9th WORLD BUFFALO CONGRESS
ARGENTINA 2010

NUTRITION AND FEEDING

07

Barley silage in dairy buffalo nutrition: study on volatile fatty acids production of ten cultivars by the in vitro gas production technique

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Abstract

In south of Italy, whole-crop barley harvested at milky-waxy maturation of grain yields 10.5-12.5 t of dry matter/hectare and its silage has been thought as substitute of corn silage in dairy buffalo nutrition. However, quality of silage is strongly influenced also by other factors, like cultivar. In a previous study the fermentation characteristics of ten cultivars of whole-crop barley silage were studied by means of the in vitro gas production. As a whole, all the samples showed a good fermentative behaviour and a nutritive value comparable to other cereal silages. In the present paper the volatile fatty acids (VFA) produced after 120 h of incubation with rumen fluid collected from three buffaloes were analysed. The Boreale cultivar showed the highest ($P<0.01$) VFA content (97.8 mmol/g) compared to the others, probably due to its best nutritive value (NEl: 4.33 MJ/kg DM). Significant differences were detected among cultivars, thus suggesting gas production technique should be taken into account as a quality marker before introducing barley silage in buffalo diet.

Key words: Barley, in vitro gas production technique, volatile fatty acids.

INTRODUCTION

In South of Italy, whole-crop barley (Hordeum vulgare L.) harvested at milky-waxy maturation of grain (32-34 % DM) yields 10.5-12.5 t of dry matter/hectare and it has also been thought as convenient substitute in dairy buffalo nutrition of corn silage, which shows some weak aspects like high contamination with aflatoxins, parasites and high cost of irrigation. In addition, harvesting whole-crop cereal silage enables crop rotation and manure utilization in the fields and reduces feed production costs. However, quality of whole-crop barley silage is strongly influenced by several factors like cultivar. In a previous study1 the fermentation characteristics of ten cultivars of whole-crop barley silage were studied using the in vitro gas production technique (IVGPT). However, it was pointed out that attention should be given to the other products of the incubation as well2. Degraded feeds may be incorporated directly into microbial biomass, or they may be fermented to produce volatile fatty acids (VFA) and gas. Measuring both the yield of gas and total VFA can be used to predict the amount of substrate that has been partitioned to microbial biomass production. In the present paper, the VFA were analysed.

MATERIALS AND METHODS

The VFA produced after 120 h of incubation with rumen fluid collected from three Italian Mediterranean Buffalo (Bubalus bubalis) were analysed using the gas-chromatography (GC)3. In particular, when fermentation was stopped (cooling the culture bottles at 4°C) a sample (5ml) from each bottle was collected and centrifuged twice at 12000 g for 10 minutes at 4°C; 1 ml of supernatant was taken and 1 ml of oxalic acid 0.06 M was added. The VFA were measured by GC including acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate as external standards.

The influence of cultivar on the volatile fatty acids production was statistically assesses4.

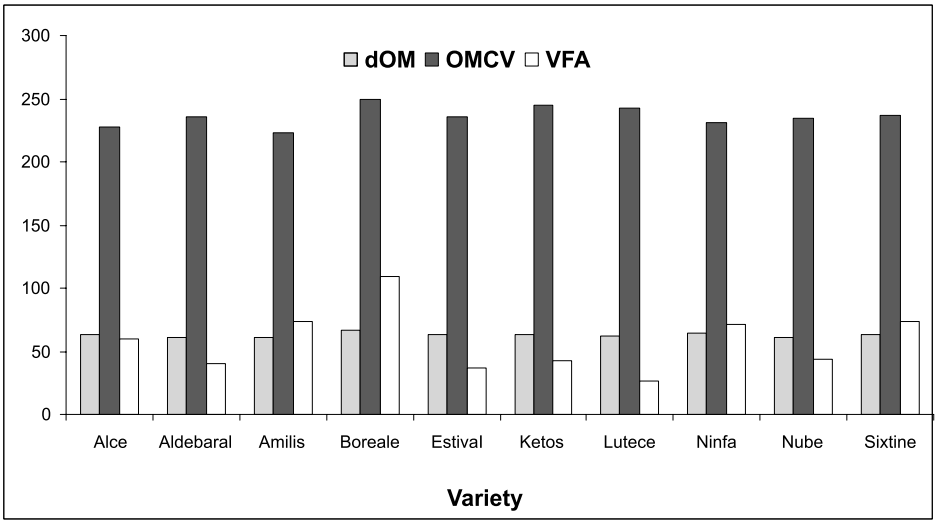
RESULTS AND DISCUSSION

The VFA production is reported in table 1. According to the highest gas production and organic matter degradability found in our previous trial1, Boreale cultivar showed the highest (P<0.01) acetic (67.23 mM/g) and butyric (7.137 mM/g) productions. This cultivar showed also high total VFA production (109.2 mM/g), even if the value was not statistically different from Amilis, Ninfa and Sixtine cultivars. Lutece cultivar showed the lowest (26.13 mM/g) VFA production. In figure 1 the in vitro end-products are illustrated. Using the data related to gas produced and organic matter degraded (dOM), obtained in the previous paper1, the VFA/dOM (mM/mg) and gas/VFA (mM/mM) ratios were 0.090 ± 0.0040 and 1.963 ± 1.015, respectively. These values are consistent with those found in other trial5, validating the scientific assumption of the gas production technique. In conclusion, the significant differences detected among cultivars, suggesting gas production technique should be taken into account as a quality marker before introducing barley silage in buffalo diet.

Table 1: In vitro VFA (mM/g) production of barley silages.

Variety	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	tVFA
Alce	34.00^{B}	19.41	0.730	4.762^{A}	0.656	0.431	59.99^{B}
Aldebaral	55.93	30.13	0.924	2.750	0.587	0.355	40.17^{B}
Amilis	45.27^{B}	21.14	0.916	5.504^{A}	0.700	0.459	73.95^{A}
Boreale	67.23^{A}	30.83	2.452	7.137^{A}	1.006^{A}	0.503	109.2^{A}
EstivaI	50.31	22.98	0.985	0.972^{B}	0.192^{B}	0.175	36.29^{B}
Ketos	58.61	26.27	0.962	2.659	0.690	0.183	42.25^{B}
Lutece	34.73^{B}	16.94	1.015	2.027	0.372	0.274	26.13^{B}
Ninfa	43.49	20.60	0.893	4.885^{A}	0.678	0.502	71.05^{A}
Nube	56.01	25.02	1.009	3.418	0.816^{A}	0.391	43.41^{B}
Sixtine	46.22	20.99	0.782	4.744^{A}	0.625	0.515	73.87^{A}
MSE	141	34.4	0.674	2.33	0.049	0.028	222

tVFA: total volatile fatty acids; in the same column, A,B: P < 0.01; MSE: mean square error



dOM: organic matter degradability (%); OMCV (ml/g): gas produced after 120 h of fermentation. VFA (mM/g): volatile fatty acids.

Figure 1: In vitro fermentation products of the ten Barley varieties

Acknowledgements. The authors wish to thank the Barchiesi Farm, (Cassino, FR – Italy) where the trial was performed. The research was supported by the MIPAAF Project (2006): "Influenza del rapporto foraggi: concentrati della dieta per bufale in lattazione sul benessere animale e la microbiologia ambientale" (Prof. F. Infascelli).

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Comparative Efficiency of Different Calf Starter Rations in Young Nili Ravi Buffalo Calves

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Abstract

Three calf starter concentrates containing Maize, Oats and Maize + Oats as a source of energy having 20% crude protein and 80% total digestible nutrients were formulated and fed to three groups of young calves A, B & C, respectively for a period of 90 days. The daily consumption of milk was 3.30, 3.70 and 3.32 litres while daily intake for starter ration and berseem hay was 0.410 & 1.80; 0.320 & 2.25 and 0.372 & 2.00 kg in groups A, B and C, respectively. The feed efficiency for milk was 5.66, 9.44 and 6.99 for concentrates 0.703, 0.816 and 0.783 and for berseem hay 3.09, 5.74 and 4.21 in respective groups. The daily weight gain was 0.583, 0.392 and 0.475 kg in groups A, B and C, respectively. The results revealed that the animals fed on starter ration containing maize as energy source gained 18.57 % and 13.71% more weight and showed better feed efficiency as compared to the calves reared on starter concentrates having Oats or Maize + Oats as a source of energy.

Key words: Calf starter concentrate, av. daily weight gain, feed efficiency, buffalo calves

INTRODUCTION

The survival of young calves is an important economic proposition in dairying and is associated with a number of factors. The number of calves born alive influence the fertility rate