic profile are outside the reference interval, it is a clear manifestation that the herd should be studied more extensively, to establish feeding corrections or herd management, thus avoiding a decrease in production, fertility and therefore the profitability of the livestock business. The veterinarian or technician in charge of the herd will judge the significance they may have detected or the changes in relation to the problems presented, taking into account the background, production and management of the herd.

Another utility, perhaps outside its application for the clinical veterinarian, and within the field of research, is aimed at setting the reference values in autochthonous or not specially studied breeds, and which may be of great interest in the future for its application. In that same breed or in other cattle groups. In most developed countries, and for several decades, an important concern has been raised about the disappearance of native animals, raising important efforts from both civil society and public administrations to ensure their preservation. The establishment of the physiological constants of these racial groupings is of utmost importance to compare the values obtained with those animals that can be separated from normality and thereby know and establish the pathological situations.

The request and performance of metabolic profiles are especially recommended when any of the following circumstances exist:

- high incidence of metabolic disorders, and especially if there is no justification for it
- problems in the quantity or quality of the milk produced, by an animal or by the whole herd



-control of metabolic balance: in protein and energy

-diagnosis and evaluation of possible mineral deficiencies

-investigation of fertility problems, especially if there is no infectious, parasitic or management justification that can justify their cause.

#### **4. Components and interpretation of a metabolic profile**

There is no rigid rule to determine what a certain metabolic profile should be composed of, but rather each professional must determine which is the one that best suits the species studied, a specific breed, and even a certain problem that has appeared in that livestock.

Thus we speak of a general (or basic) profile, or of specific profiles (renal, geriatric, hepatic, gastrointestinal, pancreatic, thyroid, presurgical, etc.), and especially in companion animals; while in production animals it is usually spoken of the complete profile, the profile of downer cow syndrome, impaired fertility in cattle, mineral profile (with different versions), muscular profile, etc.

In most cases, a general profile is requested rather than individual tests, especially when the animal (or collective) is treated for the first time. In general, it is more cost effective to request a general profile rather than running individual tests.

##### ***4.1. Components of a metabolic profile***

Although traditionally metabolic profile is synonymous with biochemical profile, in our opinion the term metabolic profile is broader and should include, in addition to a quantification of haematological parameters, the determination of various biochemical values, including the determination of substrates, enzymatic parameters, macro and microminerals and even the determination of other parameters such as gases and vitamins. In my opinion, we should always start with haematology, later moving on to those simpler parameters at the biochemical level and including macrominerals. Later, and if necessary, we will go to more complicated tests (and sometimes much more expensive), such as certain enzymes, micro-minerals or vitamins.

As we said, the profiles to be evaluated can be multiple, and depend on each specific case. In general, a complete profile should assess some or more of the following parameters:

-haematological analysis: assessing both red and white cells and platelets.

-check the energy balance: glucose,  $\beta$ -hydroxybutyrate, non-esterified fatty acids (NEFA) and cholesterol.

-evaluation of protein (and kidney) metabolism: total proteins, albumin, blood urea nitrogen, creatinine, and CK.

-evaluation of liver function: AST, ALT, GGT, LDH, IDH, OCT, GDH, direct and total bilirubin, and cholesterol.

-evaluation of macrominerals: Ca, P, Mg, Na, K, Cl and S.

-evaluation of trace elements: even though a lot of them can be evaluated, it is at least recommended to measure Se, Cu, Co, Mn, Mo, Fe, Zn, I.

-evaluation of the acid-base balance: pH, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, TCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and anion gap, as well as lactate.

-sometimes it is necessary to assess the level of vitamins (fat-soluble and water-soluble), although this assessment is complicated and there is not always availability for it.

#### ***4.1.1. Hematological parameters***

The complete blood count, or hemogram, is a test that provides great help to the clinician, since it obtains:

-cells of the red series (erythrocytes), which provide information about the red cell count, the percentage of hemoglobin and the hematocrit, to determine if the patient is anemic. In addition, it allows calculating the red cell indices, to classify the type of anemia. Another important aspect is that the shape of red blood cells can be observed, making it possible to detect diseases that produce alterations in their morphology.

-cells of the white series (leukocytes), both in absolute and differential value, and whose increase or decrease could allow the etiology of the process.

-platelet cells, their count allows establishing the diagnosis, and the stage in which the disease is, for example in parasitosis and also knowing the cause of excessive bleeding or blood clotting.

#### ***4.1.2. Biochemical and mineral parameters***

Although there are some variations between laboratories, a biochemical profile in ruminants generally includes the following analytes and enzymes:

-parameters such as glucose, serum proteins (total protein and albumin), lactate, blood urea nitrogen (BUN), creatinine, electrolytes (sodium, chloride and potassium), total carbon dioxide (TCO<sub>2</sub>), enzymes (alkaline phosphatase [ALP], gamma-glutamyl transferase [GGT], aspartate aminotransferase [AST or GOT], alanine aminotransferase [ALT, or GPT], L-idoitol dehydrogenase [IDH or sDH], ornithine carbamoyltransferase [OCT], glutamate dehydrogenase [GDH] L-lactate dehydrogenase

[LDH], creatine kinase [CK]) bilirubin, minerals (calcium, phosphorus and magnesium, and other trace elements).

-other analytes that can be analyzed by certain laboratories but are not always part of a routine biochemical profile may include bile acids, ammonia, cholesterol, ketone bodies (BOHB), and non-esterified fatty acids (NEFA).

-gas and electrolyte analyzes are useful to assess acid-base balance, and indirectly cardiopulmonary function and kidney function.

Although there is some overlap with certain analytes, a practical approach to assessing a metabolic profile is to group the analytes by body system and interpret the results together.

Wittwer (2012) suggests as an analysis to assess the energy balance in dairy cows the observation of the body condition score, together with the determinations of BHB and urea in blood and milk samples, and, in addition, cholesterol and serum AST. For his part, Conteras (2012) think that the most widely used metabolites are urea, total proteins, albumin (sometimes globulins), hemoglobin and/or hematocrit (PCV).

#### ***4.2. Interpretation of a metabolic profile***

In our opinion, the correct interpretation of a metabolic profile is the most difficult aspect to carry out, since the veterinary professional must judge the significance of the alterations found, and considering the antecedents, production and management of the herd, relating them to the problems presented. According to Wittwer (1995) it is necessary to have an adequate knowledge of the physiology and biochemistry of the metabolites evaluated, and that it is necessary to take into account that the presence and concentration of different metabolites have no meaning unless their origin and function are correctly understood.

An alteration is considered significant when:

- the average of a variable in a group is twice the standard deviation of the population mean,
- the percentage of individuals in a group of animals with abnormal values ??in one variable is greater than 19%,
- the standard deviation is larger than the reference, due to the high variance of the group.

One of the most important concepts in laboratory medicine is the so-called reference interval. The reference interval represents the values that would be expected in a healthy animal and is necessary to correctly interpret the results found in the patient. The use of terms such as normal, normal range, or normal value is discouraged for several reasons. A value may fall within the reported reference

interval (ie, it appears to be "normal"), but may not be appropriate for that patient's specific condition and may indicate a possible disease state. In addition, there is difficulty in defining what is truly normal.

Some clinical pathologists also discourage the use of the reference range, because the statistical definition of "range" is the difference between the lowest and highest number; therefore, in fact that difference may represent a unique number, which is not appropriate for its interpretation.

The reference interval is based on a large number of samples obtained from healthy animals (the reference population) and is theoretically calculated to include 95% of a healthy population. In general it is recommended that at least 60 clinically healthy animals have been used to establish a reference interval. This reference population should be selected based on predetermined clinical criteria, such as species, race, age, sex, stage of gestation or lactation, etc. and it must be representative of the investigated animals.

However, it must be taken into account that they are sometimes of limited use, such as many reference intervals published in textbooks or scientific articles, and that they have been established for "cattle" or "sheep. They often do not distinguish between dairy farmers or meat animals, sex, age or stage of lactation or pregnancy. However, if reference intervals, specific to a certain group of animals, are not available there is no other option than to use those intervals.

Sometimes it is very interesting to take into account those values previously obtained from a certain patient (or a group of animals), which are especially useful to assess those found at a later time.

The professional must decide whether or not the detected alterations are transcendent for all the animals and assess all the parameters measured as a whole, or even in all the animals sampled. We cannot forget that some metabolites present variations in the reference values depending on multiple circumstances, such as breed, time of year, production phases or management. And there are even increases, or decreases, which should not be given importance in the context of the disease we are trying to diagnose, and those changes are "almost anecdotal." Therefore, it is necessary to consider when an alteration is significant, interpreting the test result in the context of that case and never in isolation.

When performing a panel test, some value may be outside the reference values, but caution is advised when interpreting a single value (or a few values) that falls slightly outside of that interval. And furthermore, if this value does not seem reasonable in the context of the case, it may not be correct and may have been modified due to poor handling of the sample, inadequate conservation or even a failure in the measurement technique.

It should always be borne in mind that the reference interval is intended to include 95% of the healthy population; potentially 5% of the population have values outside the interval for a single test. When running a panel of tests, the probability that at least one test is outside the reference interval is much greater than 5%; therefore, caution is advised in overinterpreting a single value that falls slightly outside the reference interval. Therefore, if a laboratory value does not seem reasonable in the context of the case, it may not be correct, and it is advisable to call the laboratory, repeat the measurement or present a new sample. It is even possible that a certain value has been modified due to poor handling of the sample, inadequate conservation or even a failure in the measurement technique. A clear example is glucose: if we find low blood glucose values in several samples, it is possibly due to glucose consumption by erythrocytes, other cells or bacteria, and they do not allow us to diagnose hypoglycemia at all. It is clearly a defect in the handling of the samples. Something similar happens when different parameters, which should evolve in parallel, do not, indicating a bad management or an incorrect valuation technique.

Another very important aspect is to assess all the parameters measured together, or even in the group of animals sampled. Sometimes alterations in a parameter can be seen in isolation, which should not lead us to make a certain decision.

Changes in the blood concentration of an element are caused not only by variations in its contribution, but also by the effect of other elements, due to the close metabolic interrelationships that exist in the body. Thus, for blood glucose and calcium levels, the hormonal mechanisms of their homeostasis keep their concentration in the blood constant, therefore they are not very sensitive.

Therefore, each value must be interpreted in the context of that clinical case and the results of a certain parameter in a metabolic profile must be interpreted in the context of other panel tests, and incorporating the information obtained into the clinical history and physical examination. Of course we should never make a clinical decision based on the value of a laboratory test that we have trouble believing.

An important aspect is to differentiate the role of the laboratory technician and the role of the veterinarian, or any other professional who interprets these values. The laboratory technician should only make a correct assessment (in the aspect of measuring) these parameters. Always warning of any possible error or failure detected and that is where its function ends. However, the veterinarian must interpret the measured parameters, assessing both the increase and the decrease, both in isolation and as a whole, always taking into account all the circumstances mentioned above.

## **5. Cost-benefit**

In our opinion, the use of laboratory analysis, and therefore metabolic profiles, are always positive, and have a clearly positive impact on the farmer. It is possible that initially it may seem costly

financially, both to the veterinarian and to the producers. Sometimes it is necessary to make an economic outlay that only in some circumstances can be high. Most situations will be considerably less than initially expected if we are able to select the appropriate parameters and animals.

But on the other hand, the advantages of an accurate diagnosis and above all the possibility of establishing measures aimed at solving, or at least minimizing, the morbid process will be highly rewarding and above all with a significant impact on animal production.

We must not forget that Production Medicine is directed towards two fundamental aspects, on the one hand towards a preventive aspect, and on the other hand towards the derived economic aspect:

-prophylactic purpose. In ruminants, metabolic imbalances should not be considered sporadic, but collective and should be based on production control (liters of milk/protein concentration, fat/cow and year, lambs or calves born, calving interval, etc.). For this reason, resources must be oriented towards prophylaxis, since the metabolic profiles establish a series of markers that indicate the suitability of the ration at each productive moment.

-economic purpose. It is very important, since we must not forget that to achieve higher economic returns it is necessary to optimize the acquisition of nutritional principles, and that many times malnutrition can border on both excess and defect.

## **6. Differences between parameters and differences between species**

As we have indicated, the differences between the measured parameters could be explained by multiple factors, including the existence of a certain disease, but we must not forget that differences may appear even within the same species. Therefore, we must take the reference values based on other variables such as age, sex, management or feed.

There are several publications where the values of most of the haematological and biochemical parameters are recorded. It is essential to take into account the species in which they are measured, since the general values do not apply to any species. For example, the glycemia of ruminants is totally different from monogastric ones, or certain parameters such as NEFA are evaluated in ruminants with little importance in monogastric ones. The activity of the enzyme alanine aminotransferase (ALT, formerly serum glutamic-pyruvic transaminase or GPT), is commonly used to categorize a possible hepatocellular lesion in companion animals, while in ruminants it is low and, therefore, in these species, not it should be used to evaluate liver disease.

One aspect, in my opinion determining, is how that measured variable participates in the physiology of that particular animal. In monogastric animals it is important to assess blood glucose (and if possible fasting), while in adult ruminants it is usually much more interesting to assess NEFA, ketone bodies or even cholesterol. With regard to enzymes, we must bear in mind that these are released into the blood

when the cells that contain them are “broken”. Thus, and incorrectly, we speak of liver enzymes (AST, ALT, GGT, FA, LDH, etc.) or muscle enzymes (AST, LDH, CK, etc.). Therefore, these names "liver" or "muscular" should not be used, since they are not only specific to those locations. AST and LDH are released into the plasma as a result of muscle injury, but they are not muscle-specific enzymes, whereas serum CK, on the contrary, is a sensitive and specific indicator of muscle injury, and subtle or very high increases may occur. extensive activity as a result of intramuscular injections, intense exercise, and in trauma and muscle disease.

## **7. How to interpret these values in ruminants?**

### **7.1. Hematological parameters**

Changes in the components of circulating blood cells can support suspicious diagnoses and can sometimes provide a definitive diagnosis, for example, in hemoparasites. They are frequently used to indicate inflammatory processes and suspected anaemia. The total red and white blood cells, the number of each cell type, the percentages and some calculated indices can be easily evaluated in blood samples collected using EDTA as an anticoagulant.

Red blood cell count (RBC), haemoglobin or packed cell volume (PCV), as well as red blood cell indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW), and morphological changes of red blood cells are useful for diagnosing and evaluating anaemia. Anaemia is defined as a reduction in total blood cells red blood cells, PCV or haemoglobin concentration, while the red cell indices allow to qualify the type of anaemia. Generally speaking, there are two common causes of anaemia in cattle: blood loss and haemolysis. Traumatic injuries, including obstetric complications, gastric bleeding are responsible for most cases of blood loss and it is usually clinically evident. In contrast, haemolysis is often clinically inapparent and may be associated with the common copper toxicity in sheep or with certain hemoparasitoses, such as *Trypanosoma spp*, *Babesia spp* and *Anaplasma spp*.

Polycythaemia is rare in cattle and sheep, and is usually associated with dehydration, hypoxia, tumours, etc. In dehydrated animals, increased red blood cell counts, Hb and PCV values may occur, the evidence of which is clinically confirmed by persistence of the skin fold, dry mucous membranes, and bulbar retraction and, biochemically, by an increase in total protein (TP) and urea.

Although the total leukocyte count provides invaluable information, the differential white blood cell count (WBC) is more important than the total number, since increases and decreases in the different types of individual cells can occur simultaneously, and thus does not change the total count.

The blood smear is the essential complement for interpreting cell counts, since it allows the detection of morphological abnormalities and the presence of haemoparasites.

### **7.2. Glucose**

Glucose metabolism is unique in ruminants, as these animals do not directly absorb preformed glucose from the intestine. Thus the reference intervals for serum glucose in ruminants are lower than in monogastric ones, and they are also lower in adult ruminants compared to young animals. Red blood cells metabolize glucose *in vitro* at a rate of approximately 10% per hour at room temperature, so the serum must separate from the clot within 30 minutes. Another possibility is to use tubes with sodium fluoride when rapid clot separation is not possible. The reference interval for glycemia in sheep and cattle is [50-80] and [45-75] mg/dl, respectively.

A hyperglycemia appears after stress (including self-sampling management, excitement, fear, and intense pain); or after the administration of drugs such as xylazine, corticosteroids or solutions containing dextrose; and in dying animals. Hyperglycemia can also be seen in sheep with transport enterotoxemia, listeriosis, or tetany. Hyperglycemia can be accompanied by transient glycosuria. Although quite rare, diabetes mellitus has been reported in cattle and sheep with persistent hyperglycemia and glycosuria. Unlike other species, postprandial hyperglycemia is minimal or absent in adult ruminants due to the continuous fermentation of carbohydrates in the feed, due to the effect of ruminal microorganisms.

Hypoglycemia can be observed with ketosis, pregnancy toxemia in ewes, as well as in malnourished states, in septicemia and in terminal states, and mainly in calves and young lambs. In ovine pregnancy toxemia, hyperglycemia has also been described in some stages of the disease.

### **7.3. Serum proteins**

Besides the absolute value of proteinemia, the two main protein components are albumin and globulin. Albumin is synthesized in the liver and is primarily responsible for the oncotic pressure of plasma. A large part of the globulin fraction is made up of immunoglobulins, which are synthesized by lymphoid cells and the liver.

Chemical profiles usually include total serum proteins and albumin concentration; the globulin fraction is calculated by subtracting albumin from total proteinemia. The reference values for total proteins are [60-70] and [67.4-74.6], for albumin [24-30] and [30.3-35.5] and for globulins [35-57] and [30-34.8] g/L, in sheep and cattle respectively. The albumin-globulin ratio (A/G ratio) is usually constant in healthy cattle (interval reference: 0.84-0.94). Since most of the established reference values are for serum proteins rather than plasma proteins, serum is the sample of choice to evaluate this



parameter. It is important to remember that serum does not contain fibrinogen or other clotting factors; however, plasma does.

Although hyperproteinemia is theoretically the result of increased levels of albumin, globulin, or both, the only cause of hyperalbuminemia is dehydration. In a dehydrated animal, albumin and globulin concentrations increase, but whether they exceed the reference values is determined both by the degree of dehydration and by the initial proteinemia. Hyperproteinemia without concurrent dehydration is almost always the result of hyperglobulinemia. Common causes of hyperglobulinemia include chronic antigenic stimulation (ie, chronic inflammatory disease) and liver disease. With chronic antigenic stimulation, the A/G ratio tends to decrease, because the increase in the globulin fraction is usually accompanied by a small decrease in the albumin fraction. The decrease in albumin concentration may be more substantial in chronic liver disease, causing a more substantial decrease in the A/G ratio.

Hypoproteinemia, in adult ruminants, is usually the result of hypoalbuminemia or panhypoproteinemia. Hypoalbuminemia occurs when liver production of albumin is insufficient or when there is an excessive loss of albumin. Underproduction occurs in animals with severe chronic liver disease or as a result of inadequate protein intake, digestion, or absorption. Since albumin has a half-life of around 20 days and the reserve capacity of liver tissue is very large, liver disease must be chronic and severe to produce hypoalbuminemia. Hypoalbuminemia is also often attributed to loss of albumin through the kidney (particularly the glomerulus), the gastrointestinal tract, or in bleeding and exudation. In many cases, there may be a simultaneous loss of albumin and globulin, resulting in panhypoproteinemia. When hypoproteinemia is detected, further evaluation should include a urinalysis to look for proteinuria or haematuria.

Newborn ruminants, especially those that have not ingested colostrum, tend to have lower total protein concentrations than adults. In colostrum-deprived infants, endogenous globulin production begins immediately and serum globulin concentration increases steadily from birth. Although inappropriate passive transfer should be investigated in neonates with a serum protein less than 5.2 g/dL.

#### ***7.4. Ketone bodies ( $\beta$ -OHB) and unesterified fatty acids***

Beta-hydroxybutyrate ( $\beta$ -OHB) is a ketone body that is produced mainly in the liver, and that has its origin in the degradation of long-chain fatty acids through the tricarboxylic acid cycle. It is generally used to assess energy status in ruminants, and its increase is associated with a negative energy balance, and clinical ketosis, multiple gestations or ovine pregnancy toxemia occurs at concentrations above the reference interval.

Non-esterified fatty acids (NEFAs), also called free fatty acids (FFAs), are long-chain monocarboxylic acids, which generally contain 16 or 18 carbon atoms, although they can have as few as six carbon atoms. NEFAs should not be confused with volatile fatty acids (VFAs), which have short chains and

mainly include acetic, propionic, and butyric acids. Most NEFAs originate from fatty deposits, and their presence in the blood in high concentrations reflects an excessive mobilization of stored fat. They are a particularly useful measure in cows to indicate a negative energy state, and in cows with metabolic problems, for example, cows with left displacement of the abomasum, fatty liver disease or in sheep after periods of prolonged fasting, in advanced gestation (or multiplex) or in pregnancy toxemia.

The interval values cited for  $\beta$ -OHB are [5.73  $\pm$  0.42 mg/dl (0.55  $\pm$  0.04 mmol/L)] and [9.90  $\pm$  1.88 mg/dl (0.41  $\pm$  0.03 mmol/L)] in sheep and cattle respectively, while NEFAs are [30-100 mg/dl] in cattle.

### ***7.5. Electrolytes, anion gap and acid-base balance***

Serum electrolytes are useful in evaluating various body systems and in formulating and monitoring fluid and electrolyte therapy. Those serum electrolytes that are usually included in a biochemical profile are sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ) and  $\text{TCO}_2$ , which is used to calculate the concentration of bicarbonate ( $\text{HCO}_3^-$ ). Although there is a nominal difference between  $\text{TCO}_2$  and  $\text{HCO}_3^-$  the latter being slightly less, they can be considered equivalent. From these values, the GAP anion can be calculated, which is the difference between cations and anions and is calculated using the following formula:  $AG = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{HCO}_3^-])$

These parameters, always in mmol/L, and respectively in sheep and cattle are  $\text{pCO}_2$ : [41,3 $\pm$ 4,7] and [35-44];  $\text{TCO}_2$ : [21-28] and [21,2-32,2];  $\text{HCO}_3^-$ : [17-29] and [17-29];  $\text{Cl}^-$ : [95-103] and [97-11]; lactate: [0,56-2,22] and [1,0-1,33(9-12 mg/dL)]; Na: [3,9-5,4] and [3,9-5,8], and K: [139-146] y [132-146].

Sodium is the main extracellular cation and is responsible for most of the osmotic pressure that maintains the size of the extracellular fluid space (ECF). The sodium concentration, determined from serum, reflects total body sodium because this cation is essentially confined to the ECF. The interpretation of serum sodium should be done with knowledge of the hydration status of the animal. Potassium, the main intracellular cation, is present in the ECF but is tightly regulated, because small changes can have marked effects on organ function. Severe changes can endanger the life of the animal, since both hyperkalaemia and hypokalaemia cause muscle weakness that affects skeletal, cardiac and smooth muscle. Chloride is the main anion in ECF, being an important component in many secretions (sweat, saliva and gastric secretions) such as sodium chloride ( $\text{NaCl}$ ), potassium chloride ( $\text{KCl}$ ) or hydrogen chloride ( $\text{HCl}$ ).

Concurrent hypernatremia and hiperchloridemia generally occur with excessive water loss or sodium retention, as seen with water deprivation or salt toxicity. These electrolyte abnormalities are not

usually present in cases of dehydration, because fluid loss in ruminants often occurs with simultaneous loss of electrolytes.

Hyponatremia and concurrent hypochloridaemia occur with excessive loss of sodium-rich fluids (diarrhoea), kidney failure, or a blocked or ruptured urinary tract. In addition to being hyponatraemic, ruminants with renal impairment tend to be hypokalemic, and it is believed to be the result of a combination of decreased dietary intake, increased saliva excretion, and metabolic alkalosis.

Hyperkalaemia often occurs as a consequence of metabolic acidosis due to the loss of  $\text{HCO}_3^-$  as potassium exits the ICF to the ECF. Therefore, serum potassium is an unreliable index of total body potassium. Calves, lambs, and kids with diarrhea often have metabolic acidosis and may be hyperkalemic, but actually have total body potassium depletion due to loss of fecal potassium.

Severe hypokalemia should be suspected in weak, decubitus adult dairy cattle. These animals are generally in early lactation, have a history of prolonged anorexia associated with refractory ketosis, and have been treated with mineralocorticoids.

Hypochloridemia, hypokalemia, metabolic alkalosis and, to a lesser extent, hyponatremia are typical findings in obstructive gastrointestinal diseases, such as displacement of the abomasum, vagal indigestion, volvulus of the abomasum, intestinal intussusception and cecal torsion, since HCl is sequestered in the abomasum, leading to hypochloridemia, metabolic alkalosis, and secondary hypokalemia.

The  $\text{TCO}_2$  concentration is a reflection of the patient's metabolic acid-base status. Historically, GA, along with  $\text{TCO}_2$ , was used to determine the cause of acid-base imbalance (ie, loss of  $\text{HCO}_3^-$ , accumulation of acids [lactate, keto acids, uremic acids, and toxic metabolites] or a combination of both).

Decreased  $\text{TCO}_2$  concentration indicates the presence of metabolic acidosis and can result from hyponatremia (loss of sodium or excess water balance), hyperchloridemia, hyperproteinemia, or an increase in unmeasured anions. Secretory diarrhoea often results in loss of sodium (in the form of  $\text{NaHCO}_3$ ) in the intestine and is one of the main causes of metabolic acidosis in calves with diarrhoea.

Increased  $\text{TCO}_2$  concentration indicates the presence of metabolic alkalosis. The most common cause of metabolic alkalosis in ruminants is hypochloridemia attributable to chloride sequestration (in the form of HCl) in the gastrointestinal tract, such as right abomasum displacement, abomasal torsion, intestinal obstruction, enteritis or vagal syndrome.

### ***7.6. Lactate***

Lactate is sometimes part of the clinical profile. Lactic acid is produced during anaerobic metabolism, and its accumulation can cause metabolic acidosis with elevated AG.

In adult ruminants, the fermentation of large amounts of carbohydrates induces an increase in the concentration of organic acids that leads to a decrease in intraruminal pH, favouring the excessive growth of some bacteria, which are capable of producing high amounts of D- and L- lactate. Hyperlactatemia occurs in acute ruminal overload, hypovolemic shock, septic shock and when there is a decrease in tissue perfusion, which leads to ischemia and necrosis. Reported reference interval for lactate concentration in cattle are 9 to 12 mg/dL (1-3,33 mmol/L), and 5 to 20 mg/dL (0.56–2.22 mmol/L) for sheep.

The age of the calf must be taken into account when assessing the severity of acidosis. In calves older than 1 week, there is a high production of lactic acid, being both isomers, D- and L-lactate, produced in the gastrointestinal tract of ruminants, by bacterial metabolism on carbohydrates, without harmful consequence for these animals. However, in calves with diarrhoea, there is a similar effect, with high production of D- and L-lactate, probably due to atrophy of the villi, nutrient malabsorption and increased intestinal fermentation by bacteria present in the intestine. Both isomers (L- and D-lactate) are absorbed into the blood, but since ruminant tissues have negligible D-lactate dehydrogenase activity, the elimination of D-lactate occurs mainly through its excretion in the urine. Thus, while L-lactate is rapidly metabolized in healthy animals, diarrheic calves develop metabolic acidosis, mainly caused by increased D-lactate concentrations. Since D-lactate can only be efficiently eliminated by the kidneys, dehydration appears to play an indirect role in increasing D-lactate toxicity. Severe acidosis can be present in sick calves with diarrhoea, with or without dehydration. In addition, if the suck reflex is weak or absent, if the calf is chewing irregularly instead of sucking, if they are down, unstable or unable to stand, or lying down or in a coma, with incomplete palpebral reflex or absent, it is easy for D-lactic acidosis to be present in these animals.

### ***7.7. Liver assessment test***

The cytosol of hepatocytes contains a high activity of the enzymes AST (formerly GOT), L-idoitol dehydrogenase (IDH, formerly sorbitol dehydrogenase [sDH]), ornithine carbamoyltransferase (OCT), glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) . They are generally known as leakage or hepatolysis enzymes, since in acute or chronic hepatocellular injury or necrosis, the serum activity of these enzymes increases because they essentially "escape" from the hepatocytes into the blood. However, increased serum activity of these enzymes is not an indicator of abnormal or impaired liver function.

The reference interval that measures the activity of these and other enzymes is indicated below, always their activity measured in IU/L and in sheep and cattle respectively. ASAT: [60-280] and [78-132], ALAT: [22-38] and [11-40], ALP: [68-387] and [0-488], GGT: [0-32] and [4.3-13.4], CK: [8-12.9] and [4.8-12.1], IDH: [5.8-27.9] and [4.3-15.3], and LDH: [238-440] and [692-1445] UI/L.

The enzymes AST, LDH, and IDH are the most commonly used enzymes to assess hepatocellular injury in ruminants. Increased activity may be observed in infectious, inflammatory, toxic, or metabolic liver disorders, since these enzymes can be sensitive indicators of acute diseases. In contrast, in chronic or slowly progressive liver disease, enzyme activity may be within or below the reference interval, because very few hepatocytes are being damaged or the hepatocellular mass is substantially reduced.

The hepatocyte activity of alanine aminotransferase (ALT, formerly GPT), which is routinely used to evaluate hepatocellular injury in companion animals, is low in ruminants and is therefore not useful in evaluating liver disease.

It is important to note that AST and LDH are found in a wide variety of tissues, the most important of which are the liver and muscles. Red blood cells also contain high activities of these enzymes. Muscle injury or necrosis, especially in lying animals, can produce marked increases in AST and LDH activities. To determine the source of tissue damage, when these enzyme levels are abnormal, they must be evaluated in conjunction with other liver-specific enzymes (eg IDH, GGT) and muscle-specific enzymes, such as CK. Increased AST or LDH activity, with CK values falling within the reference interval, invites us to think about liver disease; whereas if CK increases, a muscle injury should be suspected. When serum remains in the clot too long or the sample is haemolyzed, AST and LDH can be falsely elevated due to erythrocyte leakage.

The HDI, formerly sDH, is a sensitive and specific indicator of acute hepatocellular damage in ruminants; however, its usefulness is limited due to its relative instability. The serum IDH activity drops significantly after 24 hours if the sample is refrigerated or after 72 hours if it is frozen; therefore, the delivery of samples to the laboratory must be expeditious. IDH activity after hepatocellular injury is characterized by a rapid and steep rise, followed by a rapid decline; therefore it is not useful as an indicator of chronic liver disease. Increases in IDH activity can also be associated with obstructive gastrointestinal lesions or acute toxic enteritis. Under these conditions, bacteria or bacterial toxins from the compromised gut are absorbed into the portal circulation and can potentially lead to liver damage and release of IDH.

Cholestasis results in increased production of GGT and serum alkaline phosphatase (ALP) by hepatocytes. The activity of these enzymes increases as a result of biliary obstruction caused by conditions such as fascioliasis or cholelithiasis, which is why they are called cholestasis enzymes.

Cholestatic enzymes are likely to have higher serum activity in chronic liver disease than leakage enzymes, due to bile duct injury or obstruction secondary to fibrosis.

Although GGT is found in many tissues, it is considered liver specific and is probably a better indicator of liver disease than ALP in ruminants. Essentially, all serum GGT activity originates from the bile and hepatocellular membranes and is an important indicator of hepatobiliary disorders and cholestasis in ruminants. GGT is also present in the epithelial cells of the renal tubules. Because GGT has a longer half-life, its activity tends to decline less rapidly than IDH and may be more valuable in identifying ruminants with chronic liver disease.

GGT is also present in mammary epithelial cells and colostrum from cows and sheep contains high GGT activity. Therefore, serum concentrations increase dramatically after consumption of colostrum. At 24 hours after ingestion of colostrum, serum GGT activity increases 50 to 100 times compared to serum from colostrum-deprived calves. Serum GGT is a useful estimate for the success of passive transfer, but not for the detection of liver disease in newborn calves.

ALP, being a useful indicator of liver or cholestatic disease in companion animals, is often included in the biochemical profiles of ruminants. Only slight increases in ALP activity occur in cattle with liver disease, and their reference values tend to be quite wide. For these reasons, ALP has limited diagnostic value for hepatobiliary disease in ruminants. Increases in ALP activity are associated with increased osteoblastic activity that occurs in growing young animals or, occasionally, in nutritional secondary hyperparathyroidism.

Bilirubin and bile acids have also been used to assess liver function. Bile acids are synthesized in the liver from cholesterol, secreted in the bile, reabsorbed in the intestine, eliminated from the portal circulation, and recycled by the liver. There is a prominent postprandial increase in monogastric species. In the fasting state, elevated levels of bile acids indicate liver disease and decreased hepatobiliary function. No constant increases are observed in healthy ruminants; in which a single sample is usually collected because the importance of a fasting sample is questionable. Bile acids have been shown to increase in ruminants with liver lipidosis, liver abscesses, leptospirosis, fascioliasis, and gallstones. Reference intervals in ruminants tend to be quite large, and some pathologists for this reason believe that bile acids are an insensitive indicator of liver disease.

Bilirubin is a breakdown product of haemoglobin. In the blood, unconjugated bilirubin bound to albumin is transported to the liver, where it is conjugated, to be subsequently excreted in the bile. In healthy cattle, the total bilirubin concentration is low compared to other species, and the magnitude of the increase tends to be relatively small and inconsistent, even in severe liver disease. Ruminants with cholestatic liver disease may have moderate hyperbilirubinemia; however, it is not a consistent finding either. Increases in unconjugated bilirubin are the result of rapid degradation of haemoglobin, which

can be seen with acute haemolysis. Conjugated or direct bilirubin accumulates in plasma with intra- or extrahepatic biliary obstruction. Significant bilirubinaemia and jaundice in ruminants are almost always the result of haemolysis.

Ammonia is produced in the gastrointestinal tract from the digestion of proteins and amino acids, it is absorbed in the intestine and transported to the liver through the portal circulation. In the liver it is converted to urea through the hepatic urea cycle. Although it is a good test to assess liver function, the measurement of ammonia is not included in the usual profiles due to its volatility and the need for special handling of the sample. Because urea is made in the liver, BUN can also be used to assess liver function. In severe liver failure or partial vascular abnormality, BUN is low and ammonia is high, however, decreased BUN is not always specific for liver failure. Ruminal microbes synthesize proteins from urea, but in anorexic ruminants or with low protein diets they can use any available urea, and therefore the BUN can decrease. In cattle, increased ammonia concentration is observed in urea toxicosis.

#### **7.8. Renal assessment test**

The elimination of nitrogenous wastes, the ability to concentrate urine, and the regulation of the acid-base state are vital functions performed by the kidney. Urea is generated in the liver by the urea cycle, through the detoxification of ammonia, a by-product of protein catabolism and, therefore, is also influenced by diet and liver function.

The reference values for creatinine are [1.2-1.9 mg/dl] and [1.2-2.0 mg/dl (88.4-177  $\mu\text{mol/L}$ )], and for urea/BUN [8-20 mg/dl, BUN; 3-10 mmol/L, urea] and [20-30 mg/dl, BUN; 7.14-10.7 mmol/L, urea], in sheep and cattle respectively. Urea can be calculated by multiplying the BUN x 2.4.

Creatinine is the result of normal muscle metabolism and is a breakdown product of creatine. Creatinine is freely filtered through the glomerulus and excreted by the kidneys. Creatinine concentration is minimally affected by diet or protein catabolism but may be slightly affected by muscle injury.

Serum or blood urea nitrogen (SUN or BUN) and serum creatinine are used to estimate glomerular filtration rate. In ruminants, urea is recycled in ruminal functioning, which reduces the usefulness of BUN in kidney disease. Creatinine tends to be less influenced by foreign factors, which is why creatinine is preferred over BUN to assess the renal function of ruminants. However, due to the large reserve capacity of the kidney, elevations in BUN and creatinine are generally not observed until approximately 75% of functional renal mass is lost. The increase in BUN in ruminants is an indicator of an excess of degradable protein in the rumen and in dairy herds, a mean BUN greater than 20 mg/dL may indicate that it is being fed with high protein diets and the ration should be re-evaluated.

Abnormal urine concentrating ability occurs when about 66% of nephrons stop working; therefore, urine specific gravity (USG) is more sensitive than BUN or creatinine in assessing kidney function. A USG of 1,025 or more is considered indicative of adequate renal concentrating capacity in the face of dehydration or azotaemia. However, it is quite common for normally hydrated ruminants, and especially dairy cattle, to have a USG of less than 1,025.

Azotaemia, or accumulation of nitrogenous wastes in the blood, is reflected in the biochemical profile by increases in BUN and creatinine concentrations. Possible causes of acute kidney failure include hypoxia or ischemia, infectious agents or inflammation, toxins (metals [copper, mercury, and arsenic], plants [oak/acorn accumulators and oxalate], and ethylene glycol), or drugs (antibiotics: tetracyclines, aminoglycosides, and sulphonamides) and NSAIDs, which if overdosed or administered to a poorly hydrated animal can cause acute tubular necrosis. Sheep are the most susceptible to copper toxicity, followed by goats and cattle, and animals with copper toxicity can present a haemolytic crisis with anaemia, haemoglobinuria, and azotaemia.

Although BUN, creatinine, and USG can be used to identify kidney failure, the underlying cause cannot be identified solely from this information, requiring other techniques such as complete urinalysis and rectal palpation of the kidney. kidneys. Ultrasound and kidney biopsy can also help in the diagnosis.

### ***7.9. Muscle enzymes***

As discussed, AST and LDH are released into plasma as a result of muscle injury, but they are not muscle-specific enzymes. Serum CK, on the other hand, is a sensitive and specific indicator of muscle injury. Subtle increases in CK can occur as a result of intramuscular injections or vigorous exercise. However, adult cattle in decubitus can show increases of more than 100 times, due to permanent decubitus damage, as in drooping cow syndrome. And even extraordinarily high values have been verified in bulls after exercise and intense stress. Very high CK activities in the absence of decubitus or in recumbent young cattle suggest a primary myopathy, such as white muscle disease in lambs. The half-life of CK in serum is short, approximately 4 hours, and its activity decreases rapidly in animals as the muscle lesion disappears.

The half-life of AST is much longer (at least 20 hours), and therefore the activity of AST increases and decreases more slowly; thus AST can be used in combination with CK to stage muscle injury. In animals lying down, if the AST activity is greater than that of CK, the damage probably lasts several days.

Although ALT is quite liver-specific in companion animals, ruminants have low ALT activity in hepatocytes, resulting in increased activity in skeletal muscle, resulting in increased ALT activity in certain myopathies.



**7.10. Macrominerals (calcium, phosphorus and magnesium)**

The levels of calcium, phosphorus and magnesium are influenced by the same homeostatic mechanisms; therefore, changes in their serum concentrations may be interrelated. Certain processes that occur with decubitus or difficulties in locomotion justify the evaluation of the three minerals. The blood levels of each of them comprise only a small fraction of the total content; therefore, serum concentration is an unreliable measure of body state.

Total serum calcium, both in ruminants and in other species, is distributed in approximately 50% ionized calcium (the biologically active form), 40% non-ionized calcium (the highest bound to albumin) and 10% complexed with anions. Approximately 55% of total serum magnesium is present as an ionized or free fraction, 30% is present as a protein-bound fraction, and 15% complexes with various anions and acids. Serum phosphorus is present as dissociated phosphoric acid ( $\text{H}_2\text{PO}_4$ ) that exists in three forms:  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$  and  $\text{PO}_4^{3-}$ . Reference values for these macrominerals are Ca: [11.5-12.8 mg/dl] and [9.7-12.4 mg/dl (2.43-3.10 mmol/L)]; P: [5-7.3 mg/dl] and [5.6-6.5 mg/dl (1.81-2.10 mmol/L)] and Mg: [2.2-2.8 mg/dl] and [1.8-2.3 mg/dl (0.74-0.95 mmol/L)], the first value being indicated for sheep and the second for cattle.

The degree of hypocalcaemia cannot be used consistently to predict when clinical signs will appear. Ataxia or decubitus usually occurs when the serum concentration approaches 6 mg/dL or less. Many cows, and also postpartum dairy ewes, are "hypercalcaemic" according to the reference intervals that are established for healthy cattle, but not for these types of cattle. Hypocalcaemia is also a frequent finding in endotoxemia and gastrointestinal diseases.

Hypocalcaemia and hypophosphatemia in ewes are generally observed during the last month of gestation and not during parturition; just as it happens in cows. The increased foetal  $\text{Ca}^{2+}$  needs of lambs and the fact that there is not a great demand for calcium when the ewe begins lactation are the proposed reasons for the timing of this condition in ewes.

Hypomagnesemia, concurrent with hypocalcaemia, can lead to the development of grass tetany. Hyperkalaemia may also be present and may be a key factor in the development of hypomagnesemia. Although the correlation between clinical signs and serum magnesium is not strong, ruminants with serum magnesium concentrations below 1.0 mg/dL are at high risk of developing grass tetany.

Bovine cattle with marked hypophosphatemia ( $\text{P} < 1.0$  mg/dL) are at risk of developing postpartum haemoglobinuria, a haemolytic disease. Hypophosphatemia is believed to be the result of an insulin-induced shift of plasma phosphorus into the intracellular compartment.

Both hypercalcemia and hypermagnesemia are quite rare in ruminants, and usually occur as a result of the administration of calcium or magnesium hydroxide solutions or gels as a ruminal alkalinizers.

Excessive dietary supplementation with products containing vitamin D can cause hypercalcemia with concurrent hyperphosphatemia in cattle and severe hypercalcemia in lambs.

**7.11. Trace elements (at least Se, Cu, Co, Mo, Mn, Fe, Zn, I)**

Ruminants need trace elements to catalyse multiple biochemical reactions in tissues. Disorders can arise from deficiencies as well as excesses, although deficiencies almost always develop gradually, and often only the most vulnerable individuals are affected.

There are a variety of trace element tests that can be performed on blood samples, and it would take extremely long to discuss each one. Therefore, we will only indicate some essential aspects of the assessment of microminerals in blood.

1. Interactions between them. There is a very important relationship between the different minerals, both macrominerals and trace elements. Thus, increases in some of them can secondarily trigger a blockage in absorption or even in the use of totally different ones, for example serum Cu as they are influenced by the levels of Mo, S, Fe to even Zn, Co or Al. Thus for example, diets with high levels of Mo cause the formation of molybdates at the ruminal level that prevent the absorption of Cu.

2. The determination of these minerals can be done in blood, serum or plasma, using different table techniques such as biochemical analysis, plasma optical emission spectrometry (ICP-MS) or inductively coupled plasma spectrometry (ICP-OES). The choice depends on the availability of equipment for its measurement and the sensitivity of the method to be chosen.

3. Another possibility is the indirect determination of trace elements through “their enzymes or their substrates”, that is, those variables of which they are part, with the additional advantage that we also assess the (functional) micromineral.

Among them we can point out the following examples:

-Se: glutathione peroxidase (GSH-Px)

-Cu: ceruloplasmin, superoxide dismutase, tyrosinase, etc.

-Fe: haemoglobin, transferrin, cytochrome c-oxidase, etc.

-Iodine: thyroxine (T4), triiodothyronine (T3)

Some values collected in the references are Cu: [58-160] and [32.8-35.2] µg/dl; Fe: [57-162 mg/dl, (18–48 µmol/L)] and [73-140] µg/dl; Se: [1.0–6.3] µmol/L and [81-160] µg/L; Zn: 12–19 µmol/L and [600-1090] µg/L, the first interval for sheep and the second for cattle.

## **8. Conclusions**

Clinical profiles are a valuable diagnostic tool that can be used to evaluate various body systems. There is no substitute for a complete clinical examination; clinical chemistry and haematology are only a diagnostic adjunct and when used in conjunction with medical history, physical examination, and other laboratory tests (e.g. urinalysis, etc.) metabolic profiles may be helpful in establishing certain reference parameters in a patient, for the formulation of a diagnosis or an exclusion list, which determines the prognosis, plans the therapeutic options and the response to treatment.

Therefore, and as a final conclusion, it is important to remember that it is necessary to assess all of them together, to be able to interpret them and thus obtain the appropriate conclusions.

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## **Morphological Evaluation of Experimental Autologous Rectus Fascia Sheath Vascular Grafts Used For Arterial Replacement in A Dog Model**

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### **Introduction**

Autologous vascular replacement has been used in human surgery, and it might occasionally be indicated in the veterinary field, too<sup>3,7</sup>. Although experimental patch or tubular vascular conduit grafts made from the internal rectus fascia sheath replacing arterial or venous defects have been reported in the human and veterinary literature<sup>2,5,6,8</sup>, thorough and detailed morphological evaluation and verification of the histological arterialisation of the rectus sheath graft are lacking.

### **Material and Methods**

This study has been approved by the competent authorities acting upon the recommendation received from the Scientific Ethics Committee on Animal Experimentation (authorisation number: 73/2008). The animals were treated according to the international guidelines for the care and use of laboratory animals (Declaration of Helsinki). Four purpose-bred Beagle dogs were utilised to create 8 arterial rectus fascia sheath (ARFS) grafts. After a ventral midline abdominal incision, both external iliac arteries were dissected from the surrounding tissues. After measuring the diameter of the iliac artery, a piece of internal rectus fascia sheath (3 × 4 cm) was tailored. A standard glass rod (5 mm in diameter) was used to create the ARFS graft as a 20-mm-long tubular graft was sutured with its peritoneal layer inside, using a single layer simple continuous 6-0 USP polypropylene (Prolene) suture (Figure 1.a). Immediately before clamping the iliac artery, 200 IU/kg heparin sodium injection was given intravenously to prevent thrombosis. The external iliac artery was transected between two ‘de Bakey’ straight vascular clamps, and the ARFS graft was anastomosed with the transected arterial ends in an end-to-end manner using a single layer simple interrupted 6-0 USP polypropylene (Prolene) closure. The morphological characteristics of the graft were documented after re-establishment of circulation in the artery. The abdomen was closed in three layers. Heparin sodium (200 IU/kg BID) was given subcutaneously as a postoperative anticoagulant for two weeks. At the end of the 3-month follow-up period ARFS tubular grafts along with the anastomosed section of the iliac artery were surgically removed and the arteries proximal and distal to the two anastomosis sites were ligated. The ARFS graft and a piece of the intact rectus sheath were preserved in 8% formaldehyde solution buffered with phosphate buffered saline (pH 7.0) and embedded in paraffin. Morphological examination was performed including haematoxylin-eosin (HE) and Azan staining as well as immunohistochemistry (claudin-5,  $\alpha$ -smooth muscle actin ‘ $\alpha$ -SMA’, desmin and pancytokeratin). Tissues of 1 mm<sup>3</sup> taken

from the grafts, the artery and the rectus sheath, were fixed at 4 °C in 4% buffered formaldehyde solution for electron microscopic (EM) examination for 24 hours. The morphology of ARFS grafts was compared to that of the intact arteries and rectus sheath samples.

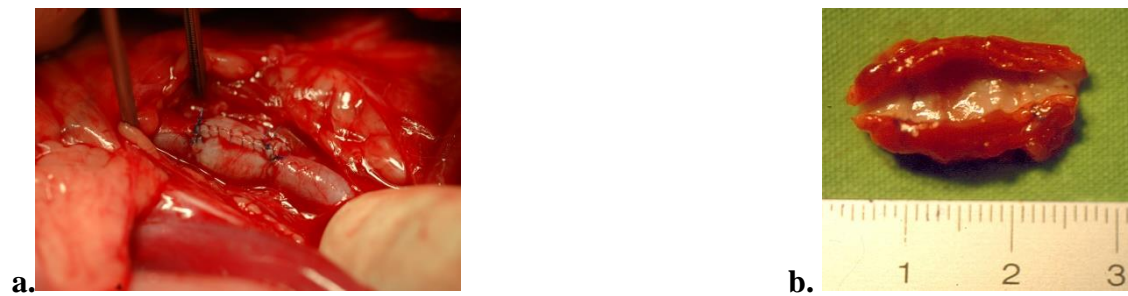
## **Results**

Four out of the 8 ARFS grafts were patent and viable with intact luminal surface and a thick and rigid wall after three months (Figure 1.b). The obstructed grafts were stenotic. HE staining showed a vessel-like layered wall of the grafts (Figure 2). Azan staining revealed large amounts of collagen fibres within the graft (Figure 3). Based on immunohistochemistry, the endothelial lining of the intact artery samples showed claudin-5 positivity and pancytokeratin negativity, the mesothelial lining of the intact internal rectus sheath samples was positive for pancytokeratin and negative for claudin-5, and the luminal surface of the graft covered by flat and fusocellular cell lining showed a linear claudin-5 positivity and pancytokeratin negativity (Table 1). Smooth muscle cells of the tunica media of the intact arteries showed  $\alpha$ -SMA and desmin positivity, the wall of the intact internal rectus fascia sheath was  $\alpha$ -SMA negative and desmin positive, and the spindle-shaped cells of the wall of the ARFS grafts showed moderate  $\alpha$ -SMA and desmin positivity (Table 2). Electron microscopic examination of the grafts revealed that the wall was rich in thick collagen fibres including large numbers of active fibroblasts. The luminal surface was covered by endothelial cell lining. In comparison with intact arteries, the membrana elastica interna and the lamina elastica externa were absent in the graft. Smooth muscle cells were not visible either.

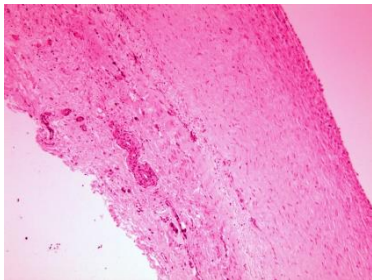
## **Discussion and Conclusions**

Fifty percent of the ARFS grafts remained patent during the follow-up period, which is a lower percentage than that obtained in our previous experimental studies<sup>5,6</sup>. Based on the histomorphological examinations, the structure of rectus sheath used as a vascular graft has changed after the implantation. Although the inner surface of the internal rectus fascia sheath was originally covered with claudin-5-negative and pancytokeratin-positive mesothelial cells, the internal cells became claudin-5 positive and pancytokeratin negative like in the intact arteries during the follow-up period<sup>4,10</sup>. Based on this, a re-endothelisation process took place by the migration of endothelial cells from the vessel to the graft (transmigration), or the mesothelial cells have differentiated into endothelial cells (transdifferentiation)<sup>1,5</sup>. Spindle-shaped cells of the wall of ARFS grafts were  $\alpha$ -SMA positive like smooth muscle cells of the intact arteries<sup>9</sup>, but  $\alpha$ -SMA immunoreactivity was negative in the intact rectus sheath. The change in the immunoreactivity of the graft shows that the fibroblast cells of the rectus sheath have changed into myofibroblast cells. The myofibroblast cell is a transdifferentiated fibroblast cell containing smooth muscle actin<sup>9</sup>. This morphological change may increase the elasticity of the graft, enabling it to accommodate to new circumstances as a vascular substitute. In conclusion, the arterial rectus fascia sheath autograft may be used as an alternative in arterial replacement, since

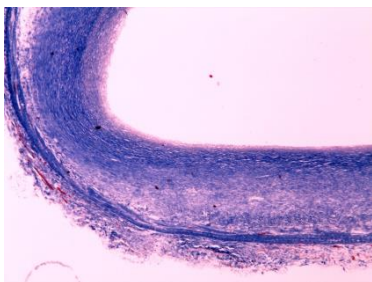
the graft becomes morphologically and functionally similar to the host vessel via arterialisation enabling it to behave as a blood vessel, and furthermore, rejection is not a possible complication. The wall of the grafts was thicker and less elastic than that of an intact vessel. Nevertheless, in our experiment only half of the grafts remained patent, and the obstructed four grafts suffered from stenosis. Further investigation of clinical behaviour is recommended before the rectus fascia sheath can be used in clinical cases as a tubular vascular substitute.



**Figure 1.** Pictures of the 20-mm-long ARFS autograft right after end-to-end anastomosis between the bisected ends of the external iliac artery (a), and after harvesting at revision surgery 3 months later (b).



**Figure 2.** The ARFS graft showed a vessel-like layered structure with a more hypocellular middle layer (HE,  $\times 100$ ).



**Figure 3.** Azan-positive blue coloured collagen fibres in the ARFS graft (Azan staining,  $\times 100$ ).

**Table 1.** Results of immunohistochemistry of the mesothelial/endothelial lining of the three types of tissue samples. (The intensity of the immunohistochemical reactions is indicated as (+++) intensive, (++) moderate, (+) weak, or (–) negative).

	<b>External iliac artery</b>	<b>Internal rectus sheath</b>	<b>ARFS graft</b>
<b>Claudin-5</b>	+++	–	++
<b>Pancytokeratin</b>	–	+++	–

**Table 2.** Results of immunohistochemistry of the tunica media/wall of the three types of tissue samples. (The intensity of the immunohistochemical reactions is indicated as (+++) intensive, (++) moderate, (+) weak, or (–) negative).

	<b>External iliac artery</b>	<b>Internal rectus sheath</b>	<b>ARFS graft</b>
<b>α-SMA</b>	+++	–	++
<b>Desmin</b>	+++	+++	+ / +++

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## **Prospective Evaluation of Correlations between Certain Clinical Findings of Perineal Hernia in 47 Dogs**

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### **Introduction**

Perineal hernia is a relatively rare type of herniation in Veterinary practice, which most commonly impacts entire male dogs<sup>1-2</sup>. The disease is usually characterised by chronic dyschezia and/or perineal swelling<sup>3</sup>. The duration of clinical signs is assessed via the history provided by the owner. Impaired defecation is essentially thought to be caused by a certain severity of rectal deviation (flexure, sacculum or diverticulum) diagnosed by rectal palpation, plain radiography as well as intra-operative assessment<sup>1</sup>. The possible role of a more proximally dilated section of the large intestine (colon) in chronic dyschezia has not been analysed. To the best of the authors' knowledge, correlations between the duration of clinical signs, the type of rectal deviation, the presence or absence of colic dilatation (megacolon) and the clinical outcome has not been studied. The aim of the study was a prospective evaluation and correlation analysis of data collected from canine patients who have undergone castration and standard surgical herniorrhaphy by transposition of the internal obturator muscle<sup>4,5</sup>. We hypothesised that there is a positive correlation between (1) the duration of clinical signs and the severity of the rectal deviation, (2) the finding by preoperative palpation and by intra-operative assessment concerning the type of the rectal deviation; (3) the presence or absence of colic dilatation (megacolon) and the dyschezia, (4) the rectal deviations and the colic dilatation, and finally (5) the rectal (and/or colic) deviation and the clinical outcome.

### **Materials and Methods**

Inclusion criterion for the prospective study was a complete clinical diagnostic workup (history, physical examination, plain radiography) of a chronic perineal hernia followed by castration, standard herniorrhaphy by transposition of the internal obturator muscle and, in case of sacculum or diverticulum, rectorrhaphy. The type (left, right caudal, lateral, dorsal or ventral) and severity of perineal muscle wall defect was graded as 1 (no defect), 2 (weakened muscle but no hernia) or 3 (muscle defect). Rectal palpation was performed to investigate the presence of a rectal flexure, sacculum or diverticulum. The O'Brien and the Lee and Leowijk formulae were used with radiographs to determine the presence or absence of megacolon evaluated by 2 blinded examiners (Figure 1)<sup>6,7</sup>. During surgery the type of the perineal hernia, the severity of rectal deviation, grade of the various perineal muscles' tone and the contents of the hernia were assessed and categorised. All dogs were surgically operated using the standard perineal herniorrhaphy with transposition of the

internal obturator muscle. Follow-up telephone interview were conducted with the owners to evaluate the outcome of treatment using a 5-grade subjective evaluation form (1:worse, 2:no change, 3:mild improvement, 4:moderate improvement, 5:significant improvement) at 2 weeks, 2 months and 6 months post-surgery. The Bland-Altman method modified by Carstensen was used for agreement analysis. Fisher's exact test was applied for the assessment of statistical correlations between various clinical findings and values ( $p < 0.05$ ). The dependence of the colic dilatation (megacolon) on the duration of signs was evaluated by logistic regression. R language and R environment was applied for all data computing and analysis.

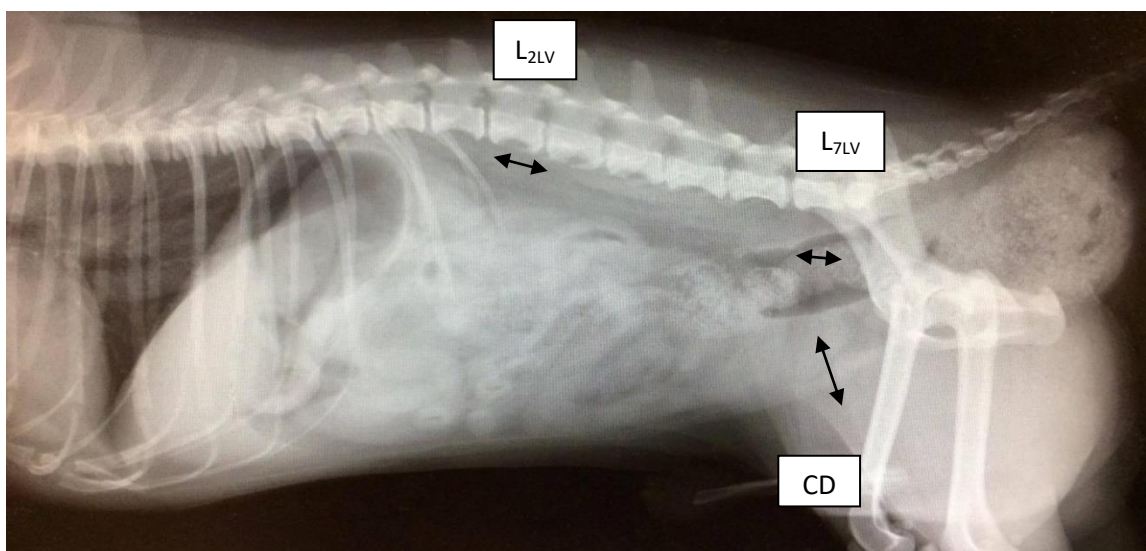
## **Results**

Forty seven perineal hernia cases were included in the study which took place over a 2-year period. The incidence of this disease was typically in older (median age of 9 years), non-castrated, small breed (32% Bichon Havanese or Bolognese), male (95.7%) dogs. Right-sided grade 2 herniations were more frequent (35/47) than left-sided (22/47). The type of the hernia on the particular side varied: 14 caudal, 2 ventral and 19 dorsal on the right, and 15 caudal, 1 ventral and 8 dorsal on the left side. There were 26 out of 47 bilateral manifestations. The anal levator muscle was the most frequently atrophied muscle within the perineal muscle wall: in 33 cases on the right and 20 cases on the left side. On comparing pre-operative rectal palpation findings to the intra-operative assessment of rectal deviations, all the cases diagnosed by rectal examination as rectal flexure were confirmed to be true positives. However, 9 out of 23 cases diagnosed as rectal sacculation on palpation were false positive; being rectal flexures when assessed intra-operatively. Concerning the radiographic analysis of the colon, only the laterolateral views were appropriate to measure the diameter of the colon (CD) and the length of the lumbar vertebrae ( $L_2$  and  $L_7$ ) due to the disturbing summation of different layers on ventrodorsal view. The median measurements of CD,  $L_2$  and  $L_7$  were 2.14 cm, 1.94 cm and 1.71 cm, respectively. Agreement analysis revealed a high correlation between the data measured by the 2 independent and blinded examiners: the assessment difference for CD,  $L_2$  and  $L_7$  was 0.15 cm, 0.05 cm and 0.07 cm, respectively. The sensitivity of the 2 radiographic measurement procedures for megacolon was significantly different. A colic dilatation was detected using the O'Brien formula in 5 cases, while 15 patients were found positive using the Lee and Leowijuk formula. Thus there were 10 out of 15 cases classified as negative for megacolon with the O'Brien formula. However, no significant correlation was found between the duration of clinical signs of the hernia and the occurrence of a radiographically positive megacolon using either the O'Brien or Lee and Leowijuk methods. Three of the O'Brien positive cases harboured a rectal deviation (all were sacculations), and 12 out of the 15 Lee and Leowijuk positive patients suffered from rectal deviation (3 flexures, 8 sacculations and 1 diverticulum) without statistical significance. There was no statistically significant correlation between the duration of clinical signs of the hernia and the severity (or absence) of the rectal deviation: the median duration of clinical signs was 20, 33 and 208 weeks in case of sacculations, flexure and

diverticulum, respectively. However, the shortest duration of signs (median 16 weeks) was found in patients without any rectal deviation. Long term (6 months) follow-up demonstrated that the owners were fully satisfied (grade 5) with the surgery in 37 out of 47 cases (78.7%). In 3 cases the assessment of surgical treatment was grade 3 (6.4%) and 2 cases were grade 2 (4.3%) regardless of the original severity of the hernia. Six owners (10.6%) were lost from follow-up.

### Discussion and Conclusion

Entire male Bichon Havanese or Bolognese breeds were overrepresented in this study compared to the other frequently predisposed breeds (e.g. German shepherd, Pekingese) maybe due to their relatively high prevalence<sup>1</sup>. A statistically significant positive correlation was not revealed between the duration of clinical signs and either the severity of the rectal (or colic) deviation or the post-operative outcome. The preoperative rectal palpation was not able to precisely diagnose the type of the rectal deviation which was later confirmed intra-operatively. There was a high correlation in the agreement between the 2 blinded examiners' measurements of parameters in a laterolateral radiographic projection. The Lee and Leowijuk method appeared more sensitive than the O'Brien formula in revealing dilation of the colon<sup>6,7</sup>. Nevertheless, there was no statistically significant positive correlation between the presence or absence of a radiologically diagnosed megacolon and either the duration of clinical signs or the severity of the rectal deviation. In conclusion, although various measurable clinical parameters did not yield significant correlations in this study as well as the clinical cases strongly vary in terms of the duration of clinical signs, the severity of dyschezia and rectal (colic) deviations, the recommended surgical approach (castration and standard herniorrhaphy by transposition of the internal obturator muscle +/- rectorrhaphy) provides a satisfactory outcome in the vast majority of cases regardless of the chronicity and severity of the disease.



**Figure 1.** Measured values for evaluation of megacolon using the O'Brien ( $CD > 1.5 \times L_{7LV}$ ) as well as the Lee & Leowijuk formula ( $CD > L_{2LV}$ ) in a plain lateral view radiograph (CD: the largest diameter of the colon;  $L_{2LV}$ : the length of the 2<sup>nd</sup> lumbar vertebra;  $L_{7LV}$ : the length of the 7<sup>th</sup> lumbar vertebra).

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## Epidemiology of Brucellosis in Ruminants and Current Control Strategy Used in Albania

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### Summary

Brucellosis is bacterial disease widely globally spread and one of major neglected zoonotic diseases and more than half million humans are infected annually. In Albania, both *Brucella melitensis* and *B. abortus* are isolated from goats, ovine, and cattle. The number of brucellosis human cases dropped significantly after implementation of the national control strategy to control caprine, sheep and bovine brucellosis in dairy herds. Despite that brucellosis is one of most studied disease, still several important questions related to pathogeneses, diagnosis, vaccination and control programs remain unexplained.

**Keywords:** Caprine and ovine brucellosis, bovine brucellosis, *B. melitensis*, *B. abortus*, Albania

### Introduction

Brucellosis is a bacterial contagious disease caused by various bacteria of the genus *Brucella*. Bovine brucellosis (*Brucella abortus*), caprine and ovine brucellosis (*B. melitensis*) and swine brucellosis (*B. suis*) are OIE listed and mandatory reported disease (Quinn *et al*, 2011). The highest incidence of brucellosis is observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico, and despite it is widespread globally, several countries in Western and Northern Europe, Canada, Japan, Australia, and New Zealand have free status (Quinn *et al*, 2011).

The main clinical sign of brucellosis in infected animals is abortion, which usually happen only once during the last trimester of pregnancy (Quinn *et al*, 2011). Infected animals may have subsequent successful pregnancies but continue to shed large numbers of bacteria in the birth fluids, milk, vaginal and uterine discharge etc (Diagram 1).

Among non-sporulating bacteria *Brucella* spp. are most resistant bacteria that persist for prolonged periods in the environment. *Brucella* spp. can be transmitted by the digestive route, and can infect animals and humans through skin abrasions, through mucous membranes and inhalation (Quinn *et al*, 2011).

Brucellosis caused by *B. melitensis*, *B. abortus* and *B. suis* is a highly infectious zoonosis disease, however, other *Brucella* spp also have zoonotic potential (Wareth *et al*, 2019). Occupational exposure

represents an important risk factor: and veterinarians, farmers, butchers, and laboratory workers are at high risk to infection (Quinn *et al*, 2011, Wareth *et al*, 2019).

Important indicators for presence of brucellosis are a) abortion storm in animals and b) human cases, but its confirmation is made through laboratory diagnosis based on serological tests, isolation and identification the bacteria and detection of *Brucella* nucleic acid. National control programmes may adopt the most appropriate control strategy according to specific circumstances (Diagram 2). In brucellosis-free countries/regions it is important to prevent the introduction of the infection, and this could be achieved by application of serological surveillance and biosecurity measures (EFSA, 2015, Bruce *et al*, 2014). The policy in endemic areas depends on prevalence rates; if prevalence rate is high, vaccination is a recommended approach accompanied with complementary measures. Standard vaccines for cattle are *B. abortus* S19 and *B. abortus* RB51 vaccinal strains, while *B. melitensis* Rev 1 strain remains the standard vaccine for sheep and goats (EFSA, 2015, Quinn *et al*, 2011, Garin-Bastuji *et al*, 2006). The *B. melitensis* Rev 1 vaccine strains can be injected subcutaneous but is recommended via the intra-conjunctival route because its desirable advantages: the protective immune response is like subcutaneous injection; the antibody titre declines rapidly, and the bacterial vaccine strain is not shed in milk. In the countries/regions where the prevalence is low, or after control programmes have reduced infection rates, a test and slaughter approach may be used until the disease is eradicated. Control of *Brucella* infection in animals prevents human brucellosis: milk pasteurisation is an important measure to minimize the risk of infection to humans (EFSA, 2015, Bruce *et al*, 2014, Quinn *et al*, 2011, Garin-Bastuji *et al*, 2006).

### **Caprine and ovine Brucellosis**

Sheep and goats in Albania represent an important sector of livestock, however, some endemic disease interfere with development of the sector. Brucellosis in small ruminants in Albania was widespread with prevalence varying from region to region. In the absence of sustained surveillance during 1990-2004, there was anecdotal evidence that the disease spread to all regions, including districts and areas considered to be free. The veterinary directory decided in 2011 to draft and enforced a national brucellosis control programme in 2012 when the first mass vaccination campaign was implemented. The mass vaccination campaigns were repeated in subsequent year (2013) and for year later (2017). According to the adopted strategy, from 2014 onwards (excluding 2017) the brucellosis control programme in small ruminants was continued by vaccination of replacement animals only. Post vaccination monitoring (PVM) was implemented to assess the efficacy of vaccination (Fero *et al*, 2019).

## **Bovine brucellosis**

Despite that majority of cattle management systems in Albania are not intensive, nevertheless dairy cattle form a most important sector for milk production. Most herds have only one or two animals per holding producing milk for households. Animals are commonly bought and sold at livestock markets, which are largely uncontrolled, or directly from farm to farm. Unauthorized movement of cattle and buying animals of unknown health status are the predominant sources of infection in the introduction of brucellosis in previously *Brucella*-free holdings (Fero *et al*, 2019). In March 2016 commenced implementation national programme to control bovine brucellosis in commercial dairy herds. The Bovine Brucellosis Control Programme (BBCP) was based on active surveillance by employing repeated quarterly bulk milk ring testing (MRT). Individual animal testing was conducted in all positive herds (MRT positive), after which the standard control measures including slaughtering of positive and in-contact animals and cleaning and disinfection were implemented. The results of the implementation of the BBCP indicated approximately 2% prevalence (Wareth *et al*, 2019). The study survey in small size dairy herds in one important district, indicate a very good epidemiological situation, where no positive animals/herds were detected. The situation in beef cattle (which a small cattle subpopulation) is not encourage and both herd and individual level was high (Ferro *et al*, 2020). This epidemiological situation indicates that there is not feasible one national control strategy for all either cattle subpopulation and or districts (Fero *et al*, 2019). Among several strategies, however, each country has specific circumstances that must considered before approving any control programme. Control of brucellosis is a multidisciplinary activity involving a range of public institutions, in particular the public health authority and authority responsible for food safety; their contributions are essential for effective control. The Ministry of Agriculture plays a lead role in coordination of the activities towards achieving the goal of control and eradication.

The highest priority for bovine brucellosis is the adoption of the most appropriate control approach adjusted to the local epidemiological conditions. The epidemiological situation in dairy herds seems to be favourable. Effective control requires full enforcement of the control measures, which will result in rapid elimination of the infection from bovine population, including enforcement of preventive measures to keep the bovine population free of the infection. The high prevalence in beef herds, in best experts' opinion, suggested control approach is by mass vaccination, using of intra-conjunctival S19 (recent introduced vaccine) is most appropriate. The encouraging trend of the decreasing number of human cases achieved by mass vaccination must be maintained and further improved by strict enforcement of veterinary public health measures. Human cases have to be investigated jointly by veterinary authorities, Public Health Authority and National Food Authority to establish epidemiological links between humans and animals, to assess the risk of transmission of infection by food and to assure that all measures in relation to food safety are in place. There is a need to introduce new diagnostic methods such as gel diffusion precipitation test and Fluorescence Polarisation Assay



(FPA), which are important for the differentiation of vaccinated and infected animals (Fero *et al*, 2019).

### **Human brucellosis in Albania**

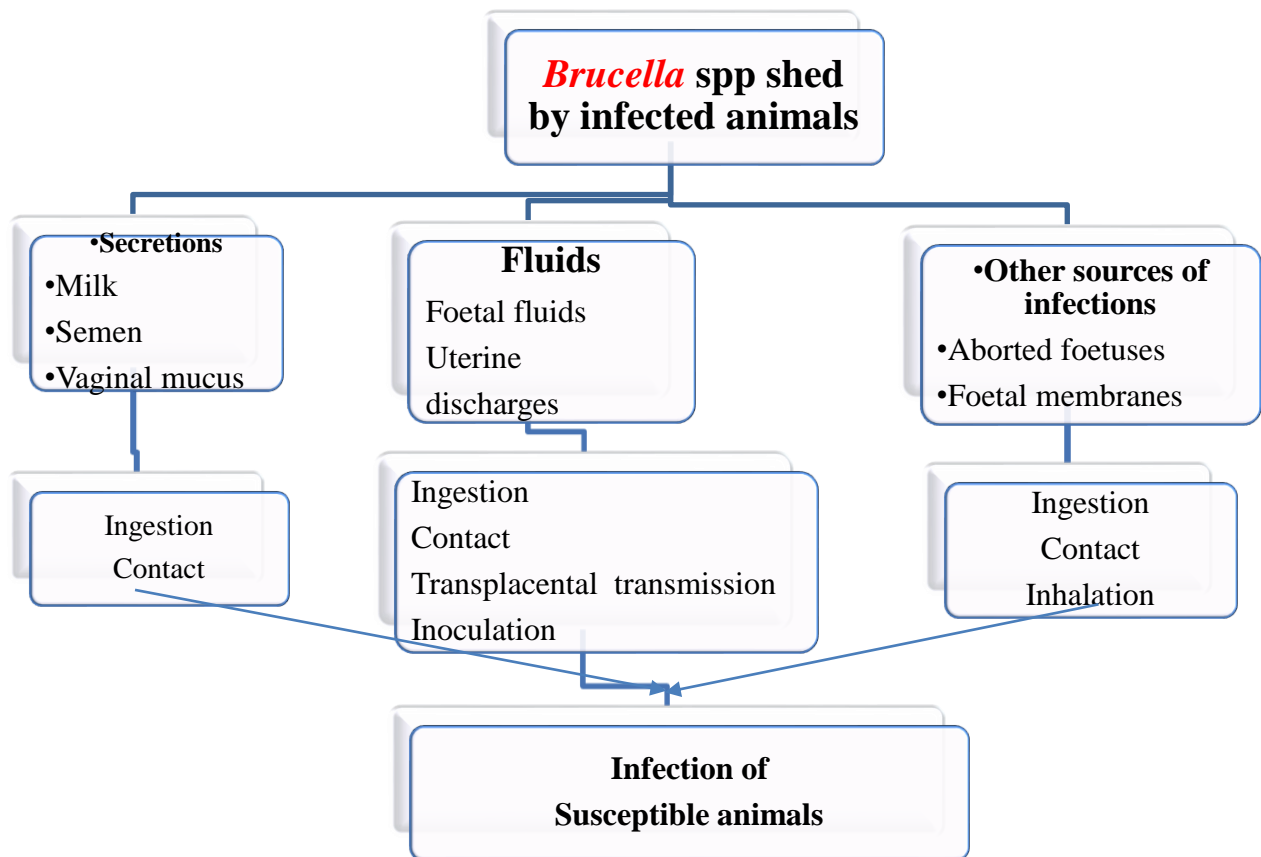
Human brucellosis in Albania was reported 96 years ago, while the positive results of *Brucella* DNA in human bones suggested that brucellosis has been endemic since at least the Middle Ages (Mutolo *et al*, 2012). The incidence of human cases reflects the prevalence of disease in the animal population and there are recorded very different incidence rate. In 1990, when democracy emerged in Albania the incidence was at a comparatively low level *i.e.*, 1.6 cases per 100,000 inhabitants (Alla&Bino, 2014). In 2004, the incidence was reported to be at 38 per 100,000 inhabitants, in 2011 the incidence dropped to 11.7 per 100,000 inhabitants again when mass vaccination of small ruminants started in 2012. In 2019, the number of cases reported only 24 human cases annually. Diagram 3 indicates the difference of human brucellosis incidence dropped. There is a significant difference before and after the control programs of brucellosis in both large and small domestic ruminants commenced.

Despite the knowledge in epidemiology of brucellosis there are identified several needs and different study projects are running for better understanding the transmission of infection from animals and their products to human population. One health philosophy may be an appropriate approach. Developing better diagnostic methodology which not only detect precisely infected/healthy animals but detect also recovered animals and vaccinated animals from infectious animals. In addition, improving vaccine safety, vaccine potency and developing vaccines which are compatible with surveillance program.

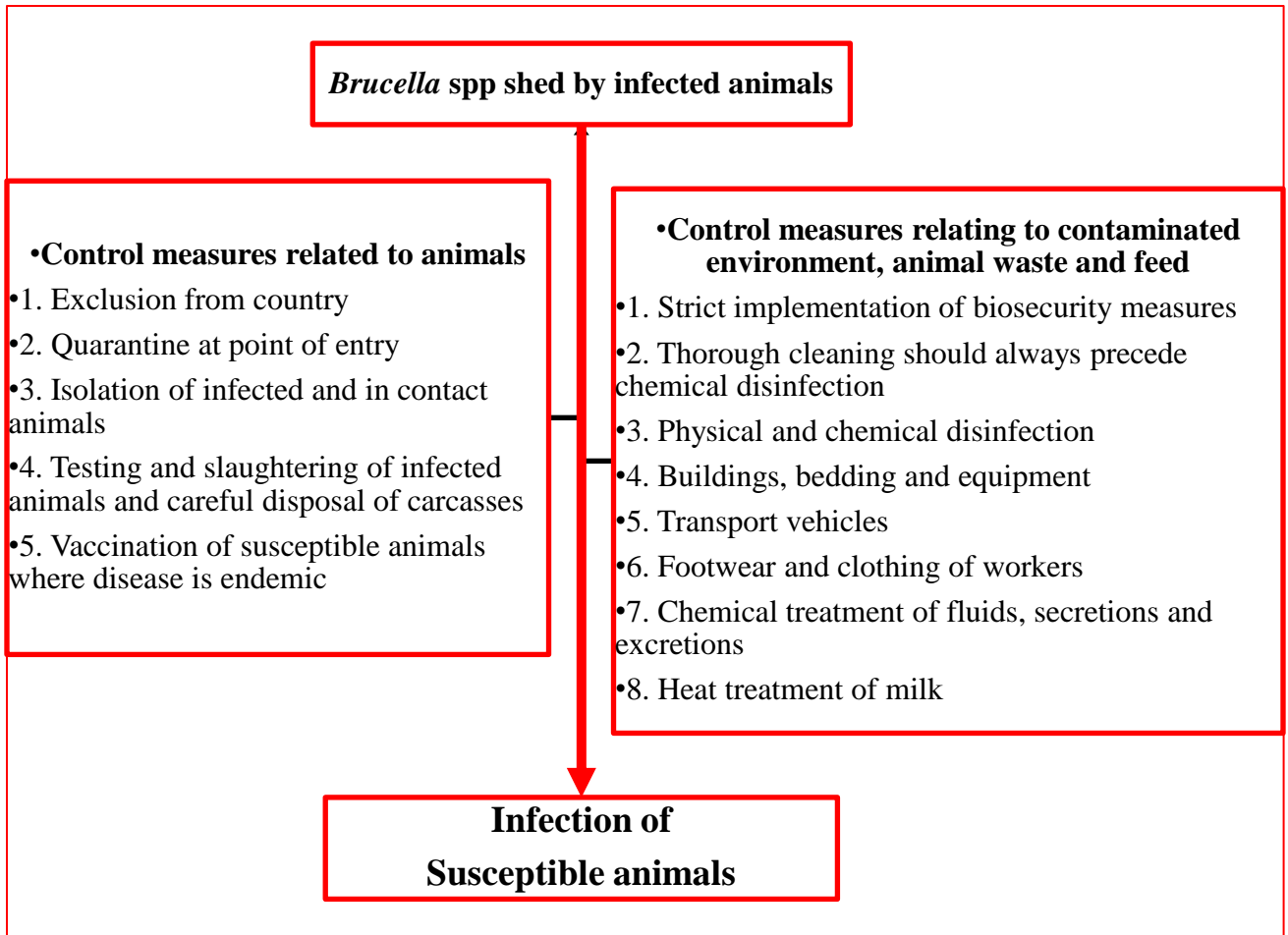
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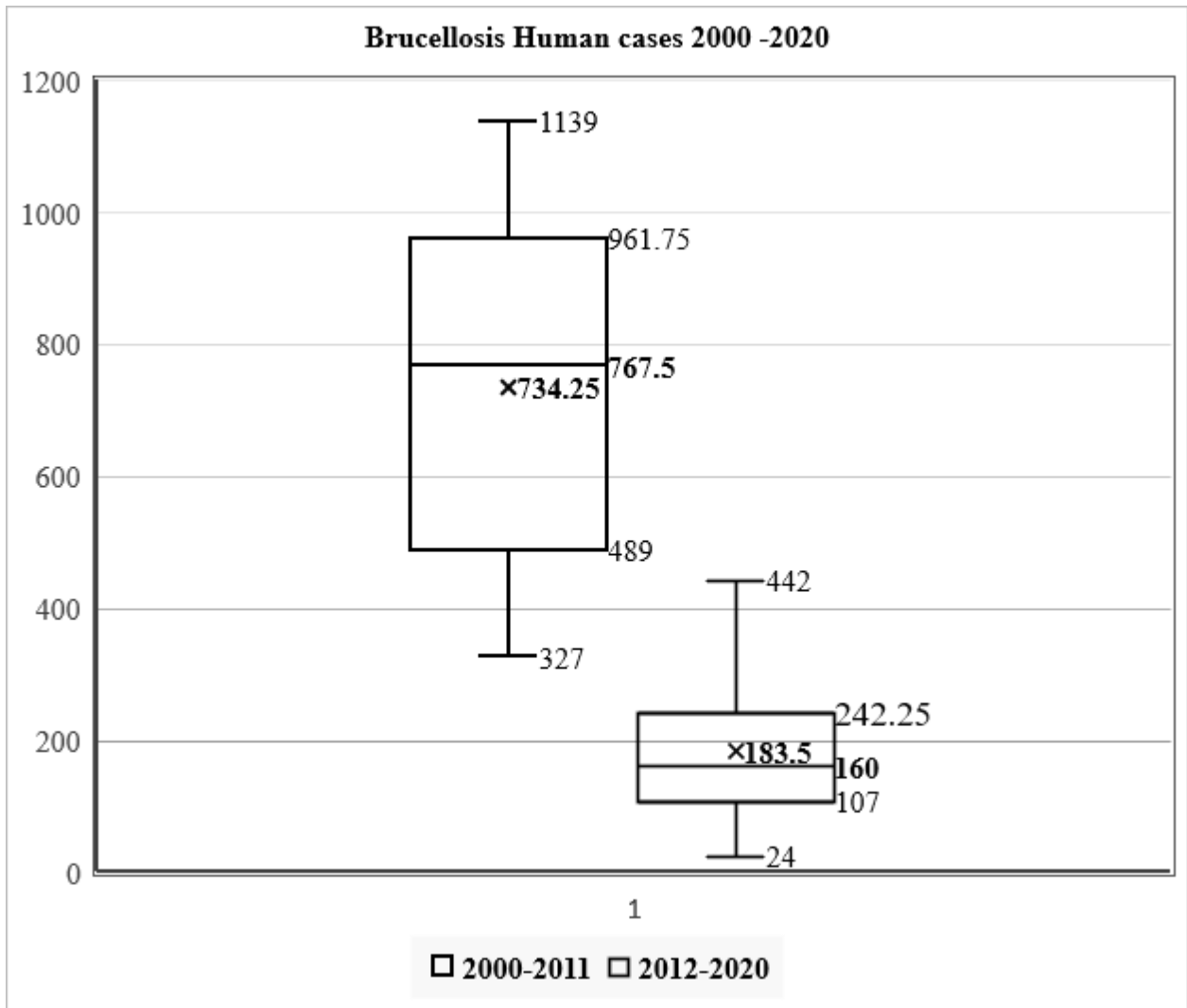
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**Diagram 1** – The main excretion and transmission route of *Brucella abortus* and *Brucella melitensis* in animals



*Diagram 2 – Potential strategy for effective control of brucellosis in ruminant animals*



**Diagram 3** – Comparison of brucellosis human cases in Albania before and after implementation the national control strategy in animals

## **Rest and Treadmill Endoscopic Findings in Race Horses Presented For Poor Performance**

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### **Practical Application**

High speed treadmill endoscopy represents an extremely valuable procedure which should be included in any diagnostic protocol for the evaluation of poor performance in race horses. It allows establishment of the clinical significance of abnormalities observed at rest and/or detection of the presence of dynamic upper airway obstructions and can be helpful in ruling out upper airway obstruction as the cause of poor performance in uncertain cases.

### **Introduction**

Several upper airway disorders may affect the ability of equine athletes to sustain high speed for a given period of time, and the complaint for poor performance may range from a slight increase in racing times up to a dramatic reduction in speed during a competition. Upper airway obstruction may be related to several disorders such as dorsal displacement of the soft palate (DDSP), mono- or bilateral vocal cord collapse, epiglottic entrapment (EE), dynamic pharyngeal collapse (DPC) and axial deviation of the aryepiglottic folds (ADAF) (Morris and Seeherman, 1990; Kannegieter and Dore, 1995; Davidson and Martin, 2003; Lane, 2006; Strand et al., 2012). Although endoscopy at rest may allow one to identify an upper airway disorder in some cases, the presence of an abnormality at rest does not necessarily indicate a clinical problem. On the other hand, the absence of abnormalities at rest does not rule out a dynamic upper airway disorder, which may only be present during strenuous exercise (Lumsden et al., 1995). Thus, when a dynamic upper airway disorder is suspected, or when the clinical significance of an abnormality observed at rest has to be evaluated, it may be necessary to resort to high speed treadmill endoscopy for a definite diagnosis.

### **Materials and Methods**

Before performing treadmill endoscopy, each patient undergoes a thorough physical examination, routine laboratory evaluation and upper airway endoscopy at rest, without sedation. The horses are then acclimatized to work on a high speed treadmill (SATO-I) by two daily training sessions, for a total of four sessions in two days (Hodgson and Rose, 1994). All horses are tacked with the same harness used for racing and a heart rate meter (Polar Horsetrainer) is used to determine heart rate (HR) during exercise at different speeds. On day three treadmill endoscopy is performed. After a warm up of 4 minutes walk (1.5 m/s) and 5 minutes trot (4.5 m/s for Thoroughbreds and 6 m/s for

Standardbreds) with 3° slope for Standardbreds and 6° slope for Thoroughbreds, a videoendoscope (ETM PVG-325) is introduced into the nasopharynx and held in position with velcro straps. A microphone is fixed to the endoscope close to the nostril in order to record respiratory noises during exercise. The treadmill is then rapidly accelerated up to the selected speed, corresponding to maximal HR ( $\geq 220$  bpm), for a distance ranging from 1600 m to 2100 m or until the horse is no longer able to maintain the belt speed. Laryngoscopic images are visualised on a monitor (Sony PVM-14N5E) and recorded on a hard disk for later slow-motion analysis and storage.

## **Results**

### ***Endoscopy at rest***

54 % of patients with poor performance show one or more abnormalities at rest (Ferrucci et al., 2004). In 30 % of the horses, endoscopy at rest shows different degrees of asynchrony in arytenoid cartilage movements (Recurrent laryngeal neuropathy, RLN). Transient DDSP at rest is present in 15 % of patients. In 11.5 % of cases there is a flaccid epiglottis, 4.6 % presents pharyngeal spasm, 2.3 % has ulceration of the free margin of the soft palate, 1.1 % has epiglottic entrapment and 1.1 % has rostral displacement of the palatopharyngeal arch (RDPA).

### ***Treadmill endoscopy***

Treadmill endoscopy shows dynamic upper airway abnormalities in 61 % of patients with poor performance (Ferrucci et al., 2004). Persistent dynamic DDSP is detected in 34.5 % of horses; in one third of these horses, DDSP is associated with other disorders, such as flutter of the aryepiglottic folds, EE, RLN and and DPC. Severe RLN, varying in degree from III to IV according to Hackett et al. (1991), is detected in 7 % of the cases. In all cases endoscopy at rest showed the presence of RLN although at a lower degree when compared with endoscopy under dynamic conditions. 19.5 % of the horses has vibration of the aryepiglottic folds or ADAF; 5.7 % of horses has DPC; 3.4 % has EE.

## **Conclusions**

Although upper airway abnormalities are frequently detected both at rest and during treadmill exercise there is poor correspondence between the two situations. Only 21% of horses were normal both at rest and during strenuous exercise. Therefore, endoscopy at rest alone would not have been sufficient to make a diagnosis in most horses, and could have been misleading in some cases. In fact, even though an elevated number of horses (30 %) had asynchronous, even pronounced, arytenoid movements at rest, the incomplete abduction worsened dramatically during strenuous exercise only in 7 % of cases. In the other horses both arytenoid cartilages were maintained in a completely abducted position during the whole treadmill session. Furthermore, only a few horses affected with persistent dynamic DDSP had shown the transient abnormality at rest. In addition, there are several other dynamic upper airway

disorders, such as DPC, ADAF, flutter of the aryepiglottic and vocal folds (Kannegieter and Dore, 1995; Parente, 1997; Roberts, 2000), which may only be detected under dynamic conditions.

The results obtained substantially agree with those reported by other workers as far as the prevalence of dynamic upper airway obstruction is concerned: DDSP shows the higher incidence (34.5 %), particularly in Standardbreds, and may be associated with other abnormalities, such as flutter of the aryepiglottic folds and 4<sup>th</sup> branchial arch defect (Lane, 1993). Vibration of the aryepiglottic folds and ADAF were frequently observed (19.5 %), although the significance was unclear; it usually indicates progressive flaccidity of pharyngeal soft tissues which, in some cases led to DDSP. Epiglottid flaccidity at rest could also be considered as a predisposing factor to DDSP, since 60 % of horses that had this abnormality at rest showed DDSP during strenuous exercise. The prevalence of RLN was lower than expected, considering the elevated number of horses that had altered arytenoid motility at rest.

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**Surgical Approach to End Stage of Otitis in Dogs and Cats**

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**Summary**

In this presentation, diagnosis of otitis and surgical approaches to end stage of otitis in dog and cats were evaluated.

## **Infertility of Horses-Stallion Perspective**

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### **Summary**

Breeding stallions are selected based on their performance results, exterior and pedigree. Although the use of individual stallions is sometimes conditioned by fashion or other preferences of the mare owner, in any case the animals are selected very rigorously and therefore very valuable. The need to enable them at the same time long-term usage in both sport and breeding, today is facilitated by the application of various ARTs methods (ART-assisted reproduction techniques). Complete system is revolutionary since it erases time and distance limitations. Semen could be stored indefinitely in frozen state and also shipped globally as chilled state since modern freight forwarding service enables transcontinental transport of chilled sperm and thus facilitates and simplifies the procedures of insemination of mares.. All of this increases the selection pressure on the most wanted stallions, so from year to year the need for the introduction of genetic tests for various inherited diseases such as WFFS (weak fragile foal syndrome) is growing. Genetic tests in particular are carried out a lot in horses bred in pure blood and ART is applied to them (e.g. Arabian purebreds) while in warm-blooded animals this is less pronounced, but all the more necessary. In the same time rapid spread of microorganisms capable to survive in frozen or chilled semen like CEM or EVA are major biosecurity concerns in equine industry and breeding. Protocols for maintaining the reproductive health of breeding stallions depend on their primary purpose, breed characteristics as well as internationally regulated regulations governing their health, welfare and usage (eg FEI Veterinary Regulations, HBLB Codes of Practice, General Regulations of the Croatian Equestrian Federation, etc.). Since fertility of particular stallion is individual variable with high impact on breeding for popular one, stallion perspective of infertility is so important, although often neglected. The reproductive health of breeding stallions can be described as a concern for health and well-being horses in general, especially the health of the locomotor system and the preservation of fertility in terms of preventive biosecurity measures (vaccinations, deworming, etc.) and control (development, spermogram, andrological examination, various serological and genetic tests, etc.)

**Keywords:** Assisted reproduction techniques, breeding stallion, reproductive health, preservation of fertility

## **Congestive Heart Failure (CHF) Diagnosis and Management**

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### **Summary**

Congestive heart failure (CHF) is a clinical syndrome characterized by congestion, oedema and/or poor peripheral perfusion. Severe heart failure produces a marked reduction in cardiac output and systemic hypotension may be present. To maintain blood pressure a series of vascular, hormonal, neural, and renal adaptations occur. Chronically activated, these compensations are considered maladaptive, and include remodeling of the heart, activation of the sympathetic nervous system and tachycardia, release of vasoconstricting sodium-retaining hormones, increased activity of endothelial vasoconstrictors, and kidney retention of sodium and water. Epidemiologically, CHF is relatively uncommon in horses and may be caused by pericardial effusion, cardiac chamber dilation, valvular lesions, or cardiac malformation. Clinical signs may vary and depend on the specific lesion and its location. Tachypnoea or coughing may be observed when the left side of the heart is involved and/or venous distention, jugular pulsation, and subcutaneous oedema in case of right-sided CHF. Electrocardiography, echocardiography, thoracic and abdominal ultrasound may be very useful in diagnosis. Although long-term prognosis for equine CHF is poor unless the primary cause can be corrected, horses are treated more commonly than previously. There are no data to recommend any specific therapy for the management of horses with asymptomatic heart disease, or information on whether any such interventions would reduce the progression of disease or have added benefit, as demonstrated in dogs with mitral valve disease. However, the value of preventive therapy in the horse may develop a robust evidence base in the future. The principles of CHF treatment useful to restore stroke volume, reduce preload, and afterload will be reviewed.

**Keywords:** Congestive heart failure, echocardiography, horse, treatment

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## **Adrenal Gland Diseases in Dogs and Cats**

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In clinical endocrinology of dogs and cats, the diseases of adrenal glands have undoubted significance. Due to their complex and varied clinical presentations and many available diagnostic tests, they still present a diagnostic challenge in small animal internal medicine. Adrenal glands in dogs and cats are composed of the inner medulla and the outer cortex. The hormones produced in the cortex, corticosteroids, are involved in the control of water and electrolytes as well as the metabolism of carbohydrates, proteins and lipids, and are essential for the maintenance of life.

In this lecture, naturally occurring hyperadrenocorticism (ie. Cushing's disease) and hypoadrenocorticism (ie. Addison's disease) in dogs and cats will be presented through a short review of their aetiology, clinical and laboratory manifestations, diagnostics and therapy. The focus will be put on the canine hyperadrenocorticism and hypoadrenocorticism, and at the end of the lecture, the feline primary hyperaldosteronism (ie. Conn's syndrome) will be presented as an underdiagnosed disease of yet unknown significance in feline medicine.

Hyperadrenocorticism is one of the most common endocrine disorders in dogs and is characterized by a chronically increased circulating cortisol concentration which causes both physical and biochemical changes. The clinical presentation in most cases consists of an almost pathognomonic constellation of clinical signs (polyuria/polydipsia, polyphagia, muscle wasting, „potbelly“, thinning of the skin and bilaterally symmetrical alopecia), but the more subtle cases of Cushing's syndrome can be difficult to diagnose. The disease has an insidious onset and is slowly progressive, but, in a few cases, clinical signs may be intermittent, with periods of remission and relapse, whereas in some dogs there may be an apparent rapid onset and progression of clinical signs. Thus, making a diagnosis requires considerable clinical insight. The naturally occurring disease can be caused by oversecretion of adrenocorticotrophic hormone from a pituitary abnormality or the result of a primary adrenocortical tumor. A presumptive diagnosis can be made from clinical signs, physical examination, routine laboratory tests, and diagnostic imaging findings, but the diagnosis must be confirmed by hormonal assay. Pituitary dependent hyperadrenocorticism can be treated by irradiation, surgically with transsphenoidal hypophysectomy or medically with trilostane. Adrenal dependent hyperadrenocorticism can be treated surgically, with mitotane or trilostane. Treatment with trilostane has become the medical treatment of choice. In cats the disease is rare to uncommon, more difficult to diagnose and treat and therefore usually has a much less favourable prognosis.

Naturally occurring canine hypoadrenocorticism is a rare to uncommon disease usually caused by immune-mediated destruction of adrenal cortex, enzyme inhibition, drug-induced adrenocortical

necrosis or infiltrative processes. There are some breeds with suspected or proven heritability (eg. Standard Poodles and Nova Scotia Duck Tolling Retrievers) in which the disease appears in significantly higher frequency. In the most common form of the disease, patients have both mineralocorticoid and glucocorticoid deficiency, much less common is the form (atypical hypoadrenocorticism) without electrolyte changes and with just signs of cortisol deficiency. Dogs with hypoadrenocorticism can be presented with a history of waxing and waning of varied clinical signs, sometimes with quite long periods of apparent normality. Clinical signs include from gastrointestinal signs, through „being unwell“, apathic or weak up to acute life-threatening collapse. As the disease has many different presentation it is often called „the great pretender“. Clinicians have to maintain high level of suspicion for this disease, and with increased testing it may prove no to be as rare as we suspected up till recently. The ACTH-stimulation test is required for establishing a definitive diagnosis. Treatment is divided into two forms: emergency and maintenance therapy. In the emergency treatment, the efforts are aimed at correcting hypovolemia, hiperkalemia, hypoglycemia and metabolic acidosis. Maintenance therapy includes replacement of glucocorticoids (prednisolone) and, in most patients, mineralocorticoids (desoxycorticosteron pivalate or fludrocortisone) too. The prognosis for patients diagnosed in a timely manner is excellent. The disease in cats is extremely rare but if diagnosed on time the prognosis is also excellent.

Primary hyperaldosteronism in cats, or so-called Conn's syndrome, is a rare disease, but still the most common adrenocortical disorder in cats. The disease is caused by bilateral idiopathic nodular hyperplasia of the zona glomerulosa or by unilateral or bilateral adenoma or adenocarcinoma. The disease affects middle-aged to older cats, and most common signs come from hypokalemia (muscle weakness) and/or from increased blood pressure (acute blindness). In this disease establishing a definitive diagnosis can be challenging, and the disease should be considered in any cat with hypertension with hypokalemia or if an adrenal mass is discovered in diagnostic imaging. Confirmation of the diagnosis can be established by obtaining high values of aldosterone in the face of hypokalemia, but the best screening test is the ratio of plasma aldosterone concentration to plasma renin activity. Treatment options include laparoscopic unilateral adrenalectomy as the treatment of choice for unilateral tumors, and in other cases, medical therapy aimed at maintenance of the normal potassium concentration and at reducing hypertension.

## **New Diagnostic Approaches in Zoonotic Diseases**

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### **Summary**

In One Health concept, human health and animal health are evaluated together. Zoonoses are diseases that can be transmitted from animals to humans. Sixty percent of existing infectious diseases and 75% of the emerging infectious diseases are zoonoses. They are caused by a wide range of pathogenic bacteria, viruses, parasites and fungi. Some of the examples of the endemic zoonoses agents are *Bacillus anthracis*, *Brucella spp*; emerging zoonoses agents are pandemic influenza H1N1 2009, Avian influenza (H5N1) and (H7N9); vector borne zoonoses agents are *Borrelia burgdorferi* sensu lato that cause Lyme disease, *Anaplasma phagocytophilum*, Tick-borne encephalitis virus (TBEV). Accurate diagnosis and timely treatment of zoonotic diseases is vital, because they are the cause of major public health problems all around the world. Some of them can cause death if not diagnosed and are untreated. Zoonotic diseases also can hamper production and trade of animal products and cause economical loss. Culture-dependent direct and immuno-diagnostic indirect detection methods have been developed to diagnose zoonotic diseases. The laboratory diagnosis are carried out traditionally by microscopy, isolation and identification of the pathogen by culture and biochemical tests. Matrix-assisted laser-desorption-ionisation time- of-flight mass spectrometry (MALDI-TOF-MS) are routinely used to identify cultured pathogens. Nucleic acid amplification techniques following the culture or from the clinical specimens are commonly used, improving the diagnostic efficiency. DNA hybridization, Real Time PCR, quantitative PCR (qPCR), multiplex PCR, reverse transcriptase PCR, restriction fragment length polymorphism analysis (RFLP), sequencing, DNA microarrays, digital PCR and Loop-mediated isothermal amplification (LAMP) have been used in diagnostics of various zoonotic diseases. For molecular typing of the microorganisms, typing methods such as multilocus enzyme typing (MLEE), multilocus sequence typing (MLST), Multi-locus variable-number tandem repeat analysis (MLVA) or single nucleotide polymorphism (SNP) have been used. Metagenomics-Next generation sequencing (NGS) based on genome sequences of different microorganisms from a microbial community have been developed for detection of the microorganisms that cannot be detected with routine methods and for the unculturable microorganisms. The immuno-diagnostic tests are based on detection of humoral immune response such as; ELISA, IFAT, magnetic bead multi analyte assays, rapid immunochromatographic tests or detection of cellular immune response such as ELISPOT. In this presentation, new laboratory diagnostic approaches in zoonotic diseases for human and domestic animals and their efficiency will be discussed focusing on selected zoonotic infectious diseases.

**Keywords:** Zoonotic diseases, new diagnostic tests

## Zoonotik Hastalıklarda Yeni Tanı Yaklaşımları

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### Özet

Tek Sağlık konseptinde insan sağlığı ve hayvan sağlığı birlikte değerlendirilir. Zoonozlar, hayvanlardan insanlara bulaşabilen hastalıklardır. Mevcut enfeksiyon hastalıklarının yüzde altmış ve yeni ortaya çıkan enfeksiyon hastalıklarının yüzde 75'i zoonozlardır. Bu hastalıklara çok çeşitli patojenik bakteriler, virüsler, parazitler ve mantarlar neden olur. Zoonoz etkenlerine verilebilecek örnekler şunlardır: Endemik zoonozlar, örn; *Bacillus anthracis*, *Brucella spp*; yeni ortaya çıkan zoonoz etkenleri, örn; pandemik influenza H1N1 2009, Avian influenza (H5N1) ve (H7N9); vektör kaynaklı zoonoz etkenleri, örn; Lyme hastalığına neden olan *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, Kene kaynaklı ensefalit virüsü (TBEV). Zoonotik hastalıkların doğru teşhisi ve zamanında tedavisi hayati önem taşımaktadır, çünkü bunlar tüm dünyada önemli halk sağlığı sorunlarına yolaçmaktadır. Bir kısmı teşhis edilemez ve tedavi edilmezse, ölümlü sonuçlanan hastalıklara neden olabilir. Zoonotik hastalıklar ayrıca hayvansal ürünlerin üretimini ve ticaretini engelleyebilir ve ekonomik kayıplara neden olabilir. Zoonotik hastalıkları teşhis etmek için kültüre dayalı-doğrudan ve immünohistokimyasal-dolaylı tespit yöntemleri geliştirilmiştir. Laboratuvar teşhisi geleneksel olarak mikroskopi, kültürde patojenin izolasyonu ve biyokimyasal testlerle tanımlanması ile gerçekleştirilir. Matris Destekli Lazer Desorpsiyon İyonizasyonu-Uçuş Zamanlı-Kütle Spektrometresi (MALDI-TOF-MS), kültürde üretilmiş patojenleri tanımlamak için rutin tanıda kullanılmaktadır. Kültür veya klinik örnekler ile yapılan nükleik asit amplifikasyon teknikleri yaygın olarak kullanılmaktadır ve bu da tanısal verimliliği artırmaktadır. DNA hibridizasyonu, Gerçek Zamanlı PZR, Kantitatif PZR, multipleks PZR, Ters Transkriptaz PZR, restriksiyon fragman uzunluğu polimorfizmi analizi (RFLP), sekans analizi ve DNA mikroarray, dijital PZR, döngü aracılı izotermal amplifikasyon (LAMP), çeşitli zoonotik hastalıkların teşhisinde kullanılmaktadır. Mikroorganizmaların moleküler tiplendirmesi için multilokus enzim tiplendirmesi (MLEE), multilokus dizi tiplendirmesi (MLST), multi-lokus değişken sayı-tekrar analizi (MLVA) veya tek nükleotid polimorfizmi (SNP) gibi tiplendirme yöntemleri kullanılmaktadır. Metagenomik-Yeni Nesil Dizileme (NGS) yöntemi, bir mikrobiyal toplulukta yer alan farklı mikroorganizmaların genom dizilerine dayalıdır ve rutin yöntemlerle tespit edilemeyen mikroorganizmaların ve kültürde üretilmeyen mikroorganizmaların tespiti için geliştirilmiştir. İmmünohistokimyasal testler, humoral immün yanıtın saptanmasına dayalı olan ELISA, IFAT, manyetik boncuk çoklu analit testleri, hızlı immünokromatografik testler veya hücresel immün yanıtın tespitine yönelik ELISPOT gibi testlerden oluşmaktadır. Bu sunumda, insanlarda ve evcil hayvanlarda zoonotik hastalıkların tanısında kullanılmakta olan yeni laboratuvar tanı yaklaşımları ve bunların etkinlikleri, bazı zoonotik enfeksiyon hastalıkları baz alınarak tartışılacaktır. **Anahtar kelimeler:** Zoonotik hastalıklar, yeni tanı testleri

**Echocardiography in Most Common Canine and Feline Cardiac Diseases**

Mara BAGARDI

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**Summary**

Echocardiography has become an indispensable tool in the specialty practice of veterinary cardiology. The Specialty of Cardiology of the American College of Veterinary Internal Medicine (ACVIM) has recognized the need to adopt profession-wide standards for nomenclature, display and recording, interpretation, communication, and publication of images obtained using this technology. Accordingly, a committee, composed of experienced veterinary cardiac ultrasonographers, was formed to produce a report of recommendations for standards in veterinary echocardiography.

The presentation “Echocardiography in most common canine and feline cardiac diseases” provides information about the most common canine and feline acquired and congenital heart disease, which each clinician most likely has to manage in daily clinical practice (canine myxomatous mitral valve disease, dilated cardiomyopathy, congenital heart diseases, pulmonary hypertension, and feline cardiomyopathies), explaining which are the patients for which it is indicated the use of an echocardiographic examination. Furthermore, the presentation resumes the main key points of the echocardiographic standard examination and then present some clinical cases, some hints to their correct clinical and therapeutic approach and their ultrasound appearance.



**Tooth Preservation with Endodontic Therapies in Equine Dentistry**

Tibor Akos HEVESI

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In this presentation, the tooth preservation with endodontic therapies in equine dentistry was evaluated.

## **Equine Myopathies: Current Classification, Diagnosis and Therapeutic Approach**

Giovanni STANCARI

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### **Summary**

Horses are superb athletes and their powerful musculature is the system that allows them to perform physical activities, including running or jumping, at a level that surpasses most other animals of similar body size.

The horse's skeletal musculature is highly physically adapted. Relative to other species, it has a greater muscle mass relative to body weight (BW), which can reach 55% in Thoroughbred. The activity of this impressive muscular mass is permitted by large intramuscular stores of energy substrates (glycogen in particular, which can reach 2% BW), a high global mitochondrial volume, a high proportion of fast-twitch muscle fibers with shortening velocities greater than would be expected for an animal of this size.

From an energetically point of view, exercise is fundamentally associated with an increase in power output achieved by contraction of muscles and associated to an increase metabolic rate, which is characterized by a continuous consumption and replace of energy stored in adenosine triphosphate (ATP). Thus, muscular movement requires the transformation of chemical energy stored in metabolic substrates to the kinetic energy of muscular contraction. All pathways integral to energy supply are concerned with the ultimate production of adenosine triphosphate (ATP), the final carrier of energy “packages” utilized by muscles for contraction. There's a finite store of energy inside the muscle, in the form of creatine phosphate and ATP, which are sufficient to restore the highly energetic phosphate group, but permitting to maintain muscular activity for only a few seconds. Therefore, for continuous muscular exertion, it is necessary to resynthesize ATP, and this is done by the pathways of aerobic (oxidative) and anaerobic phosphorylation. The first, a more efficient process, is related to oxygen availability. It's the primarily energetic pathway at a submaximal intensity of exercise, and use glucose (from muscular glycogen) and lipids as substrate. In any event, their oxidation converges at the level of the tricarboxylic acid cycle, and this implies availability of glucose for a complete lipid oxidation. When the intensity of exercise rises, the aerobic pathway is integrated by the anaerobically one which instead use only glucose as fuel. This pathway produces a metabolic derivate (i.e. lactic acid), which is the most important cause of peripherally fatigue during maximal exercise.

The energetic pathways are dependent to the exercise intensity, which moreover imply a different recruitment of muscular fiber. The latter possess different characteristics of velocity of contraction

(slow: type 1; fast: type 2 A/AX/ X), and metabolic capacity which can range from primarily aerobic (type 1) to anaerobic (type 2X). The predominantly type of fiber is genetically transmitted and is related to athletic performance in the different equine discipline. Several factors can stimulate the differentiation of the fiber, and training is one of the most important.

Exercise could be an important cause of increase muscle protein breakdown and, in some cases, can directly induce muscle damage. In fact, muscle can be exposed to several insults: traumatic, ischemic, exercise-induced or due to underlying disease. These share a common final pathway that leads to cell death—aberrant calcium cycling. Particularly, the latter induces: activation of enzyme (protease), impair energy production (mitochondrial function) and chemotaxis of neutrophils with subsequent inflammatory response. Hopefully, muscles possess a remarkable regenerative capacity primarily due to presence of stem cells in the basal membrane. These cells, activated by muscular damage, can produce a complete *restitutio ad integrum* of the damaged fibers.

In the acute phase of muscular damage horses present clinical signs, related to inflammation and pain, which can include: profuse sweating, tachycardia and tachypnea, reluctance to move, stiff gait, muscular pain by palpation and production of pigmented urine. In chronic cases history can report poor performance and intermittent sign of stiffness post exercise and the clinical examination only signs of muscular atrophy. The diagnostic approach is integrated by complementary evaluation including: serum biochemistry (CK, AST), muscular biopsy, electromyography (EMG), muscular ultrasonography, thermography, nuclear scintigraphy and genetic tests.

The myopathies are classified in exertional or nonexertional disorders. The first group include sporadic and chronic forms. The first are caused by focal or generalized trauma to muscle, dietary imbalances that affect muscle function, and exercise performed beyond any training adaptation or to the point of exhaustion. Diagnosis is obtained by history, clinical examination and result of ancillary tests. Treatment protocol for the acute phase include: rest, fluid therapy (large volumes of isotonic fluid), analgesics ( $\alpha 2$  agonist, opioids), and Acepromazine.

The chronic group of exertional disorders include forms such as: recurrent exertional rhabdomyolysis (RER), polysaccharide storage myopathy (PSSM or EPSM) type 1 and 2, myofibrillar myopathy (MFM).

RER is reported in 5-10% of Thoroughbred and Standardbred racehorses and more recently described in Arabian and Warmblood horses. Its pathogenesis is not perfectly clarified; some authors suggest a genetic basis at least for Thoroughbred. There is strong evidence that RER relates to abnormalities of skeletal muscle calcium regulation.

PSSM 1 has high prevalence in Quarter Horse and related breed, Belgian and Percherons draft horses. Affected animals present an alteration of glycogen synthase 1 gene (GYS1) and, in more severe cases,

of RYR1 gene encoding the calcium release channel of the skeletal muscle sarcoplasmic reticulum. These genetic alterations cause an intramuscular accumulation of amylase-resistant glycogen, and an impairment of the cellular energy production with subsequent rhabdomyolysis.

PSSM 2 is reported in Warmblood horses, Thoroughbred, Standardbred, Morgans and Icelandic Horses. The muscular problem is not related to genetic disorders. In this subject it is possible to identify, by means of muscular biopsy, an abnormal accumulation of amylase-sensitive glycogen.

MFM was recently identified in Arabian and Warmblood horses. The disorder shows characteristics suggestive of disturbed oxidative processes. No scientifically validated genetic test is available, and diagnosis currently relies on muscle biopsy. Many affected horses show abnormal accumulations of desmin, a cytoskeletal protein, in mature muscle fibers associated to aggregates of amylase-sensitive glycogen.

In this chronic exertional myopathy, the diagnosis, in absence of acute symptoms, is frequently obtained by muscle biopsy and genetic test (PSSM1). The objective of treatment is to prevent new episodes, so the change of management is mandatory. This includes essentially: regular daily exercise, change in diet (reduce carbohydrate and increase fat), electrolyte and antioxidant supplementation (vitamin E and Selenium).

Among the nonexertional manifestation there are: Vitamin E-deficient Myopathy, and Hypoglycin Myopathy and Immune Mediate Myositis (IMM).

Vitamin E Deficient Myopathy is related to a chronic deficiency of Vitamin E in the diet. This manifestation seems to be a precursor of Equine Motor Neuron Disease (EMND). The clinical signs are similar to EMND, and characterized by muscle weakness and diffuse muscle atrophy. Diagnosis is obtained by muscle biopsy. Unlike EMND, horses with this form of disorder are remarkably responsive to treatment with tocopherol.

Hypoglycin Myopathy is a toxic manifestation related to the assumption of Hypoglycin A. This toxic is present in seed or young shoots of Acer species trees (*Acer negundo*) and the European sycamore maple (*Acer pseudoplatanus*). The hepatic metabolite of Hypoglycin A (methylenecyclopropylacetic acid or MCPA) impairs the lipid metabolic pathways, causing accumulation of fat esters which damages muscle cell membranes and results in energy deficiency. Clinical signs include muscular weakness, sweating, fasciculations, stiffness, tachycardia, tachypnea, recumbency, which can be followed by collapse and death from respiratory or cardiac failure. High mortality rates are reported in cases with overt clinical signs. Early recognition may improve the prognosis by administration of early aggressive fluid therapy with antioxidant and antiinflammatory treatment, including dimethylsulfoxide (DMSO), vitamin E, vitamin C, and NSAIDs.

IMM affected primarily Quarter Horse and related breed, and is caused by an autosomal dominant genetic mutation in MYH1, which encodes type 2 X myosin heavy chain. Horses present symmetric atrophy which initially affects the topline muscles. Diagnosis of IMM has previously been made by identifying lymphocytic infiltrates in gluteal and epaxial muscle biopsies in early stages of atrophy. Genetic testing will soon be available to diagnose IMM in Quarter horse–related breeds. Early identification of IMM is key to halting muscle loss administering dexamethasone (0.06 mg/kg) or prednisolone (1 mg/kg).

Muscular disorders could impact performance of equine athlete as well as could be a cause of death in equine species. Their prompt identification, by a thorough clinical examination and proper ancillary diagnostic test, permit to obtain a better prognosis. In most exertional myopathy, altering diet and exercise regimes to compensate for underlying defects is often the

best available strategy to assist the affected horses.

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## **The Role of Entrepreneurship Education in Veterinary Medicine**

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### **Summary**

This conference paper focuses on providing insight into the role of entrepreneurship education and how it can be practised in the area of veterinary medicine. Although entrepreneurship education has started back in the late 1940s in the United States, it was only 30 years ago that this module began to be taught in Europe. As a result, there are very few studies conducted in this specific field, linking the outcome of entrepreneurship education and veterinary medicine. Relevant literature was reviewed, and based on the findings, the author provides the suggestion on how academics can contribute to creating a favourable environment to pursue entrepreneurial activities. The finding reveals that this study addresses an important gap in the literature on the impact of entrepreneurial actions and the process of opportunity recognition, which has relevance for university managers, academic entrepreneurs and industry. The study highlights the necessity of a collaborative network that creates synergies among different actors as one of the key success factors. An example of good practices is presented for a better understanding of the potential and the use of synergies among EU funds in the fields of research and innovation.

**Keywords:** Collaborative network, entrepreneurial education, entrepreneurial orientation, veterinary medicine

**“Wild Animals and Wildlife Medicine” in Veterinary Education in Turkey from Past to Present**

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**Summary**

Veterinary medicine education, which started in Istanbul in 1842 in Turkey, has shown continuous improvement due to the medical necessities seen in animal husbandry in the country and the world. The school, which increased the number of students due to the increase in epidemics, sent its graduates to Europe for specialization education at the beginning of the 20th century. The school, which was moved to Ankara in 1933, increased the education period to five years and served as the only faculty in our country until the 1970s. Ankara University Faculty of Veterinary Medicine, which has been serving all animal health issues in our country and nearby geography throughout its past history, established the "Aquaculture, Fisheries and Game Animals" department on May 16, 1967, which examines animal species other than domesticated. With this established department, the first academic perception and studies on wild animal species started. After this department, whose academic activities ended in the 1980s for various reasons, studies on wild animals continued with the individual efforts of academics in faculties. After a long time, the "Wildlife and Ecology Department" established within the Afyon Kocatepe University Faculty of Veterinary Medicine became the pioneer of the new era in our country, and this was followed by Faculty of Veterinary Medicine of Kafkas University. In our country, "Wild animal diseases and ecology departments" continue their activities within the body of Istanbul University Cerrahpaşa, Dicle University, Ondokuz Mayıs University and Erciyes University Veterinary Faculties. In addition to these departments in our faculties, with the joint investments made with the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, five Wildlife Rescue and Rehabilitation Centers with infrastructure compatible with the wild life of the region. These centers have been put into service at this university campuses in the last years and these are managed by academic staff. Academic interest and investments in this field are increasing day by day in our faculties, whose academic staff and geography are suitable for such activities.

**Geçmişten Günümüze Türkiye Veteriner Hekimliği Eğitiminde “Yabani Hayvanlar ve Yaban Hayatı Hekimliği”**

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**Özet**

Türkiye’de 1842 yılında İstanbul’da başlayan veteriner hekimliği eğitimi, ülke ve dünya hayvancılığında görülen tıbbi zorunluluk nedeni ile sürekli gelişim göstermiştir. Salgın hastalıkların artması sebebi ile öğrenci sayısını artıran okul 20. yüzyılın başında ihtisas eğitimi için kendi mezunlarını Avrupa’ya göndermiştir. Ankara’ya 1933’te taşınan okul eğitim süresini beş yıla çıkartmış ve 1970’lere kadar tek fakülte olarak ülkemizde hizmet vermiştir. Geçmiş tarihi boyunca ülkemiz ve yakın coğrafyadaki tüm hayvan sağlığı konularında hizmet veren Ankara Üniversitesi Veteriner Fakültesi, 16 Mayıs 1967 yılı itibarı ile evcil türler dışındaki hayvan türlerini de inceleyen “Su ürünleri, Balıkçılık ve Av hayvanları” anabilim dalını kurmuştur. Kurulan bu anabilim dalı ile yabani hayvan türleri üzerine ilk akademik ilgi ve çalışmalar da başlamıştır. Akademik faaliyetleri çeşitli gerekçelerle 1980’li yıllarda son bulan bu anabilim dalı sonrasında yabani hayvanlar üzerine olan çalışmalar fakültelerdeki akademisyenlerin bireysel çabaları ile sürdürülmüştür. Uzun bir sürenin ardından Afyon Kocatepe Üniversitesi Veteriner Fakültesi bünyesinde kurulan “Yaban hayatı ve ekoloji Anabilim Dalı” ülkemizde yeni dönemin öncüsü olmuş ve bunu Kafkas Üniversitesi Veteriner Fakültesi takip etmiştir. Ülkemizde halen İstanbul Üniversitesi Cerrahpaşa, Dicle Üniversitesi, Ondokuz Mayıs Üniversitesi ve Erciyes Üniversitesi Veteriner Fakülteleri bünyesinde “Yabani hayvan hastalıkları ve ekoloji anabilim dalları” faaliyetlerine devam etmektedir. Fakültelerimizde bu bölümlerin yanı sıra Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü ile yapılan ortak yatırımlar ile bölgenin yaban hayatı ile uyumlu altyapıya sahip beş adet Yaban Hayatı Kurtarma ve Rehabilitasyon Merkezi bulunmaktadır. Bu merkezler son yıllarda bu üniversite yerleşkelerinde hizmete açılmıştır ve akademik personel tarafından yönetilmektedir. Akademik kadroları ve bulunduğu coğrafya bu tür faaliyetlere uygun olan fakültelerimizde bu alana yönelik akademik ilgi ve yatırımlar gün geçtikçe artmaktadır.





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**ORAL  
PRESENTATIONS**

**Causes and Remedies for Non-Infectious Infertility in Sheep and Goats**

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**Summary**

In the literature, there is a wide range of information about the infectious infertility (decrease in reproductive performance or litter productivity), due to a certain microbial factor, in different breeds of breeding animals. Also, regarding infertility cases in animals, dairy breeding females (*eg.* Holstein/Simmental cow, Chios ewe and Saanen doe) come to mind first. However, non-microbial (non-infectious) infertility cases are very common in both males and females in the field. Including these 'non-infectious' factors, infertility is particularly important for sire bulls as year-round breeder or for stallions, rams and bucks that mate seasonally only during a certain period of the year (seasonal breeder). Seasonal heavy use of breeding males can lead peculiarly to their premature sexual exhaustion. Although a certain disease (Brucellosis, Maedi etc.) is not observed in male breeders in cases of 'non-infectious' infertility, which may be mainly of environmental (care-feeding) and individual origin, the regular expected semen production, libido, ejaculation and litter (lamb/kitten) yield can be seriously impaired. Even in severe infertility or advanced cases, the reproductive process may stop completely. In the solution of infertility cases, care and feeding (including flushing) in the breeder flock should firstly be arranged, and all flock-based protective measures (vaccination, parasitic control, hoof and udder care, mating-lambing/kidding-milking hygiene) should be taken. Care should also be taken in reproductive management in issues such as the use of teaser ram/buck, male/female ratio, weekly mating number, exercise and experience. In addition, inbreeding and the use of older breeders should be avoided. Especially in synchronization practices, necessary precautions should be taken against extremely hot/arid climatic conditions, together with sufficient number of rams/bucks used rotationally.

Finally, for a sustainable profit in sheep breeding, protective-preventive measures as farms and flocks should always be preferred to problem-solving (treatment) approaches. In enterprises where female animal welfare is a priority, sustainable individual yield (meat, milk, offspring) increases. Additionally, given in a small number of expensive male, the expected libido and fertility due to seasonal heavy use is only possible thanks to the well-being level and ideal reproductive management. Undoubtedly, for achieving a sustainable profit in animal husbandry, it is vital to consider animal health and welfare together. In small ruminants, the necessary interest and responsibility in production provides convenience in the ideal solution with the opportunity of early intervention against unwanted fertility problems in breeding animals.

**Keywords:** Small ruminant, management, feeding, reproduction, infertility

## Koyun ve Keçilerde Non-Enfeksiyöz İnfertilite Nedenleri ve Çözüm Yolları

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### Özet

Literatürde, değişik türlerdeki damızlık hayvanlarda belirli bir mikrobiyel etkene bağlı enfeksiyöz infertilite (üreme performansının veya yavru veriminin azalması) hakkında çok geniş bilgiler yer almaktadır. Ayrıca, hayvanlarda infertilite vakalarında ilk olarak sütçü damızlık dişiler (örneğin Holstein/Simmental inek, Sakız koyun ve Saanen keçi) akla gelmektedir. Ancak, sahada mikrobiyel kökenli olmayan (non-enfeksiyöz) infertilite vakalarına hem dişilerde hem de erkeklerde çok yaygın olarak rastlanmaktadır. Bu ‘non-enfeksiyöz’ faktörlerini kapsayan infertilite durumu özellikle yıl-boyu aktif damızlık boğalarda veya yılın sadece belli bir döneminde (sezonluk) üreme gösteren aygır, koç ve tekelerde önemlidir. Damızlık erkeklerin sezonluk yoğun kullanımı onların istemsiz olarak erken seksüel tükenmesine yol açabilir. Erkek damızlıklarda başlıca çevresel (bakım-beslenme) ve bireysel kaynaklı olabilen ‘non-enfeksiyöz’ infertilite vakalarında belli bir hastalık (Brucellosis, Maedi gibi) durumu gözlenmese de, hayvandan düzenli olarak beklenen sperma üretimi, libido, aşım, ejakulasyon ve yavru (kuzu/oğlak) verimi ciddi oranda aksayabilir. Hatta şiddetli infertilite veya ileri vakalarda üreme süreci tamamen durabilir.

İnfertilite vakalarının çözümünde, öncelikle damızlık sürüdeki bakım ve beslemenin (flushing dâhil) düzenlenmesi, sürü temelli tüm koruyucu tedbirlerin (aşılama, paraziter mücadele, tırnak ve meme bakımı, aşım-doğum-sağım hijyeni) alınması gerekir. Ek olarak, üreme yönetiminde arama koçu/tekesi kullanımı, erkek/dişi oranı, haftalık aşım sayısı, hareketlilik ve tecrübe gibi konularda hassas davranılmalıdır. Ayrıca, akrabalı yetiştirme ve yaşlı damızlıkların kullanımından sakınılmalıdır. Özellikle senkronizasyon uygulamalarında yeterli sayıda ve dönüşümlü olarak koç/teke kullanımı ile birlikte aşırı sıcak/kurak iklim koşullarına karşı da gerekli tedbirler alınmalıdır.

Son olarak, küçükbaş yetiştiriciliğinde sürdürülebilir kazanç için, çiftlik ve sürü olarak koruyucu-önleyici tedbirler her zaman için sorun çözücü (tedavi) yaklaşımlarına tercih edilmelidir. Dişi hayvan refahının öncelikli olduğu işletmelerde, sürdürülebilir bireysel verim (et, süt, yavru) artmaktadır. Ayrıca, az sayıdaki pahalı erkeklerde ise sezonluk yoğun kullanıma bağlı beklenen libido ve fertilitate elde etmek ancak refah seviyesi ve ideal üreme yönetimi sayesinde olur. Kuşkusuz, hayvancılıkta sürdürülebilir kazanç, hayvan sağlığı ve refahını birlikte dikkate almayı zorunlu kılar. Küçük ruminantlarda, üretimdeki gerekli ilgi ve sorumluluk, damızlık hayvanlarda istenmeyen fertilitate sorunlarına karşı erken müdahale fırsatıyla birlikte ideal çözümde kolaylık sağlar.

**Anahtar kelimeler:** Küçük ruminant, bakım, besleme, üreme, infertilite

## Sex Determination in Giant Anteater (*Myrmecophaga tridactyla*) Using PCR Amplification

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### Summary

The giant anteater (*Myrmecophaga tridactyla*) is the largest known anteater species. The main characteristics of this species are a long bushy tail (approximately 60-90 cm), long fur from nape to tail (resembling a mane), an elongated snout, remarkable foreclaws, and majestic body (182-217 cm in length). Sexual dimorphism is not evident in the giant anteater. Hence the genetics-based techniques are extremely necessary for sex determination in this species, especially in captive animals. Therefore, the aim of this study was to determine the sex of giant anteater species using PCR amplification. In this context, genomic DNA was extracted from hair root samples using Roche high pure PCR template preparation kit. The sex-determining region on the Y chromosome (SRY) primers were specifically designed based on the NCBI and Ensembl databases and they were verified with the BLAST program. The PCR amplification was carried out in a programmable thermal cycler, using appropriate conditions. Afterward, PCR products were run on 2% agarose gels containing 1µL GelRed and were photographed by a UV transilluminator. PCR reactions were carried out thrice with positive and negative controls. Sample profiles were determined by evaluating according to the presence or absence of the SRY region. A specific signal in the SRY amplification is diagnostic for a male individual with an SRY region. Hence it was determined that the samples belong to a male giant anteater. In this study PCR amplification, with SRY primer pair using DNA from hair roots, was shown to be a sensitive, reliable, and rapid method for identification of the sex in giant anteater because the specific PCR signal was only detected in male. Molecular genetic techniques are very effective and necessary in different applications such as animal breeding, health, and taxonomy as well as sex identification in species that do not display sexual dimorphism.

**Keywords:** Giant anteater, PCR, Sex identification, SRY

**PZR Amplifikasyonu Kullanarak Dev Karıncayiyende (*Myrmecophaga tridactyla*)  
Cinsiyet Tayini**

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**Özet**

Dev karıncayiyen (*Myrmecophaga tridactyla*), bilinen en büyük karıncayiyen türüdür. Bu türün temel özellikleri uzun, çalı gibi bir kuyruk (yaklaşık 60-90 cm), ense ile kuyruğa kadar uzanan uzun bir kürk (yeleye benzeyen), uzun bir burun, dikkat çekici ön pençeleri ve görkemli gövdesidir (182-217 cm uzunluğunda). Seksüel dimorfizm, dev karıncayiyende belirgin değildir. Bu nedenle, bu türde, özellikle tutsak hayvanlarda, genetik temelli teknikler cinsiyet tespiti için son derece gereklidir. Bu nedenle, bu çalışmanın amacı, PZR amplifikasyonu kullanarak dev karıncayiyen türüne ait cinsiyeti belirlemektir. Bu bağlamda, “Roche high pure PCR template preparation” kiti kullanılarak kıl köklerinden genomik DNA izole edilmiştir. Y kromozomu üzerindeki cinsiyet belirleme bölgesi (The sex-determining region on the Y chromosome: SRY) primerleri, NCBI ve Ensembl veritabanlarına dayalı olarak özel olarak tasarlanmış ve BLAST programı ile doğrulanmıştır. PZR amplifikasyonu, uygun koşullar kullanılarak programlanabilir bir termal döngüleyicide gerçekleştirilmiştir. Daha sonra PZR ürünleri, 1 uL “GelRed” içeren %2 agaroz jelde yürütülerek; bir UV transillüminatör ile fotoğraflanmıştır. PZR reaksiyonları, pozitif ve negatif kontrollerle üç kez gerçekleştirilmiştir. SRY bölgesinin varlığına veya yokluğuna göre değerlendirilerek örnek profilleri belirlenmiştir. SRY amplifikasyonundaki spesifik sinyal, SRY bölgesine sahip olan erkek birey için ayırt edicidir. Bu nedenle örneklerin bir erkek dev karıncayiyene ait olduğu belirlenmiştir. Bu çalışmada, kıl köklerinden DNA kullanılarak SRY primer çifti ile yapılan PZR amplifikasyonunun, dev karıncayiyenlerde cinsiyetin belirlenmesi için hassas, güvenilir ve hızlı bir yöntem olduğu gösterilmiştir çünkü spesifik PZR sinyali sadece erkeklerde tespit edilmiştir. Moleküler genetik teknikler, hayvan yetiştiriciliği, sağlığı ve taksonomi gibi farklı uygulamalarda ve aynı zamanda seksüel dimorfizm sergilemeyen türlerde cinsiyet tanımlamasında çok etkili ve gereklidir.

**Anahtar kelimeler:** Cinsiyet tayini, dev karıncayiyen, PZR, SRY

## Management of Rectal Prolapse in Animals

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### Summary

Rectal prolapse is a protrusion or eversion of the rectal mucosa from the anus. Incomplete and complete prolapse are differentiated by the tissue layers affected. An incomplete prolapse (anal prolapse) is a protrusion of anorectal mucosa through the external anal orifice. Complete prolapse (rectal prolapse) is a double-layer invagination of the full thickness of the rectal tube through the anal orifice. Rectal prolapse must be differentiated from an ileocolic intussusception that is protruding from the anus (1, 2). This study emphasizes the importance of distinguishing between rectal prolapse and prolapsed invaginated bowel, especially for correct treatment practices.

**Keywords:** Differential diagnosis, invaginated bowel, operative treatment, rectal prolapse

## Hayvanlarda Rektal Prolapsusun Yönetimi

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### Özet

Rektal prolapsus, rektumun bir veya daha fazla tabakasının anüsten dışarı çıkması durumudur. Prolapsus olguları etkilenen doku katmanları tarafından ayırt edilir. Tam olmayan bir prolapsus (anal prolapsus) anorektal mukozanın dış anal delikten dışarı çıkmasıdır. Tam prolapsus (rektal prolapsus) rektal kanalın tüm kalınlığının çift katmanlı invajinasyonudur. Rektal prolapsus, anüsten dışarıya çıkan ileokolik invajinasyondan ayırt edilmelidir (1, 2). Bu çalışmada rektum prolapsusunun, prolabe olan invagine barsak halkasından ayırımının yapılmasının, özellikle doğru tedavi uygulamaları için önemi vurgulanmaktadır.

### Materials and Methods

I have shared some of the interesting cases of rectal prolapse we have encountered so far.

Our first case is a two-month-old short-haired female cat with prolapse recti. It was determined that the prolapsed intestinal mucosa of the cat was inflamed but preserved its vitality and had no necrotic areas. Clinical examination revealed prolapsed invagination.

Our second case is a four-month-old stray dog with rectal prolapse. It was determined that the prolapsed intestinal mucosa of the dog was edematous, there was plenty of fibrous tissue on it, and there was discoloration and bleeding in the mucosa near the anus. Intestinal rings were felt within the prolapsed part on palpation. During the clinical examination, the lubricated hemostatic forceps with a blunt flat tip were advanced 5-6 cm between the rectal wall and the prolapsed tissue, and the presence of prolapsed invagination was detected.

Our third case is a five-month-old Pointer dog brought in with the complaint of long-term constipation and prolapse recti. In the clinical examination, it was observed that the general condition of the patient was poor. The prolapsed intestinal loop was found to be hyperemic and edematous. In the x-ray images, it was determined that there was plenty of gas in the intestines.

Our fourth case is a two-month-old calf. It was observed that the prolapsed bowel was traumatized and there were necrotic areas on it.

## **Results**

In the first case, median laparotomy was performed under general and epidural anesthesia. During the operation, ileum and cecum were found to be invaginated into the ascending colon. It was determined that the invaginated intestinal loops of the cat preserved their vitality and the intestinal peristalsis continued. No operative procedure was performed on the intestines.

The second case was operated under general and epidural anesthesia. It was determined that the intestinal loop, including the caecum, had lost its vitality and this part was resected. An end-to-end anastomosis was performed between the ileum and the colon using the Nipple valve method.

The third case underwent surgery after supportive treatment. Two different invaginations were detected during the operation, both ileocolic and colocolic. While the ileocolic invagination remained viable, most of the colocolic invagination was found to be necrotic, and the necrotic area was excised and an end-to-end anastomosis was performed.

Unrejected or severely traumatized prolapses require amputation. In the fourth case, prolapsed bowel containing traumatic and necrotic areas was amputated and removed under general anesthesia and epidural anesthesia.

## **Discussion**

Rectal prolapse is principally associated with endoparasitism or enteritis in young animals, and tumors or perineal hernias in middle-aged and older animals. However, any condition that causes tenesmus may cause rectal prolapse. Weakness of perirectal and perianal connective tissues or muscles,

uncoordinated peristaltic contractions, and inflammation or edema of rectal mucous membranes predisposes patients to rectal prolapse (1).

Rectal prolapse occurs in a wide range of species. It may occur at any age but is more common in young animals. Protrusion of anorectal mucosa is obvious on physical examination. The degree of prolapse may vary from a few millimeters to several centimeters. Rectal prolapse must be differentiated from an ileocolic intussusception that is protruding from the anus (1).

The invaginated bowel loop may protrude from the rectum and can be mistaken for a rectal prolapse. To distinguish rectal prolapse from protruding intussusception, the area around the protruding tissue should be palpated; the existence of a fornix indicates rectal prolapse rather than intussusception (1, 2).

Treatment and prognosis depend on the cause, the degree of prolapse, chronicity, and whether it is a recurrent prolapse. Acute rectal prolapse is easily treated, but chronic disease may require resection. For acute prolapses with minimal tissue damage and edema, manual reduction and placement of a purse-string suture around the anus are recommended. Warm saline lavage, massage, and lubrication should be applied to the prolapsed tissue prior to digital reduction. Epidural anesthesia may also help prevent additional straining and re prolapse (1, 2). Most rectal prolapse patients respond well to manual reduction when the causative agent is treated and resolved. Colopexy is indicated for recurrent prolapses. Colopexy is indicated when rectal prolapse recurs after multiple attempts at conservative management (3).

### **Conclusion**

It is very important to distinguish between rectal prolapse and prolapsed invaginated intestinal loop, especially for correct treatment practices.

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**Can We Use Probiotics in Canine Parvovirus Infections and Does It Work?**

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**Summary**

Canine parvovirus enteritis (CPV) is a very contagious and worldwide spread disease, especially in young dogs. The disease has a high mortality rate and there is no definitive treatment, yet. Therefore, successful alternatives are needed for definitive treatment. Probiotics have beneficial effects on intestinal flora and the immune system. Recovery time could be shorter in CPV infections with probiotic strain additives. In this presentation, effects of probiotics in CPV infection were evaluated.

**Keywords:** Canine parvovirus enteritis, probiotics, dog

**Köpek Parvovirus Enfeksiyonlarında Probiyotik Kullanabilir Miyiz ve İşe Yarıyor Mu?**

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**Özet**

Köpek parvovirus enteritis (Canine parvovirus enteritis, CPV), özellikle genç köpeklerde çok bulaşıcı ve dünya çapında yaygın bir hastalıktır. Hastalık yüksek bir ölüm oranına sahiptir ve henüz kesin bir tedavisi yoktur. Bu nedenle kesin tedavi için başarılı alternatiflere ihtiyaç vardır. Probiyotiklerin bağırsak florası ve bağışıklık sistemi üzerinde olumlu etkileri vardır. Probiyotik içeren katkı maddeleri ile CPV enfeksiyonlarında iyileşme süresi daha kısa olabilir. Bu bildiride, probiyotiklerin CPV enfeksiyonunda etkileri değerlendirilmiştir.

**Anahtar kelimeler:** Canine parvovirus enteritis, probiyotikler, köpek

**Introduction**

During the early 1970s, a new infectious disease of pups, Canine parvo virus (CPV) infection characterized by either gastroenteritis or myocarditis, was observed worldwide (4,5). Mortality could be as high as 91%, if treatment would not be done (7). There is still not a definitive treatment, mortality rates may be increase 4-40% in spite of aggressive supportive care. Some complications, such as sepsis, systemic inflammatory response syndrome, bacterial translocation with subsequent coliform septicaemia, endotoxaemia, multiorgan dysfunction or disseminated intravascular coagulation may cause die (6,9).

## Effects of probiotics

Probiotics are viable microorganisms have positive health effects for the prevention and treatment of some specific pathologic conditions (3). Probiotics has potential health-promoting effects which include lowering of erythrocyte fragility indexes, increased phagocytic neutrophil capacity and substantial reduction in serum endotoxin concentrations (1). But, their exact mechanism still is not clear. Probiotics has some more beneficial effects such as anti-inflammatory properties or competition for nutrients or adhesion sites with potential pathogens and immunomodulation (10).

According to previously studies, Enterobacteriales, Fusobacteriales, and Clostridiales are abundances bacteria in the intestine of dogs. Enterobacteriales have relatively higher abundance in the canine small intestine, and Clostridiales are predominant in the duodenum and jejunum (40% and 39%, respectively) (2). Whereas Proteobacteria and Actinobacteria are the dominant phyla in ileum, the colon microbiome contains a higher abundance of Firmicutes, Proteobacteria and Fusobacteria. Duodenal and jejunal ingesta samples contain 22% and 10% of Lactobacillales, respectively (8).

The effects of a probiotic possibly depend on the probiotic strain, mixture and concentration. There are several studies about effects of probiotics on dogs on particular conditions. Probiotic treatment provided a significant reduction in duration of uncomplicated acute diarrhoea in dogs. Treated with probiotics normalization of dysbiosis can be effective for the recovery in dogs with idiopathic inflammatory bowel disease. In addition, the effect of probiotic treatment on dogs with haemorrhagic diarrhea due to parvovirus has been investigated and a more rapid improvement in clinical signs as well as in leukocyte counts was observed (10).

The probiotic treatment can provide an accelerated normalization of the intestinal microbiome. Dogs with aseptic Acute Haemorrhagic Diarrhea Syndrome showed a rapid decrease of *C. perfringens* encoding NetF genes and fast clinical recovery in the groups under the symptomatic treatment without antibiotics and with probiotic therapy (10).

In one of our studies, *Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii spp bulgaricus*, *Bifidobacterium longum*, *B. breve*, *B. infantis* and *Streptococcus salivarius spp thermophilus*) were orally administered ( $450 \times 10^9$  colony-forming units/day in 100 mL water) for 1-3 weeks in puppies with CPV. Finally, 90% (9 of 10 animals) puppies in the oral probiotic administered group and 70% (7 of 10 animals) puppies in the supportive and symptomatic therapy group survived. Clinical signs and leukocyte counts more rapidly improved in dogs treated with probiotics (1).

## Conclusion

In conclusion, there are still a lot of unclear points about correct probiotic strains, dosage, and exact effects of the probiotics in animals and humans. However, according to our studies probiotics can

improve the recovery period in CPV infection in dogs. A probiotic mix that includes many different strains and high colony-forming units could be more beneficial to provide balance of microbiota in dogs than other probiotic compounds. In this way recovery time could be shorter in CPV infections.

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## Investigation of Persistent Organic Pollutants in Hair Samples Collected From Domestic Cats and Dogs

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### Summary

Persistent organic pollutants (Persistent Organic Pollutants, POPs), such as organochlorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), dioxins and some organophosphate pesticides (OPs) are extremely dangerous substances for humans, animals and the environment. Because they can be transported long distances, remain in the environment for a long time and they have biomagnification and bioaccumulation features in the ecosystem. In this study, it was aimed to determine important POPs in hair samples collected from domestic cats and dogs, to evaluate exposure status and possible effects, and to examine the relationship between pollutant levels and age, gender and nutritional status. Hair samples were collected from a total of 73 animals, including 35 cats (18 females and 17 males) and 38 dogs (13 females and 25 males) in the study. In total, 32 POPs (2 OPs, 9 OCPs, 6 PAHs and 15 PCBs) were analyzed by Gas Chromatography-Mass Spectrometry method. Detection limits of the analytical method were 0.01-0.11 ng/g, recoveries were 62.51-200.54% and linearities ( $R^2$ ) were 0.993-0.999. As a result, all samples ( $n=73$ ) contained at least one pollutant. While 27 of the 32 investigated POPs were detected, 2,4-DDE, PCB81, PCB118, PCB128 and PCB208 were not found in any samples. The incidence of POPs in hair samples was found to be quite high. In cats, OPs ( $n=13$ ), OCPs ( $n=24$ ), PCBs ( $n=25$ ) and PAHs ( $n=26$ ) were detected as  $751.46\pm 213.69$ ,  $552.29\pm 142.35$ ,  $57.81\pm 14.15$  and  $11.59\pm 1.57$  ng/g, respectively. In dogs, OPs ( $n=18$ ), OCPs ( $n=31$ ), PCBs ( $n=29$ ) and PAHs ( $n=31$ ) were determined as  $548.65\pm 133.36$ ,  $312.84\pm 107.24$ ,  $46.37\pm 12.51$  and  $19.07\pm 7.07$  ng/g, respectively. There was no difference between cats and dogs in terms of POPs concentrations, as well as age, gender and nutritional status ( $p>0.05$ ). As a result, it was seen that used analytical method was satisfactory, the incidence of POPs was high in cats and dogs and concentrations of some of them were very high. Obtained data showed that although many of POPs have been banned for a long time, they are still important environmental pollutants. Therefore, POPs should be monitored periodically in hair and other samples. To the best of our knowledge, this is the first report to investigate POP levels in hair samples from cats and dogs in Turkey.

**Keywords:** Persistent organic pollutants, cat, dog, hair

## Evcil Kedi ve Köpeklerden Toplanan Kıl Örneklerinde Kalıcı Organik Kirleticilerin İncelenmesi

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### Özet

Organik klorlu (OK) pestisitler, poli klorlu bi feniller (PCB), poli aromatik hidrokarbonlar (PAH), dioksinler ve bazı organik fosforlu (OF) pestisitler gibi kalıcı organik kirleticiler (Persistent Organic Pollutants, POPs) uzun mesafelere taşınabilmeleri, çevrede uzun süre kalmaları, ekosistemde biyomagnifikasyon ve biyoakümülyasyon özelliği göstermeleri nedeniyle insanlar, hayvanlar ve çevre için son derece tehlikeli maddelerdir. Bu çalışmada evcil kedi ve köpeklerden alınan kıl örneklerinde önemli POP'ların belirlenmesi, maruziyet durumunun ve muhtemel etkilerinin değerlendirilmesi ve kirletici seviyeleri ile yaş, cinsiyet ve beslenme durumu arasındaki ilişkinin incelenmesi amaçlandı. Çalışmada 35 kedi (18 dişi ve 17 erkek) ve 38 köpek (13 dişi ve 25 erkek) olmak üzere toplam 73 hayvandan kıl örnekleri toplandı. Toplanan örneklerde toplam 32 POP (2 OF, 9 OK, 6 PAH ve 15 PCB) Gaz Kromatografi-Kütle Spektrometri yöntemi ile analiz edildi. Kullanılan analiz yönteminin temel analitik parametrelerinden tespit limitleri 0.01-0.11 ng/g, geri kazanım oranları % 62.51-200.54, doğruluk (R<sup>2</sup>) değerleri 0.993-0.999 olarak belirlendi. Yapılan analizler sonucunda bütün örneklerde (n=73) en az bir kirletici tespit edildi. İncelenen 32 POP'tan 27 adedine rastlanırken, 2,4-DDE, PCB81, PCB118, PCB128 ve PCB208 hiçbir örnekte bulunmadı. Kıl örneklerinde POP rastlanma sıklığının oldukça yüksek olduğu görüldü. Kedilerde OF pestisitler (n=13), OK pestisitler (n=24), PCB'ler (n=25) ve PAH'lar (n=26) sırasıyla 751.46±213.69, 552.29±142.35, 57.81±14.15 ve 11.59±1.57 ng/g yoğunluklarda belirlendi. Köpeklerde ise OF pestisitler (n=18), OK pestisitler (n=31), PCB'ler (n=29) ve PAH'lar (n=31) sırasıyla 548.65±133.36, 312.84±107.24, 46.37±12.51 ve 19.07±7.07 ng/g yoğunluklarda tespit edildi. POP yoğunluğu açısından kedi ve köpekler arasında ve ayrıca yaş, cinsiyet ve beslenme durumu açısından herhangi bir fark tespit edilmedi (p>0.05). Sonuç olarak, uygulanan analitik yöntemin oldukça tatmin edici olduğu, kedi ve köpeklerde POP rastlanma sıklığının fazla ve kıl örneklerinde bazı kirleticiler için oldukça yüksek seviyelerde bulunduğu görüldü. Elde edilen veriler, birçoğu uzun zamandır yasaklanmış olmasına rağmen POP'ların halen önemli çevresel kirletici kimyasallar olduğunu, bu nedenle POP'ların kıl ve diğer örneklerde periyodik olarak izlenmesi gerektiğini gösterdi. Yapılan literatür taramasına göre bu çalışma ile Türkiye'de kedi ve köpeklerden toplanan kıl örneklerinde POP varlığı ilk kez ortaya konuldu.

**Anahtar kelimeler:** Kalıcı organik kirleticiler, kedi, köpek, kıl

**Isolation of Canine Parvovirus from Rectal Swabs of Suspected Dogs from Various Regions in Northern India**

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**Summary**

Canine parvovirus (CPV) is a highly contagious pathogen, causing acute gastro-intestinal illness among canine population. The study was aimed to isolate canine parvovirus in Madin-Darby Canine Kidney (MDCK) cell line from rectal swabs of dogs having signs of gastroenteritis. A total of 45 rectal swabs were collected from dogs exhibiting clinical signs of canine parvovirus infection from various regions of northern India viz. Punjab, Assam, Delhi, Chandigarh and Jammu. The samples were processed and subjected to MDCK cell isolation. The isolates showing cytopathic effects (CPE) like rounding of cells, and further clumping and detachment of cells were confirmed by PCR and NPCR. Out of 45 samples, 12 isolates showed cytopathic effects as observed in MDCK cell line with percentage of isolation being 26.66%. Further it was observed that out of these 12 isolates, three isolates were from dogs which had the history of vaccination for the disease. Further molecular epidemiology was studied using nested PCR and the sequence analysis of VP2 gene from NPCR amplified products to determine the prevailing antigenic type(s). It was found that the percent positivity of CPV was 70% by Nested PCR. Sequence analysis and phylogenetic analysis of VP2 gene revealed that CPV-2a is the most prevalent antigenic type in the studied regions of northern India.

**Keywords:** Canine parvovirus, MDCK cell line, Northern India, nested PCR sequence analysis.

**Evaluation of Pulmonary Artery Stiffness in Asthma Affected Horses**

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**Summary**

Pulmonary artery stiffness (PAS), a pulmonary artery elasticity index, increases in people with asthma or sleep apnea syndrome and could be an early predictor of pulmonary hypertension (PH) [1,2]. The right ventricular systolic time intervals (STIs) are pulsed-wave Doppler parameters that decrease in humans and dogs with PH [3,4]. In equine asthma, as in humans, recurrent hypoxemia/hypercapnia and mediators of inflammation induce thickening of the pulmonary wall vessels, leading to progressive increase of pulmonary vascular resistance and consequently PH [5].

In literature, there are no studies regarding PAS in horses and only a case-report measured STIs in an asthmatic pony [6]. The purposes of our study are to evaluate PAS and STIs in asthmatic horses and to assess their repeatability and reproducibility. The study was approved by the Institutional Animal Care Committee (OPBA\_27\_2020).

The Pulsed-wave Doppler waveform of the pulmonary valve was obtained from the right parasternal short axis view at the level of pulmonary artery, with settings arranged to acquire maximal frequency shift (MFS). The acceleration time (AT) was recorded from the onset of Doppler waveform to the maximum velocity plateau. The ejection time (ET) was measure from the onset to the end of the Doppler waveform. PAS was calculated as MFS/AT ratio.

According to clinical examination and bronchoalveolar lavage fluid cytology results, horses were divided into mild equine asthma (MEA, 11 horses) and severe equine asthma (SEA, 7 horses) groups.

Bland-Altman test and linear regression analysis indicated a good repeatability and reproducibility. Mann-Whitney test showed a significantly low AT ( $p=0.0204$ ) and high PAS ( $p=0.0064$ ) values in SEA horses.

In conclusion, our preliminary results suggest that PAS and STIs can be assessed consistently in horses. Moreover, differences in PAS and AT between MEA and SEA suggest that they could be used as prognostic parameters in asthmatic horses.

**Keywords:** Asthma, horse, pulmonary artery stiffness, Pulsed-wave Doppler

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**The First Time Isolation and Characterization of Indigenous Bovine Respiratory Syncytial Virus Strains of Turkey from Respiratory Disorders Causing Deaths in Cattle**

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**Summary**

Bovine respiratory disease (BRD) is a huge economic burden on the livestock industries of countries worldwide. Bovine respiratory syncytial virus (BRSV) is one of the important pathogens that contributes to BRD. In this study, we report the isolation and molecular characterisation of BRSV from lung specimens of three beef cows in Turkey that died from respiratory distress. Results indicate that the cattle were infected with BRSV from subgroup III and were closely related to previously identified American and Turkish strains. This study highlights the urgent need to better understand the burden BRSV is placing on the Turkish agricultural sector and further molecular analysis is required to understand the pathogenesis of disease in order to identify strategies to defend against infection.

**Keywords:** BRSV, cattle, isolation, respiratory disorders, sequencing

## Sığırlarda Solunum Bozukluklarından Ölüme Neden Olan Türkiye'nin Yerli Bovine Respiratory Sinsityal Virus Suşlarının İlk Kez İzolasyonu ve Karakterizasyonu

Ahmed Eisa ELHAG IBRAHİM<sup>1</sup>, Emre ÖZAN<sup>2</sup>, Cüneyt TAMER<sup>1</sup>, Bahadır MÜFTÜOĞLU<sup>2</sup>, Hanne Nur KURUÇAY<sup>1</sup>, Semra GÜMÜŞOVA<sup>1</sup>, Harun ALBAYRAK<sup>1</sup>, Zafer YAZICI<sup>1</sup>

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### Özet

Sığır solunum yolu hastalığı kompleksi (BRDC), dünya çapındaki tüm ülkelerin hayvancılık endüstrileri üzerinde büyük bir ekonomik sorundur. Bovine respiratory sinsityal virüsü (BRSV), BRDC'ye katkıda bulunan önemli patojenlerden biridir. Bu çalışmada, Türkiye'de solunum sıkıntısından ölen üç sığırın akciğer örneklerinden BRSV'nin izolasyonu ve moleküler karakterizasyonu rapor edilmiştir. Sonuçlar sığırların alt grup III'ten BRSV ile enfekte olduğunu ve önceden tanımlanmış Amerikan ve Türk suşları ile yakından ilişkili olduğunu göstermektedir. Bu çalışma, BRSV'nin Türk tarım sektörüne getirdiği yükün daha iyi anlaşılmasına yönelik acil ihtiyacın altını çizmektedir ve enfeksiyona karşı savunma stratejilerini belirlemek amacıyla hastalığın patogenezi anlamak için daha fazla moleküler analiz yapılması gerekmektedir.

**Anahtar kelimeler:** BRSV, sığırlar, izolasyon, solunum bozuklukları, sekans

## Green Synthesis of Silver Nanoparticles Using Common Poppy (*Papaver rhoeas*) and Evaluation of Their Potential Antibacterial Activity

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### Summary

Due to their easy and low cost production and enhanced properties, noble metal nanoparticles are preferred as nano-additives in most of pharmaceutical compositions. For example, silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) possess antiseptic and antimicrobial activity and they are generally preferred for obtaining antibacterial clothing, coatings. Moreover, these nanoparticles are added to the hydrogel structure and used in wound treatment. In the present study, we report a simple, low cost and green method for the synthesis of AgNPs using the aqueous extracts of *Papaver rhoeas* L. To the best of our knowledge, no reports on AgNPs synthesis using aqueous extract of *Papaver rhoeas* L. are available. Synthesized silver nanoparticles were characterized on the basis of UV–Vis, FT-IR, SEM-EDX and XRD analysis. The synthesized AgNPs were also tested for antimicrobial activity using agar well diffusion method. The AgNPs were spherical in shape and the size of the particles ranged from 10 nm to 40 nm. According to antimicrobial the test results, the zones of microorganism growth inhibition of AgNPs were determined to be highest against *Bacillus subtilis* (10 mm), *Escherichia coli* (10 mm), *Salmonella enterica* (12 mm) and *Pseudomonas aeruginosa* (13 mm). As a result, the AgNPs synthesized using *Papaver rhoeas* will be useful in areas where antibacterial application is important.

**Keywords:** Silver nanoparticles, *Papaver rhoeas*, biosynthesis, antibacterial activity

## Gümüş Nanopartiküllerin Gelincik (*Papaver rhoeas*) Kullanılarak Yeşil Sentez Yöntemiyle Eldesi ve Potansiyel Antibakteriyel Aktivitelerinin Değerlendirilmesi

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### Özet

Soy metaller kullanılarak hazırlanan nanopartiküller, kolay ve düşük maliyetli üretimleri ve gelişmiş özellikleri nedeniyle çoğu farmasötik bileşimde nano katkı maddesi olarak tercih edilmektedir. Örneğin gümüş nanoparçacıklar (AgNP'ler) ve altın nanoparçacıklar (AuNP'ler) antiseptik ve antimikrobiyal aktiviteye sahiptir ve genellikle antibakteriyel giysi, kaplama elde etmek için tercih edilirler. Ayrıca bu nanopartiküller hidrojel yapısına katılarak yara tedavisinde kullanılmaktadır. Bu çalışmada, *Papaver rhoeas* L.'nin sulu ekstrelerini kullanarak AgNP'lerin sentezi için basit, düşük maliyetli ve yeşil bir yöntem sunulmaktadır. Yaptığımız literature taramasında, *Papaver rhoeas* L.'nin sulu ekstresi kullanılarak AgNP sentezi hakkında herhangi çalışmaya rastlanmamıştır. Sentezlenen AgNP'ler UV-Vis, FT-IR, SEM-EDX ve XRD analizi temelinde karakterize edilmiştir. Sentezlenen AgNP'ler ayrıca agar well difüzyon yöntemi kullanılarak antimikrobiyal aktivite için test edildi. AgNP'lerin küresel şekilde oldukları ve parçacık boyutlarının 10 nm ila 40 nm arasında değiştiği belirlenmiştir. Antibakteriyel test sonuçlarına göre AgNPs'lerin *Bacillus subtilis* (10 mm), *Escherichia coli* (10 mm), *Salmonella enterica* (12 mm) ve *Pseudomonas aeruginosa* (13 mm)'ya karşı en yüksek antibakteriyel etkinliği sergiledikleri saptanmıştır. Elde edilen veriler ışığında *Papaver rhoeas* kullanılarak sentezlenen AgNPs'ler antibakteriyel uygulamanın önemli olduğu alanlarda faydalı olacağı sonucuna varılmıştır.

**Anahtar kelimeler:** Gümüş nanopartikül, *Papaver rhoeas*, biyosentez, antibakteriyel

**Estimation of Milk Yield Losses from Subclinical Mastitis in Dairy Cows**

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**Summary**

The material of the study consisted of milks from 300 mammary lobe belonging to 75 cows from different breeds (Holstein and Holstein Crossbred) aged between 3-8 years in dairy cattle enterprises in Efeler district of Aydın province between December 2020 and February 2021. Multiple regression analysis was utilised to estimate the relationship between somatic cell counts (SCC) and milk yield.

In this study, the relationship between cows breed, age, number of lactation, lactation periods, daily average milk yield and SCC were examined. No significant difference was found between SCC in Holstein and Holstein Crossbred cows. It was determined that SCC increased with increasing age ( $\leq 4$  and  $5 \geq$ ). In the study, it was determined that the daily milk yield of the cows was 21.1 kg and it was found that the milk yield loss showed significant differences according to the SCC. In the analysis, there is a negative relationship between SCC and milk yield; it was estimated that milk yield loss caused by 1 unit increase in SCC was 0.71 kg/cow/day.

**Keywords:** Cow, subclinic mastitis, somatic cell counts, milk yield loss

## Süt İneklerinde Subklinik Mastitisten Kaynaklanan Süt Verim Kayıplarının Tahmini

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### Özet

Araştırmanın materyalini, Aydın ili Efeler ilçesinde Aralık 2020-Şubat 2021 tarihleri arasında faaliyette bulunan süt sığırcılık işletmelerinde, yaşları 3-8 arasında değişen farklı ırklardan (Holştayn ve Holştayn Melezi) toplam 75 ineğe ait 300 meme lobuna ait sütler oluşturdu. Örneklerin somatik hücre sayısı (SHS) tespit edilerek SHS ile süt verim ilişkisi çoklu regresyon yöntemiyle tahmin edildi.

Araştırmada ineklerin ırk, yaş, laktasyon sayısı, laktasyon dönemleri, günlük ortalama süt verimi ile SHS arasındaki ilişkileri incelendi. Holştayn ve Holştayn melezi ırkı ineklerde SHS arasında fark bulunmadı. Yaş ( $\leq 4$  ve  $\geq 5$ ) arttıkça SHS'nin arttığı tespit edildi. İneklerin günlük süt veriminin ortalama 21,1 kg olduğu belirlenmiş olup, süt verim kaybının ineklerin SHS göre önemli ölçüde farklılıklar gösterdiği tespit edildi. Yapılan analizde, SHS ile süt verimi arasında ters yönlü bir ilişki olduğu ve SHS'da meydana gelen 1 ünite artışın neden olduğu süt verim kaybının 0.71 kg/inek/gün olduğu tahmin edilmiştir.

**Anahtar kelimeler:** İnek, subklinik mastitis, somatik hücre sayısı, süt verim kaybı

**Effect of Different Age and Plumage Color on Egg Quality with Cholesterol Level in Japanese Quail Eggs**

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**Summary**

This study was carried out in order to examine the effects of different feather colors and ages on the cholesterol values as well as the internal and external quality characteristics of eggs. This study was carried out to investigate the effect of different feather colors and ages on the internal and external quality characteristics of eggs as well as cholesterol values. Also, it was aimed to investigate the quality parameters of the egg weight, which is one of the quality characteristics of quail eggs, in Japanese quails of different feather colors and ages. As the study material, the eggs both of original colored Japanese quails and Jumbo quail line 23 weeks old selected in terms of live weight with yellow feathers and the eggs of the 39-week-old Jumbo quail line were used each reared in Prof. Dr. Hümeýra ÖZGEN Research and Application Farm of Selcuk University Veterinary Faculty at Konya. Furthermore, internal and external quality characteristics of 100 randomly selected eggs from the 23-week-old original Japanese quail and Jumbo quail line and the 39-week-old Jumbo quail line (respectively 40-40-20 eggs) were investigated. In order to determine the egg weight, modeling was done by using the variables of egg width, shape index, yolk height, yolk diameter, white weight, shell weight, egg volume, and egg length. In the evaluation made between the groups, an increase in cholesterol was detected depending on the increase in age; this difference between the mean cholesterol values of the groups is statistically significant ( $p < 0.01$ ). As a result of this study, it was determined that age and feather color affect some egg quality characteristics and cholesterol amounts in quails. It can be said that original colored Japanese quails breeding are still more suitable than yellow feathered Japanese quail for demand fulfillment to low-cholesterol foods nowadays.

**Keywords:** Age, cholesterol, egg quality, plumage color, quail

## Farklı Yaş ve Tüy Renginin Japon Bildircinlarında Yumurta Kalitesi ile Kolesterol Düzeyine Etkisi

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### Özet

Bu çalışma farklı tüy rengi ile yaşın yumurta iç ve dış kalite özellikleri yanı sıra kolesterol değerlerine de etkisini incelemek amacıyla yapılmıştır. Ayrıca bildircin yumurtası kalite özelliklerinden biri olan yumurta ağırlığını farklı tüy rengi ve yaşlardaki Japon bildircinlerinde hangi kalite parametreleri ile belirlenebileceğini araştırmak amaçlanmıştır. Çalışmanın materyalini Selçuk Üniversitesi Veteriner Fakültesi'ne bağlı Prof. Dr. Hümeyra ÖZGEN Araştırma ve Uygulama Çiftliği'nde yetiştirilen 23'er haftalık yaşta orijinal renkli Japon bildircinleri ve sarı tüy rengine sahip canlı ağırlık yönünden selekte edilmiş Jumbo bildircin hattı ile 39 haftalık yaşta olan Jumbo hattı sürülerinden elde edilen yumurtalar oluşturmuştur. Ayrıca 23 haftalık orijinal Japon bildircini ve Jumbo bildircin hattından ile 39 haftalık Jumbo bildircin hattından (sırasıyla 40, 40, 20'şer adet) rastgele seçilen 100 adet yumurtanın iç ve dış kalite özellikleri incelenmiştir. Yumurta ağırlığını belirlemek için yumurta eni, şekil indeksi, sarı yüksekliği, sarı çapı, ak ağırlığı, kabuk ağırlığı, yumurta hacmi ve boyu değişkenleri kullanılarak modelleme yapılmıştır. Yumurta kolesterol analizleri için her gruptan 3'er adet numune sonucu elde edilmiştir. Gruplar arası yapılan değerlendirmede, yaş artışına bağlı olarak kolesterolde artış tespit edilmiş olup; gruplardaki kolesterol değerlerinin ortalamaları arasındaki bu farklılık istatistiksel olarak anlamlıdır ( $p<0,01$ ). Sonuç olarak bu çalışmada bildircinlerde yaş ve tüy renginin bazı yumurta kalite özellikleri ile kolesterol miktarlarını etkilediği belirlenmiştir. Günümüzde kolesterol oranı düşük gıdalara olan talebin karşılanmasında orijinal tüy renkli Japon bildircini yetiştiriciliğinin sarı tüy renkli Japon bildircinlerine göre daha uygun olduğu söylenebilir.

**Anahtar kelimeler:** Bildircin, kolesterol, tüy rengi, yaş, yumurta kalitesi



**Investigation of *Mycoplasma agalactiae* from Goats with Mastitis by Bacteriological and Molecular Methods**

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**Summary**

In this study, it was aimed to determine the causative agent of contagious agalaxia by bacteriological and molecular methods in goat populations. The animals used as research material, were obtained from goat farms and farms. A total of 10 ml milk samples were taken from 118 goats with mastitis. After the milk samples were transferred to Aydın Adnan Menderes University Department of Microbiology in cold chain, they were inoculated to *Mycoplasma* selective media for identification. A total 10 isolates identified as *Mycoplasma* sp. by bacteriological and biochemical methods were firstly subjected to *Mycoplasma* sp. 16S rRNA PCR for confirmation by molecular methods and all isolates (100%) were found to be *Mycoplasma* sp. A total of 7 (70%) isolates identified as genus *Mycoplasma* were identified as *M. agalactiae* by *M. agalactiae* 16S rRNA specific PCR. As a result, 7 (6%) *M. agalactiae* were determined from 118 milk samples.

**Keywords:** Contagious agalactiae, hair goat, *Mycoplasma agalactiae*, PCR, 16S rRNA

**Mastitisli Keçilerde *Mycoplasma agalactiae*'nın Bakteriyolojik ve Moleküler Yöntemlerle Araştırılması**

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**Özet**

Araştırmamızda bulaşıcı agalaksi etkeninin, yetiştiriciliği yapılan keçi populasyonlarında bakteriyolojik ve moleküler yöntemlerle belirlenmesi amaçlanmıştır. Araştırma materyalini oluşturan hayvanlar keçi yetiştiriciliği yapılan çiftliklerden ve işletmelerden temin edilmiştir. Toplam 118 adet mastitis şikâyeti olan kıl keçisinden 10 ml süt numunesi alınmıştır. Alınan sütler, soğuk zincirde Aydın Adnan Menderes Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalına ulaştırıldıktan sonra *Mycoplasma* selektif besiyerlerine ekimler yapılmış, izole edilen kolonilerin makroskopik, mikroskopik, bakteriyolojik ve biyokimyasal testler ile değerlendirilmesi sonucunda 10 (%8) adet izolatın *Mycoplasma* sp. olarak identifikasyonu yapılmıştır. Bakteriyolojik ve biyokimyasal yöntemlerle *Mycoplasma* sp. olarak identifiye edilen 10 adet izolat, moleküler yöntemlerle doğrulanması amacıyla öncelikle *Mycoplasma* sp. 16S rRNA PCR işlemine tabi tutulmuş ve tüm izolatların (%100) *Mycoplasma* sp. olduğu tespit edilmiştir. *Mycoplasma* sp. olarak cins bazında identifiye edilen 10 adet izolatın 7 (%70) adedi ise, *M. agalactiae* 16S rRNA spesifik PCR işlemi sonucunda *M. agalactiae* olarak tür bazında identifiye edilmiştir. Sonuç olarak 118 adet süt numunesinden 7 (%6) adet *M. agalactiae* tespit edilmiştir.

**Anahtar kelimeler:** Bulaşıcı agalaksi, kıl keçisi, *Mycoplasma agalactiae*, PCR, 16S rRNA

**Biosensors in Diagnosis of Bacterial Diseases in Veterinary Medicine**

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**Summary**

Biosensors are devices that convert a biological signal into another measurable and processable signal. Biosensors are developed as a result of multidisciplinary research and provide some reliable and fast diagnosis at the point of care. Their usage is increasingly becoming widespread in many fields, especially in medical sciences. Lately, rising epidemic diseases made use of fast diagnosis systems compulsory. Biosensors are in many ways diagnostic tools that meet these mandatory needs compared to traditional methods. In veterinary medicine, rapid diagnosis of diseases on site is important in many aspects such as time, cost and protection. Fast and accurate detection of infectious diseases, especially zoonoses such as brucellosis, leptospirosis, anthrax, animal and public deficiency is critical. We wanted to learn the diagnosis of animal diseases. These biosensors have superior aspects such as on-site diagnosis, easy use and rapid diagnosis according to diagnostic methods such as culture, serology and molecular. Biosensors are devices with veterinary intense potentials in the near future after these virtual advantages.

**Keywords:** Biosensor, contagious disease, rapid diagnosis, veterinary medicine

**Veteriner Hekimlikte Bakteriyel Hastalıkların Teşhisinde Biyosensörler**

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**Özet**

Biyosensörler, biyolojik veya kimyasal bir sinyali ölçülebilir ve işlenebilir bir başka sinyale dönüştüren cihazlardır. Biyosensörler multidisipliner çalışmalar sonucu geliştirilip bakım noktası düzeyinde güvenilir ve hızlı tanı imkanı sağlarlar. Tıbbi bilimler başta olmak üzere birçok alanda kullanımı giderek yaygınlaşmaktadır. Son zamanlarda artan salgın hastalıklar hızlı tanı sistemlerinin kullanımını zorunlu hale getirmiştir. Biyosensörler birçok yönden geleneksel yöntemlerine göre bu zorunlu ihtiyacı karşılayan teşhis araçlarıdır. Veteriner hekimlikte hastalıkların yerinde hızlı teşhisi zaman, maliyet, koruma gibi birçok yönden önemlidir. Bulaşıcı hastalıkların özellikle brusellosis, leptospirosis, anthrax gibi zoonozların hızlı ve doğru tespiti hayvan ve halk sağlığı açısından kritiktir. Hayvan hastalıklarının teşhisi amacıyla çeşitli özelliklere sahip biyosensörler geliştirilmiştir. Bu biyosensörlerin kültür, seroloji, moleküler gibi tanı yöntemlerine göre yerinde teşhis, kolay kullanım, hızlı tanı gibi üstün yönleri bulunmaktadır. Biyosensörler sağladığı bu avantajlardan dolayı yakın gelecekte veteriner hekimlikte yoğun kullanım potansiyelleri olan cihazlardır.

**Anahtar kelimeler:** Biyosensör, bulaşıcı hastalık, hızlı tanı, veteriner hekimlik

**Investigation of The Microbiological Effect of Chitosan Based Peanet Pine Resin  
Added Hydrogel**

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**Summary**

Peanut pine resin whose wound-healing properties have been known since ancient times, is a secretion produced by the tree to repair itself. The resin, which has a large number of chemical content, has the feature of protection against parasites and microorganisms. One of the most important features desired in hydrogel wound dressings that can be used on humans and animals is that they have an antibacterial effect. Re-exposure to bacteria and microbes during the healing process of a wound causes infection and is an undesirable situation. Chitosan, which is frequently used in the production of hydrogel wound dressings, can also be used with different naturel and synthetic additives in order to give it antibacterial properties. In this study, peanut pine resin was prepared and added chitosan based hydrogels to see the antibacterial effect. The antibacterial effect of the resin was also tested by comparing it with silver nanoparticle added samples. Disk diffusion test was applied to measure the effect on *E. coli* (*Escherichia coli*) bacteria.

**Keywords:** Peanut pine resin, hydrogel

## Fıstık amı Reinesi Katkılı Kitosan Temelli Hidrojellerin Mikrobiyolojik Etkisinin Arařtırılması

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### Özet

Yara iyileřtirici özellikleri eski zamanlardan beri bilinen fıstık amı reinesi, ağacın zarar gören kısımlarının kendini onarması için ürettiđi salgıdır. Olduka fazla sayıda kimyasal içeriđe sahip olan reine, parazit ve mikroorganizmalara karşı dirence sahiptir. İnsanlar ve hayvanlarda kullanılabilen hidrojel yara bandajlarında istenilen en önemli özelliklerden birisi antibakteriyel etkiye sahip olmalarıdır. Bir yaranın iyileřme sürecinde tekrar bakteri ve mikroplara maruz kalması enfeksiyona sebebiyet vermekte olup istenmeyen bir durumdur. Hidrojel yara bandajların üretiminde sıklıkla kullanılan kitosana, antibakteriyel özellik kazandırmak amacıyla dođal veya sentetik katkılar ilave edilmektedir. Bu alıřmada kitosan temelli hidrojellere antibakteriyel özellik kazandırması amacıyla fıstık amı reinesi uygun kořullarda hazırlanarak eklenmiřtir. Reinenin antibakteriyel etkisi, gümüş nanoparacık katkılı numuneler ile de karşılařtırılarak test edilmiřtir. E. coli (Escherichia coli) bakterisi üzerinde etkisi ölçülmek üzere disk difüzyon testi uygulanmıřtır.

**Anahtar kelimeler:** Fıstık amı reinesi, hidrojel

**Plants with Repellent and Acaricidal Activity on Ticks Growing in Turkey**

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**Summary**

Ticks are arthropods that infect humans and animals with extremely dangerous diseases such as Babesiosis, Theileriosis, Lyme disease, Anaplasmosis, Ehrlichiosis, Tick-borne typhus, Crimean-Congo Hemorrhagic Fever. Personal protection of humans and animals, repellent substances and acaricides against ticks on animals are recommended for effective tick control. However, very important health effects of repellent and acaricides, such as acute and chronic toxicity and allergic reaction, changes in blood parameters and triglyceride level, neurotoxicity, oxidative stress, and genotoxicity have been reported. For this reason, interest in the use of herbal raw materials for control of ticks has increased in recent years. The presence of 12000 plant species in Turkey and the fact that 4000 of them are endemic are very promising for obtaining herbal medicines that can be used against ticks. In this paper, plants that grow in Turkey and have repellent and acaricidal properties on ticks were evaluated.

**Keywords:** Tick, plant, repellent, acaricide

**Türkiye’de Yetişen ve Keneler Üzerinde Kaçırıcı ve Öldürücü Etkinliği Olan Bitkiler**

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**Özet**

Keneler, insan ve hayvanlara Babesiosis, Theileriosis, Lyme hastalığı, Anaplasmosis, Ehrlichiosis, Kene kaynaklı tifüs, Kırım Kongo Kanamalı Ateşi gibi son derece tehlikeli hastalıkları bulaştıran artropodlardır. Etkili bir kene mücadelesi için insan ve hayvanların kişisel olarak korunması, kaçırıcı-kovucu (repellent) maddeler ve hayvanlar üzerindeki kenelere karşı akaricidler tavsiye edilmektedir. Ancak bu repellent ve akaricid etkili kimyasal bileşiklerin akut, kronik toksisite ve alerjik reaksiyon, kan parametrelerinde ve trigliserid seviyesinde değişim, nörotoksisite, oksidatif stress, genotoksisite gibi çok önemli zararları bildirilmiştir. Bu nedenle son yıllarda kene ile mücadelede bitkisel hammaddelerin kullanımına ilgi artmıştır. Türkiye’de 12000 bitki türünün bulunması ve bunlardan

4000 adedinin endemik tür olması kene ile mücadelesinde kullanılabilir bitkisel kökenli ilaçların elde edilmesi için oldukça umut vaad edicidir. Bu bildiri Türkiye’de yetişen ve keneler üzerinde repellent ve akarısit özelliği olan bitkiler incelenmiştir.

**Anahtar kelimeler:** Kene, bitki, repellent, akarısit

### **Kene ile mücadelede kullanılan bitkiler**

#### ***Allium sativum (Sarımsak)***

Dünyanın birçok yerinde olduğu gibi Türkiye’de de yetiştirilen sarımsak tarih boyunca şifalı bitki olarak kullanılmıştır. TÜİK (Türkiye İstatistik Kurumu) 2019 verilerine göre ise Türkiye’nin 61 ilinde özellikle Kastamonu, Gaziantep, Kahramanmaraş, Aksaray ve Tokat’ta üretimi gerçekleştirilmektedir (33). Sarımsağın içeriğindeki allisin’den dolayı antiparaziter ve antiprotozoal etki gösterdiği belirlenmiştir. Ayrıca sarımsak yağının etanolik ve metanolik ekstratlarının 24 saat içerisinde larva ve yetişkin keneler üzerinde öldürücü etki gösterdiği bildirilmiştir (1, 24).

#### ***Cuminum cyminum (Kimyon)***

Kimyon, Apiaceae (Umbelliferae) familyasından tek yıllık, bölge iklimine göre değişmekle birlikte boyu 20-50 cm civarında olan, ince ve tüysüz gövdeli bir bitkidir. TÜİK 2020 verilerine göre kimyon üretiminin Ankara, Konya, Kayseri, Afyonkarahisar, Sivas gibi İç Anadolu illerinde daha fazla yapılmaktadır. Anadolu mutfağında yeri çok eski olan kimyon aynı zamanda protein ve esansiyel yağlardan zengin içeriğinden dolayı ilaç ve parfüm sektöründe de kullanılan bir hammaddedir (5). *C. cyminum* esansiyel yağlarının 100-200 mg/mL konsantrasyonları *Rhipicephalus (Boophilus) microplus* keneleri üzerinde % 100, 50 mg/mL yoğunlukta ise % 85 akarısidal etkinlik göstermiştir (38). Başka bir çalışmada ise % 10’luk esansiyel yağı bile % 95.5 oranda etkili bulunmuştur (10).

#### ***Nerium Oleander (Zakkum)***

Zakkum, Apocynaceae ailesinde yer alan, dünyada yaygın dağılım gösteren, 6 metre uzunluğa ulaşabilen ve kışın yaprak dökmeyen çok yıllık zehirli bir bitkidir. Bahar ve yaz döneminde beyaz, pembe renkli çiçekler açmaktadır. Ülkemizde başta Ege, Akdeniz ve Karadeniz olmak üzere birçok bölgede yetişebilen zakkum, zehirli olduğu bilinmesine rağmen, yapılan çalışmalarda biyoaktif bileşiklere sahip olduğu görülmüştür (12). *N. oleander*’den elde edilen yağların, *Ixodes ricinus* niflerine % 60 oranında repellent etki gösterdiği bildirilmiştir (13).



***Matricaria chamomile (Mayıs papatyası)***

Compositae ailesine ait, Mayıs ve Ağustos ayları arasında beyaz çiçek açan tek yıllık otsu bir bitki olan Mayıs papatyası, ülkemizde aynı zamanda adi papatya veya tıbbi papatya olarak da bilinmektedirler. Özellikle Marmara, Ege, Trakya, Güneybatı Anadolu'da dağılım gösteren bir bitkidir (11, 36). Papatya çiçeği ekstraktlarının % 8 yoğunluğu, doymuş dişi *Rhipicephalus annulatus* kenelerine karşı akarisit (% 26.67) ve yumurtlama inhibisyonu (%46.67) etkisi gösterdiği bildirilmiştir (32).

***Mentha piperita (Bahçe nanesi)***

Dünyada yaygın olarak yetişen *Mentha spp.* Akdeniz Bölgesinde yayılım gösteren çok yıllık aromatik bir bitkidir. Tanen, pulegon, izomenton, metil asetat, menton ve mentol bileşikleri içeren nane, gıda ve ilaç sektöründe önemli kullanımlara sahiptir (4). *M. piperita* uçucu yağının % 5, 10 ve 20 yoğunluklarının larva ve yetişkin *Amblyomma hebraeum* kenelerine karşı sırasıyla 60 dakika, 40 dakika ve 20 dakika süreyle % 100 repellent etki gösterdikleri kaydedilmiştir (27). Başka bir çalışmada ise *A. americanum* kenelerine karşı 0.5 mL yoğunlukta 4. ve 6. saatlerde % 50 ve % 10 oranda repellent özelliği gösterdiği bildirilmiştir (26). Ayrıca *M. piperita* uçucu yağının *Rhipicephalus (Boophilus) microplus* kenelerine karşı akarisit etkisi de (LC90: 53.8) gösterilmiştir (8).

***Artemisia absinthium (Pelin otu)***

Ülkemizin doğu bölgesinde yetişen *Artemisia absinthium*'un, antihepatotoksik, antibakteriyel, antifungal, antioksidan özellikte olduğu çeşitli çalışmalar ile gösterilmiştir. Terpenoid, flavonoid, kumarin, sterol ve asetilen içeriği ile birçok alanda kullanılmaktadır (6). *A. absinthium* etanolik ekstraktı % 20 yoğunlukta *Hyalomma anatolicum* larvaları ve yetişkinlerine karşı % 100 ve 93.32 oranlarda akarisit etki göstermiş ve % 59.11 oranda yumurtlamada azalma sağlamıştır (16, 29).

***Pelargonium graveolens (Itır)***

*Pelargonium* cinsi *Geraniaceae* ailesine ait çok yıllık odunsu bitkilerdir ve Güney Yarımkürede yayılım göstermekle birlikte ülkemizde de doğal olarak yetişmektedirler (25). *P. graveolens*'ın % 10'luk konsantrasyonlarının, *Rhipicephalus microplus* kenelerinin üreme etkinliklerini % 97 oranında azalttığı görülmüştür (30).

***Lavandula angustifolia (Lavanta)***

Lamiaceae ailesine ait genellikle Akdeniz orijinli olan 39 kadar lavanta türü bulunmaktadır. Dünyada her yıl 200 ton kadar lavander yağı ve 1000 ton kadar lavandin yağı elde edilmektedir. Türkiye'de lavanta türlerinden sadece Karabaş lavanta (*L. stoechas ssp. cariensis*) doğal olarak yetişmekte, bunun yanında özellikle Isparta yöresinde kültürü yapılmaktadır. Lavanta yağları birçok alanda kullanılmakta

ve lavantanın içeriğindeki linalil asetat'ın narkotik, linalool'ün ise yatıştırıcı etkisinin kuvvetli olduğu bilinmektedir (18).

Lavantanın çeşitli zararlılara karşı repellent ve öldürücü etkilerine dair birçok yayın vardır. Keneler üzerindeki etkinliklerine bakıldığında, *L. angustifolia* esansiyel yağlarının doymuş dişi *Rhipicephalus (Boophilus) annulatus* kenelerine karşı %8 yoğunlukta 1 dakika maruziyet sonrası % 100 akarisit etkinlik ve % 4 yoğunlukta yumurta üretme kapasitesinde önemli ölçüde azalmaya neden olduğu gözlenmiştir. Lavanta yağlarının aynı kenelerde % 6-8 yoğunluklarda yumurtlama inhibisyonuna neden olduğu gözlenmiş ve LC<sub>50</sub> ve LC<sub>99</sub> değerleri sırasıyla % 2.76 ve % 8.84 olarak belirlenmiştir (31, 35). Başka bir çalışmada ise % 5, 10 ve 20 yoğunluklardaki lavanta esansiyel yağlarının *Hyalomma marginatum rufipes* yetişkin keneleri üzerinde tüm yoğunluklarda yüksek düzeyde repellent (%70-100) etki gösterdiği ve % 5'lik yoğunlukta repellent etkinin 40 dakika boyunca devam etmiştir bildirilmiştir (23). Mkolo ve ark. (2007) lavanta esansiyel yağları ile yapılan bir diğer çalışmada % 10 yoğunluklarının dişi yetişkin *Ixodes ricinus* türlerine karşı uygulamadan 80 dakika sonra % 45 repellent etkili olduğu belirlemiştir (26).

#### ***Ocimum basilicum* (fesleğen)**

*Ocimum basilicum* L. türü, Lamiaceae ailesine ait olup Fransa, İtalya ve İspanya gibi ülkelerde ticari ekimi yapılan bir bitkidir. Asya, Afrika ve Güney Amerika gibi sıcak ve ılıman bölgelerinde yaygındır ve ülkemizde doğal yayılım göstermemektedir. Türlerine göre değişmekle birlikte fesleğen esansiyel yağları estragol, metil öjanol, linalol, metil sinname gibi önemli bileşenlere sahiptir (34). *O. basilicum* buhar distilatlarının *Ixodes ricinus* nimflerine karşı % 64.5 repellent etki gösterdiği bildirilmiştir (14). Başka bir çalışmada *Rhipicephalus (Boophilus) microplus* kenelerine karşı *O. basilicum* kloroform ekstraktlarının % 6 ve 10'luk konsantrasyonlarında % 70 ve % 100 akarisit etkinliği görülmüştür (37).

#### ***Laurus nobilis* (Akdeniz Defnesi)**

Defnegiller (Lauraceae) ailesi, *Laurus* cinsi içerisinde yer alan *Laurus nobilis*, yuvarlak tepeli, sık dallı bir ağaççıktır. Kısa ve bodur olabileceği gibi 10 metreye kadar uzayabilmektedir. Genellikle Balkanlar, Anadolu ve Akdeniz Havzasında yayılım gösteren akdeniz defnesi, *Pinus brutia*'nın (kızılçam) bulunmadığı alanlarda yetişmektedir (20, 41). Defne yapraklarından elde edilen esansiyel yağların antibakteriyel, ağrı kesici, antiseptik, diyabeti tedavi edici ve migreni önleyici olarak kullanıldığı belirtilmiştir (20). *L. nobilis* esansiyel yağlarının doymuş *R. microplus* dişilerine karşı % 5-10 konsantrasyonları 24. saatin sonunda % 80.5 ölüme tol açarken, 3. gün sonunda % 100 ölüm şekillendirmiştir (38).

***Morus alba (Beyaz Dut)-Morus nigra (kara dut)***

Urticales takımının *Morus* cinsi içerisinde yer alan *Morus alba* ve *Morus nigra*, dünyanın ılıman iklim bölgelerine yayılmıştır. *M. alba*, soğuk ve sert iklim şartlarına uyum sağlamış olup, boyları 24 metreye, kökleri 20 metre derinliğe kadar ulaşabilmektedir. *M. nigra* ise diğer dut türlerine göre daha bodur özelliktedirler. *Morus* türleri Japonya, Endonezya, Arabistan, Kafkasya, Batı Afrika, Güney Amerika gibi dünyanın birçok alanında yetiştirilmektedir. Ülkemizde ise her bölgede yetiştirilmesi yapılmaktadır (19). *M. alba* etanolik ekstraktlarının yetişkin *Hyalomma anatolicum* kenelerine karşı LC<sub>50</sub> değeri 92.95 ppm olarak belirlenmiştir (3). *M. nigra* etanolik ekstraktlarının 25 mg/mL kloroform konsantrasyonu ise dişi *Rhipicephalus microplus* keneleri üzerinde % 62.6 yumurtlama inhibisyonu sağlamış ve yumurtadan çıkışı % 65.4 oranında engellemiştir (7).

***Syzygium aromaticum (karanfil)***

Dünya ticaretinde önemli baharatlardan biri olan *Syzygium aromaticum*, Myrtaceae ailesine aittir. *Caryophyllus aromaticus*, *Caryophyllus silvestris*, *Eugenia caryophyllus*, *Jambosa caryophyllus* *Myrtus caryophyllus* gibi farklı birçok karanfil türü de vardır. Karanfil, Endonezya Kuzey Maluku Adalarına özgüdür. Karanfilin başlıca yetiştirici ülkeleri Zanzibar, Endonezya ve Madagaskar'dır. Ancak ülkemizde de kültürü yapılmaktadır (2, 21). *E. Caryophyllus*'un % 15'lik yoğunluğu *Argas spp.* yetişkinlerine karşı % 80 akarısit etkinlik göstermiş ve LC<sub>50</sub> değeri % 3.15 olarak hesaplanmıştır (17). Ferreira ve ark. (2018) clevenger yöntemi ile karanfil esansiyel yağlarını elde etmişler ve ana komponentleri Gaz Kromatografi-Kütle Spektrometri (GC-MS) yöntemi ile analiz ederek içeriğinde öjenol, öjenol asetat, karyofillen ve humulen olduğunu belirlemişlerdir. Daha sonra etanol/su (%50 h/h) ile hazırlanan ve esansiyel yağ, hidrolat ve öjenol içeren formülasyonların farklı konsantrasyonlarda *R. microplus* larva ve dişilerine karşı akarısitidal etkinlikleri araştırılmış ve öjenol ve uçucu yağ, sırasıyla 2.5 mg/mL ve 5.0 mg/mL'lik konsantrasyonlarda % 100 larva ölümüne neden olmuştur (15).

***Citrus aurantium var bergamia (Bergamot)***

*Citrus bergamia*, Rutaceae ailesine ait olup, kanatlı yaprak sapı ve dikdörtgen yumurta benzeri oval yaprakları bulunan küçük ağaçlardır. Yeşil ve sarı renkli 7.5-10 cm çapında meyvelere sahip olan bu ağaç, meyve kabuğundan elde edilen esansiyel yağlar için yetiştirilmektedir. Bergamot yağının gıda, kozmetik ve ilaç endüstrilerinde yaygın olarak kullanılmasından dolayı bergamot yetiştiriciliği Türkiye, Kamerun, Brezilya, Arjantin ve Uruguay'da oldukça fazladır. Bergamot Türkiye'nin güneyinde özellikle Bodrum ve Antalya kıyılarında yetiştirilmektedir (22). *C. aurantium* yağının % 1, % 5 ve % 10'luk hazırlanan konsantrasyonları doymuş dişi *Rhipicephalus microplus* kenelerine karşı tüm konsantrasyonlarda yumurtlama ve yumurta ağırlığını azaltabilmiş, ancak kuluçka performansında

bir deęişikliğe neden olmamıştır. Bergamot yağlarının keneler üzerindeki % 10'luk konsantrasyonda etkinlikleri % 90 olarak belirlenmiştir (35).

### *Citrus limonum* (limon)

Limon, Rutaceae ailesinin önemli bir tıbbi bitkisidir. Asya'ya özgü olan *Citrus limonum*, oval sarı meyvesi bulunan yaprak dökmeyen bir ağaçtır. Limon üretiminin en fazla olduğu ülkeler Hindistan, Meksika, Arjantin, Brezilya, İspanya, Çin Halk Cumhuriyeti, Amerika Birleşik Devletleri, Türkiye, İran ve İtalya'dır. Türkiye'de limon üretiminin en fazla sağlandığı yöreler Mersin, Adana, Muğla, Antalya ve Hatay'dır. Kullanımının çoğu gıda sektöründe olan limon, antikanser aktiviteleri olan alkaloidleri için de yetiştirilmekte olup yapraklar, gövde, kök ve çiçeklerinden elde edilen ekstraktlarının klinik olarak önemli bakteri suşlarına karşı antibakteriyel özellik gösterdiği bildirilmiştir (28). *C. limonum* ekstraktının GC-MS analizi sonucunda esansiyel yağının ana komponentleri limonene,  $\beta$ -pinene ve  $\gamma$ -terpinene olarak belirlenmiş ve *Rhipicephalus* (*Boophilus*) *microplus* kenelerine LC<sub>90</sub> değeri % 4.9 olarak belirlenmiştir (40).

### **Türkiye'de yetişen bitkilerin keneler üzerinde etkinliklerine ilişkin çalışmalara bir örnek- Doktora tez çalışması: Farklı bitki ekstraktları ve bitkilerden sentezlenen nanopartiküllerin keneler üzerine in vitro etkilerinin incelenmesi (Özge Marangoz)**

Yukarıda belirtildiği gibi kenelere karşı doğal, güvenilir, etkili ve ucuz mücadele yöntemleri geliştirilmesine ihtiyaç duyulmaktadır. Bu tez çalışmasında ülkemiz kaynaklı bitkilerden elde edilecek ekstraktların ve bu bitkilerden elde edilecek nanopartiküllerin kenelere karşı akarisit ve kaçırmacı etkilerinin olup olmadığı araştırılmaktadır. Ülkemizde yaygın şekilde yetişen bu bitkilerin kenelere karşı etkinlikleri belirlense bile yapılarındaki esansiyel yağlardan dolayı uygulandıkları canlı doku üzerinde istenilen yoğunluğa ulaşamayıp etki sürelerinin oldukça kısa olma ihtimali vardır. Bu bitkilerden elde edilen nanopartikül formülasyonları ile canlı üzerinde istenilen etkiye daha fazla ve uzun süreli ulaşılması beklenmektedir. Bu kapsamda, keneler ile mücadelede faydalı olacağı düşünülen bu tezin amacı, Samsun ve diğer Karadeniz illerinden toplanan *Rhododendron luteum* (Orman gülü), *Lavandula angustifolia* (Lavanta) ve *Satureja spicigera* (kekik) ekstraktlarının ve bu bitkilerden elde edilen nanopartiküllerin köpeklerden toplanmış kan emmemiş ergin keneler (*Rhipicephalus bursa* / *R. turanicus* / *R. sanguineus*) üzerine etkinliklerinin in vitro olarak incelenmesidir. Proje kapsamında bitki ekstraktlarının etkinlikleri tek başlarına ve farklı nanopartikül formülasyonları (gümüş ve katı lipid nanopartiküller) ile birlikte piyasada yaygın olarak kullanılan ilaçlarla (repellent etki için DEET, akarisit etki için fipronil) karşılaştırmalı olarak değerlendirilecek ve en uygun ilaç adayı belirlenecektir.

İlaç araştırma-geliştirme faaliyetinin ilk aşamasını oluşturacak bu çalışmanın deneysel aşamaları devam etmekte olup, ülkemizde hem hayvan hem de insan sağlığı açısından son derece tehlikeli

zararlılar olan kenelerle mücadelede doğal, etkili ve güvenli bir alternatif ilaç adayı geliştirilmesi, bu sayede insan ve hayvan sağlığının korunmasına katkı sağlanması, ülkemiz kaynakları ile elde edilecek ürün ile önemli bir katma değer oluşturulması ve bu konuda literatürdeki eksik kalan konulara katkıda bulunarak ileride yapılacak in vivo etkinlik ve güvenlik çalışmalarına temel oluşturulması hedeflenmektedir.

### Sonuç

Kene mücadelesinde kullanılan kimyasal repellent ve akarisitlerin ciddi toksisiteleri olduğu bilinmektedir. Bu nedenle bitkisel kökenli, doğal ürünlere ihtiyaç duyulmakta ve bütün dünyada bu yönde çalışmalar yapılmaktadır. Ülkemiz, barındırdığı bitki sayısı ve çeşitliliği ile bu yönde çok önemli bir potansiyele sahiptir. Yapılan çalışmalar, Türkiye’de yetişen bazı bitkilerin keneler üzerinde kaçırmacı, öldürücü, yumurtlama engelleyici etkiler meydana getirdikleri göstermiştir. Ancak farklı bitkilerle, farklı türlerde ve farklı dozlarda ayrıntılı çalışmalara ihtiyaç vardır. Önümüzdeki yıllarda bitkisel kökenli maddelerin çok daha yaygınlaşacağı ve piyasada kullanılan mevcut kimyasal ürünler için ticari anlamda alternatif oluşturabileceği öngörülmektedir.

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**Development of ELISA Kit for Serological Diagnosis of Q Fever in Anatolian Buffaloes**

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**Summary**

In this study, it was aimed for development and comparatively evaluation of ELISA diagnostic kit that can be used in the serological investigation of Q fever disease caused by *C. burnetii* in Anatolian buffaloes. For this purpose, buffalo sera were tested with a commercial ELISA kit; and according to the obtained results home-made ELISA kit was developed with chessboard titration method using two different conjugates. Of the 92 Anatolian buffalo blood serum samples tested, 22 (23.9%) were found positive by commercial ELISA kit, 7 (7.6%) and 29 (31.5%) were positive by protein-A conjugated ELISA and anti-bovine IgG conjugated ELISA, respectively. In conclusion, within the scope of the study, an alternative home-made ELISA kit was developed for the serological diagnosis of Q fever for buffalo. Thus, the home-made ELISA kit, which can be used in researches aimed at detecting seropositivity, especially in buffalo sera, was made ready for use. It was concluded that the data obtained would contribute to wider ranging epidemiological studies.

**Keywords:** Anatolian Buffaloes, ELISA, Q Fever, sera



## **Mandalarda Q Hummasının Serolojik Teşhisi İçin ELISA Kiti Geliştirilmesi**

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### **Özet**

Bu çalışmada, mandalarda *C. burnetii*'nin neden olduğu Q humması hastalığının serolojik incelemesinde kullanılmak üzere ELISA tanı kiti geliştirilmesi ve ticari ELISA kiti ile karşılaştırmalı değerlendirilmesi amaçlanmıştır. Bu amaçla manda serumları ticari bir ELISA kiti ile test edilmiş ve elde edilen bulgular doğrultusunda iki farklı konjugat kullanılarak satranç tahtası titrasyon metodu ile home-made ELISA kiti geliştirilmiştir. Test edilen 92 adet manda kan serumu örneğinin ticari ELISA kiti ile 22'si (% 23,9), protein-A konjuge ELISA ile 7'si (% 7,6) ve anti-sığır IgG konjuge ELISA ile 29'u (% 31,5) pozitif bulundu. Sonuç olarak çalışma kapsamında Q hummasının serolojik teşhisi için alternatif home-made ELISA kiti geliştirilmiştir. Böylece, özellikle manda serumlarında seropozitifliği tespit etmeye yönelik araştırmalarda kullanılabilecek home-made ELISA kiti kullanıma hazır hale getirilmiş ve elde edilen verilerin daha geniş kapsamlı epidemiyolojik çalışmalara katkı sağlayacağı sonucuna varılmıştır.

**Anahtar kelimeler:** ELISA, manda, Q Humması, serum

**Evaluation of Rooster Semen Frozen With Shilajit Containing Extender**

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**Summary**

The high PUFA content in cock semen is essential for normal semen function as it forms semen's fluidity. Therefore, rooster semen is more susceptible to lipid peroxidation (LPO) by reactive oxygen species (ROS) during in vitro use and storage. Antioxidants are the primary defense mechanisms against oxidative stress. Shilajit has an antioxidant effect by protecting it from the damage of free radicals. This study aims to determine the effects of shilajit-containing extender on the freezing of rooster semen. 20 adult Barred Plymouth Rock roosters kept in individual cages at the age of 49 weeks were used in the study. The semen taken from 20 roosters with the abdominal massage technique was pooled at 37 °C. After the preliminary examination, the semen was divided into 6 equal parts. 3% glycerol (v/v) was added as a cryoprotectant to the Beltsville Poultry Semen Extender (BPSE) diluent. The prepared BPSE diluent was divided into 6 equal parts, including 1 control and 5 experimental groups (S5, S10, S15, S20,S25), and shilajit was added to the experimental groups at different rates (5, 10, 15, 20, 25 g/ml) as an antioxidant. Reconstituted semen were allowed to equilibrate for 2 hours at 4 °C. The prepared straws were placed on an aluminum rack and frozen in liquid nitrogen vapor for 7 minutes at the height of 4 cm on the liquid nitrogen surface. Frozen semen straws were thawed at 37 °C in 30 seconds. Motility examination was performed with the CASA system (Microptic Sperm Class Analyzer, SCA). At the end of the solution, Progressive motility values were 7.30% in the control group, 14.36% in the S5 group, 14.15% in the S10 group, 18.83% in the S15 group, 14.36% in the S20 group, and 17.00% in the S25 group.

**Keywords:** Rooster, freezing, shilajit, semen

## **Shilajit İçeren Sulandırıcı ile Dondurulan Horoz Spermalarının Değerlendirilmesi**

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### **Özet**

Horoz spermasındaki yüksek PUFA içeriği, spermanın akışkanlığının temelini oluşturduğu için normal sperma fonksiyonu için gereklidir. Bu nedenle, horoz sperması in vitro kullanım ve depolama sırasında reaktif oksijen türleri (ROS) tarafından lipid peroksidasyonuna (LPO) daha duyarlıdır. Antioksidanlar, oksidatif strese karşı primer savunma mekanizmalarıdır. Shilajit serbest radikallerin hasarından koruyarak antioksidan etki göstermektedir. Bu araştırmada amaç shilajit içeren sulandırıcının, horoz spermasının dondurulmasına etkilerinin belirlenmesidir. Çalışmada 49 haftalık yaşta bireysel kafeslerde tutulan 20 adet yetişkin Barred Plymouth Rock ırkı horoz kullanıldı. Abdominal masaj tekniği ile 20 adet horozdan alınan spermalar 37°C'de pooling yapıldı. Ön muayeneden sonra sperma 6 eşit parçaya ayrıldı. Beltsville Poultry Semen Extender (BPSE) sulandırıcısına kryoprotektan olarak %3 gliserol (v/v) eklendi. Hazırlanan BPSE sulandırıcısı 1 kontrol, 5 deney grubu (S5, S10, S15, S20,S25) olmak üzere 6 eşit parçaya bölündü ve deney gruplarına antioksidan olarak shilajit farklı oranlarda (5, 10, 15, 20, 25 µg/ml) ilave edildi. Sulandırılmış spermalar 4°C'de 2 saat boyunca ekilibrasyona bırakıldı. Hazırlanan payetler alüminyum rafa dizildi ve sıvı azot yüzeyine 4 cm yükseklikte 7 dakika boyunca sıvı azot buharında donduruldu. Dondurulmuş sperma payetleri 37°C'de 30 saniye sürede çözdürüldü. CASA sistemi ile (Microptic Sperm Class Analiser, SCA) ile motilite muayenesi yapıldı. Çözüm sonu progresif motilite değerleri kontrol grubunda %7,30, S5 grubunda %14,36, S10 grubunda %14,15, S15 grubunda %18,83, S20 grubunda %14,36 ve S25 grubunda %17,00 olarak tespit edildi.

**Anahtar kelimeler:** Horoz, dondurma, shilajit, sperma

### **Giriş**

Dondurma ve çözdürme sürecinde sperma oksidatif, ozmotik ve termal hasarlara maruz kalmaktadır. Bu hasarlar da sperma kalitesini ve fertilizasyon yeteneğini düşürmektedir (1). Dondurma hasarının arkasında yatan nedenler; ozmotik stres, soğuk şoku, hücre içi kristal oluşumu, aşırı miktarda reaktif oksijen türleri (ROS) antioksidan koruma sistemindeki değişiklikler ve bu durumların birlikte görülmesidir (2). ROS seviyesinin yükselmesi oksidatif strese neden olarak proteinlerin ve lipidlerin bozunması ile sonuçlanmaktadır (3). ROS üretimi ile antioksidanlar arasındaki denge spermatozoanın yaşamsal faaliyetlerinin korunması için kesinlikle gereklidir (4). Kryoprezervasyon sırasında, ROS ile

doğal antioksidanlar arasında dengesiz bir durum söz konusu olup, bu dengesizlik oksidatif stres ile sonuçlanmaktadır. Kryoprezervasyonun spermatozoa üzerine olan oksidatif stresi en aza indirmek amacıyla, sulandırıcılara antioksidan katılması gerekmektedir (5). Antioksidanlar, oksidatif strese karşı primer savunma mekanizmalarıdır (6). Horoz spermatozoon plazma membranı, memeli spermatozoonlarına göre çok daha fazla çoklu doymamış yağ asitleri (PUFA) içermektedir (7). Horoz spermasındaki yüksek PUFA içeriği, spermanın akışkanlığının temelini oluşturduğu için normal sperma fonksiyonu için gereklidir (8, 9). Bu nedenle, horoz sperması in vitro kullanım ve depolama sırasında reaktif oksijen türleri (ROS) tarafından lipit peroksidasyonuna (LPO) daha duyarlıdır (10). Horoz sperması normalde glutatyon peroksidaz, katalaz (CAT), süperoksit dismutaz ve ayrıca ROS'a karşı E vitamini (VitE) ve C vitamini gibi antioksidan sistem içermekte, ancak kriyoprezervasyondan etkilenmektedir (11). Bu amaçla Katalaz ve Vitamin E (12), L-karnitin (13), Hyalüronik Asit (14), Mito-TEMPO (15), Vitamin E ve Selenyum nanopartikülleri (16), Glutatyon (17) gibi çeşitli antioksidanlar horoz spermasına eklenmiştir. Shilajit, spermatojenik aktiviteyi artırıcı etki göstermekte (18), testosteron seviyesini artırmakta (19) ve vücut hücrelerini serbest radikallerin hasarından koruyarak antioksidan etki göstermektedir (20).

Bu araştırmada amaç shilajit içeren sulandırıcının, horoz spermasının dondurulmasına etkilerinin belirlenmesidir.

## **Materyal- Metot**

### ***Spermanın Alınması***

49 haftalık yaşta bireysel kafeslerde tutulan, günde 16 saat ışık/8 saat karanlık uygulanan ve ad-libitum beslenen 20 adet yetişkin Barred Plymouth Rock ırkı horoz kullanıldı.

Tüm horozlardan Burrows and Quinn (21)'in açıkladığı şekilde abdominal masaj tekniği ile 20 adet horozdan sperma alındı. Alınan spermaların tümü ısıtma tablalı mikroskop ile 37°C'de muayene edildi. Motilite değerleri %90 ve üzeri olarak tespit edilenler çalışmaya dahil edildi. Horozlardan alınan spermalar 37°C'de pooling yapıldı. Ön muayeneden sonra sperma 6 eşit parçaya ayrıldı.

### ***Sulandırıcının hazırlanması ve deney gruplarının oluşturulması***

Sperma sulandırıcısı olarak Beltsville Poultry Semen Extender (BPSE) (BPSE; dipotasyum fosfat (12,7 g/L), sodyum glutamat (8,61 g/L), fruktoz (5 g/L), sodyum asetat.3H<sub>2</sub>O (4,3 g/L), TES (1,9 g/L), monopotasyum fosfat (0,6 g/L), potasyum sitrat (0,64 g/L) ve magnezyum klorit.6H<sub>2</sub>O (0,34 g/L) karışımınının 100 mL distile su, pH 7,1-7,5 ve osmolarite 310 – 340 mOsm/kg) kullanıldı (7). Sulandırıcıya kryoprotektan olarak %3 gliserol (v/v) eklendi. Hazırlanan BPSE sulandırıcısı 1 kontrol, 5 deney grubu olmak üzere 6 eşit parçaya bölündü ve deney gruplarına antioksidan olarak shilajit farklı oranlarda ilave edildi.

Çalışma grupları; Kontrol (0 µg/ml Shilajit), S5 (5 µg/ml Shilajit), S10 (10 µg/ml Shilajit), S15 (15 µg/ml Shilajit), S20 (20 µg/ml Shilajit), S25 (25 µg/ml Shilajit) olarak dizayn edildi. Hazırlanan sulandırıcılar sperma ile kademeli olarak karıştırıldı ve 4°C’de 2 saat boyunca ekilibrasyona bırakıldı. Ekilibrasyon sonrası çalışma gruplarındaki tüm sulandırılmış spermalar 0,25 ml’lik payetlere dolduruldu ve payetlerin uçları polivinil alkol ile kapatıldı. Hazırlanan payetler alüminyum rafa dizildi ve sıvı azot yüzeyine 4 cm yükseklikte 7 dakika boyunca sıvı azot buharında donduruldu. Dondurma süresinin sonunda payetler -196°C’lik sıvı azot içerisine bırakıldı. Dondurulmuş sperma payetleri 37°C’de 30 saniye sürede çözündürüldü. CASA sistemi ile (Microptic Sperm Class Analiser, SCA) ile sperma motilite muayenesi yapıldı

### **Bulgular**

Çözüm sonu total motilite değerleri kontrol grubunda %59,16, S5 grubunda %68,76, S10 grubunda %72,80, S15 grubunda %77,21, S20 grubunda %68,76 ve S25 grubunda %71,62 olarak tespit edildi.

Çözüm sonu progresif motilite değerleri kontrol grubunda %7,30, S5 grubunda %14,36, S10 grubunda %14,15, S15 grubunda %18,83, S20 grubunda %14,36 ve S25 grubunda %17,00 olarak tespit edildi.

### **Tartışma**

Zaniboni ve arkadaşlarının 2014 yılında yaptıkları çalışmada sulandırıcı ve kryoprotektan olarak sırasıyla Lake sulandırıcısı ve DMA kullandıkları çalışmada ekilibrasyon sonrası horoz spermasının motilite değerini %48,5 olarak tespit etmişlerdir (22).

Shahverdi ve arkadaşlarının 2015’de yaptıkları çalışmada BPSE sulandırıcısı ve farklı oranlarda düşük dansiteli lipoprotein (LDL) kullandıkları çalışmada çözüm sonu horoz spermasının total motilite değerlerini %22,7, %24,1, %32,2, %43,1, %32,1 ve %19,8 olarak tespit etmişlerdir. Progresif motilite değerlerini ise %5,4, %4,2, %6,4, %5,8, %5,2 ve %4,6 olarak tespit etmişlerdir (23).

Fattah ve arkadaşlarının 2017’de horoz spermasında BPSE sulandırıcısı ve farklı oranlarda L-karnitin kullandıkları çalışmada çözüm sonu total motilite değerleri %52,7, %55,7, %68,2, %69,1, %43,1 ve 35,8 olarak tespit etmişlerdir. Progresif motilite değerlerini ise %19,4, %21,2, %28,4, %29,8, %16,2. ve %9,6 olarak tespit etmişlerdir (13).

Çalışmamızda elde edilen değerler ile görülen farklılıklar kullanılan antioksidan farklılıklarından kaynaklandığı düşünülmektedir.

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## **The Importance of Volatile Compounds in Detecting Heat in Cows**

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### **Summary**

Pheromones play an important role in animal movements and reproductive physiology. Sexual attraction, mother-young interactions, anger symptoms, initiation of anger, acceleration in adolescence, reduction in postpartum anestrus, hormonal stimulation, and increasing penile erection are some of the classic events affected by pheromones. Since they are secreted directly from the animal's own body, they are a reliable indicator of an animal's physiological state. In recent years, important pheromones have been identified on reproductive physiology in urine, feces, vaginal secretion and saliva in many mammal species. It has become possible to determine the preovulatory, ovulatory and postovulatory periods with these secretion samples, which are analyzed especially by gas chromatography-mass spectrometry (GC-MS) analysis in cows. The most prominent of these pheromones were di-n-propyl phthalate and 1-iodoundecane detected in urine. In researches on this subject, the responses of male animals have also given supportive data. Based on the available data, bovine pheromones can be used as practical tools to improve reproduction and management. Finding more practical ways to detect heat (such as kit, odor analysis, etc.) will reduce the costs that will occur due to the failure to detect heat, which causes economic losses, and production and livestock will be able to reach higher quality points.

**Keywords:** Cow, estrus, volatile compound



## İneklerde Kızgınlık Saptamada Uçucu Bileşiklerin Önemi

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### Özet

Feromonlar hayvan hareketlerinde ve üreme fizyolojisi üzerinde önemli rol oynar. Cinsel çekim, anne-geç etkileşimleri, kızgınlık belirtileri, kızgınlık başlatılması, ergenlikte hızlanma, doğum sonrası anöstrus olgusunu azaltma, hormonal uyarılma ve penis ereksiyonunu artırma feromonların etkilediği klasik olaylardan bazılarıdır. Hayvanın direk olarak kendi vücudundan salgılandıkları için bir hayvanın fizyolojik durumunun güvenilir bir göstergesi durumundadırlar. Geçtiğimiz yıllarda birçok memeli türünde idrarda, dışkıda, vajinal salgıda, tükürükte üreme fizyolojisi üzerinde önemli feromonlar tespit edilmiştir. İneklerde özellikle gaz kromatografisi kütle spektrometresi (GC-MS) ile analizi tercih edilerek analiz edilen bu salgı örnekleri ile preovulator, ovulator ve postovulatory dönemlerin tespit edilmesi olanaklı hale gelmiştir. Bu feromonlarda en çok göze çarpanları idrarda saptanan di-n-propyl phthalate and 1-iodoundecane olmuştur. Bu konuda yapılan araştırmalarda erkek hayvanların verdiği tepkiler de destekleyici cevap niteliğinde data vermiştir. Mevcut verilere dayanarak, sığır feromonları üreme ve yönetimi iyileştirmek için etkili araçlar olarak kullanılabilir. Kızgınlığı tespit etmede (kit, koku analizi vs. gibi) daha pratik yollar bulunması ile başta ekonomik kayıplara yol açan kızgınlık saptanamamasına bağlı oluşacak maliyetler azaltılacak ve üretim ile hayvancılık daha kaliteli noktalara gelebilecektir.

**Anahtar kelimeler:** İnek, kızgınlık, uçucu bileşik

**Effect of Sodium Selenite (Na<sub>2</sub>SeO<sub>3</sub>) on The Motility Parameters of Cryopreserved Dog Spermatozoa\***

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**Summary**

Selenium (Se) is a trace mineral that is required by both humans and animals. In other animals, such as rats, mice, chickens, pigs, lambs, and cattle, selenium deficiency has been associated with reproductive issues and poor sperm quality. Therefore, the effect of sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) on the cryopreservation of dog spermatozoa was examined in this study. Six healthy dogs (3 ejaculates from each dog) were used in the study. Each ejaculate was diluted at 37°C with a Tris-based extender containing 1 and 2 µg mL<sup>-1</sup> sodium selenite, cooled to 4 °C and allowed to equilibrate for 1 hours. Then, the semen was packed in 0.25 mL straws and frozen in liquid nitrogen vapor. Later, the semen was thawed at 37°C for 25-30 s and sperm motility parameters (motility, progressive motility, and kinematic parameters) were analyzed. Results showed that adding 1 µg mL<sup>-1</sup> selenium to the semen extender increased the sperm motility parameters of cryopreserved dog semen compared to the control and the group in which 2 µg mL<sup>-1</sup> selenium was added to the semen extender. These results indicated that supplementation of 1 µg mL<sup>-1</sup> sodium selenite as an antioxidant to the Tris-based extender improved the motility of cryopreserved dog spermatozoa.

**Keywords:** Dog, motility, spermatozoa, sodium selenite

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## Sodyum Selenitin ( $\text{Na}_2\text{SeO}_3$ ) Dondurulmuş Köpek Spermasının Motilite Parametreleri Üzerine Etkisi\*

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### Özet

Selenyum (Se) hem insanlar hem de hayvanlar için gerekli olan bir iz elementtir. Selenyum eksikliği, sıçan, fare, horoz, domuz, koç ve sığır gibi diğer hayvanlarda üreme sorunları ve düşük spermatozoa kalitesiyle ilişkilendirilmiştir. Bu nedenle, sodyum selenitin ( $\text{Na}_2\text{SeO}_3$ ) dondurulmuş köpek sperması üzerindeki etkisi bu çalışmada incelenmiştir. Çalışmada 6 adet sağlıklı köpek (her köpektan 3 ejakülat alınmak üzere) kullanılmıştır. Her ejakülat  $37\text{ }^\circ\text{C}$ 'de 1 ve  $2\text{ }\mu\text{g mL}^{-1}$  sodyum selenit içeren Tris bazlı bir sulandırıcı ile sulandırılmış,  $4\text{ }^\circ\text{C}$ 'ye soğutulmuş ve 1 saat süreyle ekilibrasyona bırakılmıştır. Daha sonra spermalar  $0,25\text{ mL}$ 'lik payetlerde paketlenmiş ve sıvı nitrojen buharında dondurulmuştur. Daha sonra sperma  $37\text{ }^\circ\text{C}$ 'de 25-30 süreyle çözdürüldü ve sperma motilite parametreleri (motilite, progresif motilite ve kinematik parametreler) analiz edildi. Çalışma sonucunda, sperma sulandırıcısına  $1\text{ }\mu\text{g mL}^{-1}$  sodyum selenit eklenmesinin, dondurulmuş köpek spermasının sperma motilite parametrelerini kontrol ve  $2\text{ }\mu\text{g mL}^{-1}$  sodyum selenit eklenen gruba kıyasla artırdığını göstermiştir. Bu sonuçlar, Tris bazlı sulandırıcıya antioksidan madde olarak  $1\text{ }\mu\text{g mL}^{-1}$  sodyum selenit eklenmesinin, dondurulmuş köpek sperma motilite parametrelerini artırdığı gözlemlenmiştir.

**Anahtar kelimeler:** Köpek, motilite, spermatozoa, sodyum selenit

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## Treatment Principles in Calf Diarrhea

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### Summary

Calf diarrhea is a widely reported disease in cattle breeding enterprises and is one of the most important economic losses for cattle producers. The most frequent symptom of the disease in young calves is diarrhea, which is sometimes foul-smelling, may be white, black, gray or blood-stained typically a problem in the first month of calf life. In neonatal calves, infectious and non-infectious agents cause diarrhea. Infectious agents include corona, rotavirus, cryptosporidia, coccidian, salmonella and *enterogenic* E. coli. Non-infectious agents are poor care and accommodation conditions. Regardless of the source of diarrhea, the intestinal mucosal barrier system of the calf is compromised in diarrhea. As a result of the disrupted intestinal mucosal system, it causes a significant amount of fluid loss from the intestines. As a result, dehydration and / or hypovolemic shock and death may occur due to diarrhea. The purpose of diarrhea treatment in calves should be to restore deficient body fluids, correct electrolyte imbalance and provide energy need. In this study, detailed information will be given about the etiological factors and diagnostic methods that cause diarrhea in calves, fluid loss and elimination of fluid loss, appropriate fluid selection, the amount of fluid to be given and the use of antibiotics.

## Buzağı İshalinde Tedavi Prensipleri

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### Özet

Buzağı ishali, sığır yetiştirme işletmelerinde yaygın olarak bildirilen bir hastalıktır. Sığır üreticileri için en önemli ekonomik kayıplardan biridir. Neonatal buzağılarda hastalığın en sık görülen semptomu, bazen kötü kokan, beyaz, siyah, gri veya kanlı olabilen ve tipik olarak buzağı yaşamının ilk ayında bir problem olan ishaldir. Neonatal buzağılarda ishale enfeksiyöz ve non enfeksiyöz ajanlar neden olmaktadır. Enfeksiyöz etkenler; corona, rotavirus, cryptosporidia, coccidian, salmonella ve enterojenik *E. coli* dir. Non enfeksiyöz etkenler ise kötü bakım ve barınma koşullardır. İshalin kaynağı ne olursa olsun ishalde buzağın bağırsak mukozal bariyer sistemi tehlikeye girer. Bozulan bağırsak mukozal sistem sonucunda bağırsaklardan önemli miktarda sıvısı kaybına neden olur. Sonuç olarak, İshale bağlı olarak oluşan dehidrasyon ve/veya hipovolemik şok ve ölüm meydana gelebilir. Buzağılarda ishal tedavisinin amacı; eksik vücut sıvılarını geri kazanmak, elektrolit dengesizliğini düzeltmek ve enerji ihtiyacını sağlamak olmalıdır. Bu çalışmada buzağılarda ishale neden olan etiyolojik faktörler ve tanı yöntemleri, sıvı kaybının giderilmesi, uygun sıvı seçimi, verilecek sıvı miktarı ve antibiyotik kullanımı hakkında detaylı bilgi verildi.

## **Introduction**

Calf diarrhea is a widely reported disease in cattle breeding enterprises and is one of the most important economic losses for the beef and dairy cattle industry (Von Buenau et al., 2005; Gulliksen et al., 2009; Cho and Yoon, 2014; Erkilic et al., 2016). The most frequent symptom of the disease in young calves is diarrhea, which is typically a problem in the first month of existence (Brar et al., 2015). The diarrhea, sometimes foul-smelling, may be white, black, gray or blood-stained. It may also occur in calves that are suckled by their mothers, but it is more frequent in hand-reared calves (Batmaz, 2010; Shekhar et al., 2017). Diarrhea can be caused by several species, and in one animal there can be more than one causative agent. Calf diarrhea is a multifactorial syndrome contains infective agents, non-infective and the environment or the management factors (Shekhar et al., 2017). The most frequent cause of diarrhea in young calves is viruses such as rotavirus, but protozoa such as cryptosporidia and coccidia and bacteria such as salmonella and *E. coli*, too, will cause complications (Kumar et al., 2010; Akam et al., 2011; Singh et al., 2014; Bednarski et al., 2015; Erkilic et al., 2016 ). Management factors such as hygiene (calving, sheltering), nutrition (nutritional composition and quality, feed hygiene and technique) and the animal's immune status (passive transfer of immunoglobulins, nutritional status) are non-infectious causes of diarrhea (Baumgartner, 2012). The lining of the intestine is compromised regardless of the origin of diarrhea, resulting in the loss of significant volumes of body fluid into the gut. As a consequence, the calf dehydrates rapidly, electrolytes become imbalanced, energy reserves are exhausted, and shock and death can occur in the calf. The goal of diarrhea treatment in calves should be to restore missing body fluids, correct the imbalance of electrolytes, and supply energy. Diarrhea in both beef and dairy calves is the leading cause of calf mortality before weaning. Therefore, some effort should be made by both veterinarians and farmers to create reasonable and appropriate guidelines for both diarrhea prevention and care (Millemann 2009; Çabalar, 2004; Khamees, 2015; Barua, 2018). To avoid calf diarrhea, antimicrobials have long been used and are frequently administered as a remedy. However, in order to reduce the growth of resistant bacteria, it is crucial to avoid the excessive use of antibiotics in food organisms. In adult cattle, enteric diseases are often popular, and both beef and dairy practitioners are frequently asked to establish diarrhea treatment protocols (Constable, 2009). These procedures are usually focused on understanding of the most likely pathogen and the professional expertise of the veterinarian, since diagnostic testing is frequently not possible. It is not possible to prescribe antibiotics for calves in order to avoid diarrhea. However, in order to treat select cases of calf diarrhea, the use of such antimicrobials and fluid treatment can be useful in reducing mortality and decreasing the incidence and length of diarrhea. Instead of mass-medicating vast numbers of cattle, individual animals prone to experience septicemia or have systemic symptoms of illness should be vaccinated for antimicrobial therapy.

## Infectious Agents

Many factors such as microbiological, environmental, nutritional, immunological and genetic play a role in the etiopathogenesis of diarrhea (Kuliğ and Coşkun, 2019). Diarrhea is the most important disease that causes loss of productivity and death in calves in neonatal period. Viruses, bacteria and protozoa are the most important enteric pathogens involved in the pathogenesis of the disease (Table 2) (Baker, 1995; Berchtold, 2009; Berge et al., 2006; Boileau and Kapil, 2010; Bolin et al., 1985; Çabalar, 2004; Cheetham et al., 2006; Akam et al., 2011; Cho and Yoon 2014; Cho et al., 2013; Cho and Yoon, 2014; Lorenz et al., 2011; Singh et al., 2014; Bednarski et al., 2015; Erkilic et al., 2016 ).

### 1. Viral Agents

*Rotavirus*, *Coronavirus*, *Bovine Viral Diarrhea*, *Caliciviridae*, *Toroviruses*, *Bovine norovirus* and *Neboviruses* are viral agents that cause diarrhea in calves in the neonatal period (Martella et al., 2010; Hoet and Saif, 2004; Hoet et al., 2002; Gulliksen et al., 2009; Alsaad et al., 2012; Gonzalez et al., 2021). *Rotavirus* is a non-enveloped virion with double stranded RNA segments and is in the *Reoviridae* family. There are seven serogroups (A to G) rotaviruses based on antigenic and genetic similarities of the intermediate capsid protein. *Rotavirus* is currently recognized as the primary etiological agent of calf diarrhea (Ghosh et al., 2007). Group A *rotaviruses* are the main causative agent of rotavirus infection in domestic animals. The capsid proteins of the agent play an important role in viral structure, binding to virus enterocytes and protection of antigenicity, and non-structural glycoprotein 4 (NSP4) has a special role in the release of viral enterotoxins. This protein also interferes with cellular homeostasis by increasing the calcium ion flux in the host cytoplasm. These changes in the host cause drastic changes in the movement of nutrients and water through the intestinal epithelium. These changes also constitute a more important place in the formation of histopathological lesions for viral pathogenesis. *Rotavirus* usually causes diarrhea in 1 to 2 weeks old calves (Dhama et al., 2009). The incubation period of the virus (12 ~ 24 hours) in the host is very short, causing acute diarrhea in the affected calves. Infected calves cause the virus to be transmitted to other calves by spreading a large amount of the virus through faeces for 5 ~ 7 days. The virus replicates in the cytoplasm of the epithelial cells of the small intestinal villi of the host. Destruction of mature enterocytes in the causative villa leads to villus atrophy and activation of the enteric nervous system by vasoactive components from damaged cells, causing maldigestive/malabsorptive diarrhea (Martella et al., 2010). *Coronavirus* is an enveloped virus with a single-stranded RNA genome (27 ~ 32 kb) and is a member of the Betacoronavirus 1 genus (Berge et al., 2006). Virus infection with three different clinical manifestations in cattle a) calf diarrhea in 1 to 2 weeks old calves; b) hemorrhagic diarrhea and winter dysentery in adult animals and c) bovine respiratory disease complex diseases in both young and adult cattle (Kozat and Tuncay, 2018). The spike (S) protein possessed by the virus plays an important role in the localization and pathogenesis of the virus as well as neutralizing the antibody in the host (Lin, 2000). After the agent is taken by the host, it first settles in the small intestine and then

spreads throughout the entire small intestine and colon for a short time. At the onset of the infection, the S protein of the virus and the heme-agglutinin-esterase (HE) protein bind and join the intestinal epithelial cells of the host. Microscopically, the villi of the affected small intestine and colonic crypts lead to atrophy and lamina propria necrosis. Viruses multiplying in enterocytes cause cells by a normal secretion mechanism. Mature villous epithelial cells are the primary target of the virus, but crypt enterocytes are also affected. Clinical manifestations in affected animals usually take longer due to the damage done by the virus to crypt enterocytes (Boileau and Kapil, 2010). *Bovine viral diarrhea virus* is an enveloped and single-stranded RNA virus and a member of the Pestivirus genus in the Flaviviridae family (Foster and Smith, 2009; Ridpath, 2010). The clinical symptoms of BVDV infection range from subclinical disease to fatal disease, depending on the immune status of the host, duration of pregnancy and gestation, and the presence or absence of co-infection with other pathogens. Most of the infected animals have mild clinical signs such as low-grade fever, leukopenia, anorexia and decreased milk production. Acute BVD infection is characterized by lymphopenia /leukopenia leading to diarrhea, pyrexia, depression, anorexia, decreased milk production, oral ulcers, hemorrhagic syndrome and immunosuppression (Baker, 1995). *Bovine viral diarrhea virus* can cause diarrhea in calves in two ways: 1) either persistent infection by predisposing enterocyte damage and co-infections, or 2) lesions that contribute to transient infection and diarrhea by replication in crypt enterocytes (Bolin et al., 1985; Koopmans and Horzinek, 1994). *Bovine torovirus* is an enveloped, positive-stranded RNA virus and belongs to the genus *Torovirus* in the Coronaviridae family (Koopmans and Horzinek, 1994). The virus can cause mild to moderate diarrhea in young calves younger than 3 weeks. After the virus is taken orally or nasally, it infects epithelial cells in the middle and lower parts of the intestinal villi extending to the crypt epithelium of the host and causes cell death and epithelial shedding in the small intestine with necrosis in the large intestine. Absorption/digestive disorder occurs in the intestines due to damage to villous and cryptic enterocytes, resulting in diarrhea (Hoet et al, 2002; Hoet and Saif, 2004; Haschek et al., 2006). *Norovirus* is a non-enveloped, single-stranded, positive-sensitive RNA virus belonging to the *caliciviridae* family (Mattison et al., 2007; Cho et al., 2013). The reported frequency of BNoV detection using molecular methods widely varied among different countries, ranging from 7.5 % to 49.6 %. All identified BNoVs have been phylogenetically distinct from human NoVs, suggesting that the zoonotic potential of BNoVs is very low. *Noroviruses* are a major cause of acute gastro-enteric disease in animals such as cattle, pigs, dogs, and mink. *Norovirus* infected epithelial cells of the small intestine and caused villous atrophy (in the jejunum and ileum) leading to diarrhea with virus shedding but not sero-conversion (Jor et al., 2010; Cho and Yoon, 2014). It is stated that the prevalence of *Nebovirus* in diarrheal calves ranges from 7% to 28% depending on the geographical location. *Nebovirus* similar to *Norovirus*, it mainly causes villi atrophy in the jejunum and ileum, and diarrhea due to loss of villi enterocytes (Hall et al., 1984).

## **2. Bacterial Agents**

*Escherichia (E.) coli* is an important cause of diarrhea in calves younger (< than 21 days of age) According to their virulence characteristics and clinical symptoms in the host, pathogenic *E. coli* strains; *enterotoxigenic E. coli* (ETEC), *Shiga toxin-producing E. coli* (STEC) and *enterohemorrhagic E. coli* (EHEC) (Meganck et al., 2014; Lorenz et al., 2011). *Enterotoxic E. coli* group has the ability to form two types of enterotoxins, heat labile and resistant. The most frequently isolated and especially pathogenic antigens of ETEC strains are F5 (formerly *E. coli* K99) and F41. ETEC can cause severe diarrhea in newborn calves through the production of heat-resistant enterotoxin (STa) (Fecteau et al., 1997; Foster and Smith, 2009; Gulliksen et al., 2009; Villarroel, 2009; Nguyen et al., 2011). Calves less than three weeks of age are usually infected with salmonella and *S. typhimurium* is the most causative agent of acute diarrheal disease. It causes the lesion and pseudo-membrane in the mucosa of the small intestine of calves as well as enlargement of the mesenteric lymph nodes (Mead et al., 1999). *Clostridium perfringens* is a gram-positive and spore-forming anaerobic bacterium (Meganck et al., 2014; Kumar et al., 2018). *Clostridium perfringens* can be divided into five types of toxins (A, B, C, D, and E) based on the production of four major toxins (alpha ( $\alpha$ ), beta ( $\beta$ ), epsilon ( $\epsilon$ ), and iota ( $\iota$ )), that cause a wide variety of diseases in mammals and birds (Table 1) (Ferrarezi et al., 2008; Heller and Chigerwe, 2018). While *Clostridium perfringens type A* strain alone produces  $\alpha$  toxin, B-type strains produce  $\alpha$ ,  $\beta$  and  $\epsilon$  toxins, C-type strains produce  $\alpha$  and  $\beta$  toxins, type D strains  $\alpha$  and  $\epsilon$  toxins, and type E strains,  $\alpha$  and  $\iota$  toxins. Among these strains, *Clostridium perfringens type C* strain is mostly reported to cause calf diarrhea (Barker et al., 1993; Rings, 2004).

**Table 1.** Diseases caused by *Clostridium perfringens* (Heller and Chigerwe, 2018)

<i>C perfringens</i> Type	Toxin Produced	Disease
<b>A</b>	Alpha	<ul style="list-style-type: none"> <li>• Hemorrhagic enteritis of cattle</li> <li>• Abomasal tympany and ulcers in neonatal calves</li> <li>• Gas gangrene</li> <li>• Yellow lamb diseases</li> </ul>
<b>B</b>	Alpha, beta, epsilon	<ul style="list-style-type: none"> <li>• Lamb dysentery</li> <li>• Enterotoxemia (overeating disease) of foals</li> </ul>
<b>C</b>	Alpha, beta	<ul style="list-style-type: none"> <li>• Necrotic hemorrhagic enterotoxemia of calves lambs, kids, foals, and piglets</li> </ul>
<b>D</b>	Alpha, epsilon	<ul style="list-style-type: none"> <li>• Enterotoxemia of sheep, goats, and cattle</li> </ul>
<b>E</b>	Alpha, iota	<ul style="list-style-type: none"> <li>• Abomasal tympany and ulcers in calves</li> <li>• Enteritis in rabbits</li> </ul>



***Protozoal agents***

*Cryptosporidium spp* are apicomplexan, zoonotic and cyst-forming protozoa that cause diarrhea, especially in newborn calves. *Cryptosporidium spp* is related to *Toxoplasma*, *Isopora*, *Eimeria* and *Sarcocystis spp*, belonging to the order Coccidia: *Cryptosporidium parvum* frequently can be asymptomatic or develop severe diarrhea with dehydration in neonatal calves (De Verdier et al., 2003; Boulter-Bitzer et al., 2007; Sarı et al., 2008; Villarroel, 2009; Thomson et al., 2017; Yağcı et al., 2017). In today, although there are approximately 24 *Cryptosporidium* species, generally *C. parvum*, *C. bovis*, *C. ryanae* and *C. andersoni* species can infect commonly cattle (Thompson et al., 2007). *C. parvum* is considered to be the primary causative agent and a potential zoonotic and in neonatal calf diarrhea agent (Cho and Yoon, 2014; Fayer et al., 1997; Millemann, 2009; Omidian et al., 2014; Vermeulen et al., 2017). Cryptosporidiosis is a disease controlled by age and the immune system, and the disease is common in neonates and in immature animals (Paraud and Chartier, 2012). *Cryptosporidium* can be transferred by the faecal-oral route, via either direct contact or ingestion of contaminated food or water (Cui et al., 2014).

**Table 2.** Main pathogenic agents diarrhoea in the calf

<b>Bacteria</b>	<b>Viruses</b>	<b>Parasites</b>
<i>E. coli</i> : <b>ETEC, EPEC, EHEC</b>	<b>Rotavirus</b>	<i>Cryptosporidium</i>
<b>Salmonella</b>	<b>Coronavirus</b>	<i>Giardia</i>
<b>Clostridium</b>	Torovirus (Breda)	<i>Eimeria bovis</i> , or <i>zuernii</i> (animals over 3 weeks)
<b>Campylobacter</b>	BVDV/MDV	<i>Candida</i> <i>Strongyloides</i> , <i>Toxocara vitulorum</i>

Bold characters underline the most often encountered pathogens

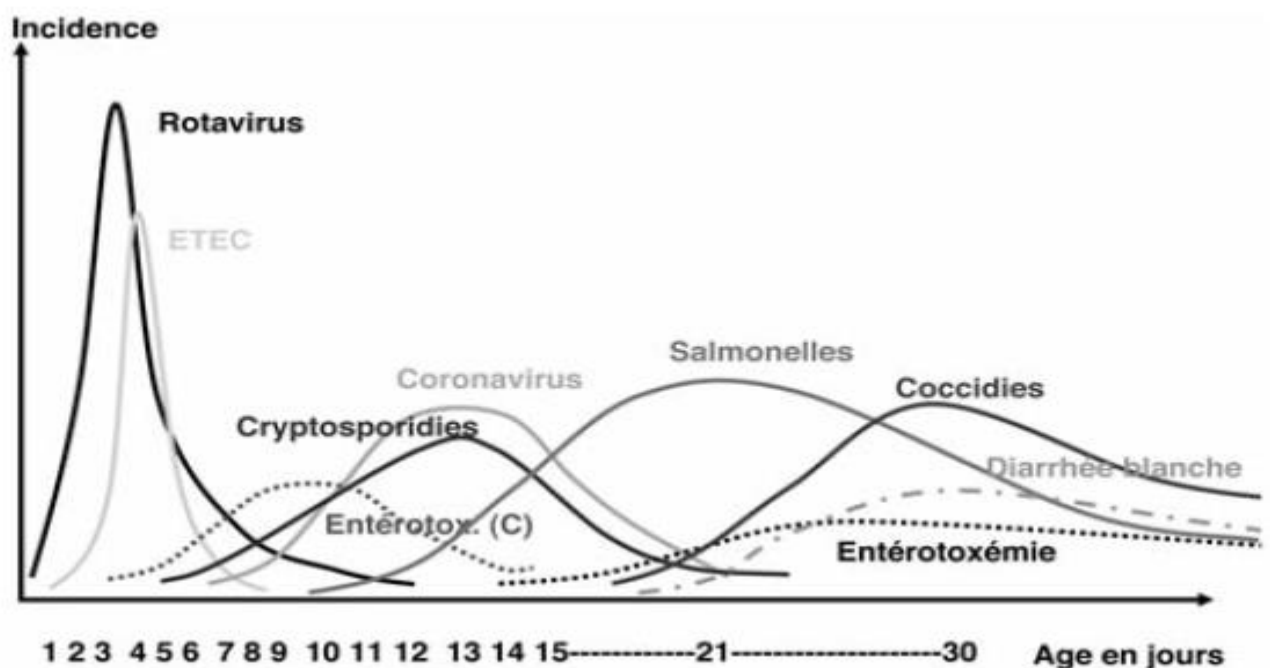
**Diagnosis of Calf Enteric Pathogens**

Diarrhea can be fatal in newborn calves due to anorexia, dehydration, and acidosis. Various pathogens or factors play a role in the development of diarrheal disease and the correct determination of the agent is very important in terms of making appropriate interventions for the disease in a timely manner. Both clinical findings and laboratory tests tests are used to determine the causative agents. Each of the factors involved in the development of the disease is discussed below (Millemann, 2009).

**Procedures for diagnosing calf diarrhea**

In order to determine the cause of diarrhea, samples are taken from the diarrhea calf for fecal and laboratory analysis, after examining the age of the patient, whether there is a vaccine record, as well as clinical findings. Generally, stool samples for *C. parvum* and *Coccidia* are determined by microscope and by bacteriological culture for *Salmonella spp.*, *E. coli* and *C. perfringens spp.*, and viral agents (for *BRV* and *B CoV*) via PCR (Mukhtar et al., 2015). In contrast, intestinal tissues are subjected to

immunohistochemistry or bacterial culturing. More recently, nucleic acid-based techniques such as PCR and an antigen-capturing enzyme-linked immunosorbent assay (Ag-ELISA) have been more commonly used for the rapid detection of various bacterial and viral pathogens in clinical specimens from diarrheic calves. When the laboratory test results are available, clinicians should consider the overall farm and clinical history in conjunction with lab results before identifying the causative pathogen. Pathogenic agents have the highest incidence at a certain age in newborn calves with diarrhea. The age-related incidence of pathogenic agents causing diarrhea is given in figure 1 (Millemann, 2009). While Colibacillosis is usually seen in newborns younger than 5 days, coccidiosis (eimeriosis) is seen in those older than 3 weeks (Eğlenti et al., 2020).



**Figure 1:** Incidence of the causes of diarrhoea according to the age of calves

**Table 3.** Helpful elements for the differential diagnosis of neonatal calf diarrhoea (Millemann, 2009).

Mean age of affected calves	Clinical signs	Probable aetiological diagnosis
1-3 days	Very liquid diarrhoea, yellow Rapid and important dehydration (eyes sunkness, diminished skin elasticity) Weakness, cold extremities	<i>Colibacillosis</i> ( <i>ETEC = F5 E. coli</i> )
4-11 days	Mucoid diarrhoea, hyperthermia, anorexia, abdominal pain, progressive dehydration	<i>Rotaviriosis</i> , <i>coronaviriosis</i> ,

		<i>cryptosporidiosis</i>
> 11 days	Very liquid diarrhoea with blood traces Severe hyperthermia (> 41°C)	<i>Salmonellosis</i>
> 18 days	Black diarrhoea, +/- blood and colic Mucoïd diarrhoea, hyperthermia Ptyalism, anorexia, epiphora	<i>Coccidiosis</i> or <i>Bovine viral diarrhoea</i>

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**Table 4.** Analyzes to be made according to suspected enteropathogen against calf diarrhea

Agent	Sample	Carried out method observations
<i>E. coli</i>	Stool, intestinal content	Elisa « BVT » Culture then slide agglutination (F5, F41, F17, CS31A) Antibiotic resistance profile. Search for genes specific for septicaemic <i>E. coli</i>
<i>Salmonella</i>	Stool on dead animal: ileo-colon, mesenteric lymphatic nodes, spleen, liver (aborted foetus)	Culture, then antibiotic resistance profiling and serotyping Serology: no diagnostic interest
<i>Clostridium</i>	Stool, intestinal content (hermetically sealed flacon), 4°C, brief delay for analysis	Culture and enumeration Possible search for genes coding for toxins (PCR) Do not forget <i>C. difficile</i> ?
<i>Rotavirus</i>	Faeces; on dead animal: ileon	Elisa « BVT » Immuno-enzymatic search, latex test Pb: only group A rotaviruses
<i>Coronavirus</i>	Stool; on dead animal: ileo-colon	Elisa « BVT » Immuno-enzymatic search, agar immuno-diffusion Sensitivity 10 <sup>6</sup> PFU /g
<i>Adenovirus</i>	Stool	Human ELISA kits useful
<i>Calicivirus</i>	Stool	RT-PCR for identification based on group primers Human ELISA kit useful because of the antigenic community.
<i>Torovirus</i>	Stool	ELISA and RT-PCR have been described Possible development of a multiplex RT PCR for cattle: calicivirus and adenovirus (+ astrovirus)
<i>Pestivirus BVD/MD</i>	<b>Stool: NO!</b>	Immuno-enzymatic test in 24h (Antigen),

	Naso-pharyngeal lavage, blood (PI only); on dead animal: ileo-colon, lymphatic nodes, spleen, abomasum, rectum, lung (aborted foetus)	cellular culture (gold standard: 8-21d), immunofluorescence on organs (1-3d delay)
<i>Cryptosporidium</i>	Stool	Microscopic examination (Ziehl Neelsen, Hein)
<i>Eimeria spp</i>	Stool	Microscopic examination

Today, rapid diagnostic commercial test kits are used to detect pathogenic factors causing calf diarrhea (kozat and Tuncay). Available commercial tests helpful in the diagnosis of Neonatal calf diarrhoea and summarized in table 4 (Millemann 2009; Çabalar, 2004; Khamees, 2015; Barua, 2018).

**Table 5.** Available commercial tests helpful in the diagnosis of calf diarrhea

Test (supplier)	Sample	Method / result	Time to obtain results
Bovine® IgG (Midland)	Stool	Immunochromatography Evidence of a quantitative failure of IgG secretion by the udder	20 minutes
Calf® IgG (Midland)	Total blood	Evidence of a quantitative failure of IgG in the calf's blood	20 minutes
Speed® Giardia (Bio Véto test)	Stool	Immunochromatography Evidence of <i>Giardia duodenalis</i> oocysts in a weight loss syndrome with intermittent diarrhoea in the young calf	5 minutes
Speed® V diar 4 and Speed® V diar 5 (Bio Véto test)	Stool	Immunochromatography Differential diagnosis of NCD for 4-5 agents (rota/coronavirus, <i>C. parvum</i> , <i>E. coli</i> K99, <i>E. coli</i> CS31A)	15 minutes
Bio K 156 « Tigettes tétravalentes » (Bio-X Diagnostics)	Stool	Immunochromatography Differential diagnosis of NCD for 4 agents (virus, bacteria parasites)	15 minutes
Bio K 195, Bio K 170, Bio K 176 (Bio-X Diagnostics)	Stool	Diagnosis of, respectively, <i>Clostridium perfringens</i> and its $\alpha$ and $\epsilon$ toxins	15 minutes

### Recommended Treatments

There are three main treatments for diarrhea calves: fluid therapy, antimicrobial use, and immunotherapy. Complications of neonatal diarrhea in calves are increased hemoconcentration, azotemia, hypoglycemia, hyponatremia, hyperkalemia, septicemia, hyper-D-lactatemia, hyper-L-

lactatemia, and the development of a strong ion (metabolic) acidosis (Trefz et al., 2017). Production of D-lactic acid results from bacterial fermentation in the gastrointestinal tract and is a common finding in neonatal calves with and without diarrhea (Constable, 2009).

### **Infusion Therapy**

Fluid therapy plays an important role in veterinary medicine in the treatment of dehydration, and in providing volume support during hypovolemic states. Both crystalloids and synthetic colloids are widely used and there are a number of guidelines for fluid resuscitation (Adamik et al., 2015). The goals of IV fluid therapy in calves were defined as:

- (1) correcting extracellular dehydration and restoring circulating blood volume,
- (2) correcting metabolic acidosis (increase blood pH >7.20),
- (3) correcting mental depression and restoring the suckle reflex,
- (4) correcting electrolyte abnormalities,
- (5) correcting the energy deficit,
- (6) improving repair of damaged intestinal surface (Berchtold, 2009).

Fluid Requirement (ml) = Fluid deficiency = Body weight X % dehydration degree X 1000 + daily maintenance fluid requirement. For these stages to be carried out effectively and rationally, the answers to the following questions must be known (Senior, 1983; Gross, 1988; Schaer, 1989).

Fluids and electrolytes are usually administered orally or parenterally to calves to correct fluid losses due to diarrhea (Sen and Constable, 2013). The method used for fluid administration in diarrheal calves should be based on the presence or absence of a breastfeeding reflex and the degree of dehydration. Calves who are breastfeeding and dehydrated to less than 6 % should be given an oral electrolyte solution (indentation of <3 mm in the orbit of the eye); If necessary, some of the fluid can be intubated using oroesophageal intubation. Calves that cannot be breastfed or are dehydrated by 8% or more (4 mm or more in the eye orbit) should be given intravenous fluids. These can be applied by inserting a jugular venous catheter or an auricular vein catheter (Roussel and Kasari, 1990; Constable, 2003, Smith, 2009).

### **1-Which Solution (s) should be given**

Crystalloids, colloids, blood products, hemoglobin-based oxygen carrier and total parenteral feeding solutions are used for fluid therapy in calves with diarrhea (Kozat and Voyvoda, 2006; Kozat, 2018). Which solution or solutions to be given in a patient requiring infusion treatment depends on the degree of volume deficiency and the quality of fluid loss. For example, the correction of intravascular volume as soon as possible in a patient in shock is the way to be followed in treatment. In the lack of volume due to blood loss, the best is blood transfusion (Clark, 1988). A colloidal solution should be used

initially if there is no blood or in the absence of volume that does not result from blood loss. Isotonic electrolyte solutions can be used to increase plasma volume, but  $\text{Na}^{++}$  is distributed to the extracellular region and only 20-25% of the volume infused remains in the intravascular part (Senior, 1983; Schaer, 1989). For this reason, the isotonic electrolyte solution is given at the start of shock, until plasma or other colloidal solution is found. Depending on the cause and severity of the fluid loss, it is necessary to regulate the defect in electrolyte and acid-base balance other than eliminating the fluid deficiency (Senior, 1983; Cullen, 1991; Kozat, 2018).

### ***Colloidal solution***

Plasma expanders are used to reinstate the circulating volume of a hypovolaemic patient. Typically, colloids are used to expand the plasma volume, although combinations of hypertonic crystalloid and colloid have recently been used (McCahon and Hardman, 2007). These solutions contain macromolecules with a molecular weight greater than 25000 daltons (Yuruk et al., 2007). Due to their high molecular size, these solutions never leave the intravascular area or leave only after they are destroyed. Dextran 40 or Dextran 60, among the colloidal solutions described as plasma expanders are the most recommended (Table 5) (Clark, 1988). These solutions, by increasing oncotic pressure, absorb fluid from the intracellular and interstitial region to the intravascular region, and it is possible to increase the blood volume rapidly (Kozat and Voyvoda 2006; Öcal and Ünsüren, 2009). Due to their colloid osmotic properties, they bind water and hold it in the intravascular area when the oncotic pressure they create exceeds arterial hydrostatic pressure (Michell, 1985). The hyperoncotic solution (6% Dextran-60 solution) draws from the extracellular space to the intravascular space with the balance of oncotic pressure and water binding capacity, thereby increasing the plasma volume (Kozat and Voyvoda, 2006; McCahon and Hardman, 2007; Kozat, 2018). Release of inflammatory mediators secondary to hypovolemia is another important factor causative to microcirculatory dysfunction. The volume replacement strategy can moderate inflammatory activation, generation of reactive oxygen, and leukocyte adhesion to the microcirculatory endothelium (Boldt, 2006). Saline solution look like to be the most pro-inflammatory fluid, whereas certain colloids (especially when dissolved in a balanced solution) may be more beneficial in controlling the inflammatory process (Kellum et al., 2006; Boldt et al., 2009; Matharu et al., 2008).

**Table 6.** Properties and suggested doses of colloidal solutions

Solution	Content	Molecule Weight (kDa)	Intravenous Half life (hour)	Volume Effects (hour)	Maximum daily dose
4.5 % Albumin	Protein	69	>24	-	-
6 % Hetastarch	Amylopectin derivative	450	>24	5-6	20 ml/kg (Max. 1.5 L)
6 % Pentastarch	Amylopectin derivative	20	10	3-4	33 ml/kg (Max. 2.5 L)
10 % Pentastarch	Amylopectin derivative	20	10	3-4	20 ml/kg (Max. 1.5 L)
6 % Tetrastarch-130	Amylopectin derivative	130	-	2-3	50 ml/kg
6 % Dextran -70	Polysaccharide	70	6-8	5	1.5 g/kg
10 % Dekstran -40	Polysaccharide	40	5	3-4	1.5 g/kg

***Crystalloid solutions***

A crystalloid solution comprises of water and various forms of electrolytes (including salt) or sugar crystals (Table 6) (Muir, 2017). Some crystalloid fluids also contain buffers (eg, acetate, gluconate, and lactate) that are metabolized to bicarbonate to increase serum pH (Mazzaferro and Powell, 2013). These solutions can be collected in two gaps, extracellular fluid regulators and maintenance solutions. Solutions used as extracellular fluid regulators are solutions that regulate the volume of extracellular fluid without changing its composition. To achieve this goal, Na<sup>++</sup>, Cl<sup>-</sup> and HCO<sub>3</sub> or precursors in solution must have plasma levels. Since the lack of volume is mostly caused by hyperkalaemia. The K<sup>+</sup> concentration in the solution should not be higher than in plasma (Clark, 1988). In particular, solutions containing HCO<sub>3</sub> provide plasma pH regulation of the kidneys in the treatment of acidosis as well as by increasing volume-decreasing renal perfusion (Cullen, 1991). Nonalkalinizing solutions are more frequently used in fluid therapy. These solutions include sodium, potassium, calcium, and chloride, which correct acidosis by increasing the ECF volume and ensuring the renal regulation of plasma pH (Berchtold, 2009). Isotonic solutions have osmolality equivalent to that in serum (280-310 mosmol/L). Of these solutions, 0.9 % NaCl, Ringer and Ringer lactate solutions can be counted. Isoionic solutions (Ringer’s solutions) contain cation combinations similar to those in serum (Schaer, 1989). Sodium-containing solutions should not be used in calves with severe hypoalbuminemia because they decrease plasma albumin concentration and oncotic pressure, which forces fluid movement into the interstitial space and exacerbates tissue edema (Constable, 2003). Before

intravenous fluid is administered to a calf with diarrhea, the calf's albumin level should be determined. If diarrhea calf hypoalbuminemia is detected, it is more effective to use colloid solutions or colloid + crystalloid (colloid /crystalloid ratio: 1/1 or 75 % crystalloid + 25% colloid) solutions (Kozat and Voyvoda, 2006) or blood transfusion instead of applying sodium-containing solutions (Constable, 2003; Berchtold, 2009).

**Table 7.** Crystalloid Fluid Types and Composition

Crystalloid	Tonicity	Sodium (mEq/L)	Chloride (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Osmolality (mOsm/L)	pH
<b>Lactated Ringer's solution</b>	Isotonic	130	109	4	3	273	6.7
<b>Plasmalyte-A</b>	Isotonic	140	103	5	0	294	7.4
<b>Normosol-R</b>	Isotonic	140	98	5	0	292	7.4
<b>Normal saline (NaCl 0.9%)</b>	Isotonic	154	154	0	0	308	5.7
<b>Ringer acetate</b>	Isotonic	130	112	1	5	276	6.0–8.0
<b>Dextrose (2.5% in 0.45 % saline)</b>	Isotonic	77	77	0	0	280	4.5
<b>Hypertonic saline (7.5%)</b>	Hypertonic	1,232	1,232	0	0	2464	5.2
<b>Normosol-M with 5% dextrose</b>	Hypertonic	40	40	13	0	363	5.2
<b>% 8.4 Sodium Bicarbonate</b>	Hypertonic	100	0	0	0	2000	7.4

***Crystalloid-free solutions***

For the addition of free water, a solution containing 5% isotonic glucose is mostly used. In this solution, after the glucose is metabolized, only water remains that do not have an osmotic effect in the fluid compartment (Table 7). In vomiting, diarrhea and burns, a fluid suitable for the change in ECF composition is selected. It has been reported that 5% dextrose solution is not sufficient to eliminate the deficiency in electrolyte losses (Clark, 1988). The patients daily energy requirement = (30 x Body weight (kg) +70) x disease factor (1.8) = kcal/24 hours) glucose to the patient by choosing the solution that is sometimes used in the treatment of fluid and closing the fluid gap but needing total parenteral



nutrition. Hypertonic total parenteral solutions consisting of amino acids and lipids should be applied (Öcal and Ünsüren, 2009).

**Table 8.** Crystalloid-free solutions Type and Composition

Crystalloid	Tonicity	Sodium (mEq/L)	Chloride (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Osmolality (mOsm/L)	pH
Dextrose (5%) in water	Hypotonic	0	0	0	0	253	5.0

**2-At what speed should be given**

The rate of development of fluid loss and the degree of clinical symptoms are important in determining the rate of delivery of the selected solution (Sensor,1983). Due to the limited uptake capacity of the therapeutically accessible intravascular region, the maximal infusion rate is limited by circulating capacity and distribution kinetics in the interstitial region. As the fluid or volume deficiency increases, the infusion rate of the fluid to be increased increases (Chandler et al., 1999). Hypertonic solutions should be given slower in isotonic solutions. In the event of sudden and severe blood loss and hypovolemic shock, correction of intravascular tissue perfusion is rapid initial intravenous application is required (Sensor, 1983). In veterinary practice, 7.2 or 8.4 % hypertonic salt solutions for resuscitation are administered to calves 6.4 ml/kg over a period of 5 minutes (Trefz et al., 2017). If fluid application is performed slowly in the specified situations, insufficiency of tissue perfusion for a long period of time, exacerbation of hypovolemic shock and makes the patient susceptible to acute renal failure. Many researchers recommend isotonic crystalloids for dehydrated calves at a maximum dose of 80 ml/kg/hr by an intravenous route (Kasari and Naylor, 1985; Kasari, 1990; Constable, 2003). But some researchers recommended that the dose be as low as 30 or 40 ml/kg/hr because of an infusion rate of 80 ml/kg/hr may lead to hypernatremia (Grove-White, 2007). However, cardiovascular and renal functions should be checked in rapid infusion. Symptoms of excessive infusion are distress, tachypnea, cough, dyspnea, lung edema, serous runny nose, ascites, polyuria, diarrhea and aexophtalmus (Cornelius, 1980; Gross,1988; Chandler et al., 1999). The first signs of excessive infusion that started were restlessness and tachypnoe in the patient with apathic status due to dehydration. It should be remembered that infusion should be stopped when cough due to pulmonary edema and dyspnoe will worsen the patient’s condition (Michell et al., 1989; Cullen, 1991). The cause of excessive infusion is the distribution of different compartments of the body. The infusion treatment first regulates the deficiency in the intravascular region. This is about 60 minutes it takes place inside. In the intravascular region, approximately 8 % of the body weight is contained with erythrocytes. While intravascularly applied electrolytes pass rapidly in the interstitial compartment, it takes time to correct the intracellular fluid loss. To maintain fluid balance in dehydrated calves, half

the amount of solution to be applied should be given within six hours. Fluid balance should be maintained in a 24-hour period. Intravenous fluid application should be done more slowly in patients with mild dehydrated. Fluid deficiency can be corrected over a period of 4-6 hours. Drip infusion 30-60 drops/min, depending on the size and body weight of the patient, 80 drops/min for calves can be given up. In severely dehydrated patients, solutions should be taken at a higher drop rate at the start of infusion therapy (Veech, 1986; Muir, 1992).

### **3-The amount of fluid to be given**

The amounts of fluid to be give determining by evaluating the anamnesis data, physical examination and laboratory test results. The degree of dehydration from skin elasticity, in other words, fluid loss from the body can be detected high in cachectic, older, and low in adipose and young animals. If patient's body weight, haematocrit, and total protein values are known the before dehydration develop, volume deficiency can be calculated more objectively (Seifi et al., 2006; Kozat, 2020). 1 kg decrease in body weight is equivalent to 1 liter of fluid loss from the body (Sensor, 1983; Schaer, 1989). Lets explain the volume of fluid required for fluid loss with an example. In dehydration due to diarrhea; fluid therapy is required to correct hypovolemia, restore vascular volume and normalize cardiac output and blood pressure. Diarrhea results in the loss of vital salts, fluid and energy necessary for the calf's survival. Treatment of diarrhea is directed at replacing these losses. Oral electrolyte solutions provide balanced sources of salts, fluids and energy, and can be fed up to six times a day. However, the energy they provide is not adequate, so continue to feed to the calf its normal milk or milk replacer. Ensure that milk feeds and electrolyte feeds are separated by at least 2 hours to allow normal milk clotting and digestion. Crystalloid solutions are best fed at body temperature. The amount of crystalloid solution required by a calf each day depends on how dehydrated the calf is and what ongoing losses are occurring Table 8 gives a guide to signs seen at different degrees of dehydration. Guide to estimating the degree of dehydration in a calf's clinical signs. The amount of electrolyte solution needed by the calf each day to correct dehydration is calculated by multiplying the weight of the calf by the percentage dehydration. The traditional recommendation for intravenous (IV) fluid therapy is predominantly based on the degree of dehydration. According to Table 8, fluid should be given orally to cases with dehydration degrees between 5-7 and diarrhea and have a sucking reflex (Berchtold, 2009). However, it should definitely be administered intravenously to calves whose dehydration is greater than 8 and do not have a sucking reflex. After the amount of fluid needed is calculated, this amount of liquid is divided into three equal amounts. It is administered intravenously in the specified amount in three stages: (1) Calves with a degree of dehydration higher than 8 % should be given hypertonic sodium bicarbonate, 1/3 of the required fluid within the first 2 hours. (2) Colloidal solution should be given at 1/3 of the remaining fluid at the 6th hour and (3) isotonic crystalloid solutions should be given at the 12 th hour. It should be taken into consideration with the criteria in scheme 9 and according to these criteria; the choice of fluid and application route should be made.

**Table 9.** Guide to estimating the degree of dehydration in a calf

Clinical signs	% Dehydration
Diarrhoea, but no other sign	5
Eyes slightly sunken, skin losing elasticity, but calf still sucking	7
Eyes sunken, skin slow to flatten if pinched, gums sticky, calf depressed	9
Eyes very sunken, skin 'tents' (won't flatten if pinched), Calf can't stant and is severely depressed.	12

Neonatal calf diarrhoea is generally observed together with other clinical signs, among them essentially dehydration with acid-base imbalance (Millemann, 2009). In calves with diarrhea, the development of metabolic acidosis causes (1) loss of bicarbonate ions ( $\text{HCO}_3^-$ ) in the faces, (2) decreased renal excretion of hydrogen ions ( $\text{H}^+$ ) associated with dehydration and reduced renal blood flow, and (3) the presence of unidentified organic acids in plasma (Groutides et al., 1990).

To maintain hydration, calves need about 10 % of their body weight in milk or milk replacer each day

**Example 1**

The daily fluid requirement for a 40 kg calf and the calculated dehydration degree is 7%.

- A). Amount of electrolyte solution needed to correct dehydration 7 % :=  $7/100 \times 40 = 2.8$  liter (L) per day.
- B). Daily amount of milk or fluid required to maintain hydration of body weight 10 % =  $10/100 \times 40 = 4$  liter per day.
- C). Therefore the estimated total amount of fluid (electrolyte+ milk or fluid) =  $2.8 + 4 = 6.8$  L per day.

**Example 2.** One week male simmental calf, body weight 70 kg, calculated degree of dehydration greater than 8 % with a history of 2 days of diarrhoea that has been getting progressively worse.

**Initial plan**

The patient requires fluids. The hypoperfusion should be addressed first as it is potentially life-threatening.

Need to calculate and sum for 24 hours.

**A-Calculatation of fluid requirement according to the degree of dehydration**

1. Replacement of hydration fluid deficite (Litter): Dehydration (%) x Body weight =  $8\% \times 70 \times 1000 = 5600$  ml
2. Maintence fluid requirement:  $50\text{ml} \times \text{kg} \times \text{day} = 50\text{ml} \times 70 = 3500$  ml/day
3. Daily fluid requirements: Replcement+maintence =  $5600 + 3500\text{ml} = 9100$  ml/day/24 hour

**B-Calculatation of Fluid Loss from Hct:**

Normal (desired) average Htc = 32%

Patient's Htc value = 40%

Increase (difference)  $40-32=8\%$

Taking into consideration that 10 ml / kg fluid is required for each unit increase above 40 % of Htc:

Total Fluid Requirement =  $8 \times 10 \times 70 = 5600$  ml.

2. Maintenance fluid requirement:  $50\text{ml} \times \text{kg} \times \text{day}$ :  $50\text{ml} \times 70 = 3500$  ml/day

3. Daily fluid requirements: Replacement+maintenance=  $5600+3500\text{ml}=9100$  ml/day/24 hour

The required amount of infusion can be found from the deficiency and meeting the daily fluid need. The amount of fluid lost can be calculated in the degree of dehydration. Mild up to 6% decrease in body weight; 60 ml in isotonic dehydration, Medium up to 8% decrease in body weight; 80 ml /kg in degree dehydration and severe 8% from in body weight; 80-120 ml /kg in severe dehydration (Cullen, 1991, Gross,1988). The amount of fluid to be given to this case and the duration of administration should be as follows. Calves with a degree of dehydration higher than 8 % should be given hypertonic sodium bicarbonate, 1/3 of the required liquid within the first 2 hours. Colloidal solution should be given at 1/3 of the remaining liquid at the 6th hour and thick liquid isotonic crystalloid solutions should be given at the 12 th hour.

**A. Correction of hypovolemia:** In all fluid losses, hypovolemia should be corrected first. The lack of volume that develops without changing the extracellular fluid (ECF) composition is the most common condition. The amount of fluid required to correct the blood volume in the circulation can be calculated from plasma loss. Plasma loss is 1/8 - 1/12 of total loss. It is ideal to deliver plasma or plasma equivalents to correct the circulation volume in shock (Veech, 1986; Schaer, 1989). This amount should be given as quickly as possible. One liter of infused crystalloid solution remains in only about 200 ml of intravascular region. Therefore, approximately 4 times the calculated plasma loss of Ringers lactate or 0.9 % NaCl solution should be given to increase blood volume. In this case, haematocrit (Hct) drops below the critical value ( $\text{Hct} < 30\%$ ). Pulmonary edema may develop with decreased mitochondrial diffusion and low oncotic pressure (Clark, 1988). It is reported that it is effective to 7.2 or 8.4 % hypertonic salt solutions for resuscitation are administered to calves 6.4 ml/kg over a period of 5 minutes in veterinary practice (Trefz et al., 2017). Hypertonic NaCl solution increases the  $\text{Na}^{++}$  concentration in the extracellular region, allowing water to pass from the intracellular region to the extracellular region. In this way, it is possible to increase the blood volume rapidly, but there is an improvement against the intracellular region. The disadvantage of this application is short. Serum Na is contraindicated in severe dehydration with increased concentration of Na and in cases where serum osmolality increases (diabetic ketoacidosis). The required amount of infusion, the amount required to meet (eliminate) the existing deficiency in the patient and meet the daily requirement can be determined from the degree of dehydration. The amount fluid required to meet the daily need consists of normal fluid losses (respiration, urine, feces, sweat) and is directly

proportional to metabolic activity, not body weight. Therefore, the larger the animal, the lesser the amount of fluid required. Mild to moderate electrolyte and acid-base disorders can be corrected by normal body compensatory mechanisms after correction of fluid volume (Schaer, 1989).

**B. Compensating the current loss:** Following the elimination of hypovolemia, the loss of the remaining fluid is provided by giving a slower rate. It is ideal to cover the total loss over 24 hours. The rest of the required amount is 1/2 in the first 6- 8 hours and the rest in 24 hours. Crucial acid- base balance disorders should be corrected early in the treatment. Metabolic acidosis is likely if the patient is known to be hyperkalemic (plasma  $K^+$  concentration  $> 6$  mmol/L). To cover the base deficit due to diarrhea, 1.3 %- 8.4%  $NaHCO_3$  solutions should be applied and the amount of  $NaHCO_3$  to be given can be calculated using the determined base deficit = live weight X 0.6 x Base deficit = mEq (Öcal et al., 2006). Dehydration and correction of metabolic acidosis  $K^+$  free fluid and  $NaHCO_3$  (1-2 mmol/kg  $HCO_3$  is unknown) and regulatory functions must be regulated. More specific treatment is required if hyperkalaemia is severe (plasma concentration  $>8$  mmol L) and cardiac arrhythmia. The 10 % calcium gluconate dose of 0.5- 1.0 ml /kg and given intravenously intravenous and corrects the effect of potassium on the heart will be removed (Veech, 1986; Chandler et al., 1999).

**4-Route of administration:** Primary fluid therapy in diarrhoeic calves involves correction of fluid and electrolytes either intravenously or orally along with antidiarrhoeals (Nowsheen et al., 2010). The way in which fluid electrolytes are administered is determined by considering some factors. In cases where rapid volume increase is required intravenous application is recommended. It can also be used intraperitoneally or subcutaneously (Kozat and Voyvoda, 2006; Öcal and Ünsüren, 2009), but absorption from the subcutaneous tissue may not be sufficient in severe dehydration. For oral fluid administration, the gastro intestinal system must be functional (Schaer, 1989). Meeting daily needs, daily normal fluid need is between 40-60 ml/kg (Öcal and Ünsüren, 2009), and it can increase up to 2 times the specified amount due to urinary excretion in disease states. This amount of fluid should be given daily until the patient can freely drink. For this purpose, hypotonic solution is used (Cornelius, 1980). Intravenous fluid therapy is most appropriate to correct dehydration and acidosis, especially in cases where dehydration is 8 % or more (ALHizab et al., 2012).

**A)** Calf with diarrhea but no systemic signs of illness. The calf is eager to stand and has a good suckle reflex. Current knowledge indicates that this calf does not need parenteral fluid. Isotonic crystalloid solutions should be given orally to these cases. The active transport of glucose and sodium in oral rehydration solutions is closely related to the absorption of water and sodium from the intestines (Meganck et al., 2014). It has been reported that the amount of glucose in oral rehydration solutions should be 110-140 nmol/L for maximum absorption of water and sodium, and an increase in water secretion in the intestines may occur when the glucose content exceeds

260 nmol/L (Şentürk, 2001). Calves fed hyperosmotic ORS solutions (600–717 mOsm/l) (HORS) have a slower abomasal emptying rate compared with calves fed iso-osmotic ORS solutions (300–360 mOsm/l) (IORS). This slower emptying rate increases the risk for bloat or abomasitis and produces a slower rate of plasma volume expansion (Meganck et al., 2014)

- B)** Calf with diarrhea and systemic signs of illness. The calf is reluctant to stand and has a weak suckle reflex. Isotonic crystalloid solutions should be given orally to these cases. Current knowledge indicates that this calf will benefit from parenteral antimicrobial administration, such as amoxicillin, ampicillin, or a third or fourth generation cephalosporin and fluoroquinolone is used. It is best to apply antibiotics simultaneously with intravenous fluid administration
- C)** Calf with profuse watery diarrhea that contains blood. The calf was admitted in lateral recumbency, and was pyrexia, markedly depressed, and inappetent. The calf was treated intravenously with 2 L of 1.4 % sodium bicarbonate solution containing glucose and was able to stand within 2 hours of treatment. It is best to apply antibiotics simultaneously with intravenous fluid administration

Current knowledge indicates that this calf should be treated with parenteral antimicrobials and a nonsteroidal anti-inflammatory agent such as meloxicam or flunixin meglumine. Parenteral fluoroquinolone or third or fourth generation cephalosporin administration may be indicated in countries where such administration is permitted because the calf needed intravenous fluid therapy.

### **Use of Antimicrobials**

Although there is controversy regarding the use of antimicrobials to treat diarrhea in calves with diarrhea (Pereira et al., 2014), there is strong and conclusive evidence that specific antimicrobials are effective in treatment (Der Verdier et al., 2003; Grove-White, 2004 Von Buenau et al., 2005). D-lactic acid development occurs from bacterial fermentation in the gastrointestinal tract and is a typical finding in neonatal calves with diarrhea. D-lactic acid is a significant component of acidemia in diarrheal calves and is preceded by systemic indications of fatigue and ataxia. In the first 3 weeks of life, this analysis focuses on adjunct diarrhea treatment. Oral or parenteral antibiotics should be used in calves with diarrhea to prevent bacterial fermentation in the gastrointestinal tract, septicemia, hyper-D-lactatemia and hyper-L-lactatemia. There are studies showing that oral administration of penicillin, chloramphenicol and neomycin in healthy calves increases the incidence of diarrhea, produces malabsorption or a decrease in growth rate (Constable, 2004).

Important points to consider when applying antimicrobials in the treatment of diarrheic calves are: (1) The drug should be administered by a specialist or veterinarian; (2) selecting an antimicrobial agent with a suitable spectrum of activity; (3) using a dosage protocol that achieves and maintains an effective therapeutic concentration at the site of infection; (4) to treat for an appropriate period of time; (5) avoid adverse local or systemic effects and residues; and (6) the transfer potential of antimicrobial

resistance genes should be minimized (Pyorala et al., 2008). Veterinarians must prescribe the most appropriate antibiotic to minimize potential adverse effects on the animal or human when applying antimicrobials to the diarrheal case (Constable, 2004). In addition, antimicrobial therapy can be effective in eliminating bacteria that produce D-lactate from the gastro-intestinal tract of calves with diarrhea (Constable, 2009), accelerating the patient recovery time period. Oxytetracycline and sulfachloropyridazine antibiotics are administered parenterally to treat colibacillosis, while amoxicillin, chlortetracycline, neomycin, oxytetracycline, streptomycin, sulfachloropyridazine, sulfamethazine, and tetracycline are administered orally (Radostits, 1975; Palmer et al., 1983; Constable, 2004). Antimicrobials should not be administered if the appetite, rectal temperature and hydration status in diarrheic calves are normal and there are no concomitant infections such as pneumonia or omphalophlebitis (Ortman and Svensson, 2004).

The core principles of antimicrobials therapy in diarrhea and systemic illness neonatal calves are:

1. To treat or eliminate gram-negative septicemia and bacteremia.
2. To decrease the amount of coliform bacteria in the proximal small intestine and abomasum.
3. To improve unspecific resistance.
4. To offer nutrients those facilitate the repair of the damaged intestine and prevent negative energy balance.

Treatment targets for all diarrhea calves are obtained by parenteral administration of mainly gram negative action range antimicrobials, short-term administration of non-steroidal anti-inflammatory drugs, such as flunixin meglumine or meloxicam, and continued feeding of milk.

### **The Use of Antibiotics to Prevent Calf Diarrhea**

The health of neonatal calves is a top priority for beef and dairy cattle holdings (Viu et al., 2000). Because it is reported that the rate of calf losses in this period is between 4 and 8 % and the most common cause of the losses is due to diarrhea (Viu et al., 2000). Therefore, protection of calves from diarrhea is of great importance in dairy and beef cattle enterprises. Three main principles of prevention of diarrhea in the neonatal period in these farms: (1) a good colostrum program should be implemented that ensures adequate immunoglobulin intake of newborn calves, (2) it should be ensured that the burden of enteric pathogens in the environment should be reduced through calf shelter cleaning, hygiene, shelter and pasture management, and (3) pregnant cows should use a vaccine containing *enterotoxigenic Escherichia coli*, *rotavirus* and *coronavirus* (Verdier et al., 2003). Ampicillin, chlortetracycline, furazolidone, neomycin, oxytetracycline and streptomycin are widely used to protect calves from diarrhea (Grove-White, 2004; Fecteau et al., 1997; Morley et al., 2005; Grove-White, 2004). However, other studies (particularly with neomycin) found increased rates of diarrhea in antibiotic-treated calves (Fecteau et al., 1997; Morley et al., 2005). Quite a few of these

older studies found that oral administration of various antibiotics did not change the incidence of diarrhea in calves when compared with untreated controls (Lofstedt et al., 1999). More recent studies have found that either oral antibiotics had no effect on decreasing calf diarrhea or in some cases diarrhea rates actually increased in calves fed antibiotics (Fecteau et al., 1997). Calves fed a medicated milk replacer had 31 % more days with diarrhea when compared with calves fed no medicated milk replacer. Medicated milk replacers should now be reserved for the treatment of bacterial enteritis (diarrhea) and bacterial pneumonia in dairy calves and not for prophylactic prevention (Guardabassi and Kruse, 2008). The use of antibiotics as a treatment in calves with diarrhea is a debated topic with strong opinions on both sides. Antibiotics are contraindicated in calves with diarrhea or that they aid no beneficial purpose (Palmer et al., 1983). In contrast, other studies have designated that antibiotics are effective in reducing mortality rate and speeding recovery in diarrheic calves (Radostits et al., 1975). Metaphylactic use of antimicrobials can only be suggested for a short period on herds actively struggling with *E. coli* diarrhoea problems (Meganck et al., 2014). The two primary treatment goals of an antibiotic in calves with diarrhea would be (1) to prevent bacteremia and (2) to decrease the number of coliform bacteria in the small intestine (Berge et al., 2006). Although many researchers have claimed that antibiotic use in calves with diarrhea is inappropriate and leads to the resistant bacteria (Radostits et al., 1997).

#### **Which Antibiotic should be used in calves with diarrhea?**

Table 9 contains a list of antimicrobials currently approved for the treatment or prevention of diarrhea in the world. At present, oxytetracycline administered parenterally and chlortetracycline, neomycin, oxytetracycline, sulfamethazine, and tetracycline administered orally are the only antimicrobials labeled for the treatment of calf diarrhea. Of these, none have been shown to be consistently efficacious in peer-reviewed studies. As discussed above, when treating calves with diarrhea the two primary goals of therapy are to (1) decrease the number of *E coli* bacteria in the small intestine and (2) treat potential *E coli* bacteremia. In addition, antimicrobial treatment has four important success criteria such as reducing mortality rate in calf diarrhea, increasing survival and growth rate, reducing the severity of diarrhea and shortening the duration of diarrhea (Constable, 2004). With these goals in mind, the target of antimicrobial therapy in calves with diarrhea should be coliform bacteria both in the blood and in the small intestine. Some efficacy has been described for oral amoxicillin in the treatment of calves with experimentally induced diarrhea (Lofstedt et al., 1999; Roussel et al., 1998) but was not effective in the treatment of naturally acquired diarrhea in beef calves (Cole et al., 2003). Amoxicillin trihydrate (10 mg/kg administered orally every 12 h) or amoxicillin trihydrate-clavulanate (12.5 mg combined drug/kg administered orally every 12 h) for at least 3 days is one antimicrobial approach that likely has some efficacy for calves with diarrhea. Amoxicillin is partially absorbed from the calf small intestine with absorption being similar in both milk-fed and fasted calves. High amoxicillin concentrations are found in bile and intestinal contents after oral administration, with



lower concentrations in serum (Rousel et al., 1988). Oral ampicillin could also be used, and its efficacy in one study was shown to be equivalent to that of amoxicillin. Most antimicrobial susceptibility studies done in the past 30 years indicate that sulfamethazine (and other sulfonamide drugs) would have poor sensitivity against coliform bacteria in the blood or small intestine. The most logical antimicrobial for parenteral treatment of calf diarrhea. Ceftiofur is given 2.2 mg/kg intramuscularly every 12 hours for at least 3 days. Ceftiofur is a broad-spectrum antibiotic that is resistant to betalactamase. The labeled dose maintains plasma concentrations of ceftiofur above the minimum concentration required to inhibit the growth of 90% of *E coli* (MIC90) in young calves (0.25 mg/mL). Furthermore, 30 % of the active metabolite (desfuroylceftiofur) is excreted into the intestinal tract of cattle providing activity in both the blood and the small intestine. Parenteral ampicillin (10 mg/kg IM every 12 h) is another antibiotic that would be likely to have efficacy in calves with diarrhea. In Europe, parenteral enrofloxacin is labeled for the treatment of calf diarrhea, and several studies have documented efficacy with using fluoroquinolone antibiotics in calves with diarrhea (wise et al. 1983; Kumar et al., 2010). In a clinical study, in total 22 calves with diarrhea; isolates of *E.coli*, 21 (96.15%), were sensitive to Ciprofloxacin, 20 (92.31%) to Norfloxain, 20 (92.31%) to Pefloxacin, 19 (84.61%) to Cotriamoxazole, 18 (80.76%) to Sparfloxacin, 18 (80.76%) to Ofloxacin, 17 (76.92%) to Amoxycillin and 3 (15.38%) to Chloromphenical. The isolates of *E.coli* were resistant to Gentamicin, Cephalexin and Furazolidone (Kumar et al., 2010). Historically, gentamicin was also considered an appropriate treatment for use in calves with diarrhea. However, parenteral administration of aminoglycosides cannot be recommended in calves with diarrhea because of the lack of published efficacy studies, prolonged slaughter withdrawal times (18 months), potential for nephrotoxicity in dehydrated calves, and availability of other drugs likely to be equally successful (ceftiofur, amoxicillin, and ampicillin). The issue of whether or not to use antibiotics in a calf with simple diarrhea (without systemic signs of disease) is a little more controversial. Although there have been studies to show that these calves gain more weight and recover faster than calves not given antibiotics, there are other studies that indicate no benefit to using antibiotics in these cases (Bywater, 1977; Fecteau et al., 1997; Fecteau et al., 2003). The clinician must weigh any potential benefit of antimicrobial therapy against the possibility of increasing the population of resistant bacteria on the farm. A fairly recent study demonstrated that individual treatment of sick calves with antibiotics increased the level of resistance to *E coli* isolates; however, the change in antimicrobial susceptibility was only transient (Johnson et al., 2004). The toxicity of aminoglycosides increases in hypovolemic conditions. In such cases, this should be taken into account during treatment with aminoglycosides and the patient should be rehydrated. Especially during treatment of gram negative (*Escherichia coli* and *Salmonella*) bacterial infections with aminoglycosides, the level of endotoxin in the blood increases and shock causes. Aminoglycosides should not be used in the treatment of such infections.

**Table 10.** Antibiotics commonly used in calf diarrhea.

<b>Antibiotic</b>	<b>Label claim</b>	<b>Dose</b>
<b>Chlortetracycline</b>	Treating and controlling to <i>E. coli</i> or <i>Salmonella spp</i>	22 mg/kg of body weight for 3-5 days orally
<b>Neomycin</b>	Treating and controlling to <i>E. coli</i>	22 mg/kg of body weight mixed in drinking water, maximum of 14 days
<b>Neomycin+Oxytetracycline</b>	Treating and controlling to <i>E. coli</i>	Mix in milk replacer to deliver 22 mg/kg of body fed continuously for a maximum of 14 days
<b>Oxytetracycline</b>	Treating to <i>E. coli</i>	6.6-11 mg/kg of body weight daily IM or SC for up to 4 days
<b>Sulfamethazine</b>	Treating to <i>E. coli</i> scours	238 mg/kg of body weight on day 1 followed by 119 mg/kg on days 2,3, and 4 mixed in water
<b>Tetracycline</b>	Treating and controlling to <i>E. coli</i> scours	Dissolve in drinking water to provide daily dose of 22 mg/kg of body weight for up to 3-5 days
<b>Sulfamethazine</b>	Treating to <i>E. coli</i> scours	352 mg/kg of body weight given orally, given once every 3 d for a maximum of 2 treatments
<b>Toltrazuril</b>	Treatment of calves naturally infected with <i>Eimeria spp</i>	15 mg/kg of body weight given orally single dose
<b>Enrofloxacin</b>	Treating for <i>E. coli</i> scours	5 mg/kg of body weight given orally for up to 3-5 days
<b>Halofuginone lactate</b>	Treating cryptosporidiosis in young calves	100 µg/kg of body weight given orally for 7 days
<b>Enrofloxacin</b>	Treating to salmonellosis scours	2.5 mg/kg of body weight daily IM or SC for up to 3 days
<b>Marbofloxacin,</b>	Treating to <i>E. coli</i> scours	1mg/kg of body weight given orally for 3 days
<b>Spectinomycin + Linkomisin ( 100 mg of Spectinomycin and 50 mg of Linkomisin per millimeter)</b>	Treating and controlling to <i>E. coli</i> scours	1ml/10 kg of body weight daily IM for up to 3 days (The first application should be twice with an interval of 12 hours and the other application once a day)
<b>Neomycin/trimethoprim/sulfadiazine</b>	Treating to salmonellosis scours	Neomycin 10mg /kg body weight and sulfadiazine 15-30mg/kg body weight should be administered orally for 3 to 5 days.

In currently, halofuginone (Halocur oral solution: 2 ml/10 kg (live weight) /once a day, 7 days in a row), Paromomycin, Decoquinat, Toltrazuril, and Azithromycin have been tested for the treatment of cryptosporidiosis. Toltrazuril is also used in the treatment and prophylaxis of apicomplexant diseases.

Toltrazuril acts on the entire range of intracellular developmental stages of all *Eimeria* and *Isospora* spp. But Toltrazuril not acts on on their oocysts. In recent years, azithromycin has been used to treat cryptosporidiosis in animals and humans. The combination of azithromycin and toltrazuril is an effective treatment option. Cryptosporidiosis in calves as it stops oocyst shedding as quickly as within nine days. Compared to azithromycin or toltrazuril alone, the combination provided a faster recovery time in naturally occurring calf cryptosporidiosis (Yağcı et al., 2017).

In addition to the additional treatment of diarrhea, adjunctive treatments should be routinely administered to all calves whose diarrhea shows signs of systemic disease in the calf as fever, loss of appetite or lethargy. In addition to parenteral applications of antimicrobials with a predominantly spectrum of gram-negative bacterial activities (especially *Escherichia coli* and *Salmonella* infections), meloxicam and flunixin meglumine should be applied in non-steroidal anti-inflammatory agents.

### **Immunotherapy**

Bovine neonates, as soon, after birth, are exposed to an environment rich in pathogenic as well as opportunistic gram-negative microbial agents. Since they are born essentially agammaglobulinemic, infectious diseases (diarrhea, bronchitis, pneumonia) endure the major cause of calf morbidity, mortality and economic losses to cattle producers. Neonatal mortality is caused by *Rotavirus*, *Coronavirus*, *Respiratory syncytial virus*, *enteropathogenic Escherichia coli*, *Pasteurella spp.*, *Salmonella spp.* and *Cryptosporidium* species, along with immunodeficiency typical of the calf, and due to seasonality, congenital defects and poor management conditions. As dairy farms raising thousands animals, usually antimicrobials drugs are added to milk or milk replacers for prophylactic or metaphylactic purpose. Because of concerns that antibiotic use in food animal production has the potential to increase antibiotic resistance in human pathogens, alternative strategies are needed. Moreover, the low Ig concentration of calf serum at birth, identified as failure of passive transfer (FPT), has been associated with a high rate of morbidity and mortality. The FPT “status” is a secondary immunodeficiency characterized by total serum protein (TP) concentration equal or lower than 50 g/L and IgG concentration equal or lower than 10 g/L at 24 hours from birth. It is therefore defined that a high quality colostrum, must submit an IgG concentration >50 g/L, but in the field, the concentration varies greatly, from 9 g/L up to 186 g/L (Bresciani et al., 2016). Passive immunity in calves in the neonatal period can be achieved either by taking colostrum immunoglobulins or by colostrum equivalents. Colostrum equivalent nutrients are used to prevent transmission of diseases that can be transmitted when not enough colostrum is available or through unpasteurized colostrum. In addition, these antibodies are also used in passive immunotherapy for the treatment of infectious diseases, especially in immunocompromised patients. Commercially dried colostrum from whey, blood / serum, normal milk and eggs can be used as an add-on / substitute for colostrum. It has been determined that successful results can be obtained when these products are used together with maternal colostrum to support passive transfer by providing antibodies at the desired level in the calf

and to reduce the risk of contamination of pathogens from colostrum (Murphy et al., 2014; Kozat 2019).

### **Prevention and Control of Calf Diarrhea**

Calf diarrhea is a multifactorial disease and factors involved in the occurrence of calf diarrhea can be summarized as ones associated with a) per partum calving management, b) calf immunity, and c) environmental stress or contamination. Characteristics of major or emerging bovine enteric pathogens were previously described in this review. There is not much of difference between the patterns of disease development and prevention of calf diarrhea according to each etiological agent. Knowing of causal pathogen(s) is important for accurately assessing the current status of the affected farm and developing further interventions. Nowadays, disease control and prevention in production animals involves animal welfare from the public or consumer's point of view, and increased productivity from the livestock producer's point of view (Uhde et al., 2008).

### **A-Per partum calving management**

Factors such as balanced feeding of the cow during pregnancy, the amount of milk production of the cow at birth, the sucking reflex of the newborn offspring and difficult birth are closely related with the catching and growth of the newborn calf diseases. Pregnant cows increase the disease morbidity and mortality rates of calves born of cows with insufficient feed intake and macro or micronutrient deficiencies in the last trimester of pregnancy (Mee, 2004; Kozat, 2018; Kozat, 2019). The quality and quantity of colostrum of the pregnant cow is related to the body condition score (BCS) and the body condition score ranges from 1 to 10. Body condition score close to 5 is acceptable for fertile cows and 6 points are required for primitive cows in calving. Balanced nutrition of pregnant cows; It is stated that the fetal growth and development of calves are affected as well as the adult life of calves (Greenwood and Café, 2007). Calves born from malnourished cows have poor growth performance, low productivity and higher susceptibility to disease (Martin, 2007). Dystocia is closely related to poor calf performance as well as increased susceptibility to environmental pathogens which frequently cause calf diarrhea (Kozat, 2018). Calves that experience dystocia may have physical symptoms such as congestion and swelling of the head and tongue, which can reduce the amount of colostrum uptake from the dam (Barrier et al., 2013). The absorption rate of colostrum-derived immunoglobulin is lower in these calves compared to healthy animals. Consequently, the affected calves cannot obtain appropriate passive immunity from the dams due to inadequate colostrum uptake during early life (*e.g.*, 2~ 6 h after birth) (Moore et al., 2005). The major causes of dystocia are associated with large calf size and small pelvic size of the dam. Large calves are more likely to have an improper position and presentation (*e.g.*, backward, breech, and mal-positioned limbs or head) in the uterus. Under these conditions, the head and legs cannot enter the birth canal. Insufficient maternal pelvic size also can

induce dystocia, especially in beef heifers. To prevent dystocia, the dam's genetic inheritance (*e.g.*, adequate pelvic size and calving ease) should be taken into consideration during heifer selection, and frequent monitoring of the calving cow is required for appropriate calving assistance (Larson and Tyler, 2005; Murray and Leslie, 2013).

### **B-Immunity and Vaccination**

Bovine placenta is structurally not allowing passive antibody to pass to the fetus. Therefore, newborn calves are very sensitive to environmental pathogens as they are born without any antibodies from their mothers. In order for newborn calves to be resistant to environmental pathogens, timely consumption of high quality colostrum in sufficient quantities within the first 2 hours after birth is very important for the calf to be resistant to pathogens and for development (Kozat, 2019). Colostrum contains antibodies (immunoglobulin G is the primary antibody is a type in bovine colostrum) immune cells (neutrophils, macrophages, T cells and B cells), fat, protein, fat-soluble vitamins (*e.g.* retinol, tocopherol,  $\beta$ -carotene), water-soluble vitamins (*e.g.* niacin, thiamine, riboflavin, vitamin B12, pyridoxal, pyridoxamine, pyridoxine), minerals (*e.g.* Ca, P, Mg, Na, K, Zn, Fe, Cu, S, Mn) and, non-specific antimicrobial factors (*e.g.* lactoferrin) (Johnson et al., 2007; Meganck et al., 2014). The newborn calf should receive colostrum, ideally 6-8 % of their body weight, within the first 6 hours after birth (Nagy, 2009). The quality of the colostrum of the cow depends on the number of calving, nutritional status and vaccination of the cow (Larson and Tyler, 2005; Cortese 2009; Kozat, 2019). In addition to colostrum, commercial multivalent vaccines are available against enteric pathogens to induce immunity in newborn calves. Most of these vaccines involve either live-modified or killed organisms or a combination of the two. Some vaccines are specific for cows while the others are designed for calves (Gonzalez et al., 2021). Passive immunity is provided in the calf by increasing the maternal antibody level in colostrum by administering combined diarrhea vaccine to pregnant cows in the last period of pregnancy. Some of them are applied subcutaneously (SC) to a healthy mother 2 months before the birth, 2 times with an interval of 2 weeks or 3 times with an interval of 1 week. Vaccination of cows with vaccines containing *Escherichia coli*, *Rotavirus* and *Coronavirus* antigens increases the amount of specific antibodies against these pathogens in colostrum and milk (Batmaz, 2010). (a) vaccination of the dams of the first 20 newborn calves against *E. coli* and *rota-* and *coronavirus* with one dose (2 ml IM) of a vaccine specific for *E. coli*, *rota-* and *coronavirus* at 3 months to 3 weeks before the expected calving date; (b) administration of halofuginone lactate to the first 20 newborn calves at a dosage of 100 micrograms/kg per day (=2 ml/10 kg) during the first 7 days of life in the milk as a metaphylactic treatment against *C. parvum* (Meganck et al., 2015).

### **C-Environmental Factors**

Weather conditions such as low temperature, rain, heavy snowfall, wind and high humidity levels of shelters cause stress in newborn calves and in such conditions calves' sensitivity to diarrhea increases.

Calves exposed to bad weather cannot regulate their body temperature effectively. As a result, it can lead to hypothermia or hyperthermia in the calf and cause the immune system to deteriorate (Mee, 2004). In order to reduce the mentioned negative factors, the calving season should be adjusted to a time when environmental conditions are more suitable by applying a controlled breeding program (Larson and Tyler, 2005). As the basic intervention concepts stated to reduce the incidence of calf diarrhea: 1) The heifers should reduce their pathogen exposure by planning the first calving, which in turn, the exposure of newborn calves to pathogens should be reduced 2) the burden of environmental pathogens should be reduced and 3) calving areas should be kept clean (Smith et al., 2003).

### **Conclusion**

Diarrhea in neonatal calves is an important disease affecting the cattle industry negatively. Herd management, preventive vaccination and drug use have an effective role in reducing animal yield losses in preventing diseases in this period. These methods are extremely reliable and have high throughput efficiency, but the importance of pathogens found in cases of calf diarrhea can often be overestimated. Therefore, the pros and cons of medical test findings and their general meaning must be properly assessed by returning clinical background to the doctor as the causative etiology is established. Cattle farmers have also ignored non-infectious risk factors that are often considered as important as infectious factors, since newborn animals are vulnerable to environmental stresses. It is more cost-effective to monitor and regulate calf diarrhea during an outbreak than to treat infected animals after the outbreak occurs. While calf diarrhea includes several enteric pathogens, infection and transmission is achieved through a fecal-oral pathway. Thus, caution must be taken to discourage the spread of pathogens. To gain an accurate diagnosis and monitor or handle risk factors associated with calf diarrhea in modernized broad production systems, advice from trained experts such as veterinarians and nutritionists is important.

In summary, three key points should be focused on the successful management of calf diarrhea. First, a detailed definition of pathogenic features is needed (e.g. the underlying pathogenicity function, field occurrence, and genetic evolution). Second, attention should be given to the benefits and drawbacks of different testing approaches and their contribution to diagnostic investigation along with clinical history. Finally, for disease prevention and control, proper cow-calf management is important.

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**The Treatment of A Calf with Chronic Pneumonia Due To *Acinetobacter baumannii*  
Complex and Identification of The Pathogen by MALDI-TOF-MS**

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**Summary**

*Acinetobacter* species, which play an important role in hospital infections, cause serious epidemics in intensive care units and are resistant to many antibiotics. Especially *Acinetobacter baumannii* have great importance due to its association with human infections. According to the anamnesis taken from the 3 month old calf which was living on the family farm, it was found that he had persistent cough, runny nose, intermittent fever, and his lethargy increased day by day. Disease treatment started at the end of July, 2.5 mg/kg enrofloxacin and 2.2 mg/kg flunixin meglumine were used for a week, but there was no recovery. On clinical examination, hyperemic conjunctiva, 56/min respiratory rate, 39.8 rectal temperature and 108/min heart rate were detected. Blood gas and blood biochemistry were performed for diagnosis, samples were taken from the nasal cavity with transport swabs for bacterial isolation. Total protein, albumin, glucose, total bilirubin, gamma glutamyl transferase, calcium, creatine, blood urea nitrogen values were measured. In the analysis, decreased protein (6.3 g/dl) and blood urea nitrogen levels (6.7 mg/dl) and increased bilirubin (0.2 mg/dl) levels were detected. For the isolation, samples were inoculated directly on blood agar plates containing 5.0% sterile defibrinated sheep blood and incubated 37.5 °C for 24-48 h at aerobic conditions. The purified colonies were described as *Acinetobacter baumannii* complex (99.9%), *Acinetobacter radioresistens* (99.9%) and *Bacillus clausii* (99.9%) by using Matrix-mediated laser desorption ionization-flight time mass spectrometry (MALDI-TOF MS) (database v2.0, bioMérieux, France) system. For treatment, colistin 4 mg/kg/day, Meloxicam 0.5 mg/kg single dose and vitamin (vitamin B complex, electrolyte, amino acid, dextrose) 30ml/5kg/day were used for two week and recovery was observed. This is the first case report in our country where *Acinetobacter baumannii* complex, *Acinetobacter radioresistens* and *Bacillus clausii* were isolated together and treated in a calf with pneumonia.

**Keywords:** *Acinetobacter*, calf, MALDI-TOF-MS, pneumonia

**Kronik Pnömonili Bir Buzağıdan MALDI-TOF-MS ile İdentifiye Edilen *Acinetobacter baumannii* Complex ve Tedavisi**

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**Özet**

Nozokomiyal enfeksiyonlarda önemli rol oynayan *Acinetobacter* türleri yoğun bakım ünitelerinde ciddi salgınlara yol açmakla birlikte birçok antibiyotiğe karşı dirençlidir. Özellikle *Acinetobacter baumannii*'nin insanlarda oluşturduğu enfeksiyonların tıbbi olarak büyük öneme sahip oldukları bildirilmiştir. Aile işletmesinde bulunan 3 aylık yaşta bir buzağıdan alınan anamnezde inatçı öksürük, nazal akıntı, aralıklı ateş olduğu ve letarjinin günden güne arttığı bilgisi alındı. Temmuz ayının sonlarında başlayan hastalık için tedavi amacıyla 2.5 mg/kg enrofloksasin ve 2.2 mg/kg flunixin meglumin bir hafta süre ile kullanıldığı ancak iyileşme olmadığı bildirildi. Klinik muayenede konjonktivalar hiperemik, solunum sayısı 56/dk, rektal sıcaklık 39.8 ve kalp frekansı 108/dk idi. Teşhis amaçlı kan gazı, kan biyokimya yapılabilen etken izolasyonu için transport besiyerli svaplar ile nazal kaviteden numune alındı. Kan gazı bulgularında pH (7.68), HCO<sub>3</sub> (30.82 mmol/L) ile referans değerlerin üzerinde bulunurken, pCO<sub>2</sub> (25.06 mm Hg) referans değerlerin altında olduğu tespit edildi. Kan biyokimyasında total protein, albumin, glukoz, total bilirubin, gama glutamil transferaz, kalsiyum, kreatin, kan üre nitrojen değerleri ölçüldü. Analizlerde protein (6.3 g/dl) ve kan üre nitrojen (6.7 m/dl) seviyesinde azalma, total bilirubinde (0.2 mg/dl) ise artış tespit edildi. İzolasyon için örnekler direkt olarak %5 steril defibrine koyun kanı katılmış kanlı agara ekilip aerob ortamda 37.5 °C'de 24-48 saat inkübasyona bırakıldı. Saflaştırılan koloniler MALDI-TOF-MS (Matrix aracılı lazer dezorpsiyon iyonizasyon uçuş zamanı kütle spektrometrisi database v2.0, bioMérieux, France) sistemi kullanılarak *Acinetobacter baumannii* complex (% 99.9), *Acinetobacter radioresistens* (%99.9) ve *Bacillus clausii* (%99.9) olarak tanımlandı. Mevcut vakada kolistin 4 mg/kg/gün, Meloksikam 0.5 mg/kg tek doz ve vitamin (vitamin B kompleks, elektrolit, amino asit, dekstroz) 30ml/5kg/gün iki hafta boyunca kullanılıp iyileşme izlendi. Bu olgu ülkemizde pnömonili bir buzağıda *Acinetobacter baumannii* complex, *Acinetobacter radioresistens* ve *Bacillus clausii*'nin birlikte izole edildiği ve tedavi edildiği ilk sunumdur.

**Anahtar kelimeler:** *Acinetobacter*, buzağı, MALDI-TOF-MS, pnömoni

**Determination of The Effect of Filgrastim Use on Hematological Parameters in Cats  
And Dogs with Leukopenia and Neutropenia: A Preliminary Study**

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**Summary**

The aim of this study was to determine the effectiveness of filgrastim (Neupogen® /Amgen) use on hematological and clinical parameters in addition to treatment in 10 cats (2 months-9 years, 6 females, 4 males) and 2 dogs (3 months- 5 months, 2 males), which were brought to the Erciyes University, Faculty of Veterinary Medicine, Training, Research and Practice Hospital, Department of Internal Medicine, with various complaints and had leukopenia and neutropenia in their hematological examinations. Clinical and hematological examinations of the cats and dogs included in the study were conducted. In addition to symptomatic treatment, filgrastim (Neupogen® /Amgen), the recombinant human granulocyte colony stimulating factors, was administered subcutaneously once a day for a total of seven days, with a dose of 6 µg/kg on the first day and 3 µg/kg on the following six days. As a result of the statistical evaluation of hematological examination results before and after treatment, it was determined that the increases in WBC, granulocyte, lymphocyte and monocyte values were significant ( $p<0.05$ ). Clinical improvement was observed in all animals included in the study. Recombinant human granulocyte colony stimulating factor was well tolerated and no adverse effects were observed. In conclusion, it was thought that recombinant human granulocyte colony stimulating factor could be applied to the treatment in cats and dogs with leukopenia and neutropenia.

**Keywords:** Filgrastim, cat, dog, leukopenia, neutropenia.

**Lökopenili ve Nötropenili Kedilerde ve Köpeklerde Filgrastim Kullanımının Hematolojik Parametreler Üzerine Etkisinin Belirlenmesi: Ön Çalışma**

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**Özet**

Bu çalışmanın amacı, Erciyes Üniversitesi Veteriner Fakültesi Eğitim, Araştırma ve Uygulama Hastanesi İç Hastalıkları Anabilim Dalı'na çeşitli şikayetlerle getirilen ve hematolojik muayenelerinde lökopeni ve nötropeni gözlemlenen 10 kedi (2 ay-9 yaş, 6 dişi, 4 erkek) ve 2 köpekte (3 ay- 5 ay, 2 erkek) tedaviye ek olarak uygulanan filgrastim (Neupogen® /Amgen) kullanımının klinik ve hematolojik parametreler üzerindeki etkinliğinin belirlenmesidir. Çalışmaya dahil edilen kedi ve köpeklerin klinik ve hematolojik muayeneleri yapıldı. Semptomatik tedaviye ek olarak, rekombinant insan granülosit koloni uyarıcı faktörler olan filgrastim (Neupogen® /Amgen), ilk gün 6 µg/kg, takip eden altı gün 3 µg/kg dozunda toplam yedi gün süreyle, günde bir kez subkutan olarak uygulandı. Tedavi öncesi ve tedavi sonrası hematolojik muayene sonuçlarının istatistik değerlendirmeleri sonucunda WBC, granülosit, lenfosit ve monosit değerlerindeki artışların önemli olduğu belirlendi ( $p<0.05$ ). Çalışmaya dahil edilen hayvanların hepsinde klinik olarak iyileşme gözlemlendi. Rekombinant insan granülosit koloni uyarıcı faktör iyi tolere edildi ve hiçbir yan etki gözlemlenmedi. Sonuç olarak, lökopeni ve nötropeni görülen kedi ve köpeklerde rekombinant insan granülosit koloni uyarıcı faktörün tedaviye ek olarak uygulanabileceği düşünüldü.

**Anahtar kelimeler:** Filgrastim, kedi, köpek, lökopeni, nötropeni

**A Case of Congenital Atrial Septal Defect in A Three- Days Old Calf**

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**Summary**

This report presents the clinical, laboratory, radiographic, and echocardiographic findings of a congenital atrial septal defect diagnosed in a calf. A three-day-old calf was presented to the Internal Medicine Department of Firat University Animal Hospital. The main complaint of the owner was severe dyspnea. On the physical examination, the calf showed severe respiratory stress findings. Thoracic auscultation findings revealed increased vesicular sound and severe systolic murmur. Also, the severe cardiac thrill was detected in both right and left thoracic areas of the heart. The hematological finding was nonspecific but blood gas-electrolyte analysis results showed severe respiratory acidosis (venous blood pH: 6.99; pCO<sub>2</sub>: 67.9 mmHg). Thoracic radiograph revealed diffuse pulmonary edema. The left and right parasternal long-axis view of the heart showed a defect between the right and left atrium, and mild pericardial effusion. Also, cardiac dimensions were measured as LA: 3.60 cm, Ao: 2.39 cm, LA/Ao: 1.51, LVIDd: 3.80 cm, LVIDs: 2.39 cm and %FS was calculated as 37.10. The calf died at the time of examination. The necropsy findings confirmed that the presence of an atrial septal defect and that there was no other congenital cardiac anomaly. In conclusion, newborn calves that show respiratory stress signs should be cautiously evaluated for the presence of congenital cardiac anomalies.

**Keywords:** Calf, dyspnea, septal defect, systolic murmur



## Üç Günlük Bir Buzağıda Kongenital Atrial Septal Defekt Olgusu

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### Özet

Bu vakada kongenital atriyal septal defekt tanısı konulan bir buzağının klinik, laboratuvar, radyografi ve ekokardiyografi bulguları sunulmaktadır. 3 günlük buzağı şiddetli dispnö şikayeti ile Fırat Üniversitesi Hayvan Hastanesi İç Hastalıkları Ruminant Kliniği'ne getirildi. Fiziksel muayenede buzağının şiddetli respiratorik stress bulguları gösterdiği dikkati çekti. Akciğer oskültasyonunda sertleşmiş veziküler sesler alınırken, kalbin sol torakstan yapılan oskültasyonunda şiddetli sistolik üfürüm belirlendi. Aynı zamanda hem sağ hem de sol toraks duvarında kardiyak yerleşim sahasında şiddetli titreşim tespit edildi. Hematolojik muayenede anormal bir bulgu görülmezken kan gazı elektrolit analizinde şiddetli respiratorik asidoz (venöz kan pH: 6.99; pCO<sub>2</sub>: 67.9 mmHg) varlığı dikkati çekti. Toraksın latero-lateral radyografisinde diffuz pulmoner ödem gözlemlendi. Kalbin 2 boyutlu sağ ve sol parasternal uzun eksen dört çember görüntülemesinde atriyal septumda 1.78 cm çapında defekt ile birlikte perikardiyal kesede hafif düzeyde efüzyon varlığı tespit edildi. Ayrıca LA: 3.60 cm, Ao: 2.39 cm, LA/Ao: 1.51, LVIDd: 3.80 cm, LVIDs: 2.39 cm olarak ölçülerek ve %FS: 37.10 olarak hesaplandı. Muayene esnasında hayatını kaybeden buzağının yapılan nekropsisinde atriyal septal defekt varlığı ve başka herhangi bir kongenital kardiyak anomalinin olmadığı doğrulandı. Sonuç olarak, solunum stresi belirtileri gösteren yenidoğan buzağuların doğuştan kalp anomalilerinin varlığı açısından dikkatlice değerlendirilmesi gerekmektedir.

**Anahtar kelimeler:** Buzağı, dispnö, septal defekt, sistolik üfürüm

**Determination of The Relationship Between Plasma B-Type Natriuretic Peptide Levels with The Hemogram and Some Biochemical Parameters in Diarrheic and Healthy Calves**

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**Summary**

The aim of this study was to determine the relationship of plasma B-type natriuretic peptide (BNP) levels with hematological and some serum biochemical parameters in healthy calves with neonatal diarrhea. The material of the study was consisted of 84 calves of the Holstein (31), Simental (47) and Brown Swiss (6) breeds, 44 with diarrhea and 40 healthy, at the age of 0-30 days, which were brought to Erciyes University Veterinary Faculty Training, Research and Application Hospital Internal Medicine Clinics for diagnosis, treatment and control purposes. Blood and serum samples were taken from the calves included in the study. Hematological parameters, plasma BNP, serum creatinine, sodium (Na), potassium (K) and total protein (TP) levels were determined from the samples taken. There was no statistically significant difference in BNP levels between diarrheic and healthy groups ( $p>0.05$ ). However, the Cohen d effect size to determine clinical significance was calculated as 0.228. This indicated that an average of 53 units (1259-1206) lower BNP value in the diarrheal group could be considered clinically significant. In addition, a statistically significant difference was found between the diarrheal and healthy groups in creatinine, Na, totally leukocytes, neutrophils and mean platelet volume (MPV) parameters ( $p<0.05$ ). The correlations between BNP levels and hemogram parameters were not statistically significant except for the mean hemoglobin concentration (MCHC) ( $p>0.05$ ). The correlation between BNP and MCHC was negative and weak ( $r=-0.238$ ). The only effective variable on BNP was determined as creatine. It was determined that if the creatine value was higher than 2.76, the BNP level would be the highest (1410.45) and if the creatinine value was less than 1.240, the BNP level would be the lowest (1268.2). As a result, it was determined that the plasma BNP level in calves with diarrhea was lower than the healthy ones and there was a correlation between the plasma BNP levels and the serum creatinine levels.

**Keywords:** Biochemistry, BNP, calf, hematology

## İshalli ve Sağlıklı Buzağlarda Plazma B-Tipi Natriüretik Peptit Seviyeleri ile Hemogram ve Bazı Biyokimyasal Parametreler Arasındaki İlişkinin Belirlenmesi

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### Özet

Bu çalışmanın amacı neonatal ishalli ve sağlıklı buzağlarda plazma B-tipi natriüretik peptit (BNP) seviyeleri ile hematolojik ve bazı serum biyokimyasal parametreleri arasındaki ilişkinin belirlenmesidir. Çalışma materyalini Erciyes Üniversitesi Veteriner Fakültesi Eğitim Araştırma Uygulama Hastanesi İç Hastalıkları Anabilim Dalı Kliniği'ne teşhis, tedavi ve kontrol amacı ile getirilen 0-30 günlük yaştaki, 44 adet ishalli ve 40 adet sağlıklı olmak üzere holstein (31), simental (47) ve montofon (6) ırkı 84 adet buzağı oluşturdu. Çalışmaya dahil edilen buzağlardan tam kan ve serum örnekleri alındı. Alınan örneklerden hematolojik parametreler, plazma BNP, serum kreatinin, sodyum (Na), potasyum (K) ve Total protein (TP) seviyeleri belirlendi. İshalli ve sağlıklı gruplar arasında BNP düzeyi bakımından istatistiksel olarak anlamlı farklılık bulunmadı ( $p>0.05$ ). Ancak klinik anlamlılığın tespiti amacıyla Cohen d etki büyüklüğü 0,228 olarak hesaplandı. Bu durum ishalli grupta BNP değerinin ortalama 53 birim (1259-1206) az olmasının klinik olarak anlamlı kabul edilebileceğini gösterdi. Ayrıca çalışmada ishalli ve sağlıklı gruplar arasında kreatinin, Na, lökosit, nötrofil ve ortalama platelet hacmi (MPV) değişkenleri bakımından istatistiksel olarak anlamlı farklılık bulundu ( $p<0.05$ ). BNP düzeyi ile hemogram sonuçları arasındaki korelasyonlar ortalama hemoglobin konsantrasyonu (MCHC) haricinde istatistiksel olarak anlamlı bulunmadı ( $p>0.05$ ) BNP ve MCHC arasındaki korelasyonda negatif yönlü ve zayıf bulundu ( $r=-0,238$ ). BNP üzerine tek etkili değişken kreatin olarak belirlendi. Kreatin değerinin 2,76'dan büyük olması durumunda BNP düzeyinin en yüksek (1410,45) değerinde ve kreatin değerinin 1,240'dan küçük olması durumunda ise BNP düzeyinin en düşük (1268,2) değerinde olacağı belirlendi. Sonuç olarak, ishalli buzağlarda plazma BNP seviyesinin sağlıklılara göre düşük olduğu ve plazma BNP düzeyi ile serum kreatinin seviyesi arasında korelasyon olduğu belirlendi.

**Anahtar kelimeler:** Biyokimya, BNP, buzağı, hematoloji

## Can Surgical Orthopedic Intervention Be Made Without General Anesthesia in New-Born Calves?

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### Summary

**Aim:** This study aims to show that the surgical treatment of metacarpus fractures can be made with sedation and local anesthesia instead of general anesthesia in new-born calves.

**Material-method:** A two-day-old, 35 kg male Simmental calf was brought to the clinic with a distal diaphyseal transversal metacarpus fracture. After intravenous xylazine HCl, injection infusion sets were applied for slowing circulation over the olecranon level. A 10 ml of blood was discharged, and 10 ml of lidocaine with epinephrine injected into the vein. A 22 G x 90 mm spinal needle was placed to the medial contralateral to the humeral articulation to the brachial plexus level, and 5 ml of lidocaine were injected. Skin and subcutaneous tissues were incised, and five cortical screws were applied with a plate onto the bone.

**Results:** Pain signs were not seen during the surgery. Slight movements appeared at the 37, 62, and 71<sup>st</sup> minutes of the surgery, and they were disappeared after 0.01 mg/kg xylazine bolus injection. The restraining ability of this triple technique was satisfying for the orthopedic surgery without any general anesthetic procedure.

**Discussion:** Although orthopedic interventions can be made under general anesthesia, complications make orthopedic surgery difficult in new-born calves. Even if the local anesthesia and sedation allow finger amputation, this does not form a sufficient anesthetic depth for long-term orthopedic initiatives in calves.

**Conclusion:** This study reported that sedation, local anesthesia, and the slowdown of the vascular circulation blocked the requirement of general anesthetic drugs and the need for long-term preoperative hunger in orthopedic surgery of calves.

**Keywords:** Bier block, intravenous regional anesthesia, sedation.

## Yeni Doğan Buzağlarda Genel Anestezi Olmadan Cerrahi Ortopedik Müdahale Yapılabilir mi?

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### Özet

**Amaç:** Bu çalışma, buzağlarda metacarpus kırıklarının cerrahi sağaltımının genel anestezi yerine sedasyon ve rejyonel anestezi ile yapılabildiğini göstermeyi amaçlamaktadır.

**Materyal-Metot:** İki günlük yaşta ve 35 kg ağırlığındaki Simmental buzağının yapılan muayenesinde distal diafiz metacarpus kırığı olduğu görüldü. Damar içi xylazine HCl enjeksiyonundan sonra sirkülasyonu yavaşlatmak için serum hortumu olekranon düzeyine bacağına sarıldı ve sıkıldı. Damar içerisinden 10 ml kan alındıktan sonra 10 ml adrenalinli lidokain damar içine verildi. Ardından 22G X 90 mm boyutunda spinal iğne humerus ekleminin medial kontrolateraline brachial plexus düzeyinde yerleştirilerek 5 ml lidokain enjekte edildi. Deri ve subkutanöz doku ensize edildikten sonra kemik üzerine beş adet kortikal vida ile plak fikze edildi.

**Bulgular:** Operasyon süresince ağrı bulgusu görülmedi. Operasyonun 37, 62 ve 71. Dakikalarında beliren ılımlı hareketlilik 0.01 mg/kg xylazine enjeksiyonundan sonra kayboldu. Bu üçlü tekniğin hareketsiz kılıcı etkisi general anestezi olmaksızın ortopedik cerrahi için tatmin ediciydi.

**Tartışma:** Buzağlarda ortopedik cerrahi girişimler genel anestezi altında yapılabilir de genel anestezi komplikasyonları ortopedik cerrahiye buzağlarda komplike hale getirmektedir. Buzağlarda lokal anestezi ve sedasyon parmak amputasyonuna izin verse de bu anestezi yöntemi uzun süreli ortopedik girişimlerde yeterli derinlikte anestezi oluşturmamaktadır.

**Sonuç:** Buzağlarda sedasyonla birlikte yapılan iki farklı rejyonel anestezi teknik genel anestezi ve uzun süreli preoperatif açlık periyoduna ihtiyaç olmaksızın ortopedik cerrahi yapmaya imkan vermektedir.

**Anahtar kelimeler:** Bier blok, intravenöz rejyonel anestezi, sedasyon.

## **Introduction**

The metacarpus is a prone bone for dystocia-related fractures in calves (1, 2). External splint coaptation and surgical reduction are the two most common osteosynthesis methods for new-born calves' metacarpus fractures (3).

Even though the amputation of the distal limbs can be done by sedation with intravenous regional anesthesia, general anesthesia is needed for the osteosynthesis of the limbs (3, 4).

Xylazine is the most common alpha-2 adrenergic agonist drug for the sedation of ruminants in the field. However, the adequate dose is unclear, and the experience is needed for the adequate dosage (5, 6). Xylazine usage in combination with a general anesthetic drug (usually ketamine) is popular in ruminant field anesthesia (5). But the complications of general anesthesia make complicated the surgery in ruminants. Regurgitation originated aspiration pneumonia, inadequate ventilation, and bloating are the most common complications of general anesthesia in ruminants (7).

Ruminants cannot control the airway and salivation during general anesthesia; therefore, the presence of consciousness is vital in ruminants during anesthesia (5, 6). Anesthesia without unconsciousness which provides surgery can only be possible with sedation with local anesthesia, but this technique is inadequate for orthopedic surgery without general anesthesia in ruminants (5, 8)

This study aims to present the satisfactory effect of sedation in combination with two different regional anesthetic methods during orthopedic surgery of a metacarpus fracture in a calf.

## **Material and Methods**

A client-owned two-days-old, 52 kg Simmental calf with a right forelimb lameness was brought to the surgery department. The history was that the leg was broken with abnormal traction during a difficult delivery. After radiologic evaluation, a unilateral distal metaphyseal complete transversal metacarpus fracture was detected. The skin was seemed thin as to be ruptured; therefore, urgent surgery was planned without preoperative fasting.

An auricular vein was catheterized with 22G IV catheter, and the sedation was maintained with slowly IV injection of 0,1 mg/kg xylazine (xylazine hydrochloride, 20 mg/ml, Xylazinbio 2% Bioveta®, Interhas Co. Ltd., Ankara, Turkey) in a 5 ml of normal saline.

An infusion set tube tourniquet encircled proximal to the distal condyle of the humerus and tightened for the intravenous regional anesthesia (Bier block) procedure. The pressure of the cephalic vein was reduced with a 22G needle and blood extracted; then 10 ml of lidocaine with adrenaline (%2, Vilcain, Vetaş, Turkey) slowly infused into the vein.

A 22G x 90 mm spinal needle was inserted between the thorax and forelimb proximal to the scapulohumeral articulation level around the brachial plexus for the brachial block; then 5 ml of lidocaine with adrenaline was injected onto the axillary plexus.

After the hair trimming, the operation site was prepared with routine surgical aseptic techniques. A dorsal incision was made; after the retraction of extensor muscle ligaments, the bone surface appeared. Periosteal layer incised and stripped. After positioning a semi-tubular T plated on the bone, the plate was fixed onto the bone with five screws. The incision line was closed with routine techniques.

Thirty-five minutes after the first xylazine injection, when the cognitive findings were seen, the second injection of xylazine was slowly infused into the auricular vein. This was repeated at the fifty-fifth and sixty-fifth minutes which depends on the appearance of the cognitive findings.

## **Results**

The animal was laterally restrained after the first xylazine injection. After the intravenous regional anesthesia and brachial plexus block, there was no mobility observed despite cutting the skin, the elevation of the periosteum, and drilling the bone. The second xylazine was injected thirty-fifth minutes after the first injection due to the appearance of awakening signs which were ear-head movements and pulling the tongue into the mouth. The third injection was made fifty-five minutes after the first xylazine infusion, and the fourth was at sixty-five after the first infusion. The operation was completed in seventy-five minutes. Excessive saliva run, regurgitation, or pedal reflex did not appear during the surgery. Despite the swallowing signs were seen, the tongue was remaining outside of the mouth. The bleeding during surgery was less in comparison with our previous clinical observations. Intramuscularly Butorphanol 0.05 mg kg<sup>-1</sup> (Butorphanol 10 mg/ml, Butomidor, Interhas, Ankara, Turkey) was injected for the postoperative pain before delivering the patient to the owner.

The recovery period was calm and without any excitement, and no reversal agent was used. After 3 hours of xylazine period, the calf was awake, standing and sucking the owner's finger.

## **Discussion**

Salivary production during the surgery cannot be ignored during the general anesthesia of ruminants. It is not easy to control their airways during general anesthesia. When over-salivation and poor airway management come together, this makes general anesthesia risky for ruminants (5, 6). Preoperative fasting is a component of surgery, but calves are not good candidates for preoperative fasting due to hypoglycemia (9).

Although fasting and general anesthetics were not considered in this orthopedic surgery, multiple-dose xylazine injections with intravenous regional anesthesia and brachial block enabled us to restrain the patient without complete unconsciousness. The encircled serum infusion hose could reduce

circulation, and this may help the reducing the pain stimulus. We believe that consciousness resulted that the calf swallowed the salivary production properly, and thus the regurgitation or aspiration complications of the general anesthesia were not developed. Open consciousness was encouraged us to discard preoperative fasting. We believe that relinquishing the preoperative fasting was blocked the hypoglycemia, which is major trouble in the field anesthesia of ruminants. Moreover, the usage of xylazine in the procedure could provide benefits due to its hyperglycaemic effect (9).

The knowledge about sedation with multiple regional anesthesia techniques could be promising for orthopedic surgery of the limbs of ruminants for the field and should be the subject of a research article.

### **Conclusions**

Xylazine sedation with intravenous regional anesthesia and brachial block provides satisfactory anesthesia for osteosynthesis of metacarpal fractures and prevents complications of general anesthesia in new-born calves.

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**Preanesthetic Evaluation of Respiratorysystem in Cats and Dogs**

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**Summary**

Preanesthetic evaluation is the clinical examination that the veterinary surgeon should perform before the anesthetic application. Preanesthetic evaluation is performed to reduce all risks associated with perioperative period. It provides to be prepared for the problems that may be encountered and to determined appropriate anesthetic protocol. The respiratory system is one of the most important system for the anesthesia in terms of both the anatomical and physiological characteristics. Since all of the anesthetic drugs suppress the respiratory system and directly affects hemodynamics, it is the most affected system. In this study; it was evaluated whether there was a respiratory system problem accompanying the existing condition of the cats and dogs that were decided to have surgery and that could pose risk during anesthesia. For that purpose, after the anamnesis and physical examination of the patients which were decided to operate, radiographic and laboratory examinations were performed. The anesthetic risk of the patients were determined by evaluating the results. Based on the findings of the preanesthetic evaluation and taking into account the individual characteristics and diseases of the patient, an appropriate anesthetic protocol was created.

**Keywords:** Cat, dog, preanesthetic evaluation, respiratory system

## **Kedi ve Köpeklerde Solunum Sisteminin Preanestezik Değerlendirilmesi**

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### **Özet**

Preanestezik değerlendirme; anestezi uygulamasından önce hekimin yapması gereken klinik incelemelerdir. Preanestezik değerlendirme perioperatif dönemle ilişkili tüm riskleri azaltmak amacıyla yapılır ve optimum koşullarda operasyonun yapılmasını sağlar. Kaşılabilir sorunlara karşı hazırlıklı olup, uygun anestezi protokolünün belirlenmesini sağlar. Solunum sistemi hem anatomik hem de fizyolojik özellikleri bakımından anestezi uygulamaları için en önemli sistemlerden biridir. Tüm anestezi ilaçlarının solunum sistemini baskılaması nedeniyle, anesteziden en çok etkilenen sistemdir ve hemodinamiyi de direkt olarak etkiler. Bu çalışmada; operasyon yapılmasına karar verilen kedi ve köpeklerin var olan durumuna eşlik eden, anestezi sırasında risk oluşturabilecek bir solunum sistemi probleminin olup olmadığı değerlendirilmiştir. Bu amaçla operasyon kararı alınan hastaların anamnez ve fiziksel muayenelerini takiben radyografik ve laboratuvar incelemeleri yapılmıştır. Sonuçlar değerlendirilerek hastaların anestezi riski belirlenmiştir. Preanestezik değerlendirme bulgularının ışığında, hastanın bireysel özellikleri ve hastalıkları göz önünde bulundurularak yapılacak operasyona uygun bir anestezi protokolü oluşturulmuştur.

**Anahtar kelimeler:** Kedi, köpek, preanestezik değerlendirme, solunum sistemi

**Assessment of Intraocular Pressurei Romanov Sheep of Different Age Groups Using Rebound Tonometry**

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**Summary**

The aim of this study was to measure the intraocular pressure (IOP) of healthy Romanov sheep using a rebound tonometer. Forty-two Romanov sheep were divided into three groups of 14 animals each, according to their age: Group I, 6 months old (7 females, 7 males, n:14), group II, 12 months old (7 females, 7 males, n:14), and group III, 24 months old (7 female, 7 male, n:14). IOP of the cases were measured on the same day (between 13-16 h). Mean IOP for groups I, II, and III were  $16.18 \pm 0.51$  mmHg,  $16.47 \pm 0.59$  mmHg, and  $16.64 \pm 0.34$  mmHg, respectively. There was no statistically significant difference between age-related IOP values ( $P > 0.05$ ). Lastly, the iCare® Rebound tonometer has also proven to be a safe diagnostic tool for IOP measurement in Romanov sheep.

**Keywords:** Age, intraocular pressure, Romanov sheep, tonometry

**Farklı Yaş Gruplarındaki Romanov Koyunlarda Rebound Tonometresi Kullanılarak  
Göz İçi Basıncının Değerlendirilmesi**

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**Özet**

Bu çalışmanın amacı, sağlıklı Romanov koyunların göz içi basıncını (GİB) rebound tonometre kullanarak ölçmektir. Kırk iki Romanov koyun, yaşlarına göre her biri 14 hayvandan oluşan üç gruba ayrıldı: Grup I 6 aylık (7 dişi, 7 erkek, n:14), Grup II 12 aylık (7 dişi, 7 erkek, n:14) ve Grup III 24 aylık (7 dişi, 7 erkek, n:14). Olguların aynı gün (13-16 saatleri arasında) göz içi basınçları ölçüldü. Grup I, II ve III için ortalama GİB sırasıyla  $16.18 \pm 0.51$  mmHg,  $16.47 \pm 0.59$  mmHg ve  $16.64 \pm 0.34$  mmHg idi. Yaşa bağlı GİB değerleri arasında istatistiksel olarak anlamlı bir fark görülmedi ( $P > 0.05$ ). Son olarak, ICare® Rebound tonometresinin Romanov koyunlarda GİB ölçümü için güvenli bir teşhis aracı olduğu da kanıtlanmıştır.

**Anahtar kelimeler:** Yaş, göz içi basınç, Romanov koyun, tonometre

## The Effect of Meloxicam on Intraocular Pressure in Horses

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**Objective:** In this study, it was aimed to investigate the effects of meloxicam use on intraocular pressure in normal horses.

**Materials and Methods:** The average age of the study material is 12.05 (6-16), the average weight of 287.85 kg (190-360 kg) 15 of the female and 5 of the male horses 13 of the British, 6 Haflinger and 1 Arab total 20 the horse was studied. The study groups were determined as meloxicam group (n = 10) and control group (n = 10). The intraocular pressures of the horses in both groups were measured by means of Tonovet®. The horses in the control group were injected with intravenous saline (15ml / 100kg). IOP measurements were performed by the same investigator in the right and left eyes of the horses in both groups. Measurements were made immediately before and after application at 5, 15, 30, 45, 60, 90, 120 minutes and 8 hours, 9 hours.

**Results:** In the meloxicam and control groups, it was evaluated whether the data obtained from the right and left eyes separately and the averages were different depending on time. Time-dependent change was significant in the meloxicam group, but the timexgrup interaction is not significant. In the control group, both time-dependent change, as well as the timexgrup interaction was statistically significant.

**Conclusion:** In conclusion, in the study performed in horses, although the IOP values were within normal limits, it was determined that meloxicam had a slightly hypotensive effect.

**Keywords:** Horse; intraocular pressure; meloxicam; rebound tonometry

## Atlarda Meloksikam'ın Göziçi Basıncına Etkisi

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**Amaç:** Bu çalışmada normal atlarda meloksikam kullanımının intraoküler basınç üzerine etkilerinin araştırılması amaçlanmıştır.

**Materyal ve Metot:** Çalışmanın materyalini yaş ortalaması 12.05 (6-16), kilolarının ortalaması 287.85 kg (190-360 kg) olan 15'i dişi ve 5'i erkek olan, 13'ü İngiliz, 6'sı Haflinger ve 1'i Arap olmak üzere toplam 20 at oluşturmuştur. Çalışma grupları, meloksikam grubu (n=10) ve kontrol grubu (n=10) olarak belirlenmiştir. Her iki gruptaki atların göz içi basınçları, Tonovet® yardımı ile ölçülerek değerlendirilmiştir. Meloksikam grubundaki tüm atlara 0.6mg/kg dozunda intravenöz meloksikam tek doz olarak uygulanmıştır. Kontrol grubundaki atlara ise intravenöz serum fizyolojik (15ml/100kg) enjekte edilmiştir. Her iki gruptaki atların sağ ve sol gözlerinde GİB ölçümleri aynı araştırmacı tarafından yapılmıştır. Ölçümler uygulamalardan hemen önce ve uygulamadan sonra 5, 15, 30, 45, 60, 90, 120. dakikalar ve 8.saat, 9. saatlerde yapılmıştır.

**Bulgular:** Meloksikam ve kontrol gruplarında hem ayrı ayrı sağ ve sol gözlerden elde edilen verilerin hem de ortalamalarının üzerinden zamana bağlı olarak farklı olup olmadıkları değerlendirilmiştir. Meloksikam grubunda zamana bağlı değişimin önemli olduğu, ancak zamanxgrup etkileşiminin önemli olmadığı görülmüştür. Kontrol grubunda ise hem zamana bağlı değişim, hem de zamanxgrup etkileşiminin istatistiksel olarak önemli olduğu belirlenmiştir.

**Sonuç:** Sonuç olarak atlarda yapılan çalışmada GİB değerlerinin normal sınırları içerisinde kalmakla beraber meloksikamın biraz hipotansif bir etkiye sahip olduğu belirlenmiştir.

**Anahtar kelimeler:** At; intraoküler basınç; meloksikam; rebound tonometri

**High Resolution Image Acquisition with 3 Tesla MRI in Sheep Brain Fixed with Formaldehyde: A Methodological Study\***

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**Summary**

**Aim:** In recent years, sheep is an animal model used in neuroscience research due to its behavioral and physiological mechanisms. Besides to this feature, it is frequently used in anatomy education due to its easy supply. In this study, it was aimed to obtain high-resolution images from sheep brain fixed with formaldehyde by 3T Magnetic Resonance Imaging.

**Method:** The sheep brain was fixed with formaldehyde solution in the cranial cavity by means of the carotid artery. In this way, the brain was fixed in its normal position. Craniotomy of the brain was performed to protect the dura mater and was removed from the cavum cranii. While dissecting of meninges, attention was paid to keep the pituitary gland on the brain. Formaldehyde negatively affects the signal intensity in MRI scanning. It is known to cause partially hypointense. For this purpose, the brain was removed from the formaldehyde solution and kept under tap water. Air bubbles between the gyri and sulci of the brain that are kept in water cause artifacts in the image. Therefore, it was immersed in vacuum pump oil for about 1-2 hours before the MRI scan. MR scanning was performed in the supine position in the 3T MR scanner to avoid any deformation of the hypothalamus.

**Results:** The highest contrast between gray and white matter was obtained in the T2-weighted image. When sagittal, coronal and axial section images were examined, structures such as lamina medullaris externa, nucleus reticularis thalami, fasciculus mammilo-thalamicus, tractus habenulo-interpeduncularis were clearly observed.

**Conclusion:** It is thought that high resolution images can be obtained from the detected brains of other animals with the method used. Scanning time is often too long to obtain high resolution images in MRI. For this purpose, it is thought that the small structures of the brain can be imaged at high resolution by preventing motion artifact in ex vivo studies that require a long scanning time.

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## Formaldehit ile Tespit Edilmiş Koyun Beyninde 3 Tesla MR ile Yüksek Çözünürlükte Görüntü Elde Edilmesi: Bir Metodolojik Çalışma

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### Özet

**Amaç:** Son yıllarda koyun; davranış özelliği ve fizyolojik mekanizmalarından dolayı sinirbilim araştırmalarında model hayvan olarak kullanılmaktadır. Bu özelliğinin yanı sıra kolay temin edilmesinden dolayı anatomi eğitiminde de sıklıkla kullanılmaktadır. Bu araştırmada 3T Manyetik Rezonans Görüntüleme ile formaldehit ile tespit edilmiş koyun beyninden yüksek çözünürlükte görüntü elde edilmesi amaçlanmıştır.

**Yöntem:** Bu amaçla arteria carotis communis'ten formaldehit solüsyonu enjekte edilen ve cavum cranii içinde tespiti sağlanan koyun beyni kullanıldı. Böylece beyin normal duruş pozisyonunda tespit edilmesi sağlandı. Dura mater korunacak şekilde beyinin kraniotomisi gerçekleştirilerek cavum cranii'den çıkarıldı. Beyin zarlarının diseksiyonu esnasında hipofizin beyin üzerinde bırakılmasına özen gösterildi. Formaldehitin MR taramasında sinyal yoğunluğunu negatif yönde etkilediği ve görüntülerde hipointense neden olduğu bilinmektedir. Bu amaçla beyin formaldehit solüsyonundan çıkarılarak belli bir süre çeşme suyu altında bekletildi. Suda bekletilen beyin gyri ve sulci'leri arasında kalan hava kabarcıklarının görüntüde artefakta neden olmaması için MR taramasından yaklaşık 1-2 saat önce vakum pompası yağında bekletildi. MR taraması 3T MR tarayıcıda facies basilaris'inde bulunan hypothalamus bölgesinin herhangi bir deformasyona uğramaması için supin pozisyonunda gerçekleştirildi.

**Bulgular:** Formaldehit ile tespit edilen koyun beyninde 3 Tesla MR kullanılarak gerçekleştirilen taramada gri ve ak madde arasındaki en yüksek kontrast T2 ağırlıklı görüntüde elde edildi. Sagittal, koronal ve axial kesit görüntüleri incelendiğinde lamina medullaris externa, nucleus reticularis thalami, fasciculus mammilo-thalamicus, tractus habenulo-interpeduncularis gibi yapılar net olarak görüldü.

**Sonuç:** Mevcut çalışmada kullanılan yöntem ile diğer canlılara ait tespit edilmiş beyinlerden yüksek çözünürlükte görüntü elde edilebileceği düşünülmektedir. MR taramalarında yüksek çözünürlükte görüntü elde etmek için çoğu zaman tarama süresi çok uzun olmaktadır. Bu amaçla uzun tarama süresi gerektiren ex vivo çalışmalarda beyni ait küçük yapıların yüksek çözünürlükte görüntülenebileceği düşünülmektedir.

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**Effect of Low and High Dose Levothyroxine on P-Glycoprotein Expression, Protein Level and Function in Mice**

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**Summary**

Levothyroxine, a synthetic form of thyroxine, is used to treat hypothyroidism. Levothyroxine exerts its effects like thyroid hormones through thyroid receptors. Thyroid hormone dysfunctions cause changes in the effects of some drugs. The function of P-glycoprotein (P-gp), which has an important effect on drug pharmacokinetics, is regulated by nuclear receptors such as thyroid receptors. In this study, we investigated the effect of different doses of levothyroxine on the expression and function of P-gp using its substrate. A total of 48 Swiss albino male mice were divided into 6 equal groups in the study. Mice were randomly divided into 6 groups as control (C), low dose levothyroxine (LL, 8 µg/kg), high dose levothyroxine (HL, 80 µg/kg), fexofenadine (F, 40 mg/kg), low dose levothyroxine + fexofenadine (LLF, 8 µg/kg+40 mg/kg), and high dose levothyroxine + fexofenadine (HLF, 80 µg/kg+40 mg/kg). Mice received levothyroxine at doses of 8 and 80 µg/kg by daily gavage for 21 days. Fexofenadine was administered once by gavage at a dose of 40 mg/kg at the 24th hour following the last levothyroxine administration. Blood, liver and small intestine tissues were collected from mice. Mdr1 mRNA levels in tissues were determined by RT-PCR and P-gp protein by Western Blot analysis. Plasma concentrations of fexofenadine were determined using HPLC-UV. At the end of the study, we determined that the effect of levothyroxine on p-gp varied depending on the dose and tissue, and that low-dose levothyroxine increased the plasma concentration of fexofenadine.

**Keywords:** Levothyroxine, mRNA, P-glycoprotein, protein level

**Düşük ve Yüksek Doz Levotiroksinin Farelerde P-Glikoprotein Ekspresyonu, Protein Seviyesi ve Fonksiyonu Üzerine Etkisi**

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**Özet**

Sentetik bir tiroksin formu olan levotiroksin, hipotiroidizmi tedavi etmek için kullanılır. Levotiroksin, tiroid hormonları gibi etkilerini tiroid reseptörleri aracılığıyla gösterir. Tiroid hormon işlev bozuklukları bazı ilaçların etkilerinde değişikliklere neden olur. İlaç farmakokinetiği üzerinde önemli bir etkiye sahip olan P-glikoprotein'in (P-gp) işlevi, tiroid reseptörleri gibi nükleer reseptörler tarafından düzenlenir. Bu çalışmada, farklı levotiroksin dozlarının substratını kullanarak P-gp'nin ekspresyonu ve işlevi üzerindeki etkisini araştırdık. Çalışmada toplam 48 adet Swiss albino erkek fare 6 eşit gruba ayrıldı. Fareler kontrol (K), düşük doz levotiroksin (DL, 8 µg/kg), yüksek doz levotiroksin (YL, 80 µg/kg), feksofenadin (F, 40 mg/kg), düşük doz levotiroksin + feksofenadin (DLF, 8 µg/kg+40 mg/kg) ve yüksek doz levotiroksin + feksofenadin (YLF, 80 µg/kg+40 mg/kg) olmak üzere 6 gruba ayrıldı. Farelere 21 gün boyunca günde bir defa gavaj yoluyla 8 ve 80 µg/kg dozlarında levotiroksin uygulandı. Son levotiroksin uygulamasını takiben 24. saatte feksofenadin gavaj yoluyla 40 mg/kg dozunda tek sefer uygulandı. Farelerden kan, karaciğer ve ince bağırsak dokuları toplandı. Dokulardaki Mdr1 mRNA seviyeleri RT-PCR ile ve P-gp proteini ise Western Blot analizi ile belirlendi. Feksofenadinin plazma konsantrasyonları HPLC-UV kullanılarak belirlendi. Çalışmanın sonunda levotiroksinin P-gp üzerindeki etkisinin doza ve dokuya bağlı olarak değiştiği ve düşük doz levotiroksinin feksofenadinin plazma konsantrasyonunu arttırdığı tespit edildi.

**Anahtar kelimeler:** Levotiroksin, mRNA, P-glikoprotein, protein düzeyi

**Prevalence of *Eimeria* Species in Calves in Burdur Region of Turkey**

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**Summary**

Bovine coccidiosis, caused by *Eimeria* species, is one of the most important diarrheal diseases of young calves and negatively affects the cattle breeding industry across the world. The present study aimed to determine the prevalence of *Eimeria* species in calves in Burdur region. As material, a total of 151 calf fecal samples were collected from eight different districts of Burdur. Flotation method with saturated salt solution was carried out to determine the positive samples and 2.5% potassium dichromate solution was used in positive samples for sporulation, then sporulated oocysts were identified according to morphological characteristics. Overall prevalence was 26.5% and six *Eimeria* species; *E. auburnensis* (55%), *E. bovis* (50%), *E. alabamensis* (37.5%), *E. zuerni* (30%), *E. ellipsoidalis* (30%) and *E. canadensis* (17.5%) were identified in positive samples. Prevalences were found higher in 0-1 month age group (38.1%), in males (28.2%) and in simental breed (50%). It's found that; 17 of 151 animals (11.25%) were infected with single species and mixed infection rate was 15.23% (23/151) with two to five species. As a result; this is the first prevalence study of *Eimeria* species in calves in Burdur region. Also it has been concluded that coccidiosis should not be ignored especially in cattle farm management and further epidemiological studies are needed for prevention and control of the disease.

**Keywords:** Burdur, calf, *Eimeria*, prevalence

## Burdur Yöresi Buzağlarında *Eimeria* Türlerinin Yaygınlığı

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### Özet

Sığır koksidiyozisi, *Eimeria* türlerinin neden olduğu, genç buzağların ishalle seyreden en önemli hastalıklarından biri olup, dünya genelinde sığır yetiştiriciliğini olumsuz yönde etkilemektedir. Bu çalışmada Burdur yöresindeki buzağlarda *Eimeria* türlerinin yaygınlığının belirlenmesi amaçlanmıştır. Materyal olarak Burdur'un sekiz farklı ilçesinden toplam 151 buzağı ve danadan dışkı örneği toplanmıştır. Doymuş tuzlu su flotasyon yöntemi kullanılarak pozitif örnekler saptanmış, bu örnekler içerisindeki ookistler % 2.5'lük potasyum dikromat solüsyonu yardımı ile sporlandırılmış ve sporlanan ookistler morfolojik özellikleri baz alınarak tür tayinleri yapılmıştır. Genel yaygınlık % 26.5 şeklinde bulunmuş ve pozitif örnekler içerisinde; *E. auburnensis* (55%), *E. bovis* (50%), *E. alabamensis* (37.5%), *E. zuerni* (30%), *E. ellipsoidalis* (30%) ve *E. canadensis* (17.5%) olmak üzere 6 farklı *Eimeria* türü tespit edilmiştir. Pozitiflik oranları; 0-1 aylık yaş grubunda (% 38.1), erkeklerde (% 28.2) ve simental ırkta (% 50) daha yüksek bulunmuştur. Tek tür ile enfekte hayvan oranının % 11.25 (17/151), iki ile beş arasında değişen sayıda tür ile miks enfekte hayvan oranının ise % 15.23 (23/151) olduğu görülmüştür. Sonuç olarak bu çalışma Burdur yöresi buzağlarında *Eimeria* türlerinin yaygınlığı ile ilgili yapılan ilk çalışma olmuştur. Ayrıca koksidiyozisin özellikle sığır yetiştiriciliğinde göz ardı edilmemesi gerektiği ve hastalığın önlenmesi ve kontrolü için daha ileri epidemiyolojik çalışmalara ihtiyaç olduğu sonucuna varılmıştır.

**Anahtar kelimeler:** Burdur, buzağı, *Eimeria*, yaygınlık

## Investigation of Prevalence of Hydatid Cyst in Cattle in Sivas Province

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### Summary

Cystic echinococcosis is one of the important parasitic diseases affecting both human and animal health. The disease, which is seen in almost all regions of the world, threatens both public and animal health in Turkey. This study was conducted to investigate the prevalence of hydatid cyst in internal organs of cattle in a slaughterhouse in Sivas and to investigate the current status of the disease in cattle in Sivas province. For this purpose, the internal organs of a total of 1,120 cattle, 945 females and 175 males, which were slaughtered in a slaughterhouse between April 1, 2019 and April 1, 2021, were macroscopically examined in terms of hydatid cysts. Hydatid cysts were detected only in the liver and lungs of the examined internal organs, while hydatid cysts were not found in other internal organs. Hydatid cysts were found in the liver and / or lungs of 15.63% (175/1120) of the animals examined in the study. It was found that 17.56% (166/945) of the female animals and 5.14% (9/175) of the male animals were infected with hydatid cysts. As a result, it was determined that the prevalence of cystic echinococcosis (CE) infections in cattle in Sivas province is quite high. It is thought that cystic echinococcosis still poses an important risk in terms of human and animal health in Sivas and therefore protection and control measures should be taken as soon as possible in order to protect humans and animals from the disease.

**Keywords:** Cyst hydatid, cattle, *Echinococcus granulosus*, Sivas.

## Sivas İlinde Sığırlarda Kist Hidatik'in Yaygınlığının Araştırılması

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### Özet

Kistik ekinokokkozis hem insan hem de hayvan sağlığını etkileyen önemli paraziter hastalıklardan birisidir. Dünyanın hemen hemen tüm bölgelerinde görülen hastalık, Türkiye'de de hem halk sağlığını hem de hayvan sağlığını tehdit etmektedir. Bu çalışma, Sivas'ta bir mezbahanedeki kesilen sığırların iç organlarında kist hidatikin yaygınlığının araştırılması ve hastalığın Sivas ilinde sığırlardaki güncel

durumunun araştırılması amacıyla yapılmıştır. Bu amaçla 1 Nisan 2019 ile 1 Nisan 2021 tarihleri arasında bir mezbahane kesilen 945 dişi ve 175 erkek olmak üzere toplam 1.120 adet sığırın iç organları makroskopik olarak kist hidatik yönünden incelenmiştir. İncelenen iç organlardan sadece karaciğer ve akciğerde hidatik kistler tespit edilirken diğer organlarda ise kist hidatike rastlanmamıştır. Çalışmada incelenen hayvanların %15,63'ünün (175/1120) karaciğer ve/veya akciğerlerinde kist hidatike rastlanmıştır. Dişi hayvanların %17,56'sının (166/945) erkek hayvanların ise %5,14'ünün (9/175) kist hidatikle enfekte olduğu tespit edilmiştir. Sonuç olarak bu çalışma ile Sivas ilinde sığırlarda kistik ekinokokkozis (CE) enfeksiyonlarının prevalansının oldukça yüksek olduğu tespit edilmiştir. Kistik ekinokokkozisin Sivas ilinde hala insan ve hayvan sağlığı açısından önemli bir risk oluşturduğu ve bu nedenle de hastalıktan insan ve hayvanların korunması amacıyla koruma ve kontrol tedbirlerinin alınması gerektiği düşünülmektedir.

**Anahtar kelimeler:** *Echinococcus granulosus*, kist hidatik, sığır, Sivas.

## **Giriş**

*Echinococcus granulosus*, Taeniidae ailesi *Echinococcus* cinsinde yer alan zoonotik bir sestoddur (1). Heteroksen yaşam çemberine sahip olan parazitin sonkonakları evcil ve yabani kanideler, arakonakları ise sığır, koyun, keçi ve insan gibi memeli canlılardır (2,3). Arakonaklar, sonkonaklar tarafından çıkarılan yumurtaları ağız yoluyla alarak parazitle enfekte olmaktadır. Yumurtaların içerisinde bulunan onkosfer arakonakların bağırsaklarında serbest kalır ve karaciğer, akciğer ve kalp gibi hayati öneme sahip birçok organ ve dokuda kist hidatikleri meydana getirir ve hastalık kistik ekinokokkozis (CE) olarak isimlendirilmektedir (2,3).

Kistik ekinokokkozis, az gelişmiş ve gelişmekte olan ülkelerde çiftlik hayvanlarındaki en önemli paraziter hastalıklardan birisidir (4). Hastalık sığır, koyun ve keçi gibi çiftlik hayvanlarında et ve süt veriminde azalmaya, yemden yararlanma kabiliyetinin düşmesine ve ayrıca kistli organ ve dokuların imhası nedeniyle ciddi ekonomik kayıplara neden olmaktadır (4,5,6).

Kistik ekinokokkozisin, zoonoz karakterli bir hastalık olması nedeniyle dünyada olduğu gibi Türkiye'de de hastalığın farklı konaklardaki yaygınlığının belirlenmesine yönelik çok sayıda çalışma yapılmıştır (7-11,15). Sığırlardaki kistik ekinokokkozisin yaygınlığının araştırılması amacıyla Türkiye'nin farklı bölgelerinde mezbahane bakısına göre yapılan çalışmalarda yaygınlığın %1.3-46.41 arasında değiştiği tespit edilmiştir (7-11). Türkiye'de sığırlarda ortalama yaygınlığın ise %7.4 olduğu tespit edilmiştir (12).

Kistik ekinokokkozis, hem halk sağlığı hem de hayvan sağlığını tehdit eden önemli paraziter hastalıklardan birisidir. Halk sağlığını ve hayvan sağlığını kistik ekinokokkozisten korumak amacıyla

hastalığın arakonak ve sonkonaklardaki yaygınlığının güncel olarak belirlenmesi ve takip edilmesi oldukça önemlidir. Bu nedenle çalışma ile Sivas ilinde bir mezbahane kesilen sığırlarda kistik ekinokokkozisin yaygınlığının belirlenmesi ve hastalığın güncel durumunun ortaya konulması amaçlanmıştır.

### **Materyal-Metot**

Çalışma materyalini 1 Nisan 2019 - 1 Nisan 2021 tarihleri arasında Sivas ilinde bir mezbahane kesimi yapılan 175 erkek ve 945 dişi olmak üzere toplam 1.120 adet sığırın iç organlarının kist hidatik yönünden incelenmesi oluşturmuştur.

### ***Organ Muayenesi***

Kesilen hayvanların iç organları öncelikle makroskopik olarak kist hidatik yönünden gözle muayene edilmiştir. Kist tespit edilen organlar, organ üzerindeki kist sayıları ve kistlerin lokasyonları kayıt altına alınmıştır. Kist tespit edilmeyen organlar ise yüzeysel olarak sıvazlamak ve basınç uygulamak suretiyle organın iç kısımlarında şüpheli herhangi bir sertlik veya şişlik olup olmadığı incelenmiştir. Şüpheli organlarda yüzeysel kesitler yapılarak kist olup olmadığı araştırılmıştır. Enfekte bulunan karaciğerler ve akciğerler kist sayısı ve enfeksiyon derecesine bakılmaksızın tamamıyla imha edilmiştir.

### ***İstatistik***

Dişi ve erkek hayvanlardaki kist hidatik yaygınlığının istatistiksel analizi Ki-Kare testi ile değerlendirilmiş ve yaygınlık arasındaki fark  $p < 0,05$  ise farkın istatistiksel olarak anlamlı olduğu değerlendirilmiştir.

### **Bulgular**

Kist hidatik yönünden incelenen 1.120 sığırın 175'inde (%15,62) kistik ekinokokkozisin olduğu belirlenmiştir. Kesimhaneye getirilen toplam 945 dişi sığırın 166'sının (%17,56) ve 175 erkek sığırın 9'unun (%5,14) karaciğer ve/veya akciğerlerinin kist hidatik ile enfekte olduğu tespit edilmiştir (Tablo 1). Hayvanların diğer iç organlarında herhangi bir kistik oluşuma rastlanmamıştır.

Bu sonuçlara göre dişi hayvanlarda hastalığın görülme oranının erkek hayvanlardan daha yüksek olduğu ve dişi ve erkek hayvanlardaki kistik ekinokokkozis yaygınlık farkının istatistiki açıdan önemli ( $p < 0,05$ ) olduğu belirlenmiştir.

**Tablo 1.** Cinsiyete göre sığırlarda kist hidatikin yaygınlığı.

Cinsiyet	Kist Hidatik Pozitif		Kist Hidatik Negatif	
	Sayı	%	Sayı	%
Dişi	166	17,56	779	82,44
Erkek	9	5,14	166	94,86
<b>Toplam</b>	<b>175</b>	<b>15.63</b>	<b>945</b>	<b>84.37</b>

### Tartışma ve Sonuç

*Echinococcus granulosus*'un arakonaklarda neden olduğu kistik ekinokokkozis, günümüzde dünyanın birçok bölgesinde olduğu gibi Türkiye'de de önemli paraziter hastalıklardan birisidir (13, 14). Dünyada kistik ekinokokkozisin kontrol altına alınması amacıyla birçok ülkede çeşitli çalışmalar yapılmasına rağmen hastalık global olarak önemini korumaktadır (14). Dünyada olduğu gibi Türkiye'de de hastalığın kontrol altına alınması amacıyla çalışmalar yapılmaktadır (15). Kistik ekinokokkozisin kontrol altına alınması amacıyla yapılacak çalışmalarda halkın, hastalık konusunda bilinçlendirilmesi, kontrolsüz hayvan kesiminin engellenmesi, muhtemel arakonak ve sonkonakların temasının en aza indirilmesi ve entegre hayvancılığın yapılması oldukça önemlidir (14,15). Hastalığın kontrolü için diğer bir önemli konu da hastalığın ülkesel ve/veya bölgesel yaygınlığı ve dağılımı hakkında güncel ve güvenilir verilerin toplanmasıdır. Türkiye'de kistik ekinokokkozisin kontrol altına alınması amacıyla yapılacak çalışmalara güncel verilerin sağlanması için Sivas ilinde sığırlarda kistik ekinokokkozisin güncel durumunun belirlenmesi amacıyla bu çalışma yapılmıştır.

Çiftlik hayvanlarının en önemli paraziter hastalıklarından birisi olan kistik ekinokokkozisin Türkiye'deki yaygınlığının belirlenmesi amacıyla çok sayıda çalışma yapılmıştır. Mezbahane kayıtlarına göre sığırlarda kistik ekinokokkozisin araştırıldığı çalışmalarda Türkiye'de yaygınlığın bölgelere göre değişmekle birlikte %1,3-46,41 arasında olduğu tespit edilmiştir (7-11). Çalışmanın yapıldığı Sivas ilinde sığırlarda kistik ekinokokkozisin yaygınlığının araştırıldığı bir çalışmada yaygınlığın %35,7 olduğu bildirilmiştir (7). Bu çalışmada ise sığırlardaki yaygınlık %15,63 olarak tespit edilmiştir. Bu çalışmada yaygınlığın daha önce Sivas ilinde yapılan çalışmadan daha düşük olduğu belirlenmiştir. Türkiye'nin farklı bölgelerinde yapılan çalışmalarda sığırlarda kist hidatikin yaygınlığının azalma eğiliminde olduğu görülmektedir. Örneğin Erzurum ilinde yapılan çalışmalarda sığırlarda kist hidatik yaygınlığı 1997 yılında %46,41 iken (9), 2010 yılında oranın %34,3'e düştüğü (18), Kars ilinde yapılan çalışmalarda ise sığırlarda kist hidatikin yaygınlığı 2004 yılında %31,25 iken



(20), 2011 yılında %5,3'e (21) düştüğü tespit edilmiştir. Bunun nedeninin son yıllarda kistik ekinokokkozisin yaygınlığının azaltılması amacıyla yapılan çalışmalar ve entegre hayvancılığın Türkiye'de giderek yaygınlaşması ile ilgili olduğu düşünülmektedir (15). Her ne kadar Sivas ilinde sığırlarda kistik ekinokokkozisin yaygınlığının zaman içerisinde azaldığı görülse de %15,63'lük oranın oldukça yüksek olduğu ve bu nedenle insan ve hayvan sağlığı için oldukça tehlikeli olan kistik ekinokokkozise karşı uygulanacak kapsamlı koruma ve kontrol tedbirleriyle daha da düşürülmesi gerekmektedir.

Türkiye'de sığır yetiştiriciliği daha çok meraya dayalı yapılmaktadır. Bu nedenle Türkiye'de kistik ekinokokkozis gibi mera kaynaklı hastalıklar sığır sürülerinde oldukça yaygındır (7-11,16-21). Sığır yetiştiriciliğinde erkek hayvanlar daha çok besi amacıyla ahırlarda beslenirken, dişi hayvanlar ise çoğunlukla merada beslenmektedir. Bu nedenle mera kaynaklı hastalıkların dişi hayvanlardaki görülme sıklığı erkek hayvanlara göre daha fazla olabilmektedir. Bu çalışmada da incelenen 945 dişi sığırın %17,56'sında kist hidatike rastlanırken, 175 erkek hayvanın ise %5,14'ünde kist hidatike rastlanmıştır. Dişi ve erkek hayvanlardaki kist hidatik yaygınlığının farkının istatistiksel olarak anlamlı olduğu tespit edilmiştir ( $p < 0,05$ ).

Sonuç olarak, insan ve hayvan sağlığı açısından oldukça önemli hastalıklardan birisi olan kistik ekinokokkozis, dünyada olduğu gibi Türkiye'de de oldukça yaygındır. Bu çalışmada da Sivas ilinde sığırlarda kistik ekinokokkozisin yaygınlığının %15,63 olduğu tespit edilmiştir. Hastalık insan ve hayvan sağlığını etkileyerek ülke ekonomilerine ciddi zararlar verebilmektedir. Hastalık kaynaklı yıllık ekonomik kaybın araştırıldığı bir çalışmada dünyada yıllık kaybın yaklaşık 3 milyar dolar olduğu tespit edilmiştir (1). Türkiye'de ise yıllık kaybın yaklaşık 89.2 milyon dolar olduğu tahmin edilmektedir (12). Kistik ekinokokkozis kaynaklı ekonomik kaybın en aza indirilmesi ve insan ve hayvan sağlığının korunması amacıyla hastalığa karşı kapsamlı korunma ve kontrol programları oluşturulması gerektiği düşünülmektedir.

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**Efficiency of Condensed Tannin in Ruminant Nutrition**

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**Summary**

Tannins present as a complex group of polyphenolic compounds in various plant species; They are divided into two groups as hydrolyzed and condensed tannins. Hydrolyzable tannins consist of a carbohydrate compound with hydroxyl groups esterified with phenolic acids. Condensed tannins (KT) are unbranched polymers of flavonoid units that are resistant to degradation by hydrolysis and generally have a higher molecular weight than hydrolyzable tannins. Depending on the type of grain consumed, its chemical structure, molecular weight, amount of consumption and animal species, its effects can be beneficial or harmful. Condensed tannins form a complex with proteins in the rumen, reducing the microbial degradation rate and increasing the amount of by-pass protein. In the pH conditions of the abomasum varying between 2.5-3.5, tannin-protein compounds are separated and the proteins are digested and absorbed in the small intestine. Thus, by reducing the degradation rate of the protein in the rumen, metabolic diseases associated with increased urea synthesis and possible negative effects on fertility are prevented, while efficient use of nitrogen is ensured. In addition, the nitrogen released into the environment is reduced and pollution is prevented. When condensed tannins are less than 50 g per kg of dry matter, they do not have adverse effects on dry matter consumption and cellulose digestion. Condensed tannins in some plants can also be effective in combating parasites in the gastrointestinal tract. Condensed tannins reduce the production of methane directly by reducing the number of methanogenic bacteria in the rumen or indirectly by reducing the population of protozoa.

**Keywords:** Condensed Tannin, methane, rumen, ruminant

## Ruminantların Beslenmesinde Kondanse Tanenlerin Etkinliđi

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### Özet

Çeşitli bitki türlerinde karmaşık bir polifenolik bileşik grubu olarak bulunan tanenler; hidrolize ve kondanse tanenler olmak üzere iki gruba ayrılırlar. Hidrolize olabilen tanenler, hidroksil grupları fenolik asitlerle esterleştirilmiş bir karbonhidrat bileşiginden oluşur. Kondanse tanenler (KT), hidrolize parçalanmaya dayanıklı flavonoid ünitelerin dallanmamış polimerleridir ve genellikle hidrolize olabilir tanenlerden daha yüksek moleküler ağırlığa sahiptirler. Tüketilen tanenin cinsine, kimyasal yapısına, moleköl ağırlığına, tüketim miktarına ve hayvan türüne bađlı olarak etkileri yararlı veya zararlı olabilmektedir. Kondanse tanenler, rumende proteinlerle kompleks oluşturarak mikrobiyel parçalanma oranını düşürür, by-pass protein miktarını arttırırlar. Abomasumun 2,5-3,5 arasında deđişen pH koşullarında tanen-protein bileşikleri ayrılarak proteinlerin ince bađırsakta sindirilmesi ve emilmesi sađlanır. Böylelikle proteinin rumende yıkılma oranı azaltılarak üre sentezinin artmasıyla ilişkili metabolik hastalıkların ve fertilité üzerine olabilecek olumsuz etkilerin önüne geçilirken nitrojenin etkin bir şekilde kullanılması da sađlanmış olur. Ayrıca çevreye salınan nitrojen azaltılarak kirliliđin de önüne geçilmiş olur. Kondanse tanenler kg kuru maddede 50 g'dan az olduğunda kuru madde tüketimi ve selüloz sindirimi üzerine olumsuz etkileri olmamaktadır. Bazı bitkilerde bulunan kondanse tanenler gastrointestinal sistemdeki parazitlerle mücadelede de etkili olabilmektedirler. Kondanse tanenler rumendeki metanojenik bakterilerin sayısını azaltarak direkt olarak ya da protozoa popölasyonunu azaltarak dolaylı olarak metan üretimini azaltmaktadırlar.

**Anahtar kelimeler:** Kondanse tanen, metan, rumen, ruminant

**Expression of The KISS1 Gene and Receptor Region (KISS1R) in Fresh and Frozen Ram Semen**

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**Summary**

Kisspeptins (Kp) are a neuropeptide involved in the transmission of melatonin signals and are secreted by the Kiss1 gene, contributing as a potent stimulator of pulsatile release of GnRH. In male animals, Kp play a role in sexual differentiation, metabolic and environmental effects, and fertility control. Melatonin, which plays an important role in animals with seasonal reproductive traits, is thought to control its effects on the reproductive system through changes in Kp levels. In this study, which is the pilot study of our proposed project, we aim to express the Kiss1 gene and receptor region (Kiss1r) in fresh and frozen-thawed semen of rams showing seasonal changes in their reproductive performance. Testes were collected from 2 same-racial 3-year-old rams slaughtered at the abattoir and brought to our laboratory at +4 °C. Spermatozoa were collected by slicing in a TRIS-based diluent at 37 °C by taking the epididymis part of the testis in a separate petri dish. The total motility of the obtained fresh spermatozoa was reported to be %81.07 in the CASA system. One of the ram spermatozoa was separated as fresh while the other was frozen by slow freezing method. The total motility of frozen and thawed sperm was %35.32. qRT-PCR was applied to show the difference in the level of gene expression due to freezing. The gene expression levels were normalized by delta Ct method and the differences between the groups were examined. For this purpose, the Gapdh gene was used as a control gene. It was found that Kiss1 gene expression level decreased 530-fold in frozen sperm samples compared to fresh sperm samples, while Kiss1r gene expression level decreased 2.4-fold. In analyzing the findings, it is found that there is a significant decrease in sperm motility. It is also found that there is a decrease in Kiss1r gene expression in parallel with the decrease in kisspeptin gene level. When all the findings are evaluated together, there are results that negatively affect fertilization in frozen semen samples. At this point, there is a need to develop freezing techniques that can be applied without disrupting sperm membrane integrity and cellular organization.

**Keywords:** Ram, Kiss1 gene, Kiss1r gene, kisspeptin sperm cryopreservation

## Taze ve Dondurulmuş Koç Spermasında KISS1 Gen ve Reseptör Bölgesinin (KISS1R) Ekspresyonu

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### Özet

Kisspeptinler (Kp), melatoninin sinyallerini taşımada görev alan ve Kiss1 geni tarafından salgılanan bir nöropeptittir ve GnRH'nin pulsatil salınımının güçlü uyarıcıları olarak katkısı bulunmaktadır. Erkek hayvanlarda Kp, seksüel farklılaşma, metabolik ve çevresel etkiler ile fertilitenin kontrolünde rol oynamaktadır. Mevsimsel üreme özelliği gösteren hayvanlarda son derece önemli olan melatonin hormonunun, üreme sistemi üzerindeki etkilerini Kp düzeylerinde değişikliklere neden olarak kontrol ettiği düşünülmektedir. Bir projenin pilot çalışması olarak planlanan bu çalışmamızda mevsimsel olarak üreme performansında değişiklik gösteren koçların taze ve dondurulmuş spermasında Kiss1 geni ve genin reseptör bölgesinin (Kiss1r) ekspresyon durumu hedeflenmektedir. Mezbahada kesilen 2 tane aynı ırklı 3 yaşındaki koçlardan testisleri alınarak +4 °C de laboratuvarımıza getirildi. Testislerin epididimis kısmını ayrı bir petri içersine alarak 37 °C de bulunan TRIS bazlı sulandırıcı içersinde dilimleme yöntemi ile spermatozoonlar elde edildi. Elde edilen taze spermanın total motilitesi CASA sisteminde %81,07 olarak kaydedilmiştir. Spermalardan bir tanesi taze olarak tutulurken, bir diğeri yavaş dondurma yöntemi ile dondurularak saklandı. Dondurulup-çözdürülmüş spermanın total motilitesi ise %35,32 olarak elde edilmiştir. Dondurma işleminin gen ekspresyon seviyesinde meydana getirdiği farkı ortaya koymak için qRT-PCR uygulandı. Gen ifade düzeyleri delta Ct yöntemi ile normalize edilerek gruplar arası farklılık araştırıldı. Bu amaçla kontrol geni olarak Gapdh geni kullanıldı. Dondurulmuş sperm örneklerinde taze sperm örneklerine göre Kiss1 gen ifade düzeyinin 530 kat azaldığı tespit edilirken, Kiss1r gen ifade düzeyinin ise 2.4 kat azaldığı tespit edilmiştir. Bulgular analiz edildiğinde sperm hareketliliğinde belirgin bir düşüş olduğu görülmektedir. Kisspeptin gen düzeyinin azalması ile paralel olarak kisspeptin reseptör gen ifade düzeyinde de azalma olduğu görülmektedir. Bütün bulgular birlikte değerlendirildiğinde, beklenildiği şekilde dondurulmuş sperm örneklerinde döllemeyi olumsuz yönde etkileyebilecek sonuçlar ortaya çıkmaktadır. Bu noktada, spermatozoon membran bütünlüğünü ve hücresel organizasyonunu bozmadan uygulanabilecek dondurma tekniklerinin geliştirilmesine ihtiyaç duyulmaktadır.

**Anahtar kelimeler:** Koç, Kiss1 geni, Kiss1r geni, Kisspeptin sperma kriyoprezervasyonu

**Isolation of Extracellular Vesicle (Exosome) From Canine Seminal Plasma Using Aqueous Two-Phase System (ATPS)**

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**Summary**

Exosomes are nanosized vesicles found in all known cells of the body and contain proteins, lipids, and nucleic acids in their structures that play a role in intercellular communication. They are actively involved in many biological processes, including vaccines, diagnosis and treatment of diseases such as cancer, especially reproductive and infertility problems, and serve as potential targets for therapeutic intervention. Mammalian seminal plasma contains exosomes, which contain large amounts of cholesterol, sphingomyelin, and complex protein compositions. Exosomes were first identified in human seminal plasma by the ultracentrifugation method, and then their presence was detected in the seminal plasma of many species. As sperm pass through the male and female reproductive tracts, they interact with exosomes produced in different segments. These interactions result in various modifications in the structure of spermatozoa and prepare them for the fertilization process and subsequent embryonic development. Exosomes in the male reproductive system ensure the transfer of various molecules to the spermatozoa and the production of fertile male gametes. With their antibacterial and antioxidant properties, they help activate and protect sperm motility. There are many different methods for isolating exosomes, but it is usually done by ultracentrifugation or with a commercial kit. In this study, complete ejaculate was obtained from healthy male dogs with known breeding characteristics that came to our clinic for seminal plasma production. Seminal plasma was separated from spermatozoa by centrifugation at  $700 \times g$  for 15 min at room temperature, and the recovered seminal plasma was centrifuged again at  $10,000 \times g$  for 10 min. The seminal plasma samples were stored at  $-80^{\circ}\text{C}$  until exosome isolation. Exosome isolation and characterization was performed from the seminal plasma obtained in the Yeditepe University Exosome and Extracellular Research Laboratory using an Aqueous Two-Phase System (ATPS). After measurement by fluorescence staining, nanoparticle tracking analysis (NTA) was performed using Nanosight NS300 and NTA 3.3 software to determine the size and concentration of exosomes. As a result, exosomes of desired sizes and characters were obtained from canine seminal plasma using the ATPS method in contrast to conventional methods.

**Keywords:** ATPS, Canine semen, exosome, seminal plasma

## Köpek Seminal Plazmasından Sulu İki Fazlı Sistem (ATPS) Kullanılarak Ekstrasellüler Vezikül (Eksozom) İzolasyonu

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### Özet

Eksozomlar vücuttaki bilinen tüm hücrelerde bulunan ve yapılarında proteinleri, lipidleri, nükleik asitleri içeren hücrelerarası iletişimde rol oynayan nano boyutlu keseciklerdir. Özellikle reproduksiyon ve infertilite problemleri olmak üzere, kanser gibi hastalıkların da tanı ve tedavisi, aşı gibi çok sayıda biyolojik olaya aktif olarak katılmakta ve terapötik müdahaleler için potansiyel hedefler olarak hizmet etmektedirler. Memeli seminal plazması, yüksek miktarda kolesterol, sfingomiyelin ve kompleks protein kompozisyonlarını bünyesinde barındıran içeren eksozomları içermektedir. Eksozomlar, ilk olarak insan seminal plazmasında ultrasantrifüj yöntemi ile tanımlanmış ve daha sonra birçok türün seminal plazmasında da varlıkları tespit edilmiştir. Spermatozoa, erkek ve dişi üreme yollarından geçerken, farklı segmentlerden üretilen eksozomlarla etkileşime girmektedir. Bu etkileşimler, spermatozoa yapısında farklı modifikasyonlara yol açarak onları fertilizasyon sürecine ve daha sonra embriyo gelişimine hazırlamaktadır. Erkek üreme sistemindeki eksozomlar farklı moleküllerin spermatozoaya aktarılmasını ve fertil erkek gametlerin üretilmesini sağlamaktadır. Antibakteriyel, antioksidan özelliği ile sperm hareketliliğinin aktivasyonu ve korunmasında yardımcı olmaktadır. Eksozom izolasyonu için birçok farklı yöntem vardır ancak yaygın olarak ultrasantrifüj yoluyla ya da ticari kit kullanılarak yapılmaktadır. Yapılan çalışmada seminal plazma eldesi için kliniğimize gelen sağlıklı ve damızlık özelliği bilinen erkek köpeklerden tam ejakülat alındı. Seminal plazma oda sıcaklığında 15 dakika süreyle  $700 \times g$ 'de santrifüjleme ile spermatozodan ayrıldı ve geri kazanılan seminal plazma,  $10.000 \times g$ 'de 10 dakika yeniden santrifüj edilerek santrifüj işlemi yapıldı. Seminal plazma örnekleri eksozom izolasyonuna kadar  $-80^{\circ}\text{C}$ 'de saklandı. Elde edilen seminal plazmadan Yeditepe Üniversitesi Eksozom ve Ekstrasellüler Araştırmaları Laboratuvarında Sulu İki-Fazlı Sistem (ATPS) kullanılarak eksozom izolasyonu ve karakterizasyonu yapıldı. Floresan boyama ile ölçüm yapıldıktan sonra eksozomların boyutunu ve konsantrasyonunu belirlemek için Nanosight NS300 ve NTA 3.3 yazılımı ile nanopartikül izleme analizi (NTA) yapıldı. Sonuç olarak, köpek seminal plazmasından geleneksel yöntemlerden farklı olarak ATPS yöntemi ile istenilen boyut ve karakterlerde eksozomlar elde edildi.

**Anahtar kelimeler:** ATPS, eksozom, köpek sperma, seminal plazma



**First Record of *Ixodes ricinus* (Acari; Ixodidae) in European Glass Lizard (*Pseudopus Apodus*; Anguidae) and Studies on Ectoparasite in Reptiles From Past to Present in Turkey**

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**Summary**

Three adult European glass lizards were caught in the garden of Faculty of Veterinary Medicine, Ondokuz Mayıs University (May 2020) and presence of tick infestation was detected. During macroscopic examination, the samples were collected from lateral groove on both sides of the lizard by using blunt tip tweezers. As a result of the microscopic analysis, all the samples were found to belong to *Ixodes ricinus* larvae and nymphs. This study is the first report of a tick species or infestation in European glass lizard (*Pseudopus apodus*). In addition, this study makes a detailed analysis of studies conducted so far on reptile ectoparasites in Turkey.

**Keywords:** *Ixodes ricinus*, *Pseudopus apodus*, veterinary herpetoparasitology

**Oluklu Kertenkele (*Pseudopus apodus*; Anguidae)'de *Ixodes ricinus* (Acari; Ixodidae)'un İlk Kaydı ve Türkiye'de Geçmişten Günümüze Sürüngenlerde Ektoparazit Çalışmaları**

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**Özet**

Ondokuz Mayıs Üniversitesi Veteriner Fakültesi bahçesinde üç ergin Oluklu kertenkele yakalanmış (Mayıs 2020) ve kene enfestasyonu varlığı tespit edilmiştir. Makroskobik muayene sırasında örnekler küt uçlu pens yardımıyla kertenkelenin her iki tarafında bulunan oluk şeklindeki girintilerin olduğu bölgeden toplanmıştır. Yapılan mikroskobik inceleme sonucu tüm örneklerin *Ixodes ricinus* larva ve nimflerine ait olduğu tespit edilmiştir. Yapılan bu çalışma, herhangi bir kene türünün veya enfestasyonun Oluklu kertenkele (*Pseudopus apodus*)'de yapılan ilk bildirimidir. Ayrıca bu çalışmada Türkiye'de sürüngen ektoparazitleri üzerine bugüne kadar yapılan çalışmalar da incelenmiştir.

**Anahtar kelimeler:** *Ixodes ricinus*, *Pseudopus apodus*, veteriner herpetoparazitoloji

**Distribution of *Heligmosomum costellatum* in *Microtus* Species in Turkey\***

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**Summary**

*Microtus* species is a rodent genus that can be seen in all regions of Turkey, lives in close areas with humans and domestic animals, and has many species. There are 62 *Microtus* species in the world and 12 species in our country. These rodents are final or intermediate hosts for many zoonotic parasites. 220 *Microtus* spp. founding Anatolia has examined the doctoral thesis study, which was carried out to determine the digestive system helminths and their prevalence rate. According to the first data obtained from the this study, this paper is the second report of *Heligmosomum costellatum* from Turkey, but the first from different *Microtus* species. *H.costellatum*, found in small rodents, is a Trichostrongylid nematode that development directly. There is no specific information about the life cycle of this nematode; however, it may be similar to other Trichostrongylidae. Infective third-stage larvae ingested orally by rodents come to the gastric glands of the stomach and form nodules, unlike many other Trichostrongylids, where they become L4. The parasites leave the nodule in the young adult period, migrate to the small intestines, and continue their life there. According to studies on *H.costellatum*, it has reported that it is seen only in *Microtus* species as the final host. *H.costellatum* was found in 35 (15.9%) of 220 *Microtus* species examined in this study, and five new host species were added; namely, *M.anatolicus*, *M.dogramaci*, *M.guentheri*, *M.levis*, *M.socialis* belonging to the *Microtus* genus. *Microtus* species examined were grouped according to their age and sex. In addition, evaluations were made based on provinces with positive results due to the collection of *Microtus* species from different geographical areas. The collected parasites were identified under the microscope by the relevant references, and essential morphological regions were measured during the diagnosis and photographed. Investigating the parasites in rodents in the wild is important for human health to detect zoonotic species and the health of rodents raised as laboratory animals.

**Keywords:** Digestive system, helminth, *Microtus*, rodent

\*This study was produced from a part of the thesis numbered OMÜ PYO.VET.1904.21.004.

**Türkiye’de *Microtus* Türlerinde Bulunan *Heligmosomum costellatum*’un Yayılışı\***

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**Özet**

*Microtus* türleri Türkiye’nin bütün bölgelerinde görülen, insan ve evcil hayvanlarla yakın bölgelerde yaşamını sürdüren ve çok sayıda türü bulunan bir kemirici cinsidir. Dünyada da 62 tür, Ülkemizde ise 12 türü bilinmektedir. Bu kemiriciler birçok zoonoz parazite konaklık veya arakonaklık yapmaktadır. Anadolu’da yayılış gösteren *Microtus* türlerinin sindirim sistemi helmintlerinin belirlenmesi ve yaygınlık durumlarının ortaya konması amacıyla yapılan doktora tez çalışmasında 220 *Microtus* spp. incelenmiştir. Bu bildiri de tez çalışmasından elde edilen ilk verilere göre Türkiye’den ikinci *Heligmosomum costellatum* bildirimini, ancak farklı *Microtus* türlerinden ilk bildirimdir. Küçük kemirgenlerde bulunan *H. costellatum*, Trichostrongylid bir nematod olup direkt gelişir. Bu parazite ait yaşam çemberinin detayları bilinmemekle birlikte ailenin genel özelliklerine benzer olduğu sanılmaktadır. Kemiriciler tarafından oral yolla alınan enfektif üçüncü dönem larvalar diğer birçok Trichostrongylid’den farklı olarak midenin gastrik bezlerine gelerek nodüller oluşturur ve burada L4 olur, parazitler genç ergin döneminde nodülü terk ederek ince bağırsaklara geçer ve orada yaşamının sürdürür. *H. costellatum* üzerine yapılmış olan çalışmalara göre son konak olarak sadece *Microtus* türlerinde görüldüğü bildirilmiştir. Çalışmamızda incelenen 220 *Microtus* türünden 35’inde (% 15.9) *H. costellatum*’a rastlanmıştır ve *Microtus* cinsine ait *M. anatolicus*, *M. dogramaci*, *M. guentheri*, *M. levis*, *M. socialis* olmak üzere beş yeni konak türü eklenmiştir. Bakısı yapılan *Microtus* türleri yaş ve cinsiyetlerine göre gruplandırılmıştır. Ayrıca farklı illerden *Microtus* türleri toplanması sebebiyle pozitif sonuç alınan iller bazında da değerlendirmeler yapılmıştır. Toplanan parazitler ilgili kaynaklar yardımıyla mikroskopta teşhis edilmiş ve tanıda önemli morfolojik bölgelerin detaylı ölçümleri yapılmış ve fotoğrafları çekilmiştir. Yabani hayatta kemirici habitatında bulunan helmint ve diğer parazitlerin bilinmesi, hem laboratuvar hayvanı olarak yetiştirilen kemiricilerde bulunan parazitler ile ilişkisinin daha iyi anlaşılması, hem de zoonoz türlerin belirlenerek insan sağlığı açısından tehdit oluşturabilecek türlerin tespit edilmesi açısından önem taşımaktadır.

**Anahtar kelimeler:** Helmint, *Microtus*, rodent, sindirim sistemi

\*Bu çalışma OMÜ PYO.VET.1904.21.004 nolu tez projesinden üretilmiştir.

**The Prevalence and Molecular Characterization of Fasciola Species in Intermediate Hosts in Samsun Region: First Results\***

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**Summary**

Fasciolosis is one of the most important diseases of sheep, goat, cattle, and buffalo breeding and has a very high prevalence in some regions. It is rarely found in other mammals and humans. *Fasciola hepatica* and *F.gigantica* are the agents of fasciolosis in our country. Both species are found in Samsun province, causing significant health and economic problems. The intermediate hosts of these species are water slugs in the *Lymnaeid* family. In Turkey, the intermediate host are known as *Galba truncatula* for *F.hepatica* and *Radix auricularia* for *F.gigantica*. However, although the parasite prefers these species, it has been demonstrated that 20 species of the *Lymneidae* family can be intermediate hosts to *Fasciola spp.* In this thesis study, it was aimed to investigate the prevalence of fasciolosis, which is a regional problem, in intermediate hosts, identification of the intermediate host species found by molecular diagnosis, examination of the seasonal relations of intermediate hosts with the host, investigation of the phylogenetic relations of the new intermediate host species with other species reported in Turkey and the world. In this paper, preliminary findings of the ongoing study will be presented. Analyzes will be made morphologically and molecularly. Until now, suitable habitats were visited in three districts of Samsun (Bafra, Çarşamba, Ladik), and 151 feces and 4613 water slug samples were collected from grasslands. No *Fasciola sp.* eggs were found in the stool samples examined. Morphological analyzes of water slugs were made, and they were separated at the genus level. Two hundred suspected trematode larval stages were collected from the intermediate hosts, and DNA extractions and molecular analyzes were started. In the investigations made so far, *Fasciola sp.* species have not been found. It is thought that parasites will begin to be seen more in the intermediate hosts in autumn, with the animals grazing on the pasture during the summer. In this year-long study, sample collection and laboratory studies continue.

**Keywords:** Intermediate hosts, Fasciola, PCR, phylogenetic analysis, Samsun

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**Samsun Yöresinde Fasciola Türlerinin Arakonaklardaki Yaygınlığı ve Moleküler Karakterizasyonları: Ön Bulgular\***

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**Özet**

Fasciolosis, başta koyun olmak üzere, keçi, sığır ve manda yetiştiriciliğinin en önemli ve bazı yörelerde çok yüksek prevalansa sahip hastalıklarından biridir. Nadiren de olsa diğer memeli hayvanlar ve insanlarda da rastlanmaktadır. Ülkemizde fasciolosis etkeni olarak *Fasciola hepatica* ve *F. gigantica* bulunmaktadır. Samsun ilinde her iki tür de bulunmakta, önemli sağlık ve ekonomik sorunlara yol açmaktadır. Bu türlerin arakonakları *Lymnaeid* ailesindeki su sümüklüleridir. Türkiye’de parazitin biyolojisindeki arakonak *F. hepatica* için *Galba truncatula*, *F. gigantica* için *Radix auricularia* olarak bilinmektedir. Fakat parazitin bu türleri tercih etmesine rağmen dünyada *Lymneidae* ailesinden 20 türün *Fasciola spp'*ye arakonaklık yapabildiği ortaya konmuştur. Yapılan bu tez çalışmasında bölgesel bir sorun olan fasciolosis’in arakonaklardaki yaygınlığının araştırılması, moleküler düzeyde teşhis yapılarak bulunan arakonak türlerinin identifiye edilmesi, arakonakların mevsimsel olarak konak ile olan ilişkilerinin incelenmesi, bulunan yeni arakonak türlerinin Türkiye ve Dünya’da bildirilen diğer türler ile olan filogenetik ilişkilerinin araştırılması amaçlanmıştır. Bu bildiriye devam eden çalışmanın ön bulguları sunulacaktır. Araştırmada *Fasciola* türlerine ait gelişim dönemi taşıyan arakonakların morfolojik analizlerinden sonra ITS2 gen bölgesi PCR ile çoğaltılarak tür tespiti yapılacaktır. Taşıdıkları gelişim dönemlerinin de *Fasciola sp.* ITS1 gen bölgesi çoğaltıldıktan sonra PCR-RFLP analizi ile gelişim döneminin hangi *Fasciola* türüne ait olduğu tespit edilecektir. Şu ana kadar Samsun’un üç ilçesinde (Bafra, Çarşamba, Ladik) uygun habitatlar gezilmiş ve otlak alanlardan 151 dışkı, 4613 su sümüklüsü örneği toplanmıştır. İncelenen dışkı örneklerinde *Fasciola sp.* yumurtasına rastlanmamıştır. Su sümüklülerinin morfolojik analizleri yapılarak cins düzeyinde ayrılmışlardır. Arakonaklardan 200 şüpheli trematod larva dönemi toplanmış, DNA ekstraksiyonları ve moleküler analizleri yapılmaya başlanmıştır. Şu ana kadar yapılan incelemelerde *Fasciola sp.* türlerine rastlanmamıştır. Hayvanların yaz boyu merada otlaması ile parazitlerin sonbaharda arakonaklarda daha fazla görülmeye başlanacağı düşünülmektedir. Yıl boyu sürececek bu çalışmada örnek toplanmaya ve laboratuvar çalışmalarına devam edilmektedir.

**Anahtar kelimeler:** Arakonak, *Fasciola*, filogenetik analiz, PCR, Samsun

\*121O118 nolu TÜBİTAK projesi tarafından desteklenmektedir.

## **Neosporosis in Turkey**

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### **Summary**

Neosporosis is a protozoan disease caused by *Neospora caninum* and is seen worldwide and causes significant economic losses. It is one of Turkey's most essential abortion factors, especially in cattle, besides yield losses. It causes substantial financial losses by causing loss of offspring, decreased milk yield, infected or dead calves. The agent causes disease in final host dogs and cattle, which are the primary, intermediate hosts, but it can also infect sheep, goats, horses and deer other than cattle. In Turkey, *N.caninum* is seen in dogs, cattle, sheep and goats, and *N.hughesi* in horses. The disease is transmitted to cattle through grass, feed and water contaminated with spore-forming oocysts in the faeces of infected dogs. These also reproduce asexually in the tissues of cattle and are transmitted to the calf via the placenta. It is transmitted to end-host dogs that eat or are fed the placenta, aborted fetus, body exudates, or postmortem carcasses of cattle and other infected animals, thus infected and develop sexually in the dog's digestive tract. The disease is more critical in calves. The most common clinical signs in calves born infected are neurological findings, including ataxia, decreased patellar reflex, contraction of hind, front or all legs, unbalanced gait and loss of consciousness. The calf weakens, fails to develop, usually dies before two months of age due to pneumonia, myocarditis and progressive paralysis. Sometimes calves are found dead without showing any clinical signs. Many studies have been carried out on this subject in Turkey. Although the prevalence of the disease varies according to the provinces, infection is encountered in every region. In high prevalence areas, cattle have been reported to use the same pasture or farm environments as dogs. If congenitally infected dogs are excluded, no clinical signs are observed in dogs, so dogs that appear healthy play a significant role in spreading infection on farms. Diagnosis of the disease is possible with IFAT, NAT, ELISA, PCR, but ELISA and PCR are preferred. The most suitable materials for diagnosis are aborted fetuses, placenta, amniotic fluid, body fluids, brain tissue and blood sera. Still, if there is autolysis in organs and tissues, blood serum is used. There is no specific treatment for the disease in cattle, so prevention methods are more important.

**Keywords:** Cattle, dog, economic loss, *Neospora caninum*, Turkey

## Türkiye’de Neosporosis

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### Özet

Neosporosis, *Neospora caninum* tarafından oluşturulan bir protozoon hastalığı olup tüm Dünya’da görülür ve büyük ekonomik kayıplara neden olur. Türkiye’de özellikle sığırlarda verim kayıpları yanında en önemli abort etkenlerinden birisidir. Etken son konak köpekler ile birincil arakonak olan sığırlarda hastalık oluşturur, ancak sığır dışında koyun, keçi, at ve geyiklerde de enfeksiyon oluşturabilmektedir. Ülkemizde köpek, sığır, koyun ve keçilerde *N. caninum*, atlarda ise *N. hughesi* görülmektedir. Hastalık sığırlara enfekte köpeklerin dışkıdaki sporlu ookistlerle bulaşık ot, yem ve sularla bulaşır. Bunlar da sığırların dokularında eşeysiz olarak çoğalır ve plasenta yoluyla buzağıya bulaşır. Bu şekilde enfekte olan sığır ve diğer hayvanların plasenta, atık fetüs, vücut akıntıları veya ölüm sonrası karkaslarını yiyen veya yedirilen son konak köpeklere bulaşır ve sindirim kanalında eşeyli olarak gelişir. Hastalık buzağılarda daha önemlidir. Enfekte doğan buzağılarda en sık rastlanan klinik belirtiler nörolojik bulgular olup bunlar ataksi, patellar reflekste azalma, arka, ön veya tüm bacakların kasılmış halde kalması, dengesiz yürüme ve şuur kaybıdır. Hayvan zayıflar, gelişemez, pnömöni, myokarditis ve ilerleyici paraliz sonucu genellikle 2 aylık olmadan ölür. Bazen de buzağılar hiçbir klinik bulgu göstermeden ölü olarak bulunurlar. Türkiye’de bu konuda çok sayıda çalışma yapılmıştır. Hastalığın prevalansı illere göre değişmekle birlikte her bölgede enfeksiyona rastlanmaktadır. Yüksek prevalans bulunan yerlerde sığırların köpeklerle beraber aynı mera veya aynı çiftlik ortamlarını kullandıkları bildirilmiştir. Konjenital enfekte köpekler hariç tutulursa, köpeklerde klinik belirtiyeye rastlanmaz, dolayısıyla sağlıklı görünen köpekler çiftliklerde enfeksiyonun yayılımında çok önemli rol oynarlar. Hastalığın teşhisi IFAT, NAT, ELİSA, PCR gibi yöntemlerle mümkündür, ancak ELİSA ve PCR tercih edilir. Teşhis amacıyla atık fetüsler, plasenta, amnion sıvısı, vücut sıvıları, kan serumu ve beyin dokusu en uygun materyaldir, ancak organ ve dokularda otoliz varsa kan serumu kullanılır. Hastalığın sığırlarda spesifik bir tedavisi yoktur, bu nedenle korunma yöntemleri daha önemlidir.

**Anahtar kelimeler:** Ekonomik kayıp, köpek, *Neospora caninum*, sığır, Türkiye



## **Puerperal Hemoglobinurea Case in a Cow**

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### **Summary**

A female Montafon cattle breed approximately 400 kg in weight with an age of 6 years old was presented to us at Erciyes University - Veterinary Faculty – Department of Internal Medicine - Education, Research & Practice Hospital – Kayseri – Turkey with a complaint of bloody urine, weakness and respiratory distress from the last 3 days. The animal had a history of given birth 1 month ago, and was being grazed on pasture for the last 2 months, also a few weeks ago another animal had died belonging to the same owner who had parturiated 1 month ago and had developed bloody urine before death. Upon further clinical examination, the rectal temperature was found to be 38.5°C, pulse rate was 76 bpm, respiratory rate was 32 breaths/min, the animal was mildly dehydrated, had developed icterus as evident from the mucous membranes and also had hemoglobinuria. Hematological analysis revealed mild leukocytosis (WBC 17.1 x 10<sup>9</sup>/L) and anemia (RBC 2.74 x 10<sup>12</sup>/L, HGB 6.0 g/dl, HCT 18.8%), while the serum biochemistry results indicated hypophosphatemia (IP <0.5 mg/dl). Microscopic analysis of the blood smear revealed it to be negative for any blood parasite. Considering the anamnesis along with the clinical and laboratory findings, a diagnosis of puerperal hemoglobinuria was established. Treatment included, Butaphosphan (Catosal®, Bayer) 100 ml intravenously on the first day, and subcutaneously on the following 2<sup>nd</sup> and 3<sup>rd</sup> days, iron-salts (Fercobsang®, Novakim) 20 ml intramuscular for 10 days, B-complex vitamin (Berovit® B12, CEVA) 20 ml for 10 days, Dexamethasone (Vetakort® 4 mg, Vetaş) 6 ml as a single dose, oxytetracycline (Primamycin-La®, Pfizer) at a dose rate of 10 mg/kg was administered twice intravenously at a 48 hour interval between them, 500 ml of Dextrose 30% was administered intravenously. Furthermore, 150 grams of dicalcium phosphate was advised as a medicinal additive to the diet twice a day. After the lapse of the planned treatment regimen, the owner was contacted telephonically and he reported that the animal had made a complete recovery. In context of this case we can safely establish that serum phosphorus levels should be measured in similar case presentations while considering puerperal hemoglobinuria as one of the differentials with patients presenting with hemoglobinuria within one month after having parturiated.

**Keywords:** Puerperal hemoglobinuria, serum phosphorus, phosphorus deficiency, intravascular hemolysis

## Bir İnekte Puerperal Hemoglobinüri Olgusu

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### Özet

Bu olguda Erciyes Üniversitesi Veteriner Fakültesi Eğitim Araştırma ve Uygulama Hastanesi İç Hastalıkları kliniğine üç gündür devam eden kanlı idrar, halsizlik, solunum güçlüğü şikâyetleri ile getirilen yaklaşık 400 kg ağırlığında, 6 yaşlı, dişi montafon ırkı sığır değerlendirildi. Anamnezde hayvanın 1 ay önce doğum yaptığı, 2 aydır merada olduğu ve birkaç hafta önce de aynı hayvan sahibinin doğumdan yaklaşık bir ay sonra 1 hayvanında kanlı idrar görüldüğü ve ineğin öldüğü öğrenildi. Yapılan klinik muayenede rektal sıcaklık 38.5 °C, nabız frekansı 76/dk, solunum sayısı 32/dk, hafif dehidrasyon, mukozalarda ikterus ve hemoglobinüri belirlendi. Yapılan hematolojik muayenede hafif lökositoz (WBC 17.1 x 10<sup>9</sup>/L) ve anemi (RBC 2.74 x 10<sup>12</sup>/L, HGB 6.0 g/dl, HCT %18.8), serum biyokimyasında hipofosfatemisi (IP <0.5 mg/dl) tespit edildi. Kan frotisinde kan parazitine rastlanmadı. Anamnez, klinik ve laboratuvar bulguları göz önüne alınarak puerperal hemoglobinüri teşhisi konuldu. Tedavi amacıyla butafosfan (Catosal®, Bayer) 100ml, ilk gün intravenöz, 2. ve 3. gün subkutan, demir (Fercobsang®, Novakim) 20 ml, intramusküler 10 gün, b kompleks vitamin (Berovit® B12, CEVA) 20 ml, 10 gün, deksametazon (Vetakort® 4 mg, Vetaş) 6 ml, tek doz, oksitetrasiklin (Primamycin-La®, Pfizer) 10mg/kg 48 saat ara ile 2 kez, %30'luk Dekstroz 500 ml intravenöz olarak uygulandı. Rasyona günde 2 kez 150 gr dikalsiyum fosfat ilave edilmesi tavsiye edildi. Tedavi süresi bittikten sonra hasta sahibi ile tekrar görüşüldü ve hayvanın tamamen iyileştiği öğrenildi. Sonuç olarak doğum sonrası bir ay içerisinde meydana gelen hemoglobinüri durumlarında puerperal hemoglobinüri göz önüne alınarak serum fosfor seviyelerinin ölçülmesi gerektiği düşünüldü.

**Anahtar kelimeler:** Puerperal hemoglobinüri, serum fosfor, fosfor eksikliği, intravasküler hemoliz

**Effects of Feeding at Different Metabolic Energy Levels on Digestibility Body Weight  
Body Condition Score and Stool Quality in Dogs**

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**Summary**

In this study, the effects of feeding at different metabolic energy (ME) levels on digestibility, body weight (BW), body condition score (BCS) and stool quality in dogs were investigated. 15 Golden retriever dogs aged 3-4 years were used in the experiment. The dogs were divided into 3 groups consisting of 5 dogs with balanced BW averages and equal numbers of females and males. The ME level of the food and ME needs of the dogs were calculated. According to the calculated ME requirement, the 1st group was fed at -50% (half of the exact need), the 2nd 100% (exact need) and the 3rd +50% (50% more than the exact need) . The experiment was continued for 15 days. As a result of BW and BCS determined on the 1st, 7th and 15th days of the study, the changes between days and groups were found to be insignificant ( $p>0.05$ ). Fresh stool samples were taken from each dog in the groups during the last 4 days of the study. Dry matter (DMD) and organic matter digestibility (OMD) were determined by the acid-insoluble ash indicator method. The lowest digestibility (DMD:68.29, OMD:72.95) was calculated in the group fed at +50% of the ME requirement ( $p<0.05$ ). Between the groups fed at -50% and 100% of ME requirement, DMD and OMD values were the highest and the difference between them was insignificant ( $p>0.05$ ). As a result of the stool scoring performed by 3 different researchers, the group fed at -50% of the ME need had the highest score (4.21), while the stools of the group fed at +50% were the lowest (3.96) ( $p<0.05$ ). There was no significant difference between fecal dry matter levels ( $p>0.05$ ). As a result, digestibility decreases with overfeeding, and more soft stools are excreted. Sometimes dogs may experience weight gain or loss when fed in the amounts written on the food package. In order to provide high digestibility, fecal quality, ideal body weight and appropriate BCS in dogs, the energy content of commercial foods should be determined by analyzes. Different formulas used in calculating the daily energy requirements of dogs should also be taken into consideration.

**Keywords:** Body weight, stool quality, dog, digestibility, metabolic energy, body condition score

## Farklı Metabolik Enerji Düzeylerinde Beslemenin Köpeklerde Sindirilebilirlik Canlı Ağırlık Vücut Kondüsyon Skoru ve Dışkı Kalitesi Üzerine Etkileri

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### Özet

Bu çalışmada farklı metabolik enerji (ME) düzeylerinde beslemenin köpeklerde sindirilebilirlik, canlı ağırlık (CA), vücut kondüsyon skoru (VKS) ve dışkı kalitesi üzerine etkileri araştırılmıştır. Denemede 3-4 yaşlarında 15 adet Golden retriever ırkı köpek kullanıldı. Köpekler canlı ağırlık ortalamaları dengeli ve eşit sayıda dişi, erkek içeren 5'er köpekten oluşan 3 gruba ayrıldı. Mamamın ME düzeyi ve köpeklerin ME ihtiyaçları hesaplandı. Hesaplanan ME ihtiyacına göre 1.gruba ihtiyacının-% 50'si (tam ihtiyacın yarısı), 2.ye %100'ü (tam ihtiyaç) ve 3.ye +%50'si (tam ihtiyacının %50 fazlası) düzeylerinde günlük besleme yapıldı. Deneme 15 gün sürdürüldü. Çalışmanın 1. 7. ve 15. günü belirlenen CA ve VKS sonucunda günler ve gruplar arası değişimlerin önemsiz düzeyde olduğu belirlendi ( $p>0.05$ ). Çalışmanın son 4 günü gruplardaki her köpekten taze dışkı numuneleri alındı. Asitte erimeyen kül indikatör metoduyla kuru madde (KMS) ve organik madde sindirilebilirlikleri(OMS) belirlendi. Me ihtiyacının +%50'si düzeyinde beslenen grupta en düşük sindirilebilirlik (KMS:68.29, OMS:72.95) hesaplandı( $p<0.05$ ). ME ihtiyacının-%50 ve %100'ü düzeyinde beslenen gruplar arasında ise KMS ve OMS değerleri en yüksekti ve aralarındaki fark önemsiz düzeydeydi ( $p>0.05$ ). Farklı 3 araştırmacı tarafından yapılan dışkı skorlama sonucunda ME ihtiyacının-%50 düzeyinde beslenen grubun skoru en yüksek(4.21), +%50'si düzeyinde beslenen grubun dışkıları ise en düşük(3.96) skorlandı ( $p<0.05$ ). Dışkı kuru madde düzeyleri arasında önemli bir farklılık belirlenmedi ( $p>0.05$ ). Sonuç olarak, köpekleri fazla besleme ile sindirilebilirlik düşmekte, bununla birlikte daha fazla ve yumuşak kıvamda dışkı atılımı gözlenmektedir. Mama paketi üzerinde yazan miktarlarda besleme ile bazen köpeklerde kilo artışı ya da kilo kaybı görülebilir. Köpeklerde yüksek sindirilebilirlik, dışkı kalitesi, ideal canlı ağırlık ve uygun VKS sağlması için ticari mamaların enerji içerikleri gerekli analizler yapılarak belirlenmelidir. Köpeklerin günlük enerji ihtiyaçlarının hesaplanmasında kullanılan farklı formüller de değerlendirilmeye alınmalıdır.

**Anahtar kelimeler:** Canlı ağırlık, dışkı kalitesi, köpek, sindirilebilirlik, metabolik enerji, vücut kondüsyon skoru

## **The Effects of Adding Waste Sesame Seeds to Diets of Male and Female Karayaka Lambs on Some Blood Parameters**

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### **Summary**

This study aims to determine the effects of the inclusion of waste sesame seed (WSS) to concentrate feed of male and female Karayaka lambs on some blood parameters. A total of twenty-three lambs were randomly assigned to one of two groups: control (11 lambs, average live weight  $23.39 \pm 0.58$  kg) and experimental group (12 lambs, average live weight  $23.55 \text{ kg} \pm 0.41$  kg), and both groups were divided into two subgroups according to their gender. For 60 days, the lambs were fed ad libitum with isonitrogenous (17% CP) and isocaloric (2650 kcal/kg ME) diets containing 0% WSS (control) or 10% WSS (experimental). A biochemistry autoanalyzer was used to test albumin, total protein, triglyceride, cholesterol, and glucose levels in blood serum on the final day of the experiment. There were no significant differences in blood serum levels between male and female lambs ( $P > 0.05$ ). However, the experimental group's male lambs' cholesterol levels were lower than those of the control group ( $P < 0.05$ ). Female lambs in the experimental group had a higher glucose level than those in the control group ( $P < 0.01$ ). On the other hand, it was determined that the addition of WSS to the diets of female and male lambs did not affect serum albumin, total protein, and triglyceride levels. In conclusion, adding 10% WSS to lamb diets had no negative effect on blood parameters.

**Keywords:** Blood, by-product, karayaka, serum, waste sesame seed

## Erkek ve Dişi Karayaka Kuzularının Rasyonlarına İlave Edilen Atık Susam Tohumlarının Bazı Kan Parametreleri Üzerine Etkisi

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### Özet

Bu çalışmada, Karayaka kuzularının konsantre yemlerine atık susam tohumları (AS) ilavesinin bazı kan parametreleri üzerine etkisinin değerlendirilmesi amaçlandı. Toplam yirmi üç kuzu, rastgele bir kontrol (11 kuzu ve ortalama canlı ağırlığı  $23.39 \pm 0.58$  kg) ve bir deneme grubuna (12 kuzu ve ortalama canlı ağırlığı  $23.55 \text{ kg} \pm 0.41$  kg) ayrıldı, her iki grup da cinsiyetlerine göre iki alt gruba bölündü. Kuzular 60 gün boyunca, AS içermeyen (kontrol grubu) ve %10 AS ilave edilen (deneme grubu) izonitrojenik (%17 HP) ve izokalorik (2650 kcal / kgME) rasyonlarla ad libitum beslendi. Denemenin son günü kuzulardan alınan kan serumlarındaki albumin, total protein, trigliserit, kolesterol ve glikoz düzeyi ölçümleri biyokimya otoanalizörü ile gerçekleştirildi. Kan serum düzeylerinde cinsiyete bağlı anlamlı bir farklılık gözlenmedi ( $P>0.05$ ). Bununla birlikte deneme grubundaki erkek kuzuların kolesterol düzeyi kontrol grubundan daha düşük olduğu tespit edildi ( $P<0.05$ ). Deneme grubundaki dişi kuzuların glikoz seviyesi kontrol grubundan daha yüksekti ( $P<0.01$ ). Diğer yandan dişi ve erkek kuzuların rasyonlarına susam atık tohumlarının ilavesinin serum albümin, toplam protein ve trigliserit düzeylerini etkilemediği belirlendi ( $P> 0.05$ ). Sonuç olarak, kuzu rasyonlarına %10 düzeyinde AS ilave edilmesinin kan parametreleri üzerinde olumsuz bir etki meydana getirmediği tespit edildi.

**Anahtar kelimeler:** Atık susam tohumu, kan, karayaka, serum, yan ürün

### Giriş

Dünya nüfusundaki artışla birlikte, insanların sağlıklı ve dengeli beslenmesinde önemli bir yeri olan hayvansal proteinlere olan talep, her geçen gün artmaktadır. Bununla birlikte, hayvansal üretim sistemlerinde tahıl gibi gıda hammaddelerinin yoğun olarak kullanılması geleceğin gıda güvenliğini konusunda endişelere yol açmaktadır. Büyüyen nüfusla birlikte gün geçtikçe azalan doğal kaynaklar ve iklim değişiklikleri gibi çevresel sorunlar hayvansal üretim sistemlerinde gıda-yem rekabetini azaltacak stratejiler geliştirme ihtiyacını ortaya çıkarmıştır. Hayvan beslemede kullanılan gıda

hammadelerinin insan tüketimine uygun olmayan sanayi yan ürünleri ve bazı diğer atık biyokütlerle ile değiştirilmesi gıda-yem rekabetini ve hayvansal üretimin çevresel etkilerini azaltabilecek en önemli potansiyel strateji olarak görülmektedir (1).

Atık susam tohumları (AS), susam soyma ve tahin üretiminden elde edilen susam işleme endüstrisi yan ürünüdür. Susamın işlendikten sonra hammaddenin %3-5'ü düzeyinde ayrılan atık susam tohumları çoğunlukla düşük kalitede (boş / kırık / küçük) susam taneleri ve bazı hasat artıklarından oluşmaktadır. Atık susamın kuzu rasyonlarına ilave edildiği bir çalışmada (2); AS'nin %33.3 ham yağ (HY), %15.5 ham protein (HP) ve 3450 kcal/kg metabolize olabilir enerji içeriğine sahip olduğunu ve kuzu rasyonlarında %10 düzeyinde AS'nin besi performansını etkilemeksizin kullanılabileceğini bildirmişlerdir. Bu çalışmada konsantre yemlerine %10 düzeyinde AS ilave edilen erkek ve dişi Karayaka kuzularının bazı kan parametreleri üzerindeki etkisinin incelenmesi amaçlanmıştır.

### **Materyal ve Metot**

Bu çalışma, Ondokuz Mayıs Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (HADYEK) izniyle yürütüldü (protokol no: 2018/11).

Toplam 23 kuzu rastgele bir kontrol grubu (6 erkek ve 5 dişi) ve deney grubu (6 erkek ve 6 dişi) olarak ayrıldı; her iki grup da cinsiyetlerine göre iki alt gruba bölündü (2x2 faktöriyel dizayn). Kontrol grubunun ortalama canlı ağırlığı 23.39 kg ( $\pm 0.58$  kg), deney grubunun ise 23.55 kg ( $\pm 0.41$  kg)'dı. Kuzulara, çalışma boyunca konsantre yem (%85) ve yonca kuru otundan (%15) oluşan izonitrojenik (%17 HP) ve izokalorik (2650 kcal/kg ME) rasyonlar ad libitum sunuldu. Kontrol grubu AS içermeyen konsantre yemle, deney grubu %10 AS içeren konsantre yemle beslendi. Bu çalışmada kullanılan atık susam tohumu ve hayvanlara sunulan konsantre yemlerin besin madde bileşimleri İsmail ve ark.(2021) (2) tarafından bildirilmiştir.

Kan numuneleri denemenin son günü sabah yemlemesinin ardından 2-4 saat içerisinde Vena jugularis'den 10 ml'lik vakumlu tüpler kullanılarak alındı. Örnekler 3000 rpm'de 10 dakika santrifüj edilerek (NF 400, Nüve, Türkiye) serumlar elde edildi. Kan serumları analizleri spektrofotometrik yöntemle otoanalizör cihazı (A25 Random Access Analyzer, Biosystems, İspanya) kullanılarak yapıldı. Ölçümler, albümin, total protein, trigliserit, kolesterol, glukoz ve kontrol serumu ticari kitleri (Biosystems, İspanya) kullanılarak gerçekleştirildi.

### **İstatistiksel analiz**

Veriler SPSS (IBM Inc., USA, version 21) ile analiz edildi. Normal dağılımı kontrol etmek için Kolmogorov-Smirnov Testi kullanıldı. Varyansların homojenliği Levene Testi ile değerlendirildi. Diyet (kontrol ve deneme) ve cinsiyet (erkek ve dişi) gruplarının kan serumu değerleri Genel Doğrusal Model (GLM) ile karşılaştırıldı.  $P < 0.05$  düzeyi istatistiksel olarak anlamlı kabul edildi.

**Bulgular**

Kontrol ve deneme gruplarından ölçülen serum albümin, total protein, trigliserit, kolesterol ve glukoz değerleri Tablo 1.'de sunulmuştur. Deneme grubundaki erkek kuzuların kolesterol düzeyi kontrol grubundan daha düşük olduğu tespit edildi ( $P < 0.05$ ). Deneme grubundaki dişi kuzuların glikoz seviyesi kontrol grubundan daha yüksekti ( $P < 0.01$ ). Diğer yandan dişi ve erkek kuzuların rasyonlarına susam atık tohumlarının ilavesinin serum albümin, toplam protein ve trigliserit konsantrasyonlarını etkilemediği belirlendi ( $P > 0.05$ ).

**Tablo 1.** Kontrol ve deney gruplarının cinsiyete göre ortalama kan serumu değerleri

Parametre	Cinsiyet	Kontrol	Deneme	P
Albumin (g/L)	Erkek	27.00±1.91	23.40±1.72	ÖD
	Dişi	24.40±0.66	24.66±1.16	ÖD
	P	NS	NS	
Total protein (mg/dL)	Erkek	70.85±6.92	55.30±0.87	ÖD
	Dişi	63.38±3.17	60.80±2.32	ÖD
	P	NS	NS	
Trigliserit(mg/dL)	Erkek	17.66±2.18	15.60±1.93	ÖD
	Dişi	21.40±2.69	21.00±3.68	ÖD
	P	NS	NS	
Kolesterol (mg/dL)	Erkek	59.16±5.64	39.40±6.67	*
	Dişi	51.40±3.58	54.33±3.77	ÖD
	P	NS	NS	
Glukoz (mg/dL)	Erkek	68.33±4.70	59.00±4.84	ÖD
	Dişi	58.80±2.22	71.00±2.69	**
	P	NS	NS	

\* $p < 0.05$ ; \*\* $p < 0.01$ ; NS:önemli değil



## **Tartışma**

Sağlıklı karayaka ırkı erkek ve dişi koyunların kan serumlarında biyokimyasal maddelerin ölçüldüğü bir çalışmada (3) dişi ve erkek koyunlar arasında istatistiksel açıdan anlamlı bir fark gözlenmezken, normal değer aralıkları albümin için 21-29 g/L, total protein için 61-84 mg/dl, kolesterol için 36 ila 87 mg/dL ve glukoz için 40 ila 84 mg/dL olarak bildirilmiştir. Mevcut çalışmada ölçülen biyokimyasal değerler ve cinsiyete bağlı farklılığın gözlenmemesi belirtilen çalışma sonuçlarıyla benzerlik göstermektedir.

Bu araştırmada deney grubu konsantre yemine mısır yerine %10 düzeyinde yüksek yağ içeriğine sahip AS ilave edilmesi sebebiyle deneme grubu rasyonunun HY düzeyi (%5.12) kontrol grubu rasyonuna (3.04) kıyasla %2.08 daha fazlaydı. Dolayısıyla deneme grubunda yer alan erkek kuzuların kolesterol konsantrasyonunda gözlemlenen düşüş susam yağında yüksek oranda bulunan doymamış yağ asitlerinin hipokolesterolemik etkisinden kaynaklanmış olabilir. (4). Bununla birlikte AS'de bulunan sesamin, kolesterolün bağırsak emilimini kısmen engelleyebilir ve kolesterol metabolizmasında anahtar olan 3-hidroksil-3-metil glutar koA (HMG-CoA) redüktazın aktivitesini azaltarak kolesterol seviyesini düşürmüş olabilir (5).

Ruminant rasyonlarına yağ ilave edilen birçok çalışmada yağ ilavesiyle serum glikoz düzeyinin yükseldiği bildirilmiştir (6,7). Bu çalışmalarla uyumlu olarak sunulan çalışmada yağ içeriği yüksek olan deneme grubu rasyonuyla beslenen dişi kuzuların glikoz konsantrasyonunda artış gözlemlendi. Diğer yandan deneme grubundaki erkek kuzuların glikoz düzeyinde rasyona bağlı bir değişiklik gözlenmedi. Bu durum erkek kuzuların pubertaya daha kısa sürede (8) ulaşması ve testosteronun glikoz konsantrasyonuna olan dengeleyici etkisinden kaynaklanmış olabilir (9).

## **Sonuç**

Dünyada hayvansal ürünlere olan talep hızla artarken, bu amaçla yetiştirilen hayvanları besleyecek yem hammaddesi temininde ciddi sıkıntılar yaşanmaktadır. Özellikle insan tüketimine uygun olan tahılların hayvan beslemede yoğun olarak kullanılması geleceğin gıda güvenliği konusunda endişelere yol açmaktadır. Bu sebeple insan tüketimine uygun olmayan endüstriyel yan ürünler ve bazı atık biyokütlelerin hayvan sağlığına zarar vermeden rasyonlara dahil edilmesi gerekmektedir. Bu araştırmada kullanılan susam yan ürününün kuzu rasyonlarının %10 düzeyinde ilave edilmesinin kan parametreleri üzerinde olumsuz bir etki meydana getirmediği belirlenmiştir. Ayrıca susam yağının hipokolesterolemik etkisinden dolayı bu ürünle beslenen hayvanların et ürünlerinin insan sağlığına olumlu yönde katkı sağlayabileceği kanısına varılmıştır.

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## Crude Protein, Crude Fat, and Ash Contents of Fish Meals Originated from The Black Sea

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### Summary

The chemical composition of the fish meal depends on the raw fish material used in production and the method applied in the process. The aim of the study was to provide information on crude protein, crude fat, and ash values of fish meal produced from anchovy and sprat during the hunting season from November 2020 to January 2021. Anchovy meal and sprat meal samples were obtained from a fish meal and fish oil factory monthly to represent production. The crude protein content of anchovy meal was about 73.40%, whereas sprat meal had 68.76%. It has been determined that anchovy meal had approximately 6% more crude protein content than sprat meal. Sprat meal had a higher value than anchovy meal in terms of crude fat content. In conclusion, crude protein, crude fat, and ash values of anchovy meal and sprat meal are generally in agreement with previous studies. **Keywords:** Anchovy meal, ash, crude fat, crude protein, sprat meal

## Karadeniz Orijinli Balık Unlarının Ham Protein, Ham Yağ ve Kül İçerikleri

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### Özet

Balık ununun kimyasal bileşimi, üretimde kullanılan ham balık materyaline ve prosese bağlıdır. Çalışmanın amacı, Kasım 2020-Ocak 2021 av sezonunda hamsi ve çaçadan üretilen balık ununun ham protein, ham yağ ve kül değerleri hakkında bilgi vermektir. Hamsi unu ve çaça unu örnekleri, üretimi temsil etmek üzere bir balık unu ve balık yağı fabrikasından aylık olarak alınmıştır. Hamsi ununun ham protein içeriği yaklaşık %73,40 iken, çaça ununun ham protein değeri %68,76 olarak bulunmuştur. Hamsi ununun çaça ununa göre yaklaşık %6 daha fazla ham protein içeriğine sahip olduğu tespit edilmiştir. Çaça ununun ham yağ içeriğinin hamsi unundan daha yüksek olduğu bulunmuştur. Sonuç olarak, hamsi unu ve çaça unu ham protein, ham yağ ve kül değerleri önceki

çalışmalarla genel olarak uyum göstermektedir. **Anahtar kelimeler:** Hamsi unu, kül, ham yağ, ham protein, çaça unu

## Introduction

Fish meal is rich in high-quality protein, and it provides long chain omega-3 fatty acids, DHA and EPA. Fish meal is a highly valuable protein source for fish or animals because of the high proportion of essential amino acids like methionine, lysine, threonine, tryptophan (1). Furthermore, fish meal is valuable feedstuff not only protein quantity or quality but also minerals such as calcium, phosphorous, magnesium, zinc, iodine, iron, cobalt. The chemical composition of fish meal depends on the raw fish material used in production and the method applied in the process. The fish meal produced from fatty fish might contain about 71% crude protein, 9% fat and 12% ash (2). The aim of the study was to provide information on crude protein, crude fat, and ash values of fish meal produced from anchovy and sprat during the hunting season from November 2020 to January 2021.

## Material and Methods

Anchovy meal and sprat meal samples were obtained from a fish meal and fish oil factory monthly from November 2020 to January 2021 to represent production. Crude protein (TS EN ISO 5983 1-2), crude fat (EC No: 152/2009), and ash (EC No: 152/2009) contents of fish meal samples were analyzed in a private laboratory.

## Results

The crude protein, crude fat and ash contents of anchovy meal and sprat meal were shown in Table 1. The crude protein content of anchovy meal was about 73.40%, whereas sprat meal had 68.76%. It has been determined that anchovy meal had approximately 6% more crude protein content than sprat meal. Sprat meal had a higher value than anchovy meal in terms of crude fat content.

**Table 1.** The crude protein, crude fat, and ash contents of anchovy meal and sprat meal.

Nutrients	Anchovy Meal ( $\bar{x}\pm Sd$ )	Sprat Meal ( $\bar{x}\pm Sd$ )
Crude protein %	73.40 $\pm$ 3.94	68.76 $\pm$ 1.44
Crude fat %	11.74 $\pm$ 1.53	14.70 $\pm$ 0.19
Ash %	12.62 $\pm$ 0.52	13.15 $\pm$ 0.72

## **Discussion**

Fish meal is the most important protein source used in fish diets. It was reported that a good quality fish meal contained between 60-72% crude protein (3). Bayraklı and Duyar (4) stated that the crude protein values of anchovy meal and sprat meal were average 72.50% and 66.68%, respectively. While Yıldırım (5) reported that the crude protein level in anchovy meal was 71%, in another study conducted by Bayraklı and Duyar (6) stated that crude protein content ranged from 68.02% to 76.63% for anchovy meal. In this study, crude protein levels of anchovy meal and sprat meal were over 60%, and this result was in agreement with other studies (3, 5, 6).

The result of the present study for crude fat values of anchovy meal and sprat meal was in agreement with Bayraklı and Duyar (4) who stated that sprat meal had higher crude fat content than anchovy meal. In the study conducted by De Koning (7), it was stated that the ash value of the fish meal was between 10-25%. Bayraklı and Duyar (6) determined that the ash content of anchovy meal varied between 10.73-11.48%. Çetiner (8) mentioned that the ash content of sprat meal varied between 13.58 and 13.75%. In the study, the ash values of anchovy meal and sprat meal were within limits reported in previous studies (6-8).

## **Conclusion**

The composition of the fish meal varies depending on many factors, such as the raw material and the production method. In conclusion, crude protein, crude fat and ash values of anchovy meal and sprat meal in the present study are generally in agreement with previous studies.

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**The Biochemical Effects of Stress Caused by The Transfer of Live Rainbow Trout  
(*Oncorhynchus mykiss*)**

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**Summary**

The world population is increasing exponentially every year. The United Nations estimates that there is a 95 % chance that the world's population will be between 8.4 and 8.7 billion by 2030, and between 9.4 and 10.2 billion by 2050. Rapid growth in the world's population has begun to drive demand for food be a problem. This growth has triggered the demand for healthy food for more people and the global demand. The upper limit of water temperature for intensively farmed rainbow trout in Turkey is 24 °C. For this reason, many dams cannot continue to produce throughout the year, especially large trout production, it is expected to be more than 1 year old or the fishing weight exceeds 1,000 grams, it is imperative to transfer the fish. To the dam with low water temperature in summer. Factors such as starvation of fish for transshipment purposes, transportation of cages to shore for aquaculture at the dam facility, mobility during loading and transport to transport trucks, and dissolved oxygen and temperature of water in transport tanks and applications will affect stress-causing fish. Although there are quantitative differences between species of aquatic organisms, it is well known that antioxidant enzymes are common. The antioxidant defense system plays a role in cellular hemostasis and helps prevent free radicals from damaging tissues and cells. Free radicals are formed in the body in a number of ways. This defense mechanism can occur both enzymatically and non-enzymatically, as in other vertebrates. The behavioral response of fish to stressful situations triggers the secretion of the hormones cortisol and adrenaline, which initiates an endocrine response. These hormones, which are transported to the tissues through the blood, accelerate the metabolic activity in the cells and cause an increase in the demand for energy. When stressors begin to affect metabolic activity, many biochemical changes will occur in cells as a physiological response. These changes can cause reversible or irreversible damage to metabolism, limit many important functions of fish, such as growth, respiration, development and reproduction, and cause death due to the destruction of hemostatic balance and the inability to restore it. In this study, changes in parameters such as stress-induced oxidative damage, serum cortisol, and blood glucose levels were evaluated.

**Keywords:** Antioxidant, oxidative damage, rainbow trout, stress, transfer

**Canlı Gökkuşığı Alabalığı (*Oncorhynchus mykiss*) Transferlerinin Oluşturduğu Stresin  
Biyokimyasal Etkileri**

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**Özet**

Dünya nüfusu her geçen yıl katlanarak artmaktadır. Birleşmiş Milletler, % 95 ihtimalle küresel nüfusun büyüklüğünün 2030'da 8,4 ile 8,7 milyar arasında, 2050'de 9,4 ile 10,2 milyar arasında olacağını tahmin etmektedir. Dünya nüfusunun hızlı artışı beraberinde gıda ihtiyacını da sorun haline getirmeye başlamıştır. Bu büyümeyle birlikte daha fazla insanı sağlıklı gıdalarla besleme ihtiyacı ve küresel talep artmaktadır. Ülkemizde yoğun olarak yetiştiriciliği yapılan gökkuşığı alabalığı için su sıcaklığının üst sınırı 24 °C dir. Bu sebeple birçok barajda üretim yıl boyunca devam edememekte, özellikle hasat yaşı 1 yaşının üstünde veya hasat ağırlığı 1000 gram üzerinde olması planlanan büyük alabalık üretiminde balıkların yaz su sıcaklıklarının düşük olan barajlara nakil edilmesini zorunlu hale getirmiştir. Transfer amaçlı uygulanan balıkların aç bırakılmaları, baraj tesisinde yapılan yetiştiricilikler için kafeslerin kıyıya taşınması, nakil kamyonlarına yükleme ve nakil sırasında oluşan hareketlilik ve nakil tanklarındaki çözülmüş oksijen ve su sıcaklığı gibi faktörler ve uygulamaların tamamı balıklarda stres oluşturmaktadır. Akuatik organizmalarda türler arasında nicelik açısından farklılıklar olmasına rağmen antioksidan enzimlerin ortak olduğu bilinmektedir. Antioksidan savunma sistemi, hücre homeostazisinde rol oynar ve vücutta çeşitli şekillerde oluşan serbest radikallerin doku ve hücrelere verdiği zararları önlemeye yardımcı olur. Bu savunma mekanizması da diğer omurgalılarda olduğu gibi, hem enzimatik hem de enzimatik olmayan şekillerde gerçekleşebilir. Balıklarda stresli durumlara karşı verilen davranışsal yanıt, kortizol hormonunun ve adrenalin hormonunun salgılanmasını tetikleyerek endokrin yanıtı başlatır. Kan yoluyla dokulara taşınan bu hormonlar hücrelerde metabolik aktiviteyi hızlandırarak enerji gereksiniminin arttırmasına neden olur. Stres unsuru, metabolik aktiviteler üzerinde etkili olmaya başladığı zaman fizyolojik cevap olarak hücrelerde birçok biyokimyasal değişimler meydana getirir. Bu değişimler metabolizmada geri dönüşümlü veya dönüşümsüz hasarlara neden olup balığın büyüme, solunum, gelişim, üreme gibi birçok hayati fonksiyonunu sınırlanmakta ve homeostatik dengenin bozulması ve tekrar kurulamaması sonucu ölümler meydana getirmektedir. Bu çalışmada, stres kaynaklı oluşan, oksidatif hasar, serum kortizol ve kan glukoz seviyesi gibi parametrelerdeki değişimler değerlendirilmektedir.

**Anahtar kelimeler:** Antioksidan, gökkuşığı alabalığı, oksidatif hasar, stres, transfer

**The Effect of Mercury, Copper and Zinc on Paraoxonase (PON) Enzyme Activity in Bonito (*Sarda sarda*) Fish**

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**Summary**

Paraoxonase (PON) is an important liver enzyme that has arylesterase and paraoxonase activities, and has a protective effect against the formation of lipid peroxides with oxidation of low-density lipoprotein (LDL). Currently heavy metal dirtiness that increase as a result of environmental pollution causes harmful effects on the organism through biochemical enzyme reactions. In this study, the effects of mercury ( $\text{Hg}^{+2}$ ), copper ( $\text{Cu}^{+2}$ ), and zinc ( $\text{Zn}^{+2}$ ) heavy metal ions, which are common in environmental pollution, on PON enzyme activity in muscle tissue of bonito (*Sarda sarda*) fish, which has an important physiological function in metabolism with its detoxification and antioxidant activity, were investigated. In the study, muscle tissues of 25 bonito (*S. sarda*) fish which an average weight of 600-800 g and a length of 40-45 cm, obtained fresh from the sea of Samsun region, were used. Solutions of Hg, Cu and Zn heavy metals as  $\text{HgCl}_2$ ,  $\text{CuCl}_2$  and  $\text{ZnCl}_2$  were prepared to be used in the analysis and the changes in PON enzyme activity were determined by adding different volumes of these heavy metal solutions. As a result of the data obtained, % activity graphs were drawn by calculating the enzyme activities of mercury, copper and zinc heavy metals. As a result of the study, when the effect of  $\text{Cu}^{+2}$  and  $\text{Zn}^{+2}$  heavy metal ions on PON enzyme activity was examined, it was determined that increasing concentrations caused a decrease in the enzyme activity, but there was no statistically significant difference between the different concentrations used. When the effect of  $\text{Hg}^{+2}$  heavy metal ion on PON enzyme activity was examined, It was determined that increasing concentrations inhibited the enzyme activity and caused a statistically significant decrease between the enzyme activities depending on the different concentrations used ( $p < 0.05$ ).

**Keywords:** Bonito (*Sarda sarda*), copper (Cu), heavy metals, mercury (Hg), paraoxonase (PON), zinc (Zn)



**Palamut (*Sarda sarda*) Balığında Paraoksonaz (PON) Enzim Aktivitesi Üzerine Civa, Bakır ve Çinkonun Etkisi**

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**Özet**

Paraoksonaz (PON), arilesteraz ve paraoksonaz aktivitelerine sahip, düşük yoğunluklu lipoprotein (LDL)'nin oksidasyonu ile lipit peroksitlerin oluşumuna karşı koruyucu etkisi olan önemli bir karaciğer enzimidir. Günümüzde çevre kirliliği sonucu artan ağır metal kirlilikleri biyokimyasal enzim reaksiyonları üzerinden organizmada zararlı etkiler meydana getirmektedir. Bu çalışmada da çevre kirliliğinde sık rastlanan civa ( $Hg^{+2}$ ), bakır ( $Cu^{+2}$ ) ve çinko ( $Zn^{+2}$ ) ağır metal iyonlarının palamut (*Sarda sarda*) balığı kas dokusunda bulunan detoksifikasyon ve antioksidan aktivitesi ile metabolizmada önemli fizyolojik fonksiyona sahip PON enzim aktivitesi üzerine etkileri araştırılmıştır. Çalışmada Samsun bölgesi denizinden taze olarak temin edilen ortalama ağırlıkları 600-800 g arası, boyu 40-45 cm olan 25 adet palamut (*S. sarda*) balığının kas dokuları kullanıldı. Analizde kullanılmak üzere Hg, Cu ve Zn ağır metallerinin  $HgCl_2$ ,  $CuCl_2$  ve  $ZnCl_2$  olarak çözeltileri hazırlandı ve bu ağır metal çözeltilerinden farklı hacimlerde alınarak PON enzim aktivitesindeki değişiklikler tayin edildi. Elde edilen veriler sonucunda civa, bakır ve çinko ağır metallerinin enzim aktiviteleri hesaplanarak % aktivite grafikleri çizildi. Çalışma sonucunda  $Cu^{+2}$  ve  $Zn^{+2}$  ağır metal iyonlarının PON enzim aktivitesi üzerine etkisi incelendiğinde artan derişimlerinin enzim aktivitesinde azalmaya neden olduğu fakat kullanılan farklı derişimler arasında istatistiksel olarak anlamlı bir fark olmadığı belirlendi.  $Hg^{+2}$  ağır metal iyonunun PON enzim aktivitesi üzerine etkisi incelendiğinde ise artan derişimlerinin enzim aktivitesini inhibe ettiği ve kullanılan farklı derişimlere bağlı olarak enzim aktiviteleri arasında istatistiksel olarak anlamlı bir azalmaya neden olduğu belirlendi ( $p < 0.05$ ).

**Anahtar kelimeler:** Ağır metal, bakır (Cu), civa (Hg), çinko (Zn), palamut (*Sarda sarda*), paraoksonaz (PON)



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**POSTER  
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**The Analysis of FSH Beta-Subunit Gene Genotypes in East Anatolian Red, East Anatolian Red×Holstein, and Zavot Bulls**

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**Summary**

The follicle-stimulating hormone (FSH) acts on the Sertoli cells in the seminiferous tubules of the testis and regulates spermatogenesis up to the secondary spermatocyte stage. Polymorphism of the FSH beta-subunit gene (*FSHB*) gene in exon 3 was significantly associated with the fresh and frozen semen quality. There is no information on this gene in Turkish native cattle breeds. Therefore, this study aimed to investigate the genotypic distribution and population genetic parameters of the single nucleotide polymorphism (SNP) at the *FSHB* gene in East Anatolian Red (EAR), East Anatolian Red×Holstein (EAR×H), and Zavot (Z) bulls. A total of 68 cattle including EAR ( $n=34$ ), EAR×H ( $n=20$ ), and Z ( $n=14$ ) bulls were used. Genomic DNA was isolated from blood samples using the phenol/chloroform method. The genotyping of the SNP was carried out by the PCR-RFLP. Deviation from Hardy–Weinberg equilibrium (HWE) was calculated by using the chi-square goodness-of-fit test. Population genetics evaluation was performed for the effective allele numbers, the polymorphism information content, theoretical heterozygosity, the fixation index, and the level of possible variability realization. Moreover, biodiversity indices including the Shannon-Weaver diversity index, Simpson Dominance, Gini-Simpson Equitability, and the True Diversity were estimated. In the present study, the AA and the AB genotypes were predominant in EAR and EAR×H bulls, respectively. Zavot breed was found to be monomorphic. There was a deviation from HWE, concerning the total cattle population. The population genetics evaluation showed that the marker was moderately informative for EAR and the crossbreeds, as well as the total population. The estimation of biodiversity indices revealed an admissible diversity for the studied locus. In conclusion, the polymorphism within exon 3 of the bovine *FSHB* can be interpreted as a genetic marker with reliable variability in EAR and the crossbreeds, but not in Zavot cattle.

**Keywords:** Cattle, *FSHB* gene, genetic marker, population genetic parameters, single nucleotide polymorphism

**Doğu Anadolu Kırmızısı, Doğu Anadolu Kırmızısı × Holştayn ve Zavot Irkı Erkek Sığırlarda FSH Beta Alt Birimi Genine Ait Genotiplerin Analizi**

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**Özet**

Folikül uyarıcı hormon (FSH), testisin seminifer tübüllerindeki Sertoli hücrelerine etki eder ve sekonder spermatozoid aşamasına kadar spermatogenezini düzenler. FSH beta alt birim (*FSHB*) geninde ekzon 3'te yer alan polimorfizm, taze ve donmuş semen kalitesi ile anlamlı düzeyde ilişkilendirilmiştir. Türk yerli sığır ırklarında bu gen hakkında bilgi bulunmamaktadır. Bu nedenle, bu çalışma Doğu Anadolu Kırmızısı (DAK), Doğu Anadolu Kırmızısı × Holştayn (DAK × H) ve Zavot (Z) ırkı erkek sığırlarda *FSHB* genindeki tek nükleotid polimorfizmine (single nucleotide polymorphism: SNP) ait genotipik dağılımı ve populasyon genetik parametrelerini araştırmayı amaçlamaktadır. DAK ( $n = 34$ ), DAK × H ( $n = 20$ ) ve Z ( $n = 14$ ) sığır ırklarından olmak üzere toplam 68 baş sığır kullanılmıştır. Genomik DNA, kan örneklerinden fenol/kloroform yöntemi kullanılarak izole edilmiştir. Genotiplendirme, PCR-RFLP yöntemiyle gerçekleştirilmiştir. Hardy-Weinberg dengesinden (HWE) sapma, ki-kare uygunluk testi kullanılarak hesaplanmıştır. Etkili alel sayıları, polimorfizm bilgi içeriği, teorik heterozigotluk, fiksasyon indeksi ve olası değişkenlik gerçekleşme seviyesi için populasyon genetiği değerlendirilmiştir. Ayrıca, Shannon-Weaver Çeşitlilik Endeksi, Simpson Dominanslığı, Gini-Simpson Eşitliği ve Gerçek Çeşitlilik gibi biyolojik çeşitlilik endeksleri hesaplanmıştır. Bu çalışmada, AA ve AB genotiplerinin sırasıyla DAK ve DAK × H sığırlarda baskın olduğu belirlenmiştir. Zavot ırkının monomorfik olduğu bulunmuştur. Toplam sığır populasyonunda HWE'den sapma olduğu görülmüştür. Populasyon genetiği parametreleri, genetik belirtecin toplam populasyonun yanı sıra DAK ve melezler için orta derecede bilgilendirici olduğunu göstermiştir. Biyoçeşitlilik endekslerinin değerlendirilmesi sonucunda, incelenen lokus için kabul edilebilir düzeyde bir çeşitlilik olduğu belirlenmiştir. Sonuç olarak, sığır *FSHB* geni ekzon 3'te yer alan polimorfizmin, Zavot sığırı hariç olmak koşuluyla, DAK ve melezlerde güvenilir değişkenliğe sahip bir genetik belirteç olarak yorumlanabileceği sonucuna varılmıştır.

**Anahtar kelimeler:** *FSHB* geni, genetik belirteç, populasyon genetik parametreleri, sığır, tek nükleotid polimorfizmi

## The Effects of Boron Supplementatation on Metabolic and Nutritional Disorders in Dairy Cattle

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### Summary

The period in which the health and productivity of dairy cattle are most affected is the transition period covering 3 weeks before and 3 weeks after calving. Physiological and metabolic changes during the transition period increase the incidence of the animal's metabolic diseases and the severity of the negative energy balance. Studies on boron mineral, which has been found to be effective in improving animal health and preventing nutritional disorders, show that boron is effective on energy metabolism, immune system and endocrine system. In previous studies, supplementation of boron to dairy cattle diets minimizes postpartum negative energy balance and improves lipotrophic effect. It was determined that the postpartum non-esterified fatty acids,  $\beta$ -hydroxybutyrate and triglyceride concentrations were significantly decreased in dairy cattle fed with boron added diets. Boron mineral, which is generally found in the organism as organic boron compounds bound to sodium and oxygen elements in the form of boric acid ( $B(OH)_3$ ) or borate ( $B(OH)_4$ ), interacts with other minerals and affects the mineral metabolism in animals. Previous studies have shown that boron increases the absorption of calcium and magnesium and reduces the amount of excretion of these minerals. As a result of these studies, it is suggested by the researchers that boron mineral can be added to the diets in order to treat hypocalcemia (milk fever), hypomagnesemia and fatty liver in lactating dairy cows. Researchers state that the use of sodium borate in lactating dairy cattle may improve the metabolic status by reducing the incidence of fatty liver. In a study where sodium borate (30 g/day) was given to pregnant cows, it was reported that serum beta hydroxy butyrate and glucagon concentrations were lower than the control group in the postpartum period. With the supplementation of sodium borate to the diet, it was determined that total triglycerides, cholesterol, low and high density lipoprotein and non-esterified fatty acids in the blood were decreased. As a result of studies examining the effects of boron on metabolic profile, it has been suggested that it can prevent metabolic diseases in dairy cattle. In this paper, articles investigating the effects of boron supplementations to dairy cattle rations on animal health and nutritional disorders were reviewed.

**Keywords:** Boron, dairy cattle, diet, metabolic disorders

## Süt Sığırlarında Rasyona Bor Eklemenin Metabolik ve Beslenme Bozuklukları Üzerine Etkileri

Cansu ÇELİK DEVECİLER, Nurcan ÇETİNKAYA

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### Özet

Süt sığırlarının sağlıkları ve verimliliklerinin en çok etkilendiği dönem doğumdan önce 3 hafta ile doğumdan sonraki 3 haftayı kapsayan geçiş dönemidir. Geçiş döneminde fizyolojik ve metabolik değişiklikler hayvanın metabolizma hastalıklarına insidansını ve negatif enerji dengesinin şiddetini artırmaktadır. Hayvan sağlığının iyileştirilmesinde ve beslenme bozukluklarının önlenmesinde etkili olduğu tespit edilen bor minerali ile ilgili yapılan araştırmalar borun enerji metabolizması, immun sistem ve endokrin sistem üzerine etkili olduğunu göstermektedir. Yapılan araştırmalarda süt sığırları rasyonlarına bor ilave edilmesinin postpartum negatif enerji dengesini minimuma indirdiği ve lipotropik etkiyi iyileştirdiği saptanmıştır. Bor ilave edilen rasyonla beslenen süt sığırlarında postpartum esterleşmemiş yağ asitleri,  $\beta$ -hidroksibütirat ve trigliserit konsantrasyonlarının önemli düzeyde azaldığı belirlenmiştir. Organizmada genel olarak borik asit ( $B(OH)_3$ ) veya borat ( $B(OH)_4$ ) formunda sodyum ve oksijen elementlerine bağlı organik bor bileşikleri halinde bulunan bor minerali, diğer minerallerle etkileşime girerek hayvanlarda mineral metabolizmasını da etkilemektedir. Yapılan araştırmalar borun, kalsiyum ve magnezyum emilimini arttırdığını ve bu minerallerin atılım miktarını azalttığını ortaya koymuştur. Bu çalışmaların sonucunda araştırmacılar tarafından laktasyondaki süt ineklerinde hipokalsemi (süt humması), hipomagnezemi ve yağlı karaciğeri tedavi etmek amacıyla rasyona bor mineralinin katılabileceği önerilmektedir. Araştırmacılar, laktasyondaki süt sığırlarında sodyum borat kullanımının yağlı karaciğer insidansını azaltarak metabolik durumu düzeltebileceğini ifade etmektedirler. Gebe ineklere sodyum borat (30 g/gün) verilen bir çalışmada, postpartum dönemde serum beta hidroksi bütirat ve glukagon konsantrasyonlarının kontrol grubuna göre daha düşük düzeyde olduğu bildirilmiştir. Rasyona sodyum borat ilavesi ile kanda total trigliserit, kolesterol, düşük ve yüksek yoğunluklu lipoprotein ve esterleşmemiş yağ asitlerinin azaldığı tespit edilmiştir. Borun metabolik profil üzerine etkilerinin incelendiği araştırmalar sonucunda, süt sığırlarında metabolik hastalıkları önleyebileceği ileri sürülmüştür. Bu bildiride, süt sığırları rasyonlarına bor eklemenin hayvan sağlığı ve beslenme bozukluklarına etkilerinin araştırıldığı makaleler derlenmiştir.

**Anahtar Kelimeler:** Bor, metabolik bozukluklar, rasyon, süt sığırları

## The Use of Essential Oils in Calf Nutrition

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### Summary

Antibiotics could be used as growth enhancers to shorten the weaning period of calves years ago. However, antibiotics used as growth promoters in livestock nutrition have been completely banned in the European Union and Turkey since 2006 (regulation 1831/2003/EC and 26056/2006 TR, respectively). After the restriction, the researchers have intensively evaluated many feed additives as an alternative to antibiotics to increase the palatability of feed, stimulate appetite and digestion, and strengthen the immune system. One of these feed additives is essential oils that are recognized as safe for animals and humans. This paper aims to review the potential effects of essential oils on calf performance and health.

**Keywords:** Calf, essential oils, performance

## Buzağı Beslemede Esansiyel Yağların Kullanımı

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### Özet

Önceki yıllarda, buzağuların sütten kesme dönemini kısaltmak için büyümeyi teşvik edici olarak antibiyotikler kullanılabildi. Bununla birlikte, 2006 yılında hayvan beslemede büyümeyi teşvik edici olarak kullanılan antibiyotikler Avrupa Birliği ve Türkiye'de (sırasıyla yönetmelik 1831/2003/EC ve 26056/2006 TR) tamamen yasaklanmıştır. Bu kısıtlamanın ardından araştırmacılar, yemin lezzetini artırmak, iştahı ve sindirimi uyarmak ve bağışıklık sistemini güçlendirmek için antibiyotiklere alternatif olarak birçok yem katkı maddesini yoğun bir şekilde değerlendirmektedirler. Bu yem katkı maddelerinden biri, hayvanlar ve insanlar için güvenli olduğu kabul edilen uçucu yağlardır. Bu makale, uçucu yağların buzağı performansı ve sağlığı üzerindeki potansiyel etkilerini gözden geçirmeyi amaçlamaktadır.

**Anahtar kelimeler:** Buzağı, esansiyel yağlar, performans

## **Introduction**

The calves are susceptible to many pathogens causing diseases during the preweaning period. Particularly, acute scours usually occur in calves aged 15 days or earlier, and they are clinically characterized by watery diarrhea, progressive dehydration, and death within a few days of its onset (1,2). After using antibiotics as feed additives were banned in European Union countries and our country in 2006, intensive efforts have been devoted to find natural and safe alternative growth promoters to antibiotics (3). This paper aims to review the potential effects of essential oils on calf performance and health.

## **Defination and major properties of essential oils**

Essential oils are not true oils, and the term of essential oil is usually come from the components of fragrance, or *Quinta essentia*, of plants. Essential oils can be extracted from many parts of aromatic plants, including the leave, flower, stem, root, seed and bark (4). The composition of essential oils extracted from the different parts of the same aromatic plant can be different (5). However, there may be some variations in chemistry among essential oil extracted from individual plants, or different varieties and age of plants, and growing environment of the plant (6).

Essential oils have a strong bactericidal activity against pathogenic bacteria such as *Escherichia coli* and *Salmonella spp.* (7). It has been reported that thymol is the main active component of thyme and one of the most examined active ingredients of essential oils with strong antimicrobial activity against a wide range of Gram (+) and Gram (-) bacteria (8). The phenols of the plants show antioxidant activity by acting as chain-breaking peroxy-radical scavengers and preventing lipid peroxidation (9, 10). Lee et al. (11) mentioned that thymol had an OH group and used it as and H transporter for peroxidation and reduced hydroxyl peroxide free radicals.

## **The potential effects of essential oils on calf performance**

Some pathogens like *Escherichia coli* and *Salmonella spp.* cause enteritis, enterotoxemia or much more severe septicemia in the calves and they can be found in the digestive tracts of newborn calves from the first day of their lives (12). It has been reported that supplementation of essential oil in calf solid starter improves growth performance (13, 14), rumen fermentation (15) and diarrhea severity (16). Kazemi-Bonchenari et al. (15) reported that supplementation of the starter diet with essential oil (1 g/kg of starter dry matter) improved weight gain, growth and feed efficiency of dairy calves, irrespective of dietary protein content. Liu et al. (14) reported that calves fed essential oil combination (EOC) at 44.1 ppm had greater dry matter intake (1.63 and 1.74 kg/d) and better nutrient digestibility compared with calves fed the control. It also was stated that blood concentrations of IgG and IgM on d 14, IgA on d 28, and total serum protein on d 42 were all greater for calves fed EOC compared with calves fed the control. Bampidis et al. (17) reported that thyme essential oil (10 mg/kg of live



weight/day) or neomycin sulfate (10 mg/kg of live weight/day) was orally administered to calves with diarrhea for treatments. It was reported that there was no difference between the thyme essential oil and the antibiotic group in terms of the number of days with diarrhea, diarrhea severity and mortality. Ünlü and Erkek (18) reported that the daily addition of 250 mg of oregano oil to full-fat milk of the calves caused a significant reduction in the total number of coliforms in the stool. In another study (19), the weaning age of suckling Holstein calves receiving control diet + 1.5% of medical plant mixture and 1.5% of medical plant mixture + 2 g probiotic showed a higher plasma antioxidant activity. Furthermore, it was suggested that 1.5% of medical plant mixture in calves' starter feed could improve performance and the immune system and also reduce the weaning age of calves.

In a study (20), sixty-six Holstein dairy calves were divided into one of six starters supplemented with: control, monensin (30 mg/kg), thyme (23 g/kg), celery (23 g/kg), eucalyptus (23 g/kg) and phytogetic feed additive containing essential oils (3 g/kg). Starter intake tended to be the highest in calves fed phytogetic feed additive containing essential oils and thyme; intermediate in calves fed control, monensin and eucalyptus; and the lowest in those fed celery. During the post-weaning period, no differences were observed among the treatments in growth, faecal score, rumen pH or ammonia-N concentration. Compared to calves fed control and monensin, those fed the plants or phytogetic feed additive containing essential oils tended to recorded higher molar proportions of acetate and butyrate, and the acetate: propionate ratio. It was also reported that three herbs and phytogetic feed additive containing essential oils were capable of modulating some rumen fermentation parameters and blood metabolites as well as eucalyptus may potentially be a better alternative to monensin for improving post-weaning performance. In another study (21), a total of 18 Murrah buffalo calves were equally divided into three groups as the control, whereas the T1 and T2 groups were supplemented with garlic powder at the dose rate of 250 and 300 mg per kg body weight, respectively. The results of the experiment revealed a significant improvement in the overall feed intake, body weight gain, average daily gain, body condition score and feed conversion efficiency in garlic supplemented buffalo calves (T1 and T2) compared with the control group, while the difference between T1 and T2 groups was not significant. Tapki et al. (22) investigated the potential use of oregano essential oil as a milk additive for reducing weaning age, promoting calf growth, and improving blood parameters and general health status of Holstein Friesian calves. Whole milk was supplemented with 0 (control), 100 mg/L (OreganoLow) and 150 mg/L (OreganoHigh) oregano essential oil. OreganoLow calves had better growth performance and earlier weaning age compared to control and OreganoHigh calves.

## **Conclusion**

Because essential oils have the potential as natural growth promoters in calf nutrition, the addition of essential oils and their mixtures to calves' diets will benefit calf health and performance. Since the information about the use of essential oils in calf nutrition is limited, detailed studies are still required to clarify their effects on calf performance and general health.

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## **Monitoring Nutritional Status of Buffaloes by Using Metabolic Profile Tests**

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### **Summary**

The total number of buffaloes in the world has been increasing over the years. The total number of buffaloes in the world according to the 2019 data of FAO is 204 million 342 thousand 419 heads. While the total number of milk buffaloes in the world is 69 million 924 thousand 520 heads, 68 million 328 thousand 457 heads of this are in Asia. In Turkey; The number of buffaloes was 184 thousand 192 heads in 2019, the number of milked buffaloes was 79 thousand 333 in 2019, and in the same year, Samsun province was shown as the province with the highest number of buffaloes in Turkey with 21 thousand 637 heads. The important features of the buffalo are that it can be breed even in regions with limited climatic and breeding conditions, its productivity characteristics are both meat and milk directions, its ability to digest poor quality forages better and more resistant to diseases. Buffalo milk is considered as a good quality milk in terms of nutritional physiology, which stands out with its high fat content and dry matter content, and the high energy it provides. For this reason, it has become a necessity for buffaloes to evaluate the relationship between diet and productivity in order to improve milk production. Metabolites measured by metabolic profile tests are parameters that provide practical application in animal nutrition in order to evaluate the productivity characteristics and nutrition of the animals. It has been preferred to use metabolic profile tests in many studies to evaluate the nutritional status of dairy cattle. Metabolic profile tests mainly include the measurement of blood glucose, urea, albumin, beta-hydroxybutyric acid (BHB), nonesterified fatty acids (NEFA) as well as minerals such as calcium, magnesium and phosphorus. The use of metabolic profile tests in evaluating the diet is emerging as an accurate method along with the environmental factors, body condition and the evaluation of nutrition. Recent studies suggest that milk metabolite profiles should be preferred because there is a high correlation between milk and blood metabolites, milk samples can be collected more easily and they do not have an invasive effect on the animal. In this presentation, the issue of using metabolic profile tests in the monitoring nutritional status of buffaloes is discussed.

**Keywords:** Buffalo, metabolic profile test, nutrition, productivity

## **Mandalarda Beslenmenin Metabolik Profil Testleri ile Kontrolü**

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### **Özet**

Dünyada toplam manda sayısı yıllar geçtikçe artış göstermektedir. Dünyada FAO'nun 2019 yılı verilerine toplam manda sayısı 204 milyon 342 bin 419 baştır. Dünyada toplam sağılan manda sayısı 69 milyon 924 bin 520 baş iken bunun 68 milyon 328 bin 457 başı Asya'da bulunmaktadır. Türkiye'de ise; manda sayısı 2019 yılında 184 bin 192 baştır, sağılan manda sayısı ise 2019 yılında 79 bin 333 baş olup, yine aynı yılda Samsun ili 21 bin 637 baş manda varlığı ile Türkiye'de en çok manda sayısına sahip il olarak gösterilmiştir. İklim ve yetiştirme şartlarının kısıtlı olduğu bölgelerde dahi yetiştirilebilmesi ile verim özelliklerinin et ve süt yönünde olması, kalitesiz kaba yemleri daha iyi değerlendirme yeteneği ve hastalıklara karşı daha dirençli olması, mandanın önemli özellikleridir. Manda sütü, içerdiği yüksek yağ oranı ve kuru madde miktarı, beraberinde sağlamış olduğu yüksek enerji ile ön plana çıkan, beslenme fizyolojisi açısından kaliteli bir süt olarak değerlendirilir. Bu nedenle, süt veriminin iyileştirilmesi amacıyla rasyon ve verimlilik ilişkisinin değerlendirilmesi mandalar için bir zorunluluk haline gelmiştir. Metabolik profil testleri ile ölçülen metabolitler, hayvanların verim özellikleri ve beslenmesini değerlendirmek amacıyla hayvan beslemede uygulama kolaylığı sağlayan parametrelerdir. Süt sığırlarının beslenme durumlarını değerlendirmek için birçok çalışmada metabolik profil testlerinin kullanılması tercih edilmiştir. Metabolik profil testleri temel olarak kan glukoz, üre, albümin, beta-hidroksibutirik asit (BHB), esterleşmemiş yağ asitlerinin (NEFA) ve ayrıca kalsiyum, magnezyum ve fosfor gibi minerallerin ölçümünü kapsar. Çevresel faktörler, vücut kondisyonu ve beslenmenin değerlendirilmesi ile birlikte rasyonun değerlendirilmesinde metabolik profil testlerinin kullanılması doğru bir yöntem olarak ortaya çıkmaktadır. Son yıllarda yapılan çalışmalar süt ile kan metabolitleri arasında yüksek bir korelasyon olduğu, süt numunelerinin daha kolay toplanabilmesi ve hayvana invaziv etkisi olmaması nedeniyle süt metabolit profillerini tercih edilmesini önermektedir. Bu bildiride mandalarda beslenmenin kontrolünde metabolik profil testlerinin kullanılması konusu tartışılmıştır.

**Anahtar kelimeler:** Beslenme, manda, metabolik profil testleri, verimlilik

**A Case of Descemetocele Accompanied by Reflex Uveitis in a Cat**

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**Summary**

Descemetocele is a corneal defect characterized by the exposure of the Descemet membrane, as a result of deep corneal ulceration. Descemet is a thin membrane and descemetocele may perform risk of perforation in the eye. The aim of this report is to evaluate descemetocele clinically and to share the treatment outcomes. A 6-month-old, male, mix cat was brought to ophthalmology unit with complaints of pain, itching and purulent discharge in his left eye. In clinical examination, there was also severe conjunctivitis and corneal ulcer. An approximately 1.5 mm diameter descemetocele was seen in the center of the deep ulceration covering almost the entire cornea. There was also a severe reflex uveitis. Tobramycin, cyclopentolate HCl, acetylcysteine eye drops were administered topically and amoxicillin was given orally. Tobramycin was changed with netilmicin on the 10th day. On the 17th day, with observation of rapid recovery, netilmicin and cyclopentolate administration stopped and hyaluronic acid eye drop was begun. After one week, fluorescein staining was negative and dexamethasone eye drop was started for 10 days. During this process, hyaluronic acid was continued. In conclusion, descemetocele, which affects a wide area of the corneal surface, was accompanied by uveitis, a dangerous inflammatory condition in this case. The cyclopentolate ability to suppress inflammation mediators, also suppressed uveitis with controlled use of it during treatment. It is pleasing that a very small scar tissue remains in the cornea with the successful and controlled treatment of these two severe conditions that can cause serious complications for the eye.

**Keywords:** Cornea, ulcer, uveitis

## Bir Kedide Refleks Uveitisin Eşlik Ettiği Desemetosel Olgusu

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### Özet

Desemetosel, derin kornea ülserinin bir sonucu olarak Desemet membranının açığa çıkması ile karakterize bir kornea defektidir. Desemet membran oldukça ince bir membrandır ve desemetosel bulunan hastalarda gözde perforasyon şekillenebilir. Bu olgu sunumunda amaç, desemetosel bulunan bir gözün klinik açıdan değerlendirilerek, sağaltım sonuçlarının paylaşılmasıdır. Sol gözünde ağrı, kaşıntı ve purulent akıntı şikayeti ile hastanemiz oftalmoloji bölümüne getirilen 6 aylık, erkek, melez kedinin yapılan klinik muayenesinde, şiddetli konjunktivitis ve kornea ülseri olduğu görüldü. Korneanın neredeyse tamamını kaplayan derin ülser alanın merkezinde yaklaşık 1,5 mm çaplı desemetosel alan dikkati çekti. Korneanın periferindeki sağlam doku kısmından göz içi muayenesi yapıldığında refleks uveitis olduğu görüldü. Sağaltımda yakalık ile kaşıma kontrolü sağlanan hastada, tobramisin, siklopentolat HCl, asetilsistein göz damlaları topikal olarak verilirken, sistemik amoksisilin uygulandı. Tobramisin göz damlası 10. gün sonunda netilmisin göz damlası ile değiştirildi. Sağaltımın 17. günü ülser alandaki hızlı gerileme sonrasında netilmisin ve siklopentolat HCl kesilerek, asetilsisteine hyaluronik asit göz damlası eklendi. Bir hafta sonra floresein boya ile yapılan muayenede korneanın epitel katmanının tamamen kapandığı görüldü ve asetilsistein göz damlası kesildi. Kornea merkezindeki vaskülarize alan için 10 gün süreyle deksametazon göz damlası başlandı. Bu süreçte hyaluronik asit göz damlasına devam edildi. Sonuç olarak, bu olguda kornea yüzeyinde geniş bir alanda etkili olan desemetosele, tehlikeli bir yangısal durum olan uveitis eşlik etmiştir. Siklopentolatın yangı mediyatörlerini baskılama özelliği, sağaltım sırasında kontrollü kullanımı ile uveitisi de baskılamıştır. Göz için ciddi komplikasyonlar oluşturabilecek bu iki şiddetli durumun başarılı bir şekilde ve kontrollü sağaltımıyla korneada oldukça küçük bir skar dokunun kalması memnuniyet vericidir.

**Anahtar kelimeler:** Kornea, uveitis, ülser

## **The Importance of Amino Acids in The Metabolizable Protein System in Dairy Cattle**

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### **Summary**

Proteins provide amino acids (AA) required for the maintenance of vital functions, reproduction, growth and lactation. Non-ruminant animals need AA in their diet. Since ruminants have the ability to synthesize AA and protein, they can use other non-protein (NPN) nitrogen sources. This is due to microorganisms exist in their rumen. In addition, ruminants have a different nitrogen mechanism. In cases where the amount of nitrogen in the diet is low, large amounts of urea return to the rumen and can be used by microorganisms in the rumen. In non-ruminant animals, urea is excreted in the urine. The last of the 20 AA found in nature, threonine, which is an essential AA, was discovered in 1935 and it was revealed that it has a serious importance for the AA requirement of animals. Although it is known that dairy cattle require EAA for a certain period after this research, animal nutritionists have continued their studies on the basis that EAA comes from microbial protein synthesized in the rumen and undegradable feed protein (RUP) in the rumen. The benefits of having a sufficient amount of limiting AA in metabolizable protein have taken many years, especially the importance of milk protein production and the EAA providing potential of RUP. Recent studies have shown that cows in transition period can benefit most from balancing restrictive AA. In addition, it has long been known that the most restrictive forms of ruminally protected AA will be needed to stabilize AA. Attempts to develop rumen protected methionine supplements; The determination of methionine as the first restrictive AA in sheep in the 1960s and indirect evidence showed that it may be the first restrictive AA in lactating dairy cattle. In this paper, the importance of amino acids in the metabolizable protein system in dairy cattle, essential amino acids and studies on the limiting characteristic of these amino acids are discussed.

**Keywords:** Amino acid, dairy cattle, essential amino acid, metabolizable protein system



## Süt Sığırlarında Metabolize Edilebilir Protein Sisteminde Amino Asitlerin Önemi

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### Özet

Hayati fonksiyonların sürdürülebilmesi, reproduksiyon, büyüme ve laktasyon için gereken amino asitleri(AA) proteinler sağlar. Ruminant olmayan hayvanlar rasyonlarında AA ihtiyaç duyarlar. Ruminantlar AA ve protein sentezlemek gibi bir özelliğe sahip olduklarından protein yapısında olmayan(NPN) diğer azot kaynaklarını kullanabilirler. Bunun nedeni rumenlerinde bulunan mikroorganizmalardır. Bunlara ek olarak ruminantlar farklı bir azot mekanizmasına sahiptir. Rasyondaki azot miktarının düşük olduğu durumlarda, büyük miktarda üre rumene geri döner ve rumende mikroorganizmalar tarafından kullanılabilirler. Ruminant olmayan hayvanlarda üre idrar ile atılır. Doğada en fazla bulunan 20 AA'den sonuncusu esansiyel bir AA olan treonin 1935 yılında keşfedilmiş ve hayvanların AA gereksinimi için ciddi bir öneme sahip olduğu ortaya konmuştur. Her ne kadar bu araştırmadan sonraki süreçte belirli bir süre süt sığırlarının EAA'ı gerektirdiği bilinmesine rağmen, hayvan beslemeciler EAA'in rumende sentezlenen mikrobiyal protein ve rumende parçalanmayan yem proteininden(RUP) geldiği temeli ile çalışmalarını devam ettirmişlerdir. Metabolize edilebilir proteinde yeterli miktarda sınırlayıcı AA bulunmasının faydaları özellikle süt proteini üretimindeki önemi ve RUP'un EAA sağlama potansiyelinin anlaşılması uzun yıllar almıştır. Son araştırmalar sınırlayıcı AA'lerin dengelenmesinden en fazla geçiş dönemindeki ineklerin yararlanabileceğini göstermiştir. Buna ek olarak AA dengelenmesi için ruminal olarak korunmuş en sınırlayıcı AA formlarına ihtiyaç olacağı uzun zamandır bilinmektedir. Rumen korumalı metiyonin takviyesi geliştirme girişimleri; 1960'larda metiyoninin koyunlarda ilk sınırlayıcı AA olduğunun belirlenmesi ve dolaylı kanıtlarla laktasyondaki süt sığırlarının ilk sınırlayıcı AA olabileceğini göstermiştir. Bu bildiride süt sığırlarında metabolize edilebilir protein sisteminde amino asitlerin önemi, esansiyel amino asitler ve bu amino asitlerin sınırlayıcı özellikleri konusunda yapılan çalışmalar tartışılmıştır.

**Anahtar kelimeler:** Amino asit, süt sığırları, esansiyel amino asit, metabolize edilebilir protein sistemi

**Biochemical Tumor Markers in Veterinary Medicine**

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**Summary**

Tumor markers are substances, usually proteins that are produced by the body in response to cancer growth or by the cancer tissue itself. Several biochemical events are performed in tumor formation and during changing of normal cell to malign cell. Some compounds that find somewhat or not find in normal cells are expressed during malign transformation of cells and can be detected in body fluids. In tumor markers, Tumor binding antigens, specific proteins, miRNAs, enzymes, some metabolites and oncogenes can be counted. An ideal tumor marker should be highly sensitive, specific, accurate, reliable and easily assayable. Detection can be done either in tissue or in body fluids like ascitic or pleural fluid or serum. Clinical uses can be broadly classified into four groups: screening and early detection, diagnostic confirmation, prognosis and prediction of therapeutic response and monitoring disease and recurrence. In addition to, tumor markers should be both sensitive and specific for the detection of cancer, to minimize both false-positive and false-negative test findings, and they should use methodology that is minimally invasive to increase acceptance by animal owners. Today, thanks to scientific developments in the field of human and veterinary medicine, a large number of biomarkers can be detected from samples such as blood, urine, and tissue. Interest in biomarkers also arises in veterinary medicine, where there is enormous potential for their development and application. The use of biochemical markers in the veterinary field is increasing, and for this purpose, many markers recommended to be used in veterinary medicine have been identified.

**Keywords:** Cancer, malign, tumor, tumor marker

## Veteriner Hekimlikte Biyokimyasal Tümör Belirteçleri

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### Özet

Tümör belirteçleri, vücut tarafından kanser büyümesine yanıt olarak veya kanser dokusunun kendisi tarafından üretilen ve genellikle protein yapısındaki maddelerdir. Tümör oluşumunda, normal hücrenin malign hücre haline geçişi sırasında birçok biyokimyasal olay gerçekleşmektedir. Normal bir hücrede az bulunan veya hiç bulunmaması gereken bazı maddeler, hücrelerin maligniteye dönüşümü esnasında üretilerek vücut sıvılarında tespit edilebilirler. Tümör belirteçleri, tümöral yapıya bağlı antijenler, spesifik proteinler, mikro RNA'lar, enzimler, bazı metabolitler ve onkogenler olarak sayılabilir. İdeal bir tümör belirteci, oldukça hassas, spesifik, doğru, güvenilir ve kolayca analiz edilebilir olmalıdır. Tespit dokuda, ascitesli veya pleural vücut sıvılarında ya da kan serumunda yapılabilir. Klinik kullanım genel olarak dört gruba ayrılır: tarama ile erken teşhis, tanısal doğrulama, prognoz ile tedaviye yanıtın tahmini ve hastalık nüksünün izlenmesi. Ayrıca tümör belirteçleri, hem yanlış pozitif hem de yanlış negatif test bulgularını en aza indirmek için kanserin saptanmasına hem duyarlı hem de spesifik olmalı ve hayvan sahipleri tarafından kabulünü artırmak için minimal invaziv metodoloji kullanılmalıdır. Günümüzde insan ve veteriner hekimliği alanındaki bilimsel gelişmeler sayesinde kan, idrar, doku gibi örneklerden çok sayıda biyobelirteç tespit edilebilmektedir. Biyobelirteçlere ilgi, bunların geliştirilmesi ve uygulanması için muazzam bir potansiyelin bulunduğu veteriner hekimliğinde de ortaya çıkmaktadır. Veteriner sahada biyokimyasal belirteç kullanımı giderek artmakta ve bu amaçla veteriner hekimliği alanına kullanımının kazandırılması önerilen çok sayıda biyobelirteç tanımlanmıştır.

**Anahtar kelimeler:** Kanser, malign, tümör, tümör belirteci

**Contraception Methods in Male Animals; Reversible Inhibition of Sperm Under Guidance (RISUG)**

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**Summary**

Controlling reproduction in animals is the most accurate strategy that can be applied in order to eliminate the problems caused by the uncontrolled increase in the animal population. There are different methods of contraception that have been continuously developed from past to present to temporarily or permanently stop reproductive activities. Permanent prevention of reproductive activities by removing male reproductive organs from the body with various operation techniques is called surgical contraception. This method has revealed the need to develop different techniques due to its disadvantages such as being irreversible, complications that may occur after the operation, and the wishes of the patient owners. In this direction, a search for a new method has been started non-surgical contraception methods such as use of hormones, intratesticular toxic agents, and immunocontraception, due to repeated dose applications and long-term undesirable side effects. Reversible Inhibition of Sperm Under Guidance (RISUG), which emerged as a result of research and is under development, is a copolymer of Styrene Maleic Anhydride (SMA) dissolved in Dimethyl Sulfoxide (DMSO) to form a gel. This gel is injected into the lumen of the vas deferens in male animals in a controlled manner, causing partial occlusion of the vas deferens. RISUG causes infertility by causing damage to the plasma membrane of spermatozoa, causing disruption of their integrity and releasing the enzymes necessary for fertilization of oocytes before fertilization. It is thought that this new method of contraception, which is reversible, non-invasive and does not contain any side effects, will make significant contributions to the control of reproduction, with further studies to be conducted on the basis of individuals and species.

**Keywords:** Contraception, fertilization, reproductive control, RISUG

## Erkek Hayvanlarda Kontrasepsiyon Yöntemleri; Rehberlik Altında Spermin Geri Dönüşümlü İnhibisyonu (RISUG)

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### Özet

Hayvanlarda üremenin denetlenmesi kontrolsüz artan hayvan popülasyonunun neden olduğu sorunları giderebilmek amacıyla uygulanabilecek en doğru stratejidir. Üreme faaliyetlerinin geçici ya da kalıcı olarak durdurulması amacıyla geçmişten günümüze kadar sürekli olarak geliştirilen farklı kontrasepsiyon yöntemleri var olmuştur. Erkek reproduktif organların çeşitli operasyon teknikleriyle vücuttan uzaklaştırılarak üreme faaliyetlerinin kalıcı olarak engellenmesi cerrahi kontrasepsiyon yöntemi olarak adlandırılır. Bu yöntem geri dönüşümsüz olması, operasyon sonrası ortaya çıkabilecek komplikasyonlar ve hasta sahiplerinin istekleri gibi dezavantajları nedeniyle farklı ve geri dönüşümlü tekniklerin geliştirilmesi ihtiyacını ortaya çıkarmıştır. Bu doğrultuda geliştirilen hormon kullanımı, intratestiküler toksik ajanların kullanımı, immünokontrasepsiyon gibi cerrahi olmayan kontrasepsiyon yöntemlerinin ise tekrarlayan doz uygulamaları ve uzun vadede istenmeyen yan etkileri nedeniyle yeni bir yöntem arayışı içerisine girilmiştir. Yapılan araştırmalar neticesinde ortaya çıkan ve geliştirilme aşamasında olan *Reversible Inhibition of Sperm Under Guidance* (RISUG), bir jel oluşturmak suretiyle Dimetil Sülfoksit (DMSO) içinde çözülmüş Stiren Maleik Anhidrit (SMA) kopolimeridir. Bu jel erkek hayvanlarda vaz deferensin lümenine kontrollü bir şekilde enjekte edilerek vaz deferensin kısmi tıkanmasına neden olur. RISUG spermatozoonların plaza membranında hasara neden olarak bütünlüğünün bozulmasına ve oositlerin fertilizasyonu için gerekli olan enzimlerin fertilizasyondan önce salınmasına neden olarak infertilite oluşturmaktadır. Birey ve tür bazında yapılacak daha fazla çalışma ile birlikte geri dönüşümlü, invaziv olmayan ve herhangi bir yan etki içermeyen bu yeni kontrasepsiyon yönteminin üremenin denetlenmesi konusuna önemli katkılar sağlayacağı düşünülmektedir.

**Anahtar kelimeler:** Fertilizasyon, kontrasepsiyon, üremenin denetlenmesi, RISUG

**Effect of Naringin on Oxidative Renal Cell Damage *In Vitro*\***

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**Summary**

Oxidative stress plays an important role in kidney diseases and suppression of oxidative stress provides success in prophylaxis and treatment of kidney diseases. Naringin, a flavone found in citrus fruits, some vegetables and herbs, has antioxidant, antiapoptotic and anti-inflammatory effects. The effect of naringin on lipopolysaccharide (LPS)-induced oxidative stress in renal cells is unknown. The aim of this study is to determine the effect of naringin on oxidative renal cell damage *in vitro*. In study, four different bovine kidney cell line groups were composed as negative control group (nc; no treatment was made), naringin group (nar; 5 µg/ml naringin for 12 hours), LPS group (lps; 10 µg/ml LPS for 4 hours) and naringin+LPS group (nar+lps; 5 µg/ml naringin for 12 hours+10 µg/ml LPS for 4 hours). The antioxidant effect of naringin on kidney cell line was evaluated with malondialdehyde (MDA), glutathione, glutathione peroxidase (GPx) and superoxide dismutase (SOD) spectrophotometric measurements. MDA level in nc, nar, lps and nar+lps groups were measured as 0.96±0.04 nmol/ml, 0.76±0.07 nmol/ml, 6.42±0.33 nmol/ml and 2.67±0.31 nmol/ml, respectively. While MDA level in the kidney cell line increased following LPS exposure, it was determined that naringin inhibited this effect (p<0.05). Glutathione level was found as 3.10±0.07 nmol/ml, 3.48±0.13 nmol/ml, 0.72±0.06 nmol/ml ve 1.65±0.07 nmol/ml in the nc, nar, lps and nar+lps groups, respectively. GPx activity was determined as 1.26±0.10 U/ml, 1.41±0.16 U/ml, 0.32±0.06 U/ml and 0.97±0.08 U/ml in the nc, nar, lps and nar+lps groups, respectively. SOD activity was measured as 2.66±0.28 U/ml, 2.87±0.11 U/ml, 0.97±0.13 U/ml, and 2.09±0.08 U/ml in nc, nar, lps and nar+lps groups, respectively. Naringin has increased the reduced GPx and SOD activities and glutathione level from LPS exposure (p<0.05). These findings showed that naringin has therapeutic potential against oxidative stress-induced damage in renal cells by exhibiting antioxidant effect.

**Keywords:** *In vitro*, lipopolysaccharide, naringin, oxidative stress, renal cell

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## Naringinin *In Vitro* Oksidatif Böbrek Hücre Hasarına Etkisi\*

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### Özet

Oksidatif stres, böbrek hastalıklarında önemli rol oynamakta ve böbrek hastalıklarının profilaksisinde ve tedavisinde oksidatif stresin baskılanması başarı sağlamaktadır. Turunçgillerde, bazı sebzelerde ve bitkilerde bulunan bir flavon olan naringin, antioksidan, antiapoptotik ve antienflamatuar etkilere sahiptir. Naringinin böbrek hücrelerinde gelişen oksidatif strese etkisi bilinmemektedir. Bu çalışmanın amacı, naringinin oksidatif böbrek hücre hasarı üzerindeki etkisinin *in vitro* belirlenmesidir. Çalışmada, negatif kontrol grubu (nk; herhangi bir uygulama yapılmadı), naringin grubu (nar; 12 saat 5 µg/ml naringin), LPS grubu (lps; 4 saat 10 µg/ml LPS) ve naringin+LPS grubu (nar+lps; 12 saat 5 µg/ml naringin+4 saat 10 µg/ml LPS) olmak üzere dört farklı sığır böbrek hücre hattı grubu oluşturuldu. Naringinin böbrek hücre hattındaki olası antioksidan etkisi malondialdehit (MDA), glutasyon, glutasyon peroksidaz (GPx) ve süperoksit dismutaz (SOD) spektrofotometrik ölçümleriyle değerlendirildi. MDA düzeyi; nk, nar, lps ve nar+lps gruplarında sırasıyla 0,96±0,04 nmol/ml, 0,76±0,07 nmol/ml, 6,42±0,33 nmol/ml ve 2,67±0,31 nmol/ml ölçüldü. Böbrek hücre hattında MDA düzeyi, LPS maruziyetini takiben artarken, naringinin bu etkiyi inhibe ettiği belirlendi (p<0,05). Glutasyon düzeyi; nk, nar, lps ve nar+lps gruplarında sırasıyla 3,10±0,07 nmol/ml, 3,48±0,13 nmol/ml, 0,72±0,06 nmol/ml ve 1,65±0,07 nmol/ml bulundu. GPx aktivitesi; nk, nar, lps ve nar+lps gruplarında sırasıyla 1,26±0,10 U/ml, 1,41±0,16 U/ml, 0,32±0,06 U/ml ve 0,97±0,08 U/ml olarak belirlendi. SOD aktivitesi; nk, nar, lps ve nar+lps gruplarında sırasıyla 2,66±0,28 U/ml, 2,87±0,11 U/ml, 0,97±0,13 U/ml ve 2,09±0,08 U/ml ölçüldü. Naringinin, LPS maruziyeti ile azalan glutasyon düzeyini, GPx ve SOD aktivitelerini artırdığı anlaşıldı (p<0,05). Bu bulgular, naringinin, antioksidan etki sergileyerek, böbrek hücrelerindeki oksidatif stres kaynaklı hasara karşı terapötik potansiyele sahip olduğunu gösterdi.

**Anahtar kelimeler:** Böbrek hücresi, *in vitro*, lipopolisakkarit, naringin, oksidatif stres

\*Bu çalışma, PYO.VET.1904.18.002 numaralı proje ile Ondokuz Mayıs Üniversitesi Proje Yönetimi Ofisi tarafından desteklenmiştir.

## **Biochemical Parameters in Colon Cancer**

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### **Summary**

Colorectal cancer (CRC) is one of the most common cancer types in our country and in the world. Today, it takes the second place in the list of diseases that cause death in many countries. The etiology of CRC is still not fully understood, but the increasing incidence of CRC in recent years can be explained by aging of the population, modification of eating habits, genetic and epigenetic variations. Biochemical parameters play an important role in the detection and treatment of patients with colorectal cancer. Biochemical parameters also have the potential to change treatment algorithms by selecting appropriate chemotherapeutic drugs for a wide range of patients. In recent years, determination of biochemical parameters in tumors has been widely used. Serum carcinoembryonic antigen (CEA) is the most widely used parameter in various cancers, including CRC, for tumor diagnosis and monitoring. Other biochemical parameters such as cancer antigen CA19-9, cancer antigen CA125, cancer antigen CA72-4, serum ferritin (SF) have been used as indicators for the diagnosis of CRC, postoperative monitoring, and monitoring of treatment effects. MicroRNAs (miRNAs) and genes are a type of non-coding RNAs that regulate the expression of target genes and play a role in cancer formation and development. Studies have confirmed that miRNAs and five hub genes (PPARGC1A, COL1A1, SYT1, 41 PGR and KCNB1) have prognostic value in CRC. Thanks to the technological developments developing better techniques day by day, the introduction of new and effective biochemical parameters will provide better results. In this study, the biochemical parameters of CRC and its effects on CRC were investigated.

**Keywords:** Biochemical mechanisms, cancer mechanism, colorectal cancer



## Kolon Kanserinde Biyokimyasal Parametreler

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### Özet

Kolorektal kanser (KRK) ülkemizde ve dünyada sık görülen kanser türlerinden biridir. Günümüzde birçok ülkede ölüme neden olan hastalıklar sıralamasında ikinci sırayı almaktadır. KRK etiyojisi hala tam olarak anlaşılamamıştır, fakat son yıllarda KRK'in artan insidansı, nüfusun yaşlanması, yeme alışkanlıklarının modifikasyonu, genetik ve epigenetik varyasyonlarla açıklanabilmektedir. Biyokimyasal parametreler kolorektal kanserli hastaların tespiti ve tedavisinde önemli bir rol oynamaktadır. Biyokimyasal parametreler ayrıca geniş bir hasta yelpazesinde uygun kemoterapötik ilaçları seçerek tedavi algoritmalarını değiştirme potansiyeline de sahiptir. Son yıllarda tümörlerde biyokimyasal parametrelerin tespiti yaygın olarak kullanılmaktadır. Serum karsinoembriyonik antijen (CEA), tümör teşhisi ve izleme için KRK dahil olmak üzere çeşitli kanserlerde en yaygın olarak kullanılan parametredir. Kanser antijeni CA19-9, kanser antijeni CA125, kanser antijeni CA72-4, serum ferritin (SF) gibi diğer biyokimyasal parametreler de KRK teşhisi, operasyon sonrası gözlemi için göstergeler olarak ve tedavi etkilerinin izlenmesinde kullanılmaktadır. MikroRNA'lar (miRNA'lar) ve genler, hedef genlerin ekspresyonunu düzenleyen, kanser oluşumunda ve gelişiminde rol oynayan bir tür kodlamayan RNA'lardır. Çalışmalar miRNA'lar ve beş hub geninin (PPARGC1A, COL1A1, SYT1, 41 PGR ve KCNB1) KRK'de prognostik değere sahip olduğunu doğrulamıştır. Teknolojik gelişmelerin her geçen gün daha iyi teknikler geliştirmesi sayesinde yeni ve etkili biyokimyasal parametrelerin kullanıma girmesi daha iyi sonuçlar alınmasını sağlayacaktır. Bu çalışmada, KRK'in biyokimyasal parametreleri ve KRK üzerindeki etkileri incelenmiştir.

**Anahtar kelimeler:** Biyokimyasal mekanizmalar, kanser mekanizması, kolorektal kanser

## Therapeutic Effects of Astaxanthin

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### Summary

It is aimed to reveal the antioxidant and anti-inflammatory effects and potential therapeutic uses of astaxanthin, which gives the natural characteristic color of aquatic animals. Astaxanthin (3,3'-dihydroxy- $\beta$ ,  $\beta$ -carotene-4,4'-dione) is a carotenoid that offers the typical color wild salmonid. It has biological functions in aquatic animals, such as protection from UV light and oxidation of fatty acids, immune response, pigmentation, communication, reproductive behavior, and improvement in reproduction. Astaxanthin is also found in birds such as flamingos and quails. This pigment has many pharmacological activities such as antioxidant, anticarcinogenic, hepatoprotective, antidiabetic and anti-inflammatory effects. Astaxanthin protects against oxidative damage through different mechanisms, such as neutralizing singlet oxygen, scavenging radicals to prevent chain reactions, protecting membrane structure by inhibiting lipid peroxidation, enhancing immune system function, and regulating gene expression. Astaxanthin has shown curative effects in cardiovascular diseases, nephrotoxicity, cancer, neuronal disorders, wound healing, and sepsis. Although the studies in cats and dogs are limited, it has been shown that astaxanthin increases the immune response. Although it is a subject that has been studied on experimental animals to determine its effects on human health, it is limited information on the impact on species other than aquatic animals. Antioxidant, anticarcinogenic, anti-inflammatory effects of astaxanthin have been determined. It is recommended to increase the studies on this pigment to benefit from its possible treatment and protective impact and gain the use of this antioxidant carotenoid in veterinary medicine with scientific studies.

**Keywords:** Anti-inflammatory effect, antioxidant effect, astaxanthin, therapeutic agent

## Astaksantinin Terapötik Etkileri

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### Özet

Bu çalışma ile, sucul hayvanların doğal karakteristik rengini veren bir pigment olan astaksantin antioksidan ve antienflamatuar etkileri ile potansiyel terapötik kullanımlarını ortaya koymak amaçlanmaktadır. Astaksantin (3,3'-dihidroksi-  $\beta$ ,  $\beta$  -karoten-4,4'-diyol), yabani salmonidlere tipik rengini veren bir karotenoiddir. Sucul hayvanlarda, UV ışıktan ve yağ asitlerini oksidasyondan koruma, immun cevap, pigmentasyon, iletişim, üreme davranışı ve üremede iyileştirme gibi biyolojik fonksiyonları bulunmaktadır. Astaksantin, suculların yanısıra flamingolar ve bıldırcınlar gibi kuşlarda da bulunmaktadır. Bu pigmentin, antioksidan, antikanserojenik, hepatoprotektif, antidiyabetik ve antienflamatuar etkileri gibi birçok farmakolojik aktivitesi bulunmaktadır. Astaksantin, singlet oksijenin nötralize edilmesi, zincir reaksiyonlarını önlemek için radikallerin temizlenmesi, lipid peroksidasyonunun inhibisyonu ile membran yapısının korunması, bağışıklık sistemi fonksiyonunun artırılması ve gen ekspresyonunun düzenlenmesi gibi farklı mekanizmalar yoluyla oksidatif hasara karşı bir koruma görevi görür. Astaksantin, kardiyovasküler hastalıklarda, nefrotoksisitede, kanserde, nöronal bozukluklarda, yara iyileşmesinde ve sepsiste iyileştirici etkileri olduğu ortaya konulmuştur. Kedi ve köpeklerde yapılan çalışmalar sınırlı olmakla birlikte immun yanıtı arttırdığı gösterilmiştir. Astaksantin insan sağlığına etkilerini belirlemek, deney hayvanları üzerinde çalışılan bir konu olsa da sucul hayvanlar dışında kalan türlerde etkileri hakkında bilgiler sınırlıdır. Astaksantin, antioksidan, antikanserojen, antienflamatuar etkileri belirlenmiş olup bu pigment üzerine çalışmalar artırılarak olası tedavi ve koruyucu etkilerinden faydalanımın artırılması ve bu antioksidan karotenoidin yapılacak bilimsel çalışmalar ile veteriner hekimliği alanında kullanımının kazandırılması önerilmektedir.

**Anahtar kelimeler:** Antienflamatuar etki, antioksidan etki, astaksantin, terapötik ajan

## The Effect of Hesperidin on Serum Insulin-Like Growth Factor-1 Level in Fructose Mediated Metabolic Syndrome Model\*

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### Summary

Insulin-like growth factor-1 (IGF-1) is a growth hormone that mediates cell growth, proliferation and differentiation. Due to its effects on carbohydrate and lipid metabolism, IGF-1 plays an important role in pathophysiology of metabolic syndrome. Maintaining physiological levels of IGF-1 has the potential to contribute to prophylaxis/treatment of metabolic syndrome. Current studies emphasize the importance of flavonoid-rich diet in the prevention, alleviation and treatment of metabolic syndrome and/or its components. Hesperidin, a flavonoid found in citrus fruits, has been scientifically proven to exhibit anti-hyperglycemic and anti-hyperlipidemic activities. The aim of this study is to evaluate the effect of hesperidin on serum IGF-1 level in fructose-mediated metabolic syndrome model. Six-week-old, male, 18 Wistar Albino rats were used in study. Control, metabolic syndrome (metS) and metabolic syndrome+hesperidin (metS+H) groups were composed as 6 rats in each group. Controls were fed standard rat chow and water *ad libitum*, while metS was fed standard chow with 20% fructose-added-drinking-water and metS+H was fed hesperidin-added chow food (1%, 10 g/kg) with 20% fructose-added-drinking-water, 10 weeks. Blood was obtained from tail vein of rats. The study protocol was approved by Gazi University Animal Experiments Local Ethics Committee (G.Ü.ET-16.064). Serum IGF-1 level was measured with rat-specific enzyme-linked immunosorbent analysis kit, following the procedure recommended by the manufacturer and samples were analyzed in duplicate. Serum IGF-1 levels were determined as 271.5±10.9 ng/ml, 109.6±12.0 ng/ml and 202.7±14.3 ng/ml in the control, metS and metS+H groups, respectively. Serum IGF-1 was significantly decreased in rats exposed to high fructose compared to the controls ( $p<0.05$ ). Decreased serum IGF-1 level in rats exposed to high fructose was increased by hesperidin treatment ( $p<0.05$ ). The findings of this study showed that hesperidin increased low IGF-1 level in rat model of metabolic syndrome. It is predicted that these results will contribute to studies on treatment of metabolic syndrome.

**Keywords:** Fructose, hesperidin, insulin-like growth factor-1, metabolic syndrome

\*This study was supported by the Project Management Office of Ondokuz Mayıs University, with the PYO.VET.1901.17.015 project number.

## Fruktoz Aracılı Metabolik Sendrom Modelinde Hesperidinin Serum İnsülin Benzeri Büyüme Faktörü-1 Düzeyine Etkisi\*

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### Özet

İnsülin benzeri büyüme faktörü-1 (IGF-1), hücre büyümesine, çoğalmasına ve farklılaşmasına aracılık eden bir büyüme hormonudur. Karbonhidrat ve lipit metabolizmasındaki etkileri nedeniyle IGF-1 metabolik sendromun patofizyolojisinde önemli bir rol oynamaktadır. IGF-1'in fizyolojik seviyelerinin korunması metabolik sendromun profilaksisine/tedavisine katkı sağlama potansiyelindedir. Metabolik sendromun ve/veya bileşenlerinin engellenmesi, hafifletilmesi ve tedavi edilmesinde güncel çalışmalar, flavonoidden zengin diyetle beslenmenin önemine vurgu yapmaktadır. Turunçgillerde bulunan bir flavonoid olan hesperidinin, anti-hiperglisemik ve anti-hiperlipidemik aktiviteler sergilediği bilimsel olarak kanıtlanmıştır. Bu çalışmanın amacı, fruktoz aracılı metabolik sendrom modelinde hesperidinin serum IGF-1 düzeyine etkisini değerlendirmektir. Çalışmada 6 haftalık, erkek, 18 adet Wistar Albino rat kullanıldı. Her grupta 6 rat olarak, kontrol, metabolik sendrom (metS) ve metabolik sendrom+hesperidin (metS+H) grupları oluşturuldu. Kontrol grubu, 10 hafta *ad libitum* standart rat yemi ve içme suyu ile beslendi. metS; 10 hafta standart rat yemi+içme sularında %20 fruktoz ile beslendi. metS+H grubu; 10 hafta hesperidin ilaveli rat yemi (% 1, 10 g/kg yem)+içme sularında %20 fruktoz ile beslendi. Ratların kuyruk venasından kan alındı. Çalışma protokolü Gazi Üniversitesi Hayvan Deneyle Yerel Etik Kurulu tarafından onaylandı (G.Ü.ET-16.064). Serum IGF-1 düzeyi rata-özü enzim bağı immunosorbent analiz kitiyle üretici firmanın belirttiği prosedür takip edilerek ölçüldü ve örnekler çift analiz edildi. Serum IGF-1 düzeyinin, kontrol, metS ve metS+H gruplarında sırasıyla 271,5±10,9 ng/ml, 109,6±12,0 ng/ml ve 202,7±14,3 ng/ml olduğu belirlendi. Serum IGF-1, yüksek fruktoza maruz kalan ratlarda kontrol grubuna göre önemli ölçüde azaldı (p<0,05). Ratların yüksek fruktoza maruz kalmasına rağmen hesperidin takviyesi serum IGF-1'i artırdı (p<0,05). Bu çalışmanın bulguları, metabolik sendrom rat modelinde hesperidinin azalmış IGF-1 düzeyini artırdığını gösterdi. Bu sonuçların metabolik sendromun tedavisine yönelik çalışmalara katkı sağlayacağı öngörülmektedir.

**Anahtar kelimeler:** Fruktoz, hesperidin, insülin benzeri büyüme faktörü-1, metabolik sendrom

\*Bu çalışma, PYO.VET.1901.17.015 numaralı proje ile Ondokuz Mayıs Üniversitesi Proje Yönetimi Ofisi tarafından desteklenmiştir.

**Evaluation of Antigen Titer and Survival Rate in Dogs with Canine Parvovirus  
Enteritis\***

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**Summary**

Canine parvovirus (CPV) is a severe viral infection that affects especially 0-6 months aged dogs all over the world. If the disease is not treated, it causes 80% mortality. This study aimed to evaluate whether the CPV antigen titer is associated with disease severity and survival rate in animals infected with CPV. In the study, animals infected with CPV were grouped separately according to antigen titer and survival rates. The patients were grouped according to their antigen titres as COI <15 (n=13) and COI >15 (n=12). There was a statistically significant difference in platelet distribution width (PDW) values between the groups. In addition, dogs with CPV were grouped as living (n=19) and non-living (n=6) according to the survival, and there was no statistical difference between the evaluated parameters. As a result, it was concluded that the changes in PDW value may be related to the severity of the disease in CPV infections.

**Keywords:** Antigen titer, canine parvovirus, PDW, canine

\*This study was supported by Project Management Office of Ondokuz Mayıs University (Project No: PYO.VET.1904.21.001)

**Kanine Parvoviral Enteritisli Köpeklerde Antijen Titresinin ve Sağkalım Oranının Değerlendirilmesi\***

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**Özet**

Kanine parvovirus (KPV) bütün dünyada yaygın olarak görülen, özellikle 0-6 aylık köpekleri etkileyen ve tedavi edilmediği takdirde %80 oranında ölümlere sebep olabilen şiddetli bir viral enfeksiyondur. Bu çalışmanın amacı, KPV ile enfekte olan hayvanlarda KPV antijen titresinin hastalığın şiddeti ve sağkalım oranı ile ilişkili olup olmadığının değerlendirilmesidir. Çalışmada KPV ile enfekte olan hayvanlar antijen titresini ve sağkalım oranlarına göre ayrı ayrı gruplandırılarak değerlendirilmiştir. Hastalar antijen titrelerine göre COI <15 olan (n=13), COI>15 (n=12) şeklinde gruplandırılmıştır. Gruplar arasında istatistiksel olarak platelet dağılım değişikliği (PDW) değerleri arasında anlamlı bir fark tespit edilmiştir. KPV tespit edilmiş köpekler sağkalım oranına göre yaşayan (n=19) ve yaşamayan (n=6) olarak gruplandırılmış ve değerlendirilen parametreler arasında istatistiksel olarak bir fark tespit edilmemiştir. Sonuç olarak, PDW değerindeki değişikliklerin KPV enfeksiyonlarında hastalığın şiddeti ile ilişkili olabileceği sonucuna varılmıştır.

**Anahtar Kelimeler:** Antijen titresini, kanine parvovirus, PDW, köpek

\*Bu çalışma Ondokuz Mayıs Üniversitesi Proje Yönetim Ofisi tarafından desteklenmiştir (Proje No: PYO.VET.1904.21.001).

## **Bocavirus Infections**

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### **Summary**

Animal Bocaviruses have been known since 1960s. Firstly It was revealed that the virus was affecting dogs and cattle, but in recent studies, it has been reported that the virus could cause infection not only in dogs and cattle but also in pigs, cats, monkeys, and humans. This review has been prepared for the purpose of informing and compiling current data about Bocavirus Infections.

**Keywords:** Bocavirus, animals



## **Bocavirus Enfeksiyonları**

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### **Özet**

Hayvan Bocavirusları 1960'lı yıllardan bu yana bilinmektedir. Virusun ilk olarak köpek ve sığırları etkilediği ortaya konulmuştur ancak yakın zamana kadar yapılan çalışmalarda sadece köpek ve sığırlarda değil domuzlarda, kedilerde, maymunlarda, insanlarda da virusun enfeksiyona neden olduğu bildirilmiştir. Bu derleme Bocavirus Enfeksiyonları hakkında bilgilendirme ve güncel verileri derleme amacıyla hazırlanmıştır.

**Anahtar kelimeler:** Bocavirus, hayvanlar

**Evaluation of The Findings and Medical Treatment Protocol in A 4-Month-Old Belgian Malinois Dog Diagnosed with Intrahepatic Portosystemic Shunt**

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**Summary**

In this case report, an intrahepatic portocystmeic shunt (PSS) case, which had been detected in a 4-month-old female Belgian Malinois breed dog admitted to Erciyes University Veterinary Faculty Training and Research Hospital with complaints of loss of weight, weakness, anorexia, stillness, diarrhea that had started for one day, hypersalivation, loss of balance, running away to dark places and leaning its head on a place, was evaluated. In the anamnesis, it was reported that the patient was given puppy dog food, wet food, and home-made food. Previously, only developmental delay and inability to gain weight had complaints, while other complaints had been seen for 1 week. When it was first brought to the hospital, its live weight was 4.7 kilograms. No abnormal finding was observed in the hemogram examination. Among the serum biochemistry findings, albumin (1.8 g/dL), BUN (4,7 mg/dL), creatinine (0,25 mg/dL) and total protein (2.65 g/dL) were found to be low, while ammonia (256 µmol /L) and ALP (504 U/L) values were found to be quite high. Ammonium urate crystals were observed in urine sediment examination. Intrahepatic portosystemic shunt was detected in the ultrasonographic examination. In the first 5 days of treatment, 0.9% NaCl, 20 mg/kg vitamin B1, 2 mg/kg vitamin B6 (Nervit/Vetaş), 20 mg/kg vitamin C (Vitce/Sanovel), 50 ml electrolyte-amino acid (Duphalyte/Zoetis) and Furosemide (Diuril/Vetaş) at a dose of 2 mg/kg was given intravenously as a slow infusion. Additionally, 50 mg/kg metronidazole (Flagyl/Sanofi) twice a day, 10 ml lactulose (Osmolak/Biofarma) 3 times a day, and 20 mg/kg S-adenosyl methionine (SAM-e/Venatura) once a day were prescribed for 5 days oral use. The dog's owner was asked to end his usual diet. Protein diet (Hills's L/D) was recommended. The clinical development and biochemical parameters of the patient were followed up regularly for two months. At the last examination performed while preparing this presentation, it was observed that clinical symptoms improved and the dog's weight was 7.8 kg. In addition, serum ammonia (293 µmol/L), ALP (326 U/L), BUN (4.6 mg/dL), creatinine (0.25 mg/dL), albumin (2.17 g/dL), and total protein (3 ,52 g/dL) values were determined. Although the weight gain was good, the patient was referred for the operation because the biochemical parameters improved very slowly. In conclusion, in this case report, it was determined that clinical improvement could be achieved in an intrahepatic portosystemic shunt case with medical and diet treatment, but complete recovery could not be achieved without operative intervention.

**Keywords:** Belgian Malinois, dog, portosystemic shunt

## İntrahepatik Portosistemik Şant Teşhisi Konulan 4 Aylık Belçika Malinois Irkı Bir Köpekte Gözlenen Bulgular ve Medikal Tedavi Protokolünün Değerlendirilmesi

İlayda ELGEZDİ, Mehmet Akif RIHTIM, Gencay EKİNCİ, İlknur KARACA BEKDİK, Emre TÜFEKÇİ, Vehbi GÜNEŞ, Mehmet ÇİTİL, İhsan KELEŞ

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### Özet

Bu olgu sunumunda; Erciyes Üniversitesi, Veteriner Fakültesi, Eğitim ve Araştırma Hastanesine zayıflama, durgunluk, iştahsızlık, bir gündür başlayan ishal, hipersalivasyon, denge kaybı, karanlık yerlere kaçma ve başı bir yere dayama şikayetiyle gelen 4 aylık, dişi, Belçika Malinois ırkı bir köpekte saptanan intrahepatik portosistemik şant (PSS) vakası değerlendirildi. Anamnezde, hastaya yavru köpek maması, yaş mama ve ev yemeklerinin verildiği, öncesinde sadece gelişme geriliği ve kilo alamama şikayetleri varken diğer şikayetlerinin 1 haftadır görüldüğü bildirildi. Hastaneye ilk geldiğinde canlı ağırlığı 4,7 kilogramdı. Yapılan hemogram muayenesinde anormal bir bulgu gözlenmedi. Serum biyokimya bulgularından albümin (1,8 g/dL), BUN (4,7 mg/dL), kreatinin (0,25 mg/dL), total protein (2,65 g/dL) değerinin düşük, amonyak (256 µmol/L), ALP (504 U/L) değerlerinin yüksek olduğu saptandı. İdrar sediment muayenesinde amonyum urat kristalleri gözlendi. Yapılan ultrasonografik muayenede intrahepatik portosistemik şant tespit edildi. Tedavide ilk 5 gün %0.9 NaCl, 20 mg/kg B1 vitamini, 2 mg/kg B6 vitamini (Nervit/Vetaş), 20 mg/kg C vitamini (Vitse/Sanovel), 50 ml elektrolit-aminoasit (Duphalyte/Zoetis) ve 2 mg/kg dozunda furosemid (Diüril/Vetaş) yavaş infüzyon şeklinde damar içi verildi. Ek olarak günde 2 kez 50 mg/kg metronidazol (Flagyl/Sanofi), günde 3 kez 10 ml laktüloz (Osmolak/Biofarma) ve günde 1 kez 20 mg/kg S-adenozil metiyonin (SAM-e/Venatura) 5 gün boyunca oral olarak kullanım için reçete edildi. Alışıl gelmiş beslenme şekline son verilmesi istendi. Protein diyeti (Hills's L/D) önerildi. İki ay boyunca hastanın klinik gelişimi ve biyokimyasal parametreleri düzenli olarak takip edildi. Bu sunu hazırlanırken yapılan son muayenede klinik semptomların düzeldiği ve kilosunun 7,8 kg olduğu görüldü. Ayrıca serum amonyak (293 µmol/L), ALP (326 U/L), BUN (4,6 mg/dL), kreatinin (0,25 mg/dL), albümin (2.17 g/dL) ve total protein (3,52 g/dL) değerleri belirlendi. Kilo artışı iyi olmasına rağmen, biyokimyasal parametrelerde çok yavaş iyileşme gözlemlendiğinden, hasta operasyona sevk edildi. Sonuç olarak, intrahepatik portosistemik şant olgularında medikal ve diyet tedavileri ile klinik düzelme sağlanabildiği ancak operatif müdahale olmadan tam iyileşme sağlanamadığı sonucuna varıldı.

**Anahtar kelimeler:** Belçika Malinois, köpek, portosistemik şant

## The Importance of Steroidogenic Enzymes in The Protection of The Epithelial Barrier

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### Summary

Epithelial barriers play an important role in protecting organism from harmful effects of pathogens. Steroidogenic enzymes are involved in synthesis of sex hormones from gonads and adrenocortical steroid hormones from adrenal cortex. Current scientific studies have determined that steroidogenic enzyme expressions and steroid hormone synthesis are also found in different tissues and organs such as brain, heart, liver, lung, pancreas, intestine, prostate, skin, mammary gland, myometrium, endometrium, muscle and adipose tissue. Steroid hormones, which are synthesized *de novo* in these tissues or organs, have a paracrine effect. Locally synthesized steroid hormones protect tissue integrity and ensure its continuity. However, there is limited information in literature about changes in steroidogenic enzyme expressions and steroid hormone levels and their roles when these tissues or epithelium acting as a barrier are damaged by various effects. The use of glucocorticoids is successful because of their anti-inflammatory effects in treatment of inflammatory damage to epithelial barriers in skin, lungs and intestines. However, expression of enzymes such as 3-beta-hydroxysteroid dehydrogenase (3 $\beta$ -HSD) and 11 $\beta$ -HSD in these tissues and *de novo* intense glucocorticoid synthesis suggest that this steroid hormone may be effective in tissue-specific protection and repair. It is stated that steroid hormones such as estradiol and progesterone stimulate synthesis of surfactant proteins in developing lungs, but their changes and effects in case of lung injury are not fully known. In veterinary medicine, the lack of scientific studies on the local synthesis of steroidogenic enzymes and their paracrine effects in diseases that cause damage to the skin and mucous membranes such as rinderpest, small ruminant plague and sheep pox indicates that this subject needs research. It is thought that steroidogenic enzymes will contribute to understanding of pathogenesis of inflammatory diseases and determination of treatment targets in veterinary medicine as well as in medicine.

**Keywords:** 3 $\beta$ -HSD, 11 $\beta$ -HSD, epithelial barrier, steroid hormone, steroidogenic enzyme

## Epitel Bariyerin Korunmasında Steroidojenik Enzimlerin Önemi

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### Özet

Epitel bariyerler, organizmanın patojenlerin yaratacağı olumsuz etkilerden korunmasında önemli rol oynamaktadırlar. Steroidojenik enzimler, gonadlardan cinsiyet hormonlarının ve adrenal korteksten adrenokortikal steroid hormonlarının sentezinde görev alırlar. Güncel bilimsel çalışmalarda, steroidojenik enzim ekspresyonlarının ve steroid hormon sentezlerinin gonadlar ve adrenler dışında beyin, kalp, karaciğer, akciğer, pankreas, bağırsak, prostat, deri, meme bezi, myometrium, endometrium, kas ve yağ doku gibi farklı doku ve organlarda da olduğu belirlenmiştir. Bu doku veya organlarda *de novo* sentezlenen steroid hormonlar, parakrin etki göstermektedirler. Lokal sentezlenen steroid hormonlar doku bütünlüğünü korumakta ve devamlılığını sağlamaktadır. Bununla birlikte, bu dokularda ya da bariyer görevi gören epitelde çeşitli etkilerle bir hasar oluştuğunda steroidojenik enzim ekspresyonlarındaki ve steroid hormon düzeylerindeki değişimler ve bunların rolleri hakkında literatürdeki bilgiler sınırlıdır. Deri, akciğer ve bağırsaklardaki epitel bariyerlerin yangısal hasarının tedavisinde anti-enflamatuar etkileri nedeniyle glukokortikoidlerin kullanımı başarı sağlamaktadır. Bununla birlikte, bu dokularda 3-beta-hidroksisteroid dehidrojenaz (3 $\beta$ -HSD) ve 11 $\beta$ -HSD gibi enzimlerin ekprese edilmesi ve *de novo* yoğun glukokortikoid sentezinin olması, dokuya özgü korunma ve onarımda bu steroid hormonun etkili olabileceğini akla getirmektedir. Gelişim aşamasındaki akciğerlerde, östradiol ve progesteron gibi steroid hormonların sürfaktan proteinlerin sentezini uyardığı belirtilmekte ancak akciğer hasarı durumunda bunlarla ilgili değişimler ve etkileri tam olarak bilinmemektedir. Veteriner hekimlikte, sığır vebası, küçük ruminant vebası ve koyun çiçeği gibi deride ve mukozada hasara neden olan hastalıklarda steroidojenik enzimlerin lokal sentezine ve parakrin etkilerine dair bilimsel çalışma bulunmaması bu konunun araştırmaya muhtaç olduğunu göstermektedir. Steroidojenik enzimlerin, tıp hekimliğinde olduğu kadar veteriner hekimlikte de yangısal hastalıkların patogenezinin anlaşılmasında ve tedavi hedeflerinin belirlenmesinde katkı sağlayacağı düşünülmektedir.

**Anahtar kelimeler:** 3 $\beta$ -HSD, 11 $\beta$ -HSD, epitel bariyer, steroid hormon, steroidojenik enzim

**Evaluation of Fractures in Wild Birds in Samsun and Surrounding Provinces**

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**Summary**

The aim of this study is to determine the types, locations, causes, treatment options, and distribution of species exposed to trauma in wild birds that were brought to OMU Veterinary Faculty Clinics during the 11 years period (2006-2016). Between 2006 and 2016, a total of 167 fractures of 35 different species of birds admitted to the faculty clinic were included in the study. The registry files of these patients were scanned and their information was recorded. When the evaluation was made according to the type of broken bone, it was determined that most of the fractures were formed in wings. When the bone fractures were evaluated according to the fracture site, humerus was the first, radius / ulna was the second and tibiotarsus was the third. When the treatment methods applied to fractured bone are evaluated, it was determined that the most bandage was applied and IM pin was performed as an operation. As a result of this study, predators were the most exposed species, the fractures were mostly formed in the wings (humerus), the injuries were mostly in the winter months and firearm injuries were the most common cause of injury. We believe that it is important to keep complete records of the patient records, diagnosis and treatments and the results obtained in order to perform such retrospective studies efficiently.

**Keywords:** Fractures, treatment, trauma, wild birds

## Samsun ve Çevre İllerdeki Yabani Kanatlılarda Şekillenene Kırıkların Deęerlendirilmesi

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### Özet

Bu çalışmanın amacı 11 yıllık süre içerisinde (2006-2016) OMÜ Veteriner Fakültesi Kliniklerine getirilen yabani kanatlılarda travmalar sonucu oluşan kırıkların tiplerinin, lokalizasyonlarının, oluşum sebeplerinin, tedavi seçeneklerinin belirlenmesi ve travmaya maruz kalan türlerin dağılımlarının ortaya konmasıdır. 2006-2016 tarihleri arasında fakülte kliniğine getirilen kuşlardaki 35 farklı türde 167 adet kırık olgusu çalışmaya dahil edildi. Bu hastalara ilişkin hasta kayıt dosyaları incelendi ve bilgiler kayıt edildi. Kırık kemiğin türüne göre değerlendirme yapıldığında yabani kanatlılarda en fazla kanat kırığının şekillendiği belirlendi. Kemik kırıkları kırık bölgesine göre değerlendirildiğinde birinci sırada humerus, ikinci sırada radius / ulna ve üçüncü sırada tibiotarsus olduğu tespit edildi. Kırık kemiğe uygulanan tedavi yöntemleri değerlendirildiğinde en fazla bandaj uygulandığı ve operasyon olarak İM pin uygulamasının yapıldığı tespit edildi. Yapılan bu çalışma sonucunda travmaya en fazla maruz kalan türün yırtıcı kuşlar olduğu, kırıkların daha çok kanatta (humerus) şekillendiği, yaralanmaların daha çok kış aylarında olduğu, yaralanma sebepleri arasında ateşli silah yaralanmalarının en fazla olduğu ortaya kondu. Bu tür retrospektif çalışmaların verimli bir şekilde yapılabilmesi için hasta kayıtlarının, teşhis ve uygulanan tedaviler ile elde edilen sonuçların kayıtlarının tam olarak tutulmasının önemli olduğu düşüncesindeyiz.

**Anahtar Kelimeler:** Kırıklar, tedavi, travma, yabani kanatlılar

**The Use of Hazelnut By-Products in Animal Feeding**

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**Summary**

Some by-products in the food and feed industry are evaluated in animal nutrition. Hazelnut is consumed as a nut in human nutrition and is used in the pastry and chocolate industry. Turkey has approximately 70% of the world hazelnut production. In the processing of hazelnuts, hazelnut oil, meal and inner membrane are obtained. Because of their nutritional and energy values, these products are added to animal feeds.

In this review, the feed value and use of hazelnut oil, meal and hull in animal nutrition will be discussed.

**Keywords:** Animal nutrition, hazelnut hull, hazelnut meal, hazelnut oil



## Fındık Yan Ürünlerinin Hayvan Beslemede Kullanımı

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### Özet

Gıda ve yem endüstrisinde ortaya çıkan bazı yan ürünler, hayvan beslemede değerlendirilmektedir. Fındık, insan beslenmesinde yemiş olarak tüketilmekte, pastacılık ve çikolata sektöründe kullanılmaktadır. Türkiye, dünya fındık üretiminde yaklaşık % 70'lık orana sahiptir. Fındığın işlenmesi sürecinde fındık yağı, küspe ve iç zarı elde edilmektedir. Besin madde ve enerji değerleri nedeniyle, bu ürünler hayvan yemlerine katılmaktadır. Bu derlemede, fındık yağı, küspesi ve iç zarının yem değeri ve hayvan beslemede kullanımı irdelenecektir.

**Anahtar kelimeler:** Hayvan besleme, fındık küspesi, fındık yağı, iç zarı

**Nutrition and Importance of Breeding Heifers (14 Months And Over) in Dairy Cow Farms**

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Heifer farming is ignored because milk yield is more important in dairy cattle breeding businesses. However, heifers are the group that determines the milk production potential of the enterprise and ensures the continuity of the herd with future fertility.

Since heifers are perceived as non-productive animals, they are generally undernourished and unbalanced. However, since heifers are not thought to be lactating animals, malnutrition may cause unhealthy heifers and low milk yields. The risk of difficult birth may increase as the development of heifers in the developmental period will not be completed as a result of malnutrition

The basis of heifer feeding is based on an economic feeding program that will ensure that the animal can reach the live weight required for use in breeding with an appropriate growth rate. While the success criterion of dairy herd management is milk production, the success criterion of breeding heifers with high productivity is optimum growth and development of heifers.

Recent research on heifers is mostly related to the speed of growth and development in heifers. In order for breeding heifers to make the expected contribution to the herd, they must show their growth values within their breed standards.

Although the age of the heifer is mentioned here, the live weight of the animal is more important than the age in insemination for the first. It is recommended that heifers be bred for the first time when they reach 65-70% of the adult body weight.

The reproductive performance of heifers is affected by the growth rate. Heifers that reach the required body weight with an appropriate growth rate are inseminated and conceived. Milk yield and live weight gain in heifers inseminated at an early age are lower than heifers inseminated after they have completed their normal development.

In the table below, live weights of different breeds of heifers are given according to age.

Yaş	Ayrshire	Esmer	Guernsey	S Alaca	Jersey
6 Hf	48	60	43	60	39
3 Ay	75	93	68	93	60
6 Ay	127	156	113	156	102
12 Ay	227	283	204	283	184
<b>15 Ay</b>	<b>279</b>	<b>345</b>	<b>250</b>	<b>345</b>	<b>222</b>
24 Ay	454	562	408	562	363

The most critical period in the heifer breeding program is the age period of 3-9 months of young animals. Breast development in cattle is isometric (consistent with general body growth) at 2-3 months of age after birth. In this period, the development of secretory tissues is not observed. After 2-3 months of age, breast allometric growth is observed (breasts grow faster than other body parts). The allometric growth phase continues until about 1 year of age, when the breast will return to isometric growth. Thus, some of the allometric growth takes place in the sexual maturity period. Acceleration of growth at 15 months of age and later has no adverse effect on mammary secretory tissues in heifers.

The target body weights of heifers in different periods are given in the table below.

İrk	Doğ Ağır. (kg)	İlkine Çiftleşme		Buzakılama		CAK* (g)	Ergin ağırlık (kg)
		CA (kg)	Yaş (Ay)	CA (kg)	Yaş (Ay)		
İri İrklar; S.Ala. Esmer	40-45	360-400	14-16	544-620	23-25	740	550-725
Orta Cüsseli ırklar	35-40	275-310	13-15	450-500	22-24	600	525-580
Küçük ırklar; Jersey	25-30	225-260	13-15	360-425	22-24	500	425-500

\*CAK: Canlı Ağırlık Kazancı

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## **Süt İneği İşletmelerinde Damızlık Düvelerin (14 Ay ve Üzeri) Beslenmesi ve Önemi**

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Süt sığırı yetiştiriciliği yapan işletmelerde süt veriminin daha çok önemsenmesi nedeniyle düve yetiştiriciliği göz ardı edilmektedir. Ancak düveler hem işletmenin süt üretim potansiyelini belirleyen hem de gelecekteki döl verimi ile sürünün sürekliliğini sağlayan gruptur.

Düveler, verim vermeyen hayvanlar olarak algılandıkları için genellikle yetersiz ve dengesiz beslenmektedirler. Ancak düvelerin sonradan süt veren hayvanlar olacağı düşünülmediğinden yetersiz beslenme düvelerin sağlıksız olmasına ve süt verimlerinin düşük olmasına neden olabilir. Henüz gelişme döneminde olan düvelerin yetersiz beslenme sonucu gelişmeleri tamamlanmayacağı için güç doğum riski artabilir.

Düve beslemenin temeli, hayvanın damızlık da kullanılabilmesi için gerekli olan canlı ağırlığa uygun bir büyüme hızı ile ulaşabilmesini sağlayacak ekonomik bir besleme programına dayanır. Sağmal sürü yönetiminin başarı ölçütü süt üretimi olurken, verim gücü yüksek düve yetiştirmenin başarı ölçütü ise düvelerin optimum düzeyde büyümesi ve gelişmesidir

Son zamanlarda düveler üzerinde yapılan araştırmalar daha çok düvelerde büyüme ve gelişmenin hızı ile ilgilidir. Damızlık düvelerin sürüye beklenen katkıyı yapabilmeleri için kendi ırk standartları içerisindeki büyüme değerlerini göstermeleri gerekir.

Burada her ne kadar düvenin yaşından bahsediliyorsa da ilkinde tohumlamada hayvanın canlı ağırlığı yaştan daha önemlidir. Düvelerin ergin canlı ağırlığın %65-70'ine ulaştıklarında ilkinde çiftleştirilmeleri önerilir.

Düvelerin döl verimine ilişkin performansları büyüme hızından etkilenir. Uygun bir büyüme hızı ile gerekli canlı ağırlığa ulaşan düvelerin tohumlanarak gebe kalması sağlanır. Nitekim erken yaşta tohumlanan düvelerde süt verimi ve canlı ağırlık artışı, gelişmesini normal olarak tamamladıktan sonra tohumlanan düvelere göre daha düşük kalmaktadır.

Aşağıdaki tabloda değişik ırk düvelerde yaşa göre canlı ağırlıklar verilmiştir.

Yaş	Ayrshire	Esmer	Guernsey	S Alaca	Jersey
6 Hf	48	60	43	60	39
3 Ay	75	93	68	93	60
6 Ay	127	156	113	156	102
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24 Ay	454	562	408	562	363

Düve yetiştirme programında en kritik dönem, genç hayvanların 3-9 aylık yaş dönemidir. Sığırlarda doğum sonrası 2-3 aylık yaşlarda meme gelişimi isometrik (genel vücut büyümesi ile uyumlu) dir. Bu dönemde salgıcı dokuların gelişmesi görülmez. 2-3 aylık yaştan sonra memelerde allometrik büyüme görülür (memeler, diğer vücut kısımlarından daha hızlı büyür). Allometrik büyüme fazı memenin tekrar isometrik büyümeye döneceği yaklaşık 1 yaşa kadar devam eder. Böylece allometrik büyümenin bir kısmı cinsi olgunluk dönemi içinde geçer. Düvelerde 15 aylık yaş ve daha sonra büyümenin hızlandırılmasının meme salgıcı dokuları üzerine olumsuz etkisi yoktur.

Aşağıdaki tabloda düvelerin değişik dönemlerdeki hedef canlı ağırlıkları verilmiştir.

İrk	Doğ Ağır. (kg)	İlkin Çiftleşme		Buzğılama		CAK* (g)	Ergin ağırlık (kg)
		CA (kg)	Yaş (Ay)	CA (kg)	Yaş (Ay)		
İri İrklar; S.Ala. Esmer	40-45	360-400	14-16	544-620	23-25	740	550-725
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## **Mesenchymal Stem Cell Therapy of Male Infertility**

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### **Summary**

Male infertility is an important clinical problem that negatively affects animal production. In recent years, mesenchymal stem cells (MSCs) have opened new possibilities for the treatment of many diseases, including infertility. Mesenchymal stem cells are multipotent cells found in many adult tissues. The ability of mesenchymal stem cells to differentiate into all cells of the three germ layers, their expandability in in vitro culture with fewer ethical concerns, their genomic stability, tissue regeneration, and immunomodulatory properties make them a very important tool in cell therapy and regenerative medicine. MSCs are believed to exert their effects mainly by differentiating into germ cells or by protecting spermatogonial stem cells (SSCs). SSCs are the basic stem cells that make up the sperm germ cell and are necessary for spermatogenesis. One of the most common causes of infertility in male patients is loss of testicular germ cells. Studies have shown that MSCs have the ability to differentiate into germ cells, Sertoli cells and Leydig cells under appropriate conditions, increase the number of germ cells in damaged testes and improve spermatogenesis. The aim of this review is to evaluate the results of important studies in which mesenchymal stem cells have been used in the treatment of infertility. MSCs are promising for the treatment of infertility and many diseases with their differentiation into various cells to replace damaged cells, suppression of apoptosis, immunomodulatory and anti-inflammatory effects.

**Keywords:** Infertility, mesenchymal stem cell (MSC), spermatogenesis

## Erkek İnfertilitesinde Mezenkimal Kök Hücre Tedavisi

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### Özet

Erkek infertilitesi hayvansal üretimi olumsuz etkileyen önemli bir klinik problemdir. Son yıllarda mezenkimal kök hücreler (MSC'ler) infertilite dahil olmak üzere birçok hastalığın tedavisi için yeni olanaklar sağlamıştır. Mezenkimal kök hücreler çok sayıda yetişkin dokuda bulunan multipotent hücrelerdir. Mezenkimal kök hücrelerin üç germ tabakasında bulunan tüm hücrelere farklılaşma yeteneğinin olması, daha az etik kaygı ile in vitro kültür genişletilebilirliğinin olması, genomik stabilite, doku rejenerasyonunu sağlayabilme, immunmodülatör özelliklerinin olması onları hücre tedavisi ve rejeneratif tıpta çok önemli bir ajan haline getirmektedir. MSC'lerin etkisini daha çok germ hücrelerine farklılaşmasıyla ya da spermatogonial kök hücreleri (SKH) korumasıyla gösterdiği düşünülmektedir. SKH'ler sperm germ hücresini oluşturan ve spermatogenezis için gerekli temel kök hücrelerdir. Erkek hastalarda en sık görülen infertilite nedenlerinden biride testiste germ hücresi kaybıdır. Yapılan çalışmalar, MSC'lerin uygun koşullar altında germ hücrelerine, sertoli hücrelerine ve leydig hücrelerine farklılaşma yeteneği olduğunu, hasarlı testislerde germ hücre sayısını arttırdığı ve spermatogenezisi düzelttiği görülmüştür. Bu derlemenin amacı infertilite tedavisinde mezenkimal kök hücre kullanılan önemli çalışmalardan elde edilen sonuçların değerlendirilmesidir. MSC'ler hasarlı hücreleri değiştirmek için değişik hücrelere farklılaşması, apoptozu baskılaması, immunmodülatör ve anti-inflamatuar etkileri ile infertilite ve birçok hastalığın tedavisi için umut vaat etmektedir.

**Anahtar kelimeler:** İnfertilite, mezenkimal kök hücre (MSC), spermatogenezis

## Antimicrobial Activity of The Silver Nanoparticles Loaded Locust Bean Gum Hydrogel

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### Summary

Green synthesized silver nanoparticles (AgNPs) are well known and recognized as potential candidate for the novel antimicrobial agents. In this study, antimicrobial activity of the silver nanoparticles loaded locust bean gum hydrogel were investigated. For this purpose, silver nanoparticles were synthesised using egg white protein extract. The locust bean gum (LBG) solution (1%) was added into the green synthesised AgNPs solution under magnetic stirring. AgNP-LBG solution was stirred almost 6 hours at room temperature to get a homogeneous AgNP loaded LBG hydrogels. The silver nanoparticles formation was confirmed by using of UV-Vis spectroscopy, Mastersizer, and SEM/STEM. Interaction between the AgNP and LBG was analysed with FTIR. STEM and Mastersizer results showed the spherical shape of AgNPs with a size distribution was from 9 to 13 nm and the hydrodynamic diameter range of 0.1-7 µm. Finally; hydrogels were tested against the plant pathogenic fungi strains (*Bipolaris sorokiniana*, *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium verticillioides*) using agar well diffusion method. The antimicrobial property of silver nanoparticles was analyzed by measuring the inhibition zone. The antimicrobial results showed that AgNP loaded-LBG solutions play an important role in the inactivation of the *Bipolaris sorokiniana* (19 ±1 mm zone of inhibition) and *Fusarium graminearum* (20 ±1 mm zone of inhibition) species.

**Keywords:** Silver nanoparticles; green synthesis; egg white; locust bean gum; antimicrobial agents.



## Keçiboynuzu Gamı Hidrojelle Yüklü Gümüş Nanopartiküllerin Antimikrobiyal Aktivitesi

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### Özet

Yeşil sentezle üretilen gümüş nanoparçacıklar (AgNP), güncel antimikrobiyal ajanlar olarak bilinmektedir. Bu çalışmada; gümüş nanoparçacık katkılı keçiboynuzu gamı (LBG) hidrojellerinin antimikrobiyal etkinlikleri araştırılmıştır. Öncelikle yumurta akı ekstraktından gümüş nanoparçacıklar sentezlenmiştir. Yeşil sentezle elde edilen AgNPlar, %1'lik LBG çözletisine eklenerek yaklaşık 6 saat oda sıcaklığında manyetik karıştırıcıda karıştırıldıktan sonra homojen AgNP yüklü LBG hidrojelleri elde edilmiştir.

Sentezlenen nanoparçacıklar UV-Vis spektroskopisi, Mastersizer ve SEM/STEM kullanılarak karakterize edildi. FTIR kullanılarak AgNP ve LBG arasında oluşan etkileşim analiz edildi. STEM sonuçları AgNPlerin küresel şekilli ve boyut dağılımının 9 -13 nm, Mastersizer sonuçları partiküllerin hidrodinamik çapının 0.1-7 µm aralığında olduğunu gösterdi. Hidrojellerin; *Bipolaris sorokiniana*, *Fusarium culmorum*, *Fusarium graminearum* ve *Fusarium verticillioides* karşı antimikrobiyal aktiviteleri disk difüzyon yöntemi kullanılarak test edildi, inhibisyon zonları ölçülerek antimikrobiyal etkinlikleri gözlemlendi. Sonuç olarak; AgNP-LBG hidrojellerinin *Bipolaris sorokiniana* (19 ±1 mm inhibisyon zonu) ve *Fusarium graminearum* (20 ±1 mm inhibisyon zonu)'a karşı önemli derecede antifungal etkisinin olduğu gözlemlendi.

**Anahtar kelimeler:** Gümüş nanoparçacık, yeşil sentez, yumurta akı, keçi boynuzu gamı, antimikrobiyal ajan

## **Use of Magnesium Oxide (MgO) in Dairy Cattle**

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### **Summary**

The pH of the environments in the organisms of animals and in the indoor environment shows very narrow indications. With the feed they consume, animals are constantly fed with acids such as chlorine, phosphorus, sulfur; they contain elements in basic characters such as sodium, potassium, calcium, magnesium. It can be designed to be a complete system to be engineered for organism-base bites. One of these mechanisms, the bicarbonate system; blood and its environment is the foremost buffer system of the media. Hemoglobin, plasma proteins and rich phosphorus in the blood also show buffer properties. Magnesium oxide is used for use for rumen acidosis resulting from the use of high concentrate feed, low fiber content uses. The rumen pH of magnesium oxide is displayed after 24 hours when it is consumed for the cultivation of foods. Magnesium oxide guides you in preventing the improvement in milk lubrication resulting from lactation with regard to the environment and diet. It is stated that magnesium oxide reduces rumen acidity, increases feed use, milk yield and fat use. It is reported that magnesium oxide increases the color transition of triglyceride to triglycerides, and its predictability is higher for MgO, which has a high value. Magnesium oxide is used in dairy cow rations at a dose of 4-8 g/kg. The positive effect of milk fat shows that MgO is absorbed from the rumen in the infrastructure of the process. Magnesium oxide is used for use for rumen acidosis resulting from the use of concentrates high in fiber, low in fiber luck. The rumen pH of magnesium oxide is displayed after 24 hours when it is consumed for the cultivation of foods. Magnesium oxide removes from lactation in preventing improvement in milk fat aspect resulting from environmental and ration. It is stated that magnesium oxide reduces rumen acidity, increases feed use, milk yield and fat use. It is reported that magnesium oxide increases the color transition of triglyceride to triglycerides, and its predictability is higher for MgO with a higher value. Magnesium oxide is used in dairy cow rations at a dose of 4-8 g/kg. The positive effect of milk fat shows that MgO is absorbed from the rumen in the infrastructure of the process.

**Keywords:** Acid base balance, dairy cow, magnesium oxid

## Süt Sığırlarında Magnezyum Oksit (MgO) Kullanımı

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### Özet

Hayvanların organizmalarında hücre içi ve hücreler arası sıvıların pH'ları çok dar sınırlar içinde değişme gösterir. Hayvanlar tükettikleri yemlerle vücutlarına sürekli olarak klor, fosfor, kükürt gibi asit; sodyum, potasyum, kalsiyum, magnezyum gibi baz karakterde elementler almaktadırlar. Organizma asit-baz dengesini koruyabilmek için başta tampon sistemi olmak üzere bazı mekanizmaları devreye sokar. Bu mekanizmalardan biri olan bikarbonat sistemi; kan ve hücreler arası sıvıların en önde gelen tampon sistemidir. Kandaki hemoglobin, plazma proteinleri ve çeşitli fosfat bileşikleri de tampon madde özelliği gösterirler. Magnezyum oksit, konsantre yem oranı yüksek, lifli madde oranı düşük rasyonların kullanılması nedeniyle ortaya çıkan rumen asidozunu önlemek için kullanılmaktadır. Magnezyum oksit rumen pH'sını artırmakta ancak çözünürlüğü düşük olduğu için etkisini 24 saat sonra göstermektedir. Magnezyum oksit laktasyon başlangıcında çevre ve rasyon şartlarına bağlı olarak ortaya çıkan süt yağ oranındaki azalmayı önlemede etkilidir. Magnezyum oksitin rumen asiditesini düşürdüğü, yemden yararlanmayı, süt verimini ve yağ oranını arttırdığı belirtilmektedir. Magnezyum oksitin trigliseritlerin meme bezlerine geçişini artırdığı, partikül büyüklüğü küçük olan MgO kaynaklarının eriyebilirliğinin daha yüksek olduğu bildirilmektedir. Magnezyum oksit süt ineği rasyonlarında 4-8 g/kg dozunda kullanılmaktadır Süt yağı üzerine olan olumlu etkisi, MgO'in rumenden ziyade sindirim sisteminin alt kısımlarında emildiğini göstermektedir. Magnezyum oksit, konsantre yem oranı yüksek, lifli madde oranı düşük rasyonların kullanılması nedeniyle ortaya çıkan rumen asidozunu önlemek için kullanılmaktadır. Magnezyum oksit rumen pH'sını artırmakta ancak çözünürlüğü düşük olduğu için etkisini 24 saat sonra göstermektedir. Magnezyum oksit laktasyon başlangıcında çevre ve rasyon şartlarına bağlı olarak ortaya çıkan süt yağ oranındaki azalmayı önlemede etkilidir. Magnezyum oksitin rumen asiditesini düşürdüğü, yemden yararlanmayı, süt verimini ve yağ oranını arttırdığı belirtilmektedir. Magnezyum oksitin trigliseritlerin meme bezlerine geçişini artırdığı, partikül büyüklüğü küçük olan MgO kaynaklarının eriyebilirliğinin daha yüksek olduğu bildirilmektedir. Magnezyum oksit süt ineği rasyonlarında 4-8 g/kg dozunda kullanılmaktadır Süt yağı üzerine olan olumlu etkisi, MgO'in rumenden ziyade sindirim sisteminin alt kısımlarında emildiğini göstermektedir.

**Anahtar kelimeler:** Asit baz dengesi, magnezyum oksit, süt sığırı

## **The Effect of Hormones in Nutrition**

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### **Summary**

Living things provide the energy necessary to perform their metabolic reactions with their feeding behavior. Following food intake, the gastrointestinal tract transmits various signals to the brain. The brain responds to incoming nutritional signals in order to maintain energy homeostasis. One of the main centers in the appetite metabolism of the hypothalamus, which regulates the amount of food to be taken into the body and appetite, is the arcuate nucleus (ARC), where hormonal signals are synthesized. The arcuate nucleus is located close to the permeable part of the blood brain barrier and provides the passage of peripheral peptides and transmits signals to the necessary regions of the hypothalamus. There are connection pathways between the ARC region and other regions of the hypothalamus that are effective in fasting and satiety metabolism. It has been reported that there are two different neuron types in the arcuate nucleus that work opposite each other, namely appetite enhancer (orexigenic) and appetite suppressant (anorexigenic). The arcuate core regulates food intake by releasing the orexigenic peptides Neuropeptide Y (NPY) and Agouti-related peptide (AgRP) and the anoresigenic peptides proopiomelanocortin (POMC). However, it also receives signals from other peptides and hormones. These peptides, which can work by activating or inhibiting each other with hunger and satiety signals, constitute the feeding behavior. Disruptions that may occur in the system can cause obesity, which has become a global problem. With this study, it is aimed to explain the physiological pathway that will provide energy homeostasis.

**Keywords:** Nutrition, energy, hormones, metabolism, peptides

## **Beslenmede Hormonların Etkisi**

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### **Özet**

Canlılar metabolik reaksiyonlarını gerçekleştirebilmek için gerekli olan enerjiyi beslenme davranışı ile sağlamaktadırlar. Besin alımını takiben gastrointestinal sistem çeşitli sinyalleri beyine iletmektedir. Beyin, enerji homeostazisini sağlayabilmek için gelen beslenme sinyallerine yanıt oluşturmaktadır. Vücuda alınacak besin miktarlarını ve iştahı düzenleyen hipotalamusun, iştah metabolizmasındaki ana merkezlerinden biri hormonal sinyallerin sentezlendiği arkuat çekirdektir (ARC). Arkuat çekirdek, kan beyin bariyerinin geçirgen kısmına yakın olarak bulunmaktadır ve periferel peptitlerin geçişini sağlayarak sinyalleri hipotalamusun gerekli bölgelerine iletmektedir. ARC bölgesi ile hipotalamusun açlık tokluk metabolizmasında etkili diğer bölgeleri arasında bağlantı yolları bulunmaktadır. Arkuat çekirdekte iştah arttırıcı (oreksijenik) ve iştah baskılayıcı (anoreksijenik) olmak üzere birbirine zıt çalışan iki farklı nöron tipleri olduğu bildirilmektedir. Arkuat çekirdek oreksijenik peptitlerden Nöropeptit Y (NPY) ve Aguti-ilişkili peptid (AgRP) ile anoresijenik peptitlerden proopiomelanokortin (POMC) salınımı yaparak gıda alımını düzenlemektedir. Bununla birlikte, diğer peptit ve hormonlardan da sinyaller almaktadır. Açlık tokluk sinyalleri ile birbirlerini aktive veya inhibe ederek çalışabilen bu peptitler beslenme davranışını oluşturmaktadır. Sistemde meydana gelebilecek aksaklıklar küresel bir sorun haline gelen obeziteye sebep olabilmektedir. Bu çalışma ile enerji homeostazını sağlayacak fizyolojik yolağın açıklanması amaçlanmıştır.

**Anahtar kelimeler:** Beslenme, enerji, hormonlar, metabolizma, peptitler

## The Retrospective Evaluation of Cat and Dog Ear Swab Samples Sent to Our Laboratory

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### Summary

Ear infections caused by bacteria or fungi are very common problems in cats and dogs. During the preparation of the treatment protocol, it is recommended to culture the examination samples taken at the right time and in the right way. Otherwise, it is inevitable to encounter unnecessary antibiotic use and the development of resistance to antibiotics. In this study, the results of bacterial and fungal identification and antibiotic susceptibility tests of ear examination samples taken from cats and dogs coming to the laboratory since January will be evaluated. Samples sent for bacteriological and mycological examination were examined with the BD Phoenix device after the isolation stage. The most frequently isolated bacteria from 138 samples (45 cats, 93 dogs) examined; *Staphylococcus intermedius*, *Staphylococcus felis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, Methicillin Resistant *Staphylococcus intermedius* and *Staphylococcus schleiferi*. While it was determined that the susceptibility rate to amikacin was high in the isolated agents, the antibiotics with the highest resistance development were determined as ampicillin and penicillin. *Malassezia pachydermatis* was the most encountered factor as a result of the identification of 21 samples isolated from fungal culture. Obtained results; clearly presents the importance of culture methods in ear infections of cats and dogs.

**Keywords:** Bacteriology, BD Phoenix, cat, dog, mycology

## Laboratuvarımıza Gönderilen Kedi ve Köpek Kulak Sıvab Örneklerinin Retrospektif Değerlendirilmesi

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### Özet

Kedi ve köpeklerde bakteri ya da mantar kaynaklı kulak enfeksiyonları sıklıkla görülmektedir. Tedavi protokolünün düzenlenmesi aşamasında, doğru zamanda ve şekilde alınan inceleme örneklerinden kültür yapılması önerilmektedir. Aksi durumda gereksiz antibiyotik kullanımı ve antibiyotiklere karşı direnç gelişimi ile karşılaşmak kaçınılmazdır. Bu çalışmada, Ocak ayından günümüze kadar laboratuvara gelen kedi ve köpeklerden alınan kulak inceleme örneklerinin, bakteri ve mantar identifikasyonu ile antibiyotik duyarlılık testlerinin sonuçları değerlendirilecektir. Bakteriyolojik ve mikolojik inceleme için gönderilen örnekler izolasyon aşamasının ardından BD Phoenix cihazı ile incelenmiştir. İncelenen 138 örnekten (45 adet kedi, 93 adet köpek) en sıklıkla izole edilen bakteriler; *Staphylococcus intermedius*, *Staphylococcus felis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, Metisilin Dirençli *Staphylococcus intermedius* ve *Staphylococcus schleiferi* olarak gözlemlenmiştir. İzole edilen etkenlerde amikasine olan duyarlılık oranının yüksek olduğu belirlenirken, en fazla direnç gelişiminin olduğu antibiyotikler ise ampisilin ve penisilin olarak belirlenmiştir. Mantar kültürü izolasyonu yapılan 21 numunenin identifikasyon sonucunda ise en çok karşılaşılan etken *Malassezia pachydermatis* olmuştur. Elde edilen sonuçlar; kedi ve köpeklerde meydana gelen kulak enfeksiyonlarında kültür yöntemlerinin önemini açık bir şekilde göz önüne sunmaktadır.

**Anahtar kelimeler:** Bakteriyoloji, BD Phoenix, kedi, köpek, mikoloji

## Hematological and Echocardiographic Findings in a Calf with Respiratory Distress

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### Summary

Respiratory system diseases are one of the important causes of mortality in calves. Respiratory system disease complex (BRD complex) in cattle is caused by many viral, bacterial, mycoplasmal and environmental reasons. Infectious agents can cause disease in the respiratory system as well as affect the heart and circulatory system. Echocardiography is a diagnostic method used in heart and circulatory diseases. As it is used in cat and dog medicine, heart diseases can be diagnosed in cattle by echocardiographic examination.

**Keywords:** Bovine respiratory disease complex, echocardiographic examination, pneumonia.

## Respiratorik Distresli Bir Buzağıda Hematolojik ve Ekokardiyografi Bulguları

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### Özet

Solunum sistemi hastalıkları buzağılarda mortalitenin önemli sebeplerinden birisidir. Sığırlarda solunum sistemi hastalığı kompleksini (BRD kompleks) viral, bakteriyel, mikoplasmal, çevresel birçok sebep oluşturmaktadır. Enfeksiyöz etkenler solunum sisteminde hastalık tablosu oluşturabilmesinin yanında kalp ve dolaşım sistemini de etkileyebilmektedir. Ekokardiyografi kalp ve dolaşım hastalıklarında kullanılan bir tanısal yöntemdir. Kedi ve köpek hekimliğinde kullanıldığı gibi sığırlarda da ekokardiyografik muayeneyle kalp hastalıklarının tanısı konulabilmektedir.

**Anahtar kelimeler:** Ekokardiyografik muayene, pnömoni, sığırların solunum sistemi hastalığı kompleksi



## **Introduction**

Bovine respiratory disease complex (BRDC) is a disease condition of calves shaped by a combination of management and pathogenic factors (1). In addition to the treatment and care costs in this disease, increased mortality despite treatment, growth retardation in recovered calves and reproductive problems in later ages are the conditions that can be seen (2). In recent years, a scoring system has been developed that the disease is categorized according to the clinical manifestations of BRDC. The respiratory scoring chart in calves includes the determination of 5 different clinical signs (rectal temperature, presence of cough, nasal drip, scoring of the eyes and ears) with a 4 level scale (3). Heart failure is the last stage of heart disease (4). Clinical signs of heart failure in cattle are increased hydrostatic pressure and peripheral edema. The presence of signs of heart failure shows that the prognosis is poor (5).

## **Material and Method**

This case consisted of a 1-month-old male Belgian blue crossbred calf brought to Ataturk University Veterinary Faculty Hospital with complaints of tachypnea, open-mouthed breathing, and fatigue. For hematological analysis, blood samples taken from the *vena jugularis* of the animals were transferred to anticoagulant (EDTA) tubes. Hematological analyzes were performed with a hemogram device (Abacus Junior Vet 5, Hungary). Echocardiographic examinations were performed using a color doppler device (GE S7 Ultrasound, USA).

## **Results**

In this case, tachypnea, tachycardia, and mild cyanosis in the mucous membranes were determined as clinical findings. Therefore, oxygen therapy was started (Figure-1). Hematological examination revealed an increase in the total leukocyte count and neutrophilia. Also, in the hematological examination, a decrease in hemoglobin, mean erythrocyte volume (MCV) and mean erythrocyte hemoglobin (MCH) values were observed. Ultrasonographic examination revealed regurgitation of the tricuspid valve in the right parasternal position (Figure-2). Also, in the echocardiographic examination, the ejection fraction (EF) was determined to be 14% (Figure-3).

## **Discussion**

Respiratory distress syndrome in neonatal calves is a condition in which, in addition to interstitial and pulmonary edema, a decrease in blood oxygen capacity and an increase in carbon dioxide level are formed (6). A number of echocardiographic indices have been used to evaluate LV systolic function, including ejection fraction (EF), fractional shortening (FS), cardiac output (CO), E point of septal separation (EPSS), pre-ejection period (PEP), ejection time (ET), and the ratio of PEP to ET (7). Among these echocardiographic markers, EF is the most used parameter to determine LV function (8).

In this case, EF was measured to determine the LV function and the EF level was found to be 14%. It showed a severe weakness in left ventricular resistance at this rate. In the echocardiographic examination of the calves, it was determined that tricuspidal valve regurgitation was formed in the right parasternal long axis image. Therefore, echocardiographic examination revealed that both right and left-sided insufficiency were formed in the calf brought with respiratory failure in this case.

### **Conclusion**

Respiratory system diseases are one of the important causes of mortality, especially in calves. In bacterial, viral and mycoplasmal infections, the heart and circulatory system may also be affected by the complication of the disease or by the effect of direct factors. In this case, it was understood that in addition to routine examinations, echocardiographic examinations are also diagnostically important in calves with intense respiratory distress.

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## Cellular Therapies in Veterinary Medicine

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### Summary

Regenerative medicine or regenerative therapy concepts have gained more scientific importance recently, which targeted to heal or repair tissue or organ damages within an interdisciplinary view. In veterinary medicine, cellular therapies are promising frequently encountered orthopedic problems or surgery cases in terms of regenerative medicine. As of now, some animal cell-based products are approved by the FDA and the cellular therapy market tends to the rapid economic growth trend. Cellular therapies consist of mainly stem cell-based applications. The division, regeneration, and differentiation abilities of stem cells in the appropriate conditions make them principal actors of regenerative medicine and therapy. Also, clinical usage is expanding in inflammatory and infectious diseases due to their high anti-inflammatory and immunomodulatory properties. The experimental animal models are a considerable source in translational medicine to research, developing and, realize novel therapeutic strategies for human medicine. Therefore, the rapidly increasing clinical stem cell applications and researches are anchored inherently joint in both human and veterinary medicine. In this review, we aimed to provide information about stem cell-based therapies and their current clinical applications.

**Keywords:** Cell therapy, clinic, stem cells, veterinary medicine

## Veteriner Hekimliğinde Hücresel Tedaviler

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### Özet

Disiplinler arası bir bakış açısıyla doku veya organ hasarlarını iyileştirmeyi veya onarmayı amaçlayan rejeneratif tıp veya rejeneratif terapi kavramları son yıllarda daha fazla bilimsel önem kazanmaya başlamıştır. Veteriner hekimliğinde sık karşılaşılan ortopedik problemlerde veya cerrahi sağaltım gerektiren durumlarda rejeneratif tıp yönünden hücresel tedaviler ümit vaat etmektedir. Günümüzde FDA tarafından onaylanan hayvan hücresi temelli ürünler bulunmakta ve hücresel tedavi pazarı hızlı bir ekonomik büyüme trendi göstermektedir. Hücresel tedaviler ile sağaltım ağırlıklı olarak kök hücre

uygulamalarından oluşmaktadır. Kök hücrelerin sahip olduğu bölünebilme, yenilenme, uygun koşullar altında birçok farklı hücre tipine farklılaşabilme yetenekleri, onları rejeneratif tıbbın önemli aktörleri haline getirmektedir. Aynı zamanda, yüksek antiinflamatuvar ve immünomodülatör özellikleri sebebiyle de inflamatuvar ve enfeksiyöz hastalıklarda klinik kullanım alanları genişlemektedir. Kök hücrelerin özelliklerini ve potansiyellerini incelemek ve geliştirmek üzerine kullanılan deney hayvanı modelleri, insan tıbbında gelecekteki olası tedavi yöntemlerini geliştirip uygulamak için önemli bir translasyonel araştırma kaynağıdır. Bu nedenle, hızla artan klinik kök hücre uygulama ve araştırmaları, beşeri ve veteriner hekimliğini özünde birbirine bağlamaktadır. Bu derlemede, veteriner hekimliğinde kök hücre temelli tedaviler hakkında bilgi vermek ve klinik kullanımları ile ilgili güncel çalışmaları sunmak amaçlanmıştır.

**Anahtar kelimeler:** Hücresel tedavi, klinik, kök hücre, veteriner hekimliği

## **Introduction**

Regenerative medicine aims to involve rapid healing and re-functionalization in damaged tissue or organs. Regenerative therapeutics use multidisciplinary scientific knowledge, including stem cells, biomaterials, biomolecules, 3D printed organoids (Ntege et al., 2020; Ashammakhi et al., 2019). Veterinary regenerative therapy strategies are mostly built on platelet-rich plasma (PRP) and cell-based products especially stem cells, stromal vascular fractions (SVFs), due to their cost-friendly and quickly applicable advantages.

Cell-based therapies are medicinal products and have growing markets in the field of veterinary medicine pharmaceutical industry, the utilization as therapeutics are regulated by the national and European Union (EU Directive 2001/82/EC) laws, and according to the Food and Drug Administration (FDA) is classified as drugs. Also, European Medicines Agency's (EMA) is currently developing the guidance in accordance with scientific authorization established throughout the EU Ad Hoc Expert Group on Veterinary Novel Therapies (ADVENT) (Faltus and Brehm, 2016; EMA, 2021).

Stem cell therapy is promising, related to recent clinical studies. Innovative stem cell therapies possess significant healing power as well as promise the potential to fundamentally change the classic treatment of disease. Stem cells are biological cells of the living body that have not yet specialized into an organ or tissue. They have the ability to renew themselves, to transform into a different organ or tissue, or any cell of the organism. These kinds of cells have the capability to construct numerous tissues and manage the regeneration process of damaged cells in the tissues (Schatten and Sun, 2009).

## A Very Brief History of Cell-Based Therapies



**Figure 1** Ord. Prof. Dr. Süreyya Tahsin AYGÜN (1895-1991)

Veterinary medicine has made significant contributions to disease and treatment studies from past to present. Cell and tissue culture systems were lead researches of cell-based therapies. The pioneer of these systems in Turkey and also a part of Europe is Ord Prof Dr Süreyya Tahsin Aygün (Fig.1), who started his career as a military veterinary and continued in Ankara University Faculty of Veterinary Medicine (Dinçer, 1982). He pioneered stem cell studies by saying that the placentas that are discarded after birth contain cord cells that extend human life. Between 1950 and 1960, he conducted research in the treatment of many different diseases with fetal grafts and cord blood grafts in animals and published them in various medical journals (Şahin et al., 2005).

Although the experimental animal studies began many years ago, in 1957 first allogeneic hematopoietic stem cells were given unsuccessfully; after that, in 1969 first human bone marrow transplantation was given to a patient suffering from aplastic anemia (Sampogna et al., 2015). Regenerative medicine concept was named throughout in the late '90s and is still developing in – omics, organoids era with groundbreaking biotechnological research.

Cell-based therapies mostly focus on various types of stem cells, however, detailed studies have been conducted on mesenchymal stem cells (MSCs) due to their convenience isolation, and culturing processes and simplicity of autologous applications by clinicians. The mesenchymal stem cell therapy concept was reported by Caplan (1991) to rapid and specific repair of skeletal tissues with autologous stem cells.

In 2012 Gurdon and Yamanaka were awarded Nobel Prize in Physiology and Medicine for their discoveries on re-programmed mature cells becoming pluripotent (NobelPrize.org., 2021) which are called inducible pluripotent stem cells (iPSCs) today.

### Stem Cell Therapies in Veterinary Medicine

Stem cells are mainly divided into two groups as embryonic stem cells (ESC) and non-embryonic or adult-type stem cells. Adult-stem cells can originate from endoderm, mesoderm, and ectodermal tissues, while embryonic stem cells are derived from the inner cell mass of the blastocyst. Also, stem cells are classified as totipotent, pluripotent and multipotent according to their differentiation abilities (Fortier, 2005; De Schauwer et al., 2011; Smith, 2001; Can, 2008; Koerner et al., 2006). In addition, stem cells have several stages of specific specialization to a different tissue or cell, and adult stem cells, which are frequently used in tissue regeneration, are MSCs isolated from the stroma of the bone marrow. (Chanda et al., 2010).

Accompanied by developing technology, stem cells can be grown under laboratory conditions and transformed into specialized cell types with similar properties to different tissue cells such as muscle or nerve cells through cell culture techniques. In 2006, Takahashi and Yamanaka reprogrammed mouse and human fibroblast cells using four transcription factors identified as “OSKM” factors (Oct 3/4, Sox-2, Klf-4, c-Myc). Thus, induced pluripotent stem cell (iPSc) was obtained and a new era started in stem cell research. Since the obtained iPSc cells have the same functions as embryonic stem cells, a new horizon has been opened in stem cell research. In addition, it is highly specific to obtain individual and disease-specific iPSc cells. By enabling disease modelling in the laboratory for drug research, a new field has been created for cell-based researches.

Veterinary mesenchymal stem cells can be produced from non-self (allogeneic or xenogenic) and self (autologous) sources. Frequently, bone marrow concentrate is isolated from tissue sources such as pure bone marrow, peripheral blood, umbilical cord blood, umbilical cord tissue, stromal vascular fraction of adipose tissue. Autologous mesenchymal stem cells are mostly used in cell-based therapies. However, De Schauwer et al. (2014) reported that the use of allogeneic mesenchymal stem cells may be more advantageous compared to autogenic mesenchymal stem cells in providing a product that can be obtained quickly, standardized and easily available, especially considering the immunosuppressive abilities of mesenchymal stem cell. Nevertheless, mesenchymal stem cells obtained from different tissues and organs have differences within themselves. In particular, neonatal-derived MSCs may be preferred due to their much higher proliferative capacity compared to adult-derived mesenchymal stem cells (De Schauwer et al., 2014; Stubbendorff et al., 2013; Deuse et al., 2011). Also, MSCs represent a very small fraction of the number of cells derived from bone marrow harvested from cats (Martin et al., 2002), humans (Pittenger et al., 1999), and are thought to be the same in other species, including horses (Barberini et al., 2014).

Although the mechanisms involved in the repair processes of stem cell therapy are similar, the indication may be different in human and veterinary patients. Animal models used to study and develop the properties and potential of stem cells are an important resource for developing and applying possible future treatment modalities in human medicine. Therefore, the rapidly increasing clinical stem cell practice and research is intrinsically linking human and veterinary medicine. When developing new human-specific treatment methods, Borjesson and Peroni (2011) recommended using a rodent species together with a large non-rodent animal species with characteristics more similar to humans to evaluate the efficacy and safety tests of preclinical treatment. Compared to rodents, dogs have common similarities with humans in terms of pathogenesis, anatomy, athletic performance and more developed joints, allowing dogs to be used in studies. This similarities have provides an opportunity to analyze more detailed the therapeutic effects (Bendele, 2001; Hytonen and Lohi, 2016). In veterinary medicine, stem cells application is still on growing demand, and there are no standardized therapy algorithms. However, clinical trials and researches are conducted on musculoskeletal system disorders especially in ligament (Renzi et al., 2013), tendon injuries (Smith et

al., 2003) and osteoarthritis (Yun et al., 2016), wound healing (Martinello et al., 2018), eye (Davis et al., 2019), liver (Yan et al., 2019), cardiac (Pogue et al., 2013), neuromuscular (Bach et al., 2019), respiratory (Barussi et al., 2016), renal (Quimby et al., 2011) systems injuries, diseases and disorders.

The translational researches on musculoskeletal system, aspiring to improve stem cell technology, are most commonly performed in equine medicine according to literature data. The first documented autologous stem cell application in equine medicine was reported by Herthel (2001), from 1991 to 1998 the group was treat ligament injuries that are frequently encountered in horses by injecting a large amount of pure bone-marrow derived stem cells and components obtained from the sternum directly into the damaged ligament, functional recovery was achieved in the damaged area compared to normal treatment methods after application, and announced to get approximately %90 success rate. After the therapeutic usage of equine mesenchymal stem cells applied to relieve equine orthopedic disorders was reported in 2003, the clinical use of MSCs in many equines around the world has increasingly continued (Borjesson and Peroni, 2011).

Osteoarthritis (OA) is a rapidly progressive, debilitating, degenerative, painful disease that leads to joint failure and impairs quality of life. Many factors such as fragmented coronoid process, cranial cruciate ligament rupture, femoral head necrosis, and hip dislocation are among the important factors in the clinical management of patients with osteoarthritis. The impact of the disease can be reduced if treated early. Dogs are more susceptible to arthritis compared to cats. Also, MSCs therapies for osteoarthritis are both attractive and highly successive for many reasons. In particular, the direct differentiation of MSCs into chondrocytes or the potential of stem cells to form a new cartilage constitute a reason for treatment. Kriston-Pal et al. (2017) announced in a long-term monitoring study that treatment only with adipose-derived MSCs had a highly regenerative effect on cartilage tissue in dogs suffering from elbow dysplasia and osteoarthritis. In another study conducted by Srzentic Drazilov et al. (2018) reported autologous adipose-tissue derived MSCs on the management of OA had beneficial effects on functionality and life quality in 10 various dog breeds.

Stem cell therapies restore the cardiac functions and contribute to cardiac remodeling and regeneration processes. MSCs therapies have been used in cardiac diseases in dogs as an ischemic damage model for translation research to humans (Silva et al., 2005). Perin et al. (2008) reported injection to the transendocardial route of the allogenic bone-marrow derived MSCs in canine acute myocardial infraction model led to improvement functional capacity of ischemic area.

Stem cell transplantation is a key concept for eye diseases in human medicine (Blenkinsop et al., 2012). Lots of animals have been suffering from visual problems due to neurologic, viral or mechanical ophthalmic diseases. Villatoro et al. (2015) were applied allogeneic adipose-derived MSCs to dogs suffered from keratoconjunctivitis sicca, an immune-mediated multifactorial disease with high prevalence among human and dogs, and reported significant improvement in all ocular clinical parameters.

Recurrent and obstructive airway diseases have trouble both patients and owners in veterinary medicine. These oxygen-deprived distressful conditions trigger the inflammatory response in the lung. MSCs have serious immunomodulatory effects by suppressing T cell proliferation and regulation the homeostasis of TH1 cells, lowering proinflammatory cytokines, supporting anti-inflammatory cytokines and suppressing cytokine storm (Debosschere et al., 2020; Akkoc, 2020; Weiss and Dahlke, 2019). Barussi et al. (2016) reported the intratracheal administration of bone marrow-derived mononuclear cells (BMMCs) could alleviate the inflammatory response better than dexamethasone in equine recurrent airway obstruction. Trzil et al. (2016) conducted a pilot study in feline asthma by infused intravenously allogenic adipose-derived MSCs, thereafter 9 months treatment period, obtained unclarified data about the immunological response, however, emphasized that MSCs therapy have a beneficial effect and delayed with reducing the airway inflammation, hyper-responsiveness and remodelling in asthma.

MSCs have also beneficial effects against acute and chronic kidney diseases into different stages. Lee et al. (2017) showed the canine umbilical cord blood (cUCB) derived MSCs therapy in dogs were lowered blood urea nitrogen and creatinine levels, and extended life span with contributed life quality in acute kidney injury. Quimby et al. (2013) evaluated MSCs therapy in cats suffered by chronic kidney disease; while feline adipose-derived MSCs were improved renal function parameters, effective and safely on intravenous administration, the frozen and thawed MSCs were found ineffective. The literature have contradictory data about the efficiency of MSCs therapy on renal diseases. Thus, more studies must conduct among various breed types and different stages to clarify the MSCs therapy in renal diseases.

There are significant differences in studies of cell-based products among different species related to their bio-composition and many factors including the cell preparation technique and the source of the cell (Bogers, 2018). As an example, cell therapies for the cornea in veterinary medicine are really limited due to lacking of detailed knowledge over the the limbal region anatomy, the regional reservoir of stem cells, and their identification across species (Patrino et al., 2017). Furthermore, many basic questions about mesenchymal stem cells, which are clinically applied in veterinary medicine, are still unanswered. In particular, there are many questions marks such as when to apply stem cell therapy, how effective it will be, the method of application, the optimal number of cells, and whether stem cells should be chosen as autologous or allogeneic (Borjesson and Peroni, 2011; Fortier, 2005; Whitworth and Banks, 2014). Briefly, critical questions regarding stem cell therapies in veterinary patients must be answered urgently, not only for motivation but also to develop a standard and accurate treatment of stem cells to be applied clinically in both veterinary and human medicine.

The geriatric studies are generally limited in veterinary medicine, whereas the life spans are extended related to modern life and quality care. Most stem cell studies on the aging process involve mouse and human studies. According to literature data the amount releasing stem cells from the bone-marrow decrease drastically in elderly (Dimmeler and Vasa-Nicotera, 2003). Aging period can be delayed by



increasing the amount and release of stem cells (Scheubel et al., 2003; Rauscher et al., 2003). Recent studies show that the more stem cells that participate in the circulation, the greater the ability of damaged tissues or organs to regenerate and heal themselves.

### **Conclusion**

Pharmaceutical biotechnology is canalized ultra-fast developing stage in the last decade. Cell-based therapy strategies will be dominant in personalized medicine in the near future. A growing transformation is waiting to veterinary medicine and veterinary therapeutics market. In light of obtained literature data veterinarians have to concentrate on cell-based therapy strategies with deep knowledge.

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## **Feeding Methods Applied to Calves in Dairy Cow Farms Factors to Consider**

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### **Summary**

Care conditions and feeding strategies of calves are of vital importance in dairy farms. The healthy raising of calves is the main factor affecting the sustainability and profitability of businesses. Shelter style of calves significantly affects their development characteristics and health. Adequate areas of calf huts or groups and the bedding material used as well as hygiene conditions are among the factors that shape the future of calves. After the calf is born, rapid and adequate consumption of colostrum promotes calf health by increasing passive immunity. Colostrum feeding methods and levels differ significantly between businesses. Whole milk or milk replacer feeds are used during the liquid feeding period of the calves. The growth and development of the animal varies according to the amount and duration of feeding with liquid feeds. Consumption of calf starter feed enables the development of the rumen and digestive system of the animal, thus making the weaning period earlier. Knowing the care conditions and feeding conditions of the calves will increase the growth performance of the calves and contribute to the creation of new strategies and measures to reduce losses.

**Keywords:** Calf, maintenance conditions, feeding methods, feeding with solid feeds, liquid feed sources

## Süt İneği İşletmelerinde Buzağılara Uygulanan Besleme Metotlarında Dikkat Edilmesi Gereken Faktörler

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### Özet

Süt ineği işletmelerinde buzağuların bakım şartları ve besleme stratejileri hayati öneme sahiptir. Buzağuların sağlıklı olarak büyütülmesi işletmelerin sürdürülebilirlikleri ve karlılıklarını etkileyen ana faktördür. Buzağuların barınma şekli gelişme özellikleri ile sağlıklarını önemli düzeyde etkilemektedir. Buzağı kulübeleri veya gruplarının alanlarının yeterli olması ve kullanılan yataklık materyalinin yanı sıra hijyen şartları buzağuların geleceğini şekillendiren etmenler arasındadır. Buzağının doğduktan sonra hızlı ve yeterli miktarda kolostrum tükettirilmesi pasif bağışıklığı artırarak buzağı sağlığını teşvik etmektedir. Kolostrum besleme metotları ve düzeyleri işletmeler arasında önemli derecede farklılaşmaktadır. Buzağuların sıvı besleme dönemlerinde tam süt veya süt ikame yemleri kullanılmaktadır. Sıvı yemlerle besleme miktarı ve süresine göre hayvanın büyüme ve gelişmesi değişiklik göstermektedir. Buzağı başlangıç yemi tüketimi hayvanın rumen ve sindirim sistemi gelişimini sağlayarak süttan kesme süresini erkene çekmektedir. Buzağuların bakım şartları ve besleme durumlarının bilinmesi buzağuların büyüme performansını artıracak ve kayıpları azaltacak önlemlerin alınması ve yeni stratejilerin oluşturulmasına katkıda bulunacaktır.

**Anahtar Kelimeler:** Buzağı, bakım şartları, besleme metotları, katı yemlerle besleme, sıvı besleme kaynakları

## **The Use of Nanoparticles Obtained by The Green Synthesis Method as Pesticides**

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### **Summary**

Pesticides are chemicals used in agriculture, environmental health, veterinary and human medicine fields to control all kinds of pests such as weeds, insects, rodents and to obtain more productive products. Depending on the use of pesticides, skin, eye and nervous system damage may occur in humans and animals, and serious problems such as cancer may occur in long-term exposure. In addition, excessive and unregulated use of pesticides leads to a decrease in the population of non-target organisms. Intensive studies are carried out all over the world in order to obtain more effective and safe pesticides, and among these studies, nanoparticles are very promising. It is thought that by formulating pesticides with nanoparticles, their early decomposition will be prevented, the solubility of poorly soluble active ingredients will increase, and therefore, their duration of action in the targeted tissue will be prolonged when they are applied to plants, animals or humans. In particular, the effectiveness of nanoparticles obtained from plants against many arthropod pests has been determined. Nanoparticles can be obtained from different sources and methods. Among these, the green synthesis method comes to the fore as it is much less harmful and environmentally friendly. In this method, nanoparticles are obtained from natural sources such as various plants, yeast, bacteria, fungi and algae. In this paper, the use of important nanoparticles obtained by green synthesis as pesticides was evaluated.

**Keywords:** Pesticide, nanoparticle, green synthesis

## **Yeşil Sentez Yöntem ile Elde Edilen Nanopartiküllerin Pestisit Olarak Kullanımı**

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### **Özet**

Pestisitler, tarım, çevre sağlığı, veteriner ve beşeri hekimlik alanlarında yabancı otlar, böcekler, kemirgenler gibi her türlü zararlıyı kontrol altına alabilmek ve daha verimli ürün elde edebilmek için kullanılan kimyasal maddelerdir. Pestisitlerin kullanımına bağlı olarak insan ve hayvanlarda cilt, göz, sinir sistemi ile ilgili hasarlar oluşabilmekte ve uzun süreli maruziyette kanser gibi ciddi sorunlar ortaya çıkabilmektedir. Bunun yanında, pestisitlerin aşırı ve düzensiz kullanımı hedef dışı organizmaların popülasyonunun azalmasına neden olmaktadır. Daha etkili ve güvenli pestisitler elde edilebilmesi için bütün dünyada yoğun çalışmalar yapılmakta ve bu çalışmalar arasında nanopartiküller oldukça umut vadetmektedir. Pestisitlerin nanopartiküller ile formüle edilmesi sayesinde erken bozunmalarının önleneceği, çözünürlüğü zayıf aktif bileşenlerin çözünürlüğünün artacağı ve dolayısıyla bitki, hayvan veya insanlara uygulandıklarında hedeflenen dokuda etki sürelerinin uzayacağı düşünülmektedir. Özellikle bitkilerden elde edilen nanopartiküllerin birçok eklembacaklı zararlıya karşı etkinlikleri belirlenmiştir. Nanopartiküller farklı kaynak ve metotlarla elde edilebilmektedir. Bunlar arasında, çok daha az zararlı ve çevreci olması ile yeşil sentez yöntemi ön plana çıkmakta, bu yöntemde nanopartiküller çeşitli bitki, maya, bakteri, mantar, alg gibi doğal kaynaklardan elde edilmektedir. Bu bildiriye yeşil sentez ile elde edilen önemli nanopartiküllerin pestisit olarak kullanımları değerlendirilmiştir.

**Anahtar kelimeler:** Pestisit, nanopartikül, yeşil sentez



## Relationship between Nutritional Differences and Biochemical Parameters in Cats and Dogs

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### Summary

In this study, it was aimed to compare the effects of good and bad quality food use on biochemical parameters in cats and dogs of similar age range. Six healthy dogs (average age  $7.00 \pm 0.58$  years) and five cats (average age  $11.00 \pm 1.79$  years) brought to the veterinary clinic for vaccination and general control purposes, fed with good quality dry food, and five dogs (mean age  $10.20 \pm 0.73$  years) and four cats (mean age  $8.00 \pm 1.08$  years) which are fed with poor quality dry food/home meal with complaints of general condition disorder, constituted the study materials. Serum glucose (GLU), urea (BUN), creatinine (CREA), phosphorus (PHOS), calcium (CA), total protein (TP), albumin (ALB), globulin (GLOB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), total bilirubin (TBIL), cholesterol (CHOL), lipase (LIPA) and amylase (AMY) levels were determined with commercial test kits. GLU, BUN, ALT, ALP, TBIL, AMY, CREA and PHOS values of cats have not fed professional food (Group II) were found to be higher than reference values. Only GLOB value was high in healthy cats (Group I). Meanwhile, a statistically important difference between CREA and PHOS values was determined between two groups in cats ( $p < 0.01$ ). In dogs, BUN, CREA, PHOS, GLOB, ALT and ALP values were higher than reference values in Group II. All biochemical parameters were within reference ranges in Group I animals. A statistically significant difference in BUN, CREA, CA, ALB ( $p < 0.01$ ), PHOS and ALP ( $p < 0.05$ ) values was determined between Group I and II. It was concluded that balanced and adequate nutrition may have positive effects on health and metabolic status and quality of life of elderly animals.

**Keywords:** Nutrition, biochemical parameters, cat, dog

## Kedi ve Köpeklerin Beslenme Farklılıklarının Biyokimyasal Parametreler ile İlişkisi

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### Özet

Bu çalışmada, benzer yaş aralığındaki kedi ve köpeklerde, iyi ve kötü kalitedeki mama kullanımlarının biyokimyasal parametreler üzerine olan etkilerinin karşılaştırılması amaçlandı. Veteriner kliniğine aşı ve genel kontrol amacıyla getirilen, iyi kalite kuru mama ile beslenen sağlıklı altı köpek (ortalama yaş 7.00±0.58) ve beş kedi (ortalama yaş 11.00±1.79) ile genel durum bozukluğu şikayeti olan, kötü kalite kuru mama/ev yemeği ile beslenen beş köpek (ortalama yaş 10.20±0.73) ve dört kedi (ortalama yaş 8.00±1.08) çalışma materyalini oluşturdu. Serum glikoz (GLU), üre (BUN), kreatinin (CREA), fosfor (PHOS), kalsiyum (CA), total protein (TP), albumin (ALB), globülin (GLOB), alaninaminotransferaz (ALT), alkalenfosfataz (ALP), gama glutamiltransferaz (GGT), total bilirubin (TBIL), kolesterol (CHOL), lipaz (LIPA) ve amilaz (AMY) düzeyleri ticari test kitleri kullanılarak belirlendi. Profesyonel mama ile beslenmeyen kedilerde (Grup II) GLU, BUN, ALT, ALP, TBIL, AMY, CREA ve PHOS değerlerinin referans değerlerden yüksek olduğu saptandı. Sağlıklı kedilerde (Grup I) ise sadece GLOB değerinin yüksek olduğu görüldü. Aynı zamanda, iki grubun CREA ve PHOS değerleri arasında istatistiksel olarak fark olduğu tespit edildi ( $p<0.01$ ). Köpeklerde ise Grup II'de BUN, CREA, PHOS, GLOB, ALT ve ALP değerleri referans değerlerin üzerinde ölçülürken, Grup I'de bütün değerlerin referans değerler içinde olduğu belirlendi. BUN, CREA, CA, ALB ( $p<0.01$ ), PHOS ve ALP ( $p<0.05$ ) değerleri açısından Grup I ve II arasında istatistiksel olarak anlamlı düzeyde fark olduğu görüldü. Sonuç olarak dengeli ve yeterli beslenmenin özellikle yaşlı hayvanların sağlık ve metabolik durumları ile yaşam kaliteleri üzerinde pozitif etkileri olabileceği kanısına varıldı.

**Anahtar kelimeler:** Beslenme, biyokimyasal parametreler, kedi, köpek

### Introduction

Aging is often related to the body structure, requirements for energy, declining organ and system functions and metabolic differences. Therefore nutrition plays a crucial role to delay such chances and to prevent the progression especially in senior pets (2). In this study, it was aimed to compare the effects of good and bad quality food use on biochemical parameters in cats and dogs of similar age range.

## **Materials and Methods**

In total, 20 animals (9 cats and 11 dogs) were used in the study. Animals were divided into two groups. The control group (Group I) consisted of five healthy cats which were 5-16 years old (average age  $11.00 \pm 1.79$ ) and six healthy dogs which were 5-8 years old (average age  $7.00 \pm 0.58$  years old). The control animals were brought to the veterinary clinic for vaccination and general control purposes and they were fed with good quality dry food through their whole life. The study group (Group II) consisted of four cats which were 5-10 years old (average age  $8.00 \pm 1.08$  years) and five dogs which were 8-12 years old (average age  $10.20 \pm 0.73$  years). The animals in the study group were with complaints of general condition disorder and fed with homemade food and/or poor quality pet foods.

Blood samples were collected from all animals into empty tubes and serum samples were obtained by centrifuging at 3000 rpm for 10 minutes. Serum glucose (GLU), urea (BUN), creatinine (CREA), phosphorus (PHOS), calcium (CA), total protein (TP), albumin (ALB), globulin (GLOB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), total bilirubin (TBIL), cholesterol (CHOL), lipase (LIPA) and amylase (AMY) levels were determined with commercial test kits.

## **Results**

Ages and determined biochemical parameters in cats and dogs are shown in Tables 1 and 2, respectively. GLU, BUN, ALT, ALP, TBIL, AMY, CREA and PHOS values of cats have not fed professional food (Group II) were found to be higher than reference values. Only GLOB value was high in healthy cats (Group I). Meanwhile, a statistically important difference between CREA and PHOS values was determined between two groups in cats ( $p < 0.01$ ). In dogs, BUN, CREA, PHOS, GLOB, ALT and ALP were higher than reference values in Group II. All biochemical parameters were within reference ranges in Group I animals. A statistically significant difference in BUN, CREA, CA, ALB ( $p < 0.01$ ), PHOS and ALP ( $p < 0.05$ ) values was determined between dogs fed with poor quality food (Group II) and those fed with professional food (Group I).

**Table 1.** Age and biochemical parameters of cats\*.

		Group I (Control) n=5	Group II (Study) n=4	
	Reference	Mean±S.E.M.	Mean±S.E.M.	P
AGE		11.00±1.79	8.00±1.08	
GLU	74-152mg/dL	107.40±6.68	<b>203.00±57.45</b>	
BUN	15.0-37.0mg/dL	22.14±1.70	<b>84.40±46.75</b>	
CREA	0.7-2.1mg/dL	1.34±0.09	<b>5.90±1.10**</b>	<0.01
PHOS	2.6-6.4mg/dL	3.70±0.27	<b>10.65±0.15**</b>	<0.01
CA	8.9-12.6mg/dL	10.52±0.25	10.85±0.65	
TP	5.8-9.1g/dL	7.10±1.58	7.35±0.60	
ALB	2.2-4.1g/dL	3.24±0.15	3.03±0.14	
GLOB	2.6-5.1g/dL	<b>5.28±0.46</b>	4.30±0.69	
ALT	13-109U/L	44.60±7.09	<b>172.75±88.34</b>	
ALP	9-109U/L	36.60±5.02	<b>109.25±75.66</b>	
GGT	0-2U/L	<5	<5	
TBIL	0.00-1.00mg/dL	0.20±0.09	<b>3.83±3.68</b>	
CHOL	50-230mg/dL	212.80±27.75	168.00±31.00	
LIPA	0-32U/L	<20	<20	
AMY	500-1400U/L	1323.00±199.60	<b>1697.00±178.00</b>	

\*Bold numbers are higher than the reference values.

\*\*There is a statistical difference between groups.

**Table 2.** Age and biochemical parameters of dogs.

		Group I (Control) n=6	Group II (Study) n=5	
	Reference	Mean±S.E.M	Mean±S.E.M	P
AGE		7.00±0.58	10.20±0.73	
GLU	74-146 mg/dL	96.00±5.92	114.00±22.11	
BUN	7.0-29.0 mg/dL	14.38±1.70	<b>94.54±25.18**</b>	<b>&lt;0.01</b>
CREA	0.3-1.5 mg/dL	0.83±0.11	<b>8848.78±8846.56**</b>	<b>&lt;0.01</b>
PHOS	2.0-6.0 mg/dL	3.95±0.13	<b>8.27±1.42**</b>	<b>&lt;0.05</b>
CA	9.0-13.4 mg/dL	10.50±0.12	9.60±0.12**	<b>&lt;0.01</b>
TP	5.3-8.4 g/dL	7.28±0.31	7.64±0.80	
ALB	2.2-3.9 g/dL	3.10±0.13	2.54±0.10**	<b>&lt;0.01</b>
GLOB	2.1-4.9 g/dL	4.16±0.20	<b>5.12±0.76</b>	
ALT	12-101 U/L	44.17±8.43	<b>135.50±65.64</b>	
ALP	18-214 U/L	73.00±26.03	<b>255.00±80.45**</b>	<b>&lt;0.05</b>
GGT	0-7 U/L	<5	<5	
TBIL	0.00-1.00 mg/dL	0.15±0.02	0.50±0.22	
CHOL	100-330 mg/dL	179.75±12.95	237.33±35.22	
LIPA	0-155 U/L	47.75±20.52	132.50±105.50	
AMY	500-1400 U/L	680.25±147.66	916.67±134.69	

\*Bold numbers are higher than the reference values.

\*\*There is a statistical difference between groups.

## Discussion

The geriatric pets need to special and regular care by veterinarians. The periodic examinations should include general physical and behavioural examination (vision changes, hearing changes, skin changes, mobility changes, and restlessness at night). In addition, hematologic and biochemical examination must be check (3). Aging encompasses a complex set of processes that gradually leads to increased vulnerability and damage at the cellular and organ level, and eventual death of the organism (2). Due to unbalanced nutrients of home-prepared diet, cats and dogs could have organ failures (4).

In our study, general condition and biochemical parameters were evaluated in geriatrics cats and dogs according to diet types. In animals fed with poor quality food, GLU, BUN, ALT, ALP, TBIL, AMY, CREA and PHOS values of cats and BUN, CREA, PHOS, GLOB, ALT and ALP values of dogs were

higher than physiological values. Also, PHOS and ALP values in cats and BUN, CREA, CA, ALB, PHOS and ALP values in dogs were statistically different between animals fed with good and poor quality foods. These results compatible with complete nutrition improves health over the long term with less damage on the animals. Right feeding in all life stages especially in senior age, can help prevent diet-associated diseases and also may help in the control of other diseases (1)

### **Conclusion**

Geriatric cats and dogs need to particular care and regular examination. Most important point for health is balanced diet for prevent diet-associate disease, organ failures and cell-damages. It may not extend the life duration of the pets but cats and dogs can be lived more comfortable life without pain, anxiety. In the light of our findings, it was concluded that balanced and adequate nutrition may have positive effects on metabolic status, quality of life and lifespan of elderly animals.

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## Pemphigus Foliaceous in a Cat

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### Abstract

In this case report, Pemphigus foliaceus (PF) was evaluated in a female, 3 years old, Scottish fold cat. In this case report, a 3-year-old, female Scottish fold cat was evaluated. The cat was 3.4 kg and gave birth to 5 puppies two months ago. The cat was presented with an acute onset of non-pruritic crusted lesions on the pinnae, lateral mouth, ears, and nail beds, and nasal area, especially after birth. She had prolapse nail in paws and xanthic–crusted lesions and this situation happened since first birth. Also, she had a one-week history of anorexia and weakness. There were some haematological changes, as well. In the histopathological examination, discrete, subcorneal pustules formed by the presence of a limited number of individualized, round to polyhedral acantholytic keratinocytes with a viable nucleus, singly or small clumps, were free-floating, were determined. From these findings, it is tentatively diagnosed as feline PF. Prednisolone tablet (Deltacortril tablet, 4 mg/kg/day PO for 6 weeks) provided clinical recovery, amoxicillin-clavulanate (8.75 mg/kg/day PO for 7 days) and supportive therapy were administered. The cat has recovered after 6 weeks.

**Keywords:** Pemphigus Foliaceous, cat, glucocorticoids, prednisolone.

## Bir Kedide Pemfigus Foliaceous Olgusu

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### Özet

Bu bildiriye, 3 yaşlı, dişi bir Scottish fold ırkı bir kedide Pemfigus Foliaceous (PF) olgusu değerlendirildi. Klinik muayenede, özellikle doğumdan sonra kulak kepçesi, labial komisura, kulaklar, tırnak kökleri ve burun bölgesinde akut başlangıçlı, kaşıntılı olmayan kabuklu lezyonlar; doğum yaptığından itibaren mevcut olan patilerde tırnak sarkması ve ksantik-kabuklu lezyonlar; bir haftadır devam eden anoreksi ve halsizlik; titreme ve kulaklarda kaşıntı ve kulaklardan sarımtırak, yoğun kıvamlı akıntı olduğu görüldü. Ayrıca hematolojik parametrelerde bazı değişiklikler belirlendi. Histopatolojik incelemede subkorneal püstüllerin içerisinde nötrofiller ile birlikte, sınırlı sayıda,

akantolitik keratinositlerin bulunduđu görüldü. Bu bulgular PF ile uyumlu bulundu. Prednisolon tablet (Deltacortril tablet, 5 mg; oral yolla 6 hafta süresince 4 mg/kg/gün), amoksisilin-klavulonik asit (7 gün süresince oral yolla 8.75 mg/kg/gün) ve destekleyici tedavi uygulandı. Kedi tedaviye başlanmasından 6 hafta sonar iyileşti.

**Anahtar kelimeler:** Pemphigus Foliaceous, kedi, glikokortikoidler, prednisolone.

### **Introduction**

Pemphigus foliaceus (PF) is the most common form of pemphigus in cats, as well as the most common autoimmune dermatosis (1, 2). The incidence of PF rate has been reported as 5/1000 (1).

Pustules and crusted lesions are seen most commonly on the pinnae, nasal planum, periocular area, chin, and feet of affected cats. This disease has an acantholytic cell population that characterized cell-cell interaction break off. A definitive diagnosis is made based on histopathology showing subcorneal pustules with nondegenerate neutrophils and acantholytic cells. Pemfigus foliaceus is treated corticosteroids, such as prednisolone and immune-suppressive drugs, such as chlorambucil (4).

### **Case definition and clinical findings**

In this case report, a 3-year-old, female Scottish fold cat was evaluated. The cat was 3.4 kg and gave birth to 5 puppies two months ago. The cat was presented with an acute onset of non-pruritic crusted lesions on the pinnae, lateral mouth, ears, and nail beds, and nasal area, especially after birth. She had prolapse nail in paws and xanthic-crusted lesions and this situation happened since first birth. Also, she had a one-week history of anorexia and weakness. The cat had the shaking and itching and there was a yellow flow in the ears.

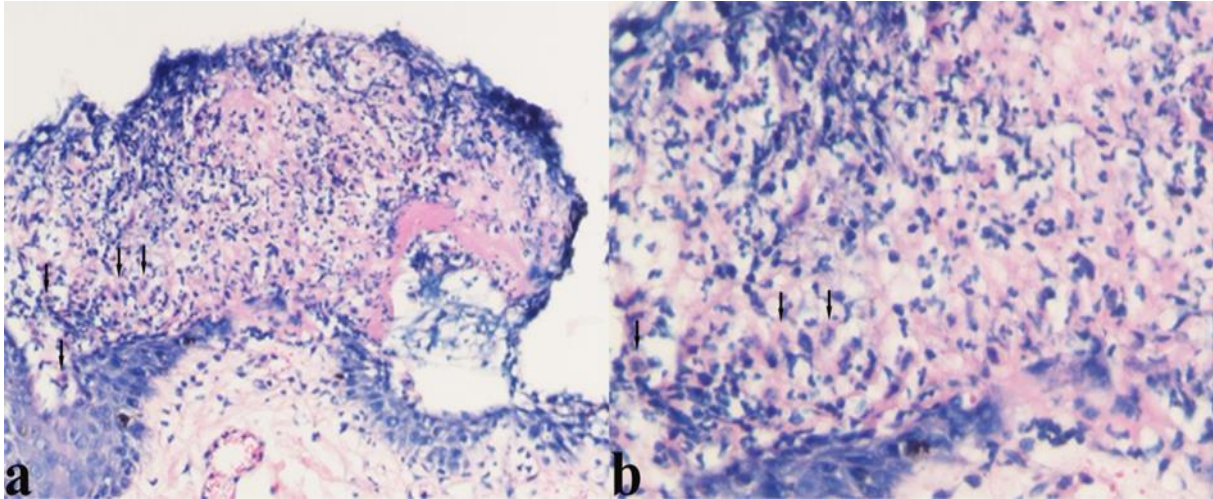
### **Haematological parameters**

In the complete blood count examination, leucocytosis, neutrophilia and monocytosis (WBC: 28.99  $10^9/L$ , Neu: 25.20  $10^9/L$ , Mon: 2.13  $10^9/L$ ) and decreased HGB (7.9 g/dL), HCT (0.163) and MCV (30.4 fL) were detected. Serum biochemical parameters were normal. The cat had mild dehydration, as well.

### **Histopathology**

Microscopical analysis showed discrete, subcorneal pustules formed by the presence of a limited number of individualized, round to polyhedral acantholytic keratinocytes with a viable nucleus, singly or small clumps, were free-floating. These acantholytic keratinocytes were intermingled with well-preserved neutrophils (Figure 1). From these findings, it is tentatively diagnosed as feline pemphigus foliaceus.





**Figure 1.** (a) Subcorneal pustule with limited number of free-floating (arrows) acantholytic cells. HEx20. (b) Higher magnification of previous figure. Presence of free-floating, round to polyhedral acantholytic epithelial cells with viable nucleus (arrows) in a pustule containing well preserved neutrophils HEx40.

### Treatment

In the first examination, cephalexin (Sef tablet/Mustafa Nevzat), povidone-iodine solution, Centella Asiatica extract (Madecassol pomade, Bayer), nitrofurans (Furacine pomade, Zentiva), veterinary amino acid solution (Duphalyte, Fako veterinary drugs) were prescribed. After two weeks, the lesions were enlarged and treatment was changed. Afterward, prednisolone tablet (Deltacortril tablet, 5 mg; 4 mg/kg/day PO for 6 weeks), amoxicillin-clavulanate (8.75 mg/kg/day PO for 7 days) were administered. In addition, lactated ringers solution (20 mL/kg) and amino acid solution (5 mL/kg) were applied intravenously for the supportive therapy. The cat has recovered after 6 weeks started the treatment.

### Discussion

PF is an autoimmune skin disease and characterized by the breakdown of epidermal intercellular connections (6). Eighty per cent of cats were reported pruritic. At the time of biopsy, the distribution of lesions varied, but included some combination of face/head, paws, dorsum or ventrum and consisted of crusts, erosions, scale and alopecia (5).

The diagnosis of the PF depends on histopathological findings, the presence of intact or degenerate intraepidermal pustules containing granulocytic cells and acantholytic keratinocytes (6). Similar findings were determined in this case.

There are some haematological changes in PF and 17% of cats with PF have high WBC count (20-30 K/ $\mu$ L). In this case report, the cat has 28.99 K/ $\mu$ L, as well. Therefore antibiotic was used (2).

Glucocorticoids (GCs) are valuable and effective treatment alternative in PF in cats. GCs has effects on both humoral and cell-mediated immunity, inhibition of inflammatory mediators and suppression of autoantibody levels. Previous studies reported different GC types and dosage for GCs in PF treatment in cats. Prednisolone dose of 4-5 mg/kg/day can provide 62% remission but 2 mg/kg/day is effective, too. Also, 6.6 mg/kg/day dosage of prednisolone provided remission in the past reports (6). Some other studies reported that GCs in cats with PF are not always successful, and it is common to need additional immunomodulating agents to manage the disease (3). In this case, 4 mg/kg daily prednisolone was used. In six weeks, significant clinical recovery was observed. The dose were decreased gradually  $\frac{1}{2}$  every two weeks and the treatment was finished.

Although there are some different alternatives to treatment of the PF in cats, solely prednisolone treatment was effective in this case.

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**Regulation of The Immune System Against Viral Infections in Honey Bees**

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**Summary**

Beekeeping is breeding activity and bee products are a valuable food group in terms of balanced and healthy human nutrition. regions Viral infections, one of the important factors affecting bee health, are important for the longevity and productivity of bees. In this poster, is contribute to the understanding of antiviral defense include RNA interference (RNAi), endocytosis, melanization, encapsulation, autophagy and conserved immune pathways including Jak/STAT (Janus kinase/signal transducer and activator of transcription), JNK (c-Jun N-terminal kinase), MAPK (mitogen-activated protein kinases) and the NF-KB mediated Toll and Imd (immune deficiency) pathways and the virus-limiting immune response triggered by dsRNA, which is not sequence specific in honey bees.

**Keywords:** Honey bee, antiviral defense

**Bal Arılarında Viral Enfeksiyonlara Karşı İmmun Sistemin Regülasyonu**

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**Özet**

Arıcılık bir yetiştiricilik faaliyeti olup, arı ürünleri dengeli ve sağlıklı insan beslenmesi açısından değerli bir gıda maddesidir grubudur. Arı sağlığını etkileyen önemli faktörlerden biri olan viral enfeksiyonlar arıların uzun ömürlülüğü ve verimleri açısından önemlidir. Bu poster arı antiviral savunma mekanizmaları olan RNA interferansı (RNAi), endositoz, melanizasyon, kapsülleme ve Jak / STAT (Janus kinaz / sinyal dönüştürücü ve transkripsiyon aktivatörü), JNK (c-Jun N-terminal kinaz), MAPK (mitojenle aktive olan protein kinazlar) dâhil olmak üzere korunmuş bağışıklık yolları ile NF-kB aracılı Toll ve Imd (immün yetmezlik) yolları ve bal arılarında sekansa özgü olmayan, dsRNA ile tetiklenen, virüs sınırlayıcı immün yanıt hakkındadır.

**Anahtar kelimeler:** Bal arısı, antiviral savunma

## **Tick-born Encephalitis in Ruminants**

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### **Summary**

Tick-borne diseases (TBE, CCHF, Borrelia, Babesia, Theileria) are extremely important in Turkey as well as all over the world. Tick Borne Encephalitis (TBE) is a tick-borne viral zoonotic infection of the Central Nervous System. It is a disease that is among the serious neurological infections especially seen in Asian and Northern European countries. Although the primary route of infection is the bite of an infected tick, infection has also been found to be spread through consumption of TBEV-contaminated dairy products. The main problem of infected ruminants is the transmission of infection to humans from milk and dairy products obtained from these animals. Although our country has similar climatic and ecological conditions with endemic regions in terms of TBE, information about TBEV activity is limited. This poster contains up-to-date information on TBEV infection in ruminants (bovine, sheep, goat), its vectors, and foodborne TBE infections.

**Keywords:** Sheep, goat, cattle, tick born encephalitis

## **Ruminantlarda Tick Born Encephalitis**

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### **Özet**

Kene kaynaklı hastalıklar(TBE, KKKA, Borrelia, Babesia, Theileria) tüm dünyada olduğu gibi Türkiye’de de son derece önemlidir. Tick Borne Encephalitis(TBE) Merkezi Sinir Sistemi’ nin kene kaynaklı viral zoonoz bir enfeksiyonudur. Özellikle Asya ve Kuzey Avrupa ülkelerinde görülen ciddi nörolojik enfeksiyonlar arasında yer alan bir hastalıktır. Enfeksiyonun birincil yolu, enfekte bir kenenin ısırığı olsa da, TBEV ile kontamine süt ürünlerinin tüketimi yoluyla da enfeksiyon yayıldığı tespit edilmiştir. Enfekte ruminantların ana sorunu, bu hayvanlardan elde edilen süt ve süt ürünlerinden insanlara enfeksiyonun bulaşmasıdır. Ülkemiz TBE açısından endemik bölgelerle, benzer iklim ve ekolojik şartlara sahip olmakla birlikte TBEV aktivitesi hakkında bilgiler kısıtlıdır. Bu poster ruminantlarda (sığır, koyun, keçi) TBEV enfeksiyonları, vektörleri ve gıda kaynaklı TBE enfeksiyonları hakkında güncel bilgileri içermektedir.

**Anahtar kelimeler:** Koyun, keçi, sığır, tick born encephalitis

**First record of *Pelargodacna heteromorpha* Perez & Atyeo, 1992 (Pterolichidae, Xoloptopidinae) on a Black Stork (*Ciconia nigra*) from Turkey**

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**Summary**

A dead specimen of Black Stork (*Ciconia nigra*), brought to Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Parasitology Laboratory was subjected to systemic ectoparasite examination. After the ectoparasite examination, were examined for feather mites. As a result of the examination, samples of feather mites were collected from tail and wing feathers. The feather mite samples were examined under the microscope and determined as *Pelargodacna heteromorpha* Perez & Atyeo, 1992 (Pterolichidae, Xoloptopidinae). 41 feather mite species have been identified in studies conducted in Turkey so far, but *P. heteromorpha* has been not reported in this studies. The existence of *P. heteromorpha* has been reported for the first time in Turkey as a result of this study.

**Keywords:** *Ciconia nigra*, feather mites, *Pelargodacna heteromorpha*,

**Kara Leylek (*Ciconia nigra*)’ten *Pelargodacna heteromorpha* Perez & Atyeo, 1992  
(Pterolichidae, Xoloptopidinae)’nın Türkiye’den İlk Bildirimi**

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**Özet**

Ölü olarak Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı laboratuvarına getirilen Kara leylek (*Ciconia nigra*) sistemik ektoparazit muayenesine tabi tutulduktan sonra kuyruk ve kanat telekleri tüy akarları yönünden incelenmiştir. Yapılan inceleme sonucunda toplanan tüy akarı örnekleri mikroskop altında incelenerek literatürde geçen anahtara göre *Pelargodacna heteromorpha* Perez & Atyeo, 1992 (Pterolichidae, Xoloptopidinae) olarak tanımlanmıştır. Türkiye’de şu ana kadar yapılan çalışmalarda 41 tüy akarı türü tanımlanmış olup *P. heteromorpha*’nın ise herhangi bir bildirimine rastlanılmamıştır. Yapılan bu çalışma ile Türkiye’de ilk defa *P. heteromorpha* tespit edilmiştir.

**Anahtar kelimeler:** *Ciconia nigra*, *Pelargodacna heteromorpha*, tüy akarları



## **One Health Concept and Viral Infections**

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### **Summary**

The One Health approach is defined by the One Health Commission as "the joint effort of multiple disciplines to achieve optimal health for people, animals and our environment" and plays an important role in the prevention and control of zoonoses. The Zoonoses are diseases based on many factors such as changes in human behavior, farming and trade practices, vector distributions and genetics of microorganisms and should be considered as the single most critical risk factor for human health and well-being. Virus-induced zoonotic diseases, which constitute an important part of zoonotic diseases are important for this reason and in order to solve these complex health problems at the human-animal-environment interface is needed multidisciplinary One Health application.

In this poster, viral infections that have been resolved with a single health application are explained.

**Keywords:** One health, viral zoonotic infections

## Tek Sağlık Konsepti ve Viral Enfeksiyonlar

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### Özet

Tek sağlık yaklaşımı, Tek Sağlık Komisyonu tarafından "İnsanlar, hayvanlar ve çevremiz için optimal sağlığı elde etmek için birden fazla disiplinin ortak çabası" olarak tanımlanmaktadır ve zoonozların önlenmesi ve kontrolünde önemli rol oynar. Zoonozlar insan davranışları, çiftçilik ve ticaret uygulamaları, vektör dağılımları ve mikroorganizmaların genetiğindeki değişiklikler gibi birçok faktöre dayalı hastalıklardır ve insan sağlığı ile refahı için tek ve en kritik risk faktörü olarak düşünülmelidir. Zoonoz hastalıkların önemli bir bölümünü oluşturan virus nedenli zoonoz hastalıklar bu nedenle önemlidir ve insan-hayvan-çevre arayüzündeki mevcut bu karmaşık sağlık sorunlarını çözebilmek için multidisipliner Tek Sağlık uygulamasına ihtiyaç vardır. Bu posterde tek sağlık uygulaması ile çözüme ulaşılmış viral enfeksiyonlar anlatılmıştır.

**Anahtar kelimeler:** Tek sağlık, viral zoonotik enfeksiyonlar

## The Use of Antioxidant in Cryopreservation of Rooster Semen

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### Summary

During the freezing and thawing processes, sperm are exposed to some damages that reduce spermatological parameters and fertilization ability. Since rooster semen is sensitive to these damages, decreases in sperm motility, and fertilization ability can be seen. This sensitivity of rooster semen is due to its plasma membrane rich in polyunsaturated fatty acids. Because of this feature, it is sensitive to lipid peroxidation and sperm motility, membrane integrity, and fertilization ability are adversely affected. Antioxidant additions were made to rooster semen extenders in order to eliminate this sensitivity to lipid peroxidation caused by reactive oxygen species (ROS) during in vitro studies and cryopreservation process. In recent years, some antioxidants such as Selenium, Nano-Selenium, Vitamin E, Melatonin have been added to rooster semen extenders and investigated in terms of spermatological parameters.

**Keywords:** Antioxidant, rooster, cryopreservation, semen

## Antioksidanların Horoz Spermasının Dondurulmasında Kullanımı

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### Özet

Sperma, dondurma ve çözündürme süreçlerinde spermatolojik parametreleri ve fertilizasyon yeteneğini düşürücü bir takım hasarlara maruz kalmaktadır. Horoz sperması bu hasarlara duyarlı olduğundan dolayı sperma motilitesi ve fertilizasyon yeteneğinde azalışlar görülebilmektedir. Horoz spermasının

bu duyarlılığı, çoklu doymamış yağ asitleri açısından zengin plazma membranına sahip olmasından kaynaklanmaktadır. Bu özellik yüzünden lipid peroksidasyonuna karşı duyarlıdır ve sperma motilitesi, membran bütünlüğü ve fertilizasyon yeteneği olumsuz etkilenmektedir. İn vitro çalışmalar ve kriyoprezervasyon süreçlerinde reaktif oksijen türleri (ROS) tarafından oluşturulan lipid peroksidasyonuna karşı olan bu duyarlılığın giderilmesi amacıyla horoz sperma sulandırıcılarına antioksidan ilaveleri yapılmıştır. Son yıllarda Selenyum, Nano-Selenyum, Vitamin E, Melatonin gibi bazı antioksidanlar horoz sperma sulandırıcılarına katılıp spermatolojik parametreler açısından incelenmiştir.

**Anahtar kelimeler:** Antioksidan, horoz, kriyoprezervasyon, sperma

## **Giriş**

Spermanın dondurularak saklanması, genetik kaynakların korunması ve suni tohumlama uygulamaları için değerli bir yöntemdir. Son yıllarda, kriyoprezervasyon yöntemleri memeli türlerinde ve özellikle bu tekniklerin standart hale geldiği büyükbaş hayvanlarda önemli başarılar kaydedilmiştir (1). Memeli hayvanlardakinin aksine kanatlı hayvanlarda spermanın dondurulması için standart bir prosedür oluşturulmamıştır ve kullanımı sığırlarda olduğu kadar yaygın olarak kullanılmamaktadır (2).

Kriyoprezervasyon teknolojilerinin kullanımı, spermatozoon üzerinde geri dönüşü olmayan bir takım hasarlara neden olmaktadır. Bu hasarlar spermatozoonun yapısal bütünlüğünü tehlikeye sokmaktadır. Ayrıca kriyoprezervasyonun spermatozoonda DNA hasarında artışa yol açtığına dair önemli kanıtlar bildirilmiştir (3, 4).

Spermatozoonun plazma membranındaki lipid içeriği, fertilizasyon yeteneğinde önemli bir faktördür (5). Horozların spermatozoon hücre zarlarındaki çoklu doymamış yağ asitleri (PUFA) konsantrasyonu, memeli spermatozoonundan çok daha fazla miktardadır (6). Bu nedenle, horoz sperması, in vitro işlemler ve depolama sırasında reaktif oksijen türleri (ROS) tarafından gerçekleştirilen lipid peroksidasyonuna (LPO) daha duyarlıdır (7).

Ayrıca spermatozoa, spermatogenezisin başkalaşım aşamasında sitoplazmasının bir kısmını kaybederek koruyucu antioksidanlarının önemli bir bölümünü de kaybetmektedir (8, 9). Horoz spermasının kriyoprezervasyonundan önce antioksidan takviyesi ile bu olumsuz durumların telafi edilebileceği düşünülmektedir (10). Bu amaçla, horoz spermasının dondurulmasında birçok antioksidanın kullanıldığı araştırmalar yapılmıştır. Bu metinde son yıllarda dondurma ve çözündürme sonrası sperma kalitesini artırmayı amaçlayan araştırmalarda kullanılan antioksidanların etkileri ve araştırma sonuçları özetlenmeye çalışılmıştır.

### Selenyum, Kolostrum ve Vitamin E

Łukaszewicz ve arkadaşlarının 2020 yılında yaptıkları bir çalışmada, 5 adet ISA Brown (ISA-B) ırkı horoz ve 5 adet Hubbard Flex (H-F) ırkı horoz kullanılmıştır. Çalışmadaki tüm horozlardan dorso-abdominal masaj yöntemi ile sperma alınmıştır. Sperma sulandırıcısı olarak 100 mL distile suya 0,14 g potasyum sitrat, 0,21 g sodyum dihidrojen fosfat, 0,98 g disodyum hidrojen fosfat, 0,7 g glukoz, 1,4 g sodyum glutamat, 0,2 g D-fruktoz, 0,7 g inositol, 0,1 g polivinilpirolidon, ve 0,02 g protamin sülfat katarak sperma sulandırıcısı oluşturmuşlardır. Oluşturdukları sulandırıcıya “EK” ismini vermişlerdir. Hayvanlardan alınan sperma 5 eşit gruba bölünmüştür ve 4 grup EK sulandırıcısı ile 1:3 oranında sulandırılmıştır. Çalışma grupları taze (sulandırıcı katılmamış), EK (sadece sulandırıcı katılmış), EK+Se+E (EK sulandırıcısı, 1µg/mL organik selenyum ve 8µg/mL vitamin E), EK+AS (EK sulandırıcısı, 10 mg/mL arı sütü) ve EK+KOL (EK sulandırıcısı, 0,25 g/mL liyofilize sığır kolostrumu) olarak dizayn edilmiştir. Çalışma gruplardaki spermalar 1mL’lik steril tüpler içerine konup 15 dakika 20oC-22oC’de bekletilmiş ve ardından 4oC’lik buzdolabına konup 24 saat sonra spermatolojik incelemeler için çıkartılmıştır. Tüm gruplarda motilite, kinematik parametreler, spermatozoa canlılığı ve anormal spermatozoa oranı muayeneleri gerçekleştirilmiştir. Çalışma sonucunda EK+AS ve EK+KOL gruplarında spermatolojik değerlerin daha yüksek olduğu saptanmıştır. Böylelikle horoz spermasının saklanması istenmeyen etkilerin arı sütü ve kolostrum kullanılarak azaltılabileceği anlaşılmıştır (11).

### Nano – Selenyum ve Vitamin E

Safa ve arkadaşlarının 2016’da yaptıkları bir çalışmada, 20 adet Leghorn ırkı horoz kullanılmıştır. Çalışmadaki horozlardan dorso-abdominal masaj tekniği ile sperma alınmıştır. Sperma sulandırıcısı olarak Modifiye BPSE kullanılmıştır. Sulandırılan sperma 9 eşit parçaya ayrılmış ve E<sub>0</sub>NS<sub>0</sub> (kontrol) E<sub>0</sub>NS<sub>1</sub> (vitamin E 0 µg/mL, nano-selenyum %1), E<sub>0</sub>NS<sub>2</sub> (vitamin E 0 µg/mL, nano-selenyum %2), E<sub>5</sub>NS<sub>0</sub> (vitamin E 5 µg/mL, nano-selenyum %0), E<sub>5</sub>NS<sub>1</sub> (vitamin E 5 µg/mL, nano-selenyum %1), E<sub>5</sub>NS<sub>2</sub> (vitamin E 5 µg/mL, nano-selenyum %2), E<sub>10</sub>NS<sub>0</sub> (vitamin E 10 µg/mL, nano-selenyum %0), E<sub>10</sub>NS<sub>1</sub> (vitamin E 10 µg/mL, nano-selenyum %1), E<sub>10</sub>NS<sub>2</sub> (vitamin E 10 µg/mL, nano-selenyum %2) olarak çalışma grupları tasarlanmıştır. Ön hazırlıklardan sonra ekilibrasyon ve sıvı azot buharında dondurma işlemleri gerçekleştirilmiştir. Çözüm sonu spermatolojik parametreler incelenmiştir. İncelenen parametreler; sperma motilitesi, kinematik parametreler, spermatozoa canlılık oranı, anormal spermatozoa oranı, spermatozoon plazma membran bütünlüğü ve lipid peroksidasyon seviyeleri (malondialdehit, katalaz, glutatyon peroksidaz ve superoksit dismutaz) incelenmiştir. Çalışma sonucunda, sperma sulandırıcısına Vitamin E ve nano-selenyum katılması ile spermanın lipid peroksidasyonunun azalacağı ve horoz seminal plazmasının antioksidan miktarını artırdığı tespit edilmiştir. Ayrıca horoz sperma sulandırıcılarına küçük dozlarda (E<sub>5</sub>NS<sub>1</sub> grubu) Vitamin E ve Nano-

Selenyumun kombine şekilde katılmasının, tek başlarına katılmalarına kıyasla sperma kalitesini ve morfolojisini korumada daha etkili olduğunu göstermiştir (12).

### Melatonin

Appiah ve arkadaşlarının 2019'da yaptıkları çalışmada, Arbor Acres ırkı 40 horoz kullanmışlardır. Çalışmadaki horozlardan dorso-abdominal yöntem ile sperma alınmıştır. Sperma sulandırıcısı olarak temel kryoprezervasyon medyumunu kullanılmıştır. Sulandırılan sperma 4 eşit parçaya ayrılmış ve M0 (kontrol), M1 (0,125 mg/mL melatonin), M2. (0,25 mg/mL melatonin), M3 (0,5 mg/mL melatonin) olarak çalışma grupları tasarlanmıştır. Yapılan işlemlerden sonra ekilibraasyon ve sıvı azot buharında dondurma işlemleri gerçekleştirilmiştir. Çözüm sonrası sperma motilitesi, spermatozoon plazma membran bütünlüğü, akrozom bütünlüğü, sperma antioksidan içeriği, glutatyon peroksidaz, katalaz, superoksit dismutaz ve reaktif oksijen türleri yönünden muayeneler yapılmıştır. Çalışma sonucunda sperma sulandırıcısına katılan 0,25 mg/mL melatoninin dondurulmuş horoz spermasında çözüm sonu spermatolojik parametreler üzerine olumlu etkiye sahip olduğu tespit edilmiştir. Böylece horoz spermasına sulandırma aşamasında melatonin eklenmesinin kryoprezervasyon sırasında horoz spermasının kalitesinin korunmasında yardımcı olacağı anlaşılmıştır (13).

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## Identification of DNA Damage in Rooster Sperm

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### Summary

Measurement of sperm DNA damage is a useful tool in the evaluation of male infertility, as the sperm nucleus lacks protection against oxidative stress and is vulnerable to oxidation mediated DNA damage. The Comet assay or single-cell gel electrophoresis is a relatively simple and sensitive method for measuring strand breaks in DNA in individual sperm. During this procedure, spermatozoa are embedded in a thin layer of agarose on a microscope slide and lysed with detergent under high salt conditions. This process removes protamines and histones, allowing the nucleus to form a nucleoid-like structure containing supercoiled loops of DNA. Sperm Chromatin Dispersion Assay is based on the principle that spermatozoa with DNA fragmentation cannot form a halo of dispersed DNA chains when mixed with agarose, followed by acid denaturation and removal of nuclear proteins. In this paper, some methods used in detecting DNA damage in rooster semen are described.

**Keywords:** Comet, DNA damage, oxidative stress, rooster, SCD assay

## Horoz Spermasında DNA Hasarı Tespiti

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### Özet

Spermatozoon nükleusunun oksidatif strese karşı savunma mekanizması olmadığı ve oksidasyon kaynaklı DNA hasarına karşı savunmasız olduğu için, spermatozoon DNA hasarının ölçümü erkek infertilitesinin değerlendirilmesinde oldukça faydalı bir yöntemdir. Comet testi veya tek hücreli jel elektroforezi, spermatozoon DNA'sındaki iplik kopmalarını ölçmek için nispeten basit ve hassas bir



yöntemdir. Comet testi sırasında, spermatozoa, lam üzerindeki ince bir agaroz tabakasına gömülür ve yüksek tuzlu koşullarda deterjanla parçalanır. Bu işlem protaminleri ve histonları ortadan kaldırarak çekirdeğin süper sarmallı DNA içeren nükleoid benzeri bir yapı oluşturmasını sağlar. Spermatozoon Chromatin Dispersion Testi ise DNA fragmantasyonuna sahip spermatozoonun, agaroz ile karıştırıldığında dağılmış DNA zincirlerinin ışık huzmesi oluşturamaması ve bunu takiben asit denatürasyonu ve nükleer proteinlerin uzaklaştırılması prensibine dayanmaktadır. Bu bildiride horoz spermasındaki DNA hasarı tespitinde kullanılan bazı yöntemler anlatılmıştır.

**Anahtar Kelimeler:** Comet, DNA Hasarı, Horoz, Oksidatif Stres, SCD Testi

## **Giriş**

Spermatozoon DNA hasarının etiyojisi birçok faktöre bağlıdır ve primer testiküler veya sekonder faktörlere bağlı olabilir. Spermatozoon DNA hasarının, anormal protamin ekspresyonu, reaktif oksijen türlerinin (ROS) aşırı üretimi ve spermatogenez sırasında oluşan apoptozisin sonucu olduğuna inanılmaktadır. Spermatozoon DNA hasarı ve ROS arasındaki ilişki, spermatozoon DNA hasarının tedavisinde antioksidan kullanımının temelini oluşturmaktadır (1).

Spermatozoa kaynaklı ROS seviyeleri spermatozoon DNA hasarı ile ilişkilendirilmiştir, ancak üzerinde spermatozoon DNA hasarının tespit edildiği herhangi bir ROS eşik seviyesi belirlenmemiştir (2,3,4). Spermatozoanın normal fonksiyonlarını gerçekleştirebilmesi için düşük seviyelerde ROS'un kontrollü salınımı gerekiyken, yüksek ROS seviyeleri spermatozoa disfonksiyonuna neden olabilmektedir (5).

Horozların spermatozoon hücre zarlarındaki çoklu doymamış yağ asitleri (PUFA) konsantrasyonu, memeli spermatozoonundan çok daha fazla miktardadır (6). Bu nedenle, horoz sperması, in vitro işlemler ve depolama sırasında reaktif oksijen türleri (ROS) tarafından gerçekleştirilen lipid peroksidasyonuna (LPO) daha duyarlıdır (6). Bu süreç başladığında, spermatozoa yüzeyinde lipid peroksidleri birikmekte ve spermatozoon DNA'sında oksidatif hasar meydana gelebilmektedir (7,8).

## **Comet testi**

Comet testi veya tek hücreli jel elektroforezi, spermatozoon DNA'sı üzerindeki tek ve çift sarmal hasarlarını tespit etmek için kullanılan basit yöntemlerden biridir (9). Testin prensibi, ipliklerin yükü ve boyutu tarafından kolaylaştırılan bir elektrik alanının etkisi altında hasarlı DNA ipliklerinin ayrıştırılmasına dayanmaktadır. Ayrıştırıldıktan sonra bozulmamış DNA kuyruklu yıldızın baş kısmında kalırken tek ve çift sarmallı hasarlı DNA parçaları kuyruklu yıldızın kuyruğuna doğru göç etmektedir (10). Bu nedenle, yüksek oranda DNA hasarı olan spermatozoonda, kuyruklu yıldızın kuyruk kısmında yoğunluk (11) ve uzunluk fazla miktarda olacaktır (12).

### **Horoz sperması için Comet optimizasyonu**

Gliozzi ve arkadaşlarının 2011 yılında yaptıkları çalışmada 18 adet Mericanel della Brianza ırkı horozlardan alınan sperma karıştırılmış (pool), modifiye edilmiş Lake sulandırıcısı ile sulandırılmış ve kryoprotektan olarak %6 Dimetilasetamid (DMA) kullanılmıştır. Pellet yöntemi ile dondurulan spermalar 60°C'de 10 saniye süre ile çözündürülmüştür. Comet yöntemi ile horoz spermasında DNA hasarının tespiti için kullanılan metodun uygulanmasında, sperma örnekleri 1:1 (v/v) oranında dondurma öncesi sulandırıcı ile sulandırılmış ve PBS/BSA solüsyonu ile santrifüj edilmiştir. Spermatozoa 37°C'de Low melting point agarose jel solüsyonu (LMPA) ile karıştırılmıştır. Ardından solüsyon dikkatli bir şekilde normal-melting point agarose bulunan lam üzerine yerleştirilmiş ve başka bir lam ile kapatılmıştır. 4°C'de 10 dakika kurumanın ardından kapatma lamı kaldırılmış ve üçüncü bir agaroz katmanı yapılarak tekrar lam ile kapatılıp 4°C'de 10 dakika bekletilmiştir. Ardından kapatılan lam uzaklaştırılmış ve lizis buffer solüsyonu içerisine yerleştirilip 4°C'de 1 saat bekletilmiştir. Hazırlanan lam 37°C'de içerisinde 5µg/ml proteinaz K bulunan lizis solüsyonu içerisinde 1 saat inkübasyona bırakılmıştır. Ardından PBS solüsyonu ile yıkanmış ve içerisinde elektroforez solüsyonu bulunan yatay elektroforez tankında 20 dakika boyunca ekilibrasyona bırakılmıştır. Elektroforez işlemi oda ısısında 10 V, 8 mA ve 20 dakika süre ile gerçekleştirilmiştir. İşlemlerin sonunda lam nötralize buffer solüsyonu ile yıkanmış, methanol ile fikse edilmiş ve oda koşullarında kurumaya bırakılmıştır. Analiz Leica DC 300F mikroskop ile Leica FW4000 ve CASP (Comet Assay Software Project 1.2.2) yazılımları ile yapılmıştır. Çalışma sonucunda taze spermada spermatozoon membran hasarı, motil spermatozoa ve spermatozoon DNA hasarı sırasıyla %2,94±1,94, %52,10±2,20, %6,24±2,47 olarak tespit edilmiş, dondurulup çözündürülen spermada spermatozoon membran hasarı, motil spermatozoa ve spermatozoon DNA hasarı sırasıyla %66,68±1,77, %19,82±2,20, %19,82±2,47 olarak tespit edilmiştir. Bu çalışma ile horoz spermasında comet analizi ile ilk kez DNA hasar tespiti yapılmıştır (13).

### **Spermatozoon Chromatin Dispersion Testi (SCD)**

Spermatozoon Chromatin Dispersion Testi, spermatozoon DNA fragmantasyonunun analizi için basit ve ucuz bir yöntemdir. Bu yöntem, DNA fragmantasyonuna sahip spermatozoonun, agaroz ile karıştırıldığında dağılmış DNA zincirlerinin ışık huzmesi (halo) oluşturamaması ve bunu takiben asit denatürasyonu ve nükleer proteinlerin uzaklaştırılması prensibine dayanmaktadır (14). Oluşan ışık huzmeleri Wright boyası ile boyandıktan sonra brightfield mikroskop altında görülebilmektedir (15).

### **Horoz Spermasında SCD Testi Kullanımı**

Rui ve arkadaşlarının 2017 yılında yaptıkları çalışmada 6 adet Lohman ırkı horozlardan alınan spermalar Biggers-Whitten-Wittingham sulandırıcısı ile sulandırılmıştır. SCD Testi için kullanılan metod şu şekildedir; sperma örnekleri 37°C'de low-melting agarose ile eşit hacimlerde karıştırılmış ve agaroz ile kaplanmış lamlar üzerine aktarılır. Bu aşamaların ardından hazırlanan lamlar lamel ile

kapatılır ve 5°C'de 4 dakika bekletilir. Daha sonra, lameller dikkatlice uzaklaştırılır ve lamalar yatay olarak 22°C'de 3 dakika süreyle yeni hazırlanmış denatüre edici (0.08 N HCl) solüsyon içerisine konur. Lamalar daha sonra oda sıcaklığında 5 dakika süreyle nötralize edici ve lize edici bir çözeltiye (0.4 M Tris, 0.8 M DTT, 1% SDS, 50 mM EDTA, pH 7.5) konur, 2 dakika süreyle Tris-borat-EDTA tamponunda yıkanır, kademeli olarak her birinde 2 dakika olmak üzere %70, %90 ve %100 etanolde ve son olarak oda koşullarında kurutulur. Hazırlanan lamaların boyanması için Wright boyası 1:1 oranında PBS ile karıştırılır ve hazırlanan lamaların üzerine aktarılır, 10 dakika süreyle boyanması beklenir. Son olarak lamalar yıkanır ve kurumaya bırakılır. Mikroskop altında 1000x magnifikasyon altında örnekler incelendiğinde DNA fragmentasyonu olan spermatozoonlar büyük ve orta büyüklükte ışık huzmesi gösterirler ve DNA fragmentasyonu olmayan spermatozoonlar ise küçük ışık huzmesi gösterirler veya ışık huzmesi göstermezler.

Çalışma sonucunda horoz spermasında SCD Testi ile DNA hasarının tespiti başarılı bir şekilde yapılmış ve istatistik olarak olumlu sonuçlanmıştır (16).

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## Cryptorchidism in Dogs

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### Summary

Cryptorchidism in dogs is a congenital disease caused by sex-restricted autosomal recessive genes. The incidence is higher in some breeds, particularly in smaller and inbred animals. Parents of puppies affected by cryptorchids are carriers. Unilateral cryptorchid dogs may be fertile, but cryptorchid individuals and their siblings should be excluded from breeding programs to reduce the incidence of the disease. The incidence of cryptorchidism is higher in small breed dogs than large breed dogs. The 10 breeds with the highest incidence of cryptorchidism are Toy Poodles, Pomeranian, Yorkshire Terrier, Miniature Dachshund, Cairn Terrier, Chihuahua, Maltese, Boxer, Pekingese, and English Bulldog. As a medical treatment, hCG or GnRH applications are used to increase endogenous or exogenous LH activity. In addition to medical treatment, surgical castration or laparoscopic removal of testicles is one of the most preferred methods.

**Keywords:** Dog, cryptorchidism, testes

## Köpeklerde Kriptorşidizm

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### Özet

Köpeklerde kriptorşidizm, cinsiyetle sınırlı otozomal resesif genlerden kaynaklanan konjenital bir hastalıktır. İnsidans bazı ırklarda, özellikle daha küçük ve inbred yetiştirilen hayvanlarda daha yüksektir. Kriptorşitten etkilenen yavruların ebeveynleri taşıyıcıdır. Unilateral kriptorşitli köpekler fertil olabilirler fakat hastalığın görülme sıklığını azaltmak için kriptorşitli bireyler ve kardeşleri üreme programlarından çıkarılmalıdır. Küçük ırk köpeklerde kriptorşidizm insidansı büyük ırk köpeklere

göre daha yüksektir. En yüksek kriptorşidizm insidansına sahip 10 ırk, Toy Poodles, Pomeranian, Yorkshire Terrier, Minyatür Dachshund, Cairn Terrier, Chihuahua, Maltese, Boxer, Pekinges ve English Bulldog'dur. Medikal tedavi olarak endojen veya eksojen LH aktivitesini artırmak amacıyla hCG veya GnRH uygulamaları kullanılmaktadır. Medikal tedavinin yanısıra cerrahi olarak kastrasyon veya laparoskopik yöntemlerle testislerin alınması en sık tercih edilen yöntemlerdendir.

**Anahtar kelimeler:** Köpek, kriptorşidizm, testis

## **Giriş**

Kriptorşidizm, bir tür için normal olan zamanda testislerden birinin veya her ikisinin skrotuma inmemesidir. Tipik olarak, kriptorşidizm doğumda veya doğumdan kısa bir süre sonra tespit edilir. Abdominal testis; karın boşluğu içinde, tipik olarak böbrek ve mesane arasında veya iç kasık halkasının yakınındadır. Proksimal ve yakın kasık bölgeleri arasındaki ayırım önemlidir, çünkü kriptorşid oluşumuna neden olan probleme işaret edebilir (1). İnguinal testis; iç ve dış kasık halkaları tarafından sınırlanan boşluk içindedir (2). Fertil köpeğin skrotumunda iki normal testis bulunmalıdır. Skrotumunda testis bulunmayan kastre edilmemiş bir erkek bilateral bir kriptorşid olarak adlandırılırken, skrotumunda sadece bir testisi olan bir erkek unilateral kriptorşid olarak adlandırılmaktadır. Unilateral kriptorşidizm, bilateral kriptorşidizme göre daha sık görülmektedir. Normal köpeklerde testislerin skrotuma inişi doğumdan yaklaşık 10 gün sonra tamamlanmaktadır. (3). Bazı kriptorşidli hayvan sahipleri, köpeklerindeki skrotal testislerin var olduğunu ve ardından kaybolduğunu bildirebilirler. Ancak yeni doğan köpeklerde testisler küçük ve yumuşaktır. Özellikle stres veya korku durumlarında testisler, skrotum ile inguinal kanal arasında hareket edebilmektedir (4, 5). Bir köpeğe kriptorşid teşhisi konmadan önce yaklaşık olarak 6 ay kadar beklenmelidir. Köpeklerde inguinal halkalar çoğunlukla 6 aylıkken kapanmaktadır. Kriptorşidizm kalıtsaldır; köpeklerde cinsiyetle sınırlı otozomal resesif bir özelliktir (6) Safkan köpeklerde kriptorşidizm insidansı, safkan olmayan köpeklere göre daha yüksek görülmektedir. Cocker Spaniel ve Minyatür Schnauzer ırklarında yüksek kriptorşidizm prevalansı bildirilmiştir (7, 8). Kriptorşid köpeklerde inguinal ve abdominal fitiklar, patellar luksasyon, prepisyum ve penis problemleri dahil olmak üzere diğer konjenital rahatsızlıklar sık görülmektedir. Kriptorşid testisler neoplaziye yatkındır, sertoli hücre tümörleri ve seminomlar en sık görülen tümörlerdendir (9). Unilateral kriptorşid bir hayvanda spermatogenezis devam edebilirken, bilateral kriptorşidli bir hayvan sterildir. Normal spermatozoa üretmek için testislerin sıcaklığının vücut sıcaklığından 4-5 °C daha düşük olması gerekmektedir, bu sebepten dolayı testislerin skrotum içinde lokalize olması gerekmektedir. Tek taraflı kriptorşid olgularında sperma kalitesinde ciddi anlamda bir düşüş görülmesine rağmen, kızgınlıkta olan bir dişi ile doğal çiftleşme yolları ile başarılı bir fertilizasyon görülebilmektedir. Kriptorşidizm, testosteron üretimini etkilememektedir (10, 11). Bu nedenle çoğu kriptorşid hayvanda cinsel istek ve ereksiyon gözlenir (12). Kriptorşid testisler daha küçüktür; seminifer tübüllerin çapı, skrotal testislere kıyasla %60'a kadar

küçülür (13). Küçük ırk köpeklerde kriptorşidizm insidansı büyük ırk köpeklere göre daha yüksektir (14). En yüksek kriptorşidizm insidansına sahip 10 ırk, Toy Poodles, Pomeranian, Yorkshire Terrier, Minyatür Dachshund, Cairn Terrier, Chihuahua, Maltese, Boxer, Pekingesese ve English Bulldog'dur (6).

### **Diagnoz**

Skrotum ve kasık bölgesinin muayenesi dikkatli bir şekilde yapılmalıdır. Ancak skrotal yağ ve inguinal lenf yumruları, kriptorşid testis ile karışabilir. Abdominal testislerin palpasyon veya ultrasonografik muayene ile saptanması oldukça zordur. Kan testosteron konsantrasyonlarında bir artış sağlamak için hCG veya GnRH kullanan bir stimülasyon testi önerilmektedir. Bu test için standart protokol; GnRH enjeksiyonundan önce ve GnRH enjeksiyonundan 60 dakika sonra alınan kan örneklerinde testosteron konsantrasyonlarını belirlemektir. Test sonrası alınan kan örneğinde testosteron konsantrasyonlarında önemli bir artış kriptorşidizmi göstermektedir (15).

### **Tedavi**

Kriptorşidizm, kalıtsal bir hastalıktır ve kriptorşid bir köpek üreme programlarına katılmamalıdır. Kriptorşidizmin en iyi kontrolü, kriptorşid köpeklerin, anne ve babalarının, üreme programlarından çıkarılmasıyla gerçekleştirilebilir (16). Tercih edilen tedavi türü kastrasyondur. Kriptorşidli testisi bulmak ve çıkarmak için cerrahi yaklaşım konumuna bağlıdır. En yaygın olarak standart cerrahi teknikler kullanılsa da, kriptorşid testis laparoskopisi ile alınabilir (17). Kriptorşid testisi tespit etmenin en iyi yolu, duktus deferensin belirlenmesi ve onun testise kadar takip edilmesi ile gerçekleştirilir. En yaygın medikal tedavi olarak endojen veya eksojen LH aktivitesini artırmak amacıyla hCG veya GnRH uygulamaları kullanılmaktadır (15). Laparoskopik yaklaşımlar, çoğunlukla minimal invaziv olmaları ve ilgilenilen organların daha iyi görüntülenmesini kolaylaştırmaları nedeniyle küçük hayvan cerrahisinde popüler hale gelmiştir (18).

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## Augmented Reality and Veterinary Medicine

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### Summary

Today, the dizzying development of information technologies results in the emergence of new approaches in many fields and the introduction of these innovations into our daily lives in a short time. One of the most interesting among these innovations is Augmented Reality (AG) technology. AR is used in many different areas, and AR applications in the fields of health and education are becoming more common. As in human medicine, it is obvious that AR applications will be extremely beneficial in veterinary medicine where all aspects of diseases should be addressed and theoretical knowledge should be supported by applications. In this paper, information about the features of AR technology and its usage in education, health and especially in veterinary medicine is given.

**Keywords:** Augmented reality, education, veterinary medicine

## Arttırılmış Gerçeklik ve Veteriner Hekimlik

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### Özet

Günümüzde bilişim teknolojilerinin baş döndürücü bir hızda gelişmesi, birçok alanda yeni yaklaşımların ortaya çıkması ve bu yeniliklerin kısa zamanda günlük hayatımıza girmesi ile sonuçlanmaktadır. Bu yenilikler arasında en ilgi çekicilerden birisi Arttırılmış Gerçeklik (Augmented Reality, AG) teknolojisidir. AG birçok farklı alanda kullanılmakta, sağlık ve eğitim alanında AG uygulamaları gittikçe yaygınlaşmaktadır. Beşeri hekimlikte olduğu gibi, hastalıkların her yönüyle ele alınıp teorik bilginin uygulamalarla desteklenmesi gereken veteriner hekimlikte AG uygulamalarının son derece yararlı olacağı ortadadır. Bu bildiride, AG teknolojisinin özellikleri, eğitim, sağlık ve özellikle veteriner hekimlikte kullanımı ile ilgili bilgi verilmiştir.

**Anahtar kelimeler:** Arttırılmış gerçeklik, eğitim, veteriner hekimlik

### **Arttırılmış gerçeklik (AG) teknolojisi**

Sanal ile gerçeğin eş zamanlı olarak iç içe girdiği bir dijital ortam olan Arttırılmış Gerçeklik (Augmented Reality, AG), “gerçek dünya nesnelere yerine dijital ortam ürünlerinin kullanıldığı gerçeklik ortam” şeklinde nitelendirilmektedir. Daha açık bir tanımlama ile AG ‘bir kamera ya da görüntüleme cihazı aracılığıyla çoğunlukla gömülü bir hedefi okuyup sanal olarak bilgisayarda üretilen görüntü ve gerçek dünyanın görüntüsünün bilgisayar yazılımı ile bir araya getirilmesiyle oluşan ortam’dır (4).

Bu noktada sıklıkla karıştırılan arttırılmış gerçeklik ve sanal gerçeklik (virtual reality) teknolojilerinin ayrımını yapmakta fayda vardır. Sanal gerçeklik, bilgisayar kaynaklı üç boyutlu oyunlarda karşılaşılan, kullanıcının bu ortama girdiğinde dünya ile ilişkisinin tamamen yok olduğu bir ortam olarak ifade edilebilir. Arttırılmış gerçeklik ise gerçek dünya ile bağlantısını devam ettiren, veri ve görüntülerin gerçek dünya görüntülerine eklenebildiği, gerçek ve sanal nesnelere aynı ortamda birlikte algılanmasını sağlayan bir ortam olarak ifade edilmektedir (4). Sanal gerçeklik uygulamalarında tamamen gerçek dünyadan uzaklaşılırken, bunu sağlayan gözlük gibi özel ekipmanlar kullanılmaktadır. Arttırılmış gerçeklik uygulamalarında ise gerçek dünya ile ilişki devam etmekte, sadece gerçek objeler ile bilgisayar tarafından üretilen objeler görüntü ve sesli olarak birleştirilmekte ve uygulama için PC ile tablet ve akıllı telefon gibi mobil cihazlar yeterli olmaktadır (4,7).

### **AG teknolojisinin eğitim alanında kullanılması**

AG uygulamaları, işitsel ve görsel imkânları ile sunduğu sınırsız zenginlik nedeniyle her geçen gün popülerlik kazanmakta ve birbirinden farklı birçok alanda kullanılmaktadır. Bunlar içerisinde mühendislik, tasarım, askeri faaliyetler, sağlık, gezi, müzecilik ve eğitim alanları öne çıkmaktadır. AG teknolojisinin kullanılmaya başlaması 1990’larda olmasına rağmen, eğitim alanında kullanımı çok daha yenidir. Ancak görülen yararları ve potansiyeli göz önüne alındığında arttırılmış gerçekliğin eğitim alanında kullanımı artması kaçınılmaz görünmektedir (3).

AG teknolojisinin eğitimde kullanılması, birçok avantaj sağlamıştır. Öncelikle AG teknolojisi sanal objeleri gerçek dünyadaki nesnelere birleştirerek çıplak gözle görünemeyecek olayların gözlemlenmesini sağlamakta ve öğrencilerin keşif yeteneklerini arttırmaktadır. Görsel nesnelere üç boyutlu olarak kullanılması öğrencinin/okuyucunun ilgisini çekmekte, interaktif olduğu ve teknoloji barındırdığı için öğrencinin katılımını arttırmaktadır. AG “*edutainment*” yani eğlenerek öğrenme kavramını çok iyi karşılamaktadır. Ayrıca AG anlatılması ve anlaşılması güç olan astronomi, coğrafya, tıbbi bilimler gibi konularda kavramayı arttırmaktadır (3,7).

Sanal nesnelere sabit veya manipüle edilebilir büyük bir düz ekranda veya bir baş üstü ekranda gösterilmesiyle AG, öğrencilerin fiziksel becerilerinin arttırılmasında da faydalı olmaktadır. Yapılan birçok çalışma AG uygulamaları ile desteklenen eğitim modüllerinin öğrenciler arasındaki

motivasyonu artırmaya yardımcı olduğunu ve bu uygulamaların özellikle akademik açıdan daha az başarılı olan öğrenciler arasında öğrenme deneyimlerini olumlu yönde etkilediğini göstermiştir (3,7).

### **AG ve veteriner hekimlik**

Sağlık Bilimleri içerisinde yer alan veteriner hekimlik eğitimi yoğun teorik bilginin yanında, küçük ve büyük hayvanlarda uygulamaya dayanan bir süreçtir. Bölgeler arasındaki hasta profillerinin farklılıkları nedeniyle öğrencilerin geniş spektrumlu bir hasta çeşitliliği için pratik bilgi birikiminin sınırlı olması, bütün dünyada olduğu gibi ülkemizde de önemli bir sorundur. Bu nedenle, veteriner hekimlerin öğrenim hayatları boyunca ve mezuniyetten sonra, farklı hasta ve hastalıkları canlı olarak görmeseler de bilişim teknolojilerinin yardımıyla gerçeğe yakın bir şekilde deneyimlemeleri büyük fayda sağlayacaktır (1, 6).

AG teknolojisi ile intravenöz enjeksiyon ve köpek kafatası modelleri oluşturulmuş ve laparoskopik cerrahi girişimleri deneyimlemeleri için çalışmalar gerçekleştirilmiştir (2, 5). Bunun yanında AG teknolojisi kullanılarak uzaktan eet muayenesine yönelik uygulamalar denenmiştir (1). Ancak, bu sınırlı uygulamaların yetersiz olduğu ortadadır. AG teknolojinin eğitimde meydana getirdiği pozitif etki ve kullanıldığı alanlarda sağladığı yarar ve yenilikler göz önüne alındığında, veteriner hekimlik gibi zorlu bir meslekte bu teknolojinin kullanıldığı uygulamaların ve eğitim araçlarının geliştirilmesi oldukça yararlı ve önemli olacaktır (1, 2, 6).

### **Sonuç**

Sonuç olarak AG teknolojisi beşeri hekimlikte olduğu gibi veteriner hekimlik eğitiminde de öğrencilerin konulara hakim olma seviyesini arttırmak ile kalmayıp onlara daha fazla pratik yapma imkanı da sunmaktadır. Bunun yanında mezun veteriner hekimlerin yararlanacağı AG teknolojisine dayalı uygulamaların geliştirilmesi gerekmektedir. Ülkemizde de yakın gelecekte veteriner hekimlik alanında AG teknolojisi içeren bilimsel çalışmaların yapılacağı öngörülmektedir.

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## **Wolf Teeth Sighted in Horses**

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### **Summary**

As with other animal species, nutrition occupies an important place in horses. The mouth is located at the beginning of the digestive tract, and in order to make effective use of nutrients, the structures in the mouth must be healthy. At the beginning of the ranking of the structures are teeth. After a horse has a tooth disorder, it can affect the quality of life, racing performance, also can cause a number of problems.

For this reason, this poster is intended to provide information about the tooth structure of horses and specifically about wolf teeth.

**Keywords:** Horse, wolf teeth

## **Atlarda Görülen Kurt Dişleri**

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### **Özet**

Beslenme diğer hayvan türlerinde olduğu gibi atlarda da önemli yer tutmaktadır. Ağız sindirim kanalının başında yer almaktadır ve besinlerden verimli bir şekilde yararlanabilmek için ağız içinde bulunan yapıların sağlıklı olması gerekir. Bu yapıların sıralamasının başında dişler gelmektedir. Bir atın diş bozukluğunun olması sonrasında yaşam kalitesini ,yarış performansını etkilemekle beraber bir dizi sorunlar meydana getirebilmektedir.

Bu nedenle, bu bildiri ile atların diş yapısı ve spesifik olarak kurt dişleri hakkında bilgi verilmesi amaçlanmıştır.

**Anahtar kelimeler:** At, kurt dişi

## **The Three-Dimensional World of Parasites**

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### **Summary**

Types of learning, tools and materials used in teaching and their effects on education have been researched for many years, and they are constantly developing and changing over time. The first visualization studies in education started in the 17th century, and the Magic lantern, the forerunner of the image projector, was invented by Christiaan Huygens in 1659. Then Leonhard Euler developed the Episcopo (Diasscope) in 1756, and Henry Morton developed the projection device in 1872. In the 1950s, Roger Appledorn further developed the projector and designed the Overhead Projector. With Liquid Crystal Display (LCD) production in the 1980s, projection devices have taken their final form in use today. With technology development, 3D printers have emerged, and their usage areas have started to progress rapidly, printers as part of 3D visual education. 3D printing has quickly expanded in Medicine, Dentistry, and Veterinary medicine education, where applied education is essential, and facilitated learning through modelling and visualization in these areas. Especially in medical education, 3D printers take their data from computed tomography (CT) and magnetic resonance (MR) data and transform them into 3D modelling. Today, three-dimensional modelling techniques are in various fields such as cardiovascular surgery, patient-specific prostheses, and dental restoration. In addition, 3D printing technologies in parasitology will facilitate the detailed examination of parasites' morphological structures and increase the quality of learning and education. For this purpose, *Paramphistomum* sp. and *Clonorchis sinensis* samples were designed as three-dimensional in the computer environment in the closest way to reality, based on two-dimensional visual models. Completed models were saved in ".stl" format and ready for printing in 3D printers. We aimed to investigate the effect on learning by using these models in student practice lessons. Due to the Covid-19 outbreak, the study was incomplete because courses continued online. However, it will continue to work in the coming years. We thought it would be helpful to present this work plan and the first results to improve the quality of education.

**Keywords:** *Clonorchis sinensis*, education model, *Paramphistomum* sp., 3D printing, veterinary parasitology

## Parazitlerin Üç Boyutlu Dünyası

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### Özet

Öğrenme türleri, öğrenimde kullanılacak araç ve gereçler, bunların öğrenme üzerine etkisi uzun yıllardır araştırılmakta, zaman içerisinde sürekli gelişmekte ve değişmektedir. Eğitimde ilk görselleştirme çalışmaları 17. yüzyılda başlamış ve 1659 yılında Christiaan Huygens tarafından görüntü projektörünün öncüsü olan Sihirli fener icat edilmiştir. Ardından Leonhard Euler tarafından 1756 yılında Episkop (Diaskop), 1872 yılında ise Henry Morton projeksiyon cihazını geliştirmiştir. 1950'lerde Roger Appledorn, projektörü daha da geliştirerek Tepegöz Projektörünü tasarlamıştır. 1980'lerde Likit Kristal Ekran (LCD) üretimi ile beraber projeksiyon cihazları günümüzde kullanılmakta olan son şeklini almıştır. Teknolojinin gelişimi ile birlikte 3D yazıcılar ortaya çıkmış ve kullanım alanları hızla çoğalmaya başlamıştır. Bu gelişmeler eğitim alanına da aynı şekilde yansımış ve görsel eğitimin bir parçası olarak 3D yazıcıların kullanılacağı anlaşılmıştır. Özellikle uygulamalı eğitimin önem taşıdığı Tıp, Diş ve Veteriner hekimliği eğitiminde 3D baskının kullanımı hızla artmaya başlamış, bu alanlarda modelleme ve görselleştirme yoluyla öğrenmeyi kolaylaştırmıştır. Tıp eğitiminde 3D yazıcılar, verilerini bilgisayarlı tomografi (BT) ve manyetik rezonans (MR) verilerinden alarak 3D modellemeye dönüştürmektedir. Günümüzde kalp-damar cerrahisi, hastaya özel protez yapımı, diş restorasyonu gibi çeşitli alanlarda üç boyutlu modelleme teknikleri yaygın olarak kullanılmaktadır. Veteriner hekimliğin önemli bilim dallarından biri olan Parazitoloji'de, parazitlerin 3 boyutlu baskı teknolojilerinin eğitimde kullanılacağı, parazitlerin morfolojik yapılarının detaylı incelenmesinde kolaylık sağlayacağı, öğrenmeyi ve eğitimin kalitesini yükselteceği düşünülmüştür. Bu amaçla *Paramphistomum* sp. ve *Clonorchis sinensis* örnekleri iki boyutlu görsel örnekleri baz alınarak bilgisayar ortamında gerçeğe en yakın haliyle üç boyutlu olarak tasarlanmıştır. Tasarımın ardından dosyaların stl uzantısı alınarak üç boyutlu yazıcı da baskısı yapılmıştır. Amacımız öğrenci uygulama derslerinde bu modelleri kullanarak öğrenme üzerindeki etkisini araştırmaktı, ancak Covid-19 salgını nedeniyle eğitimin online olarak devam edilmesi üzerine çalışma yarım kalmış, ancak önümüzdeki yıllarda çalışmaya devam edilecektir. Eğitim kalitesini artırmaya dönük bu çalışma planını ve ilk sonuçların sunulmasının yararlı olacağı düşünülmektedir.

**Anahtar kelimeler:** *Clonorchis sinensis*, eğitim modeli, *Paramphistomum* sp., üç boyutlu baskı, veteriner parazitoloji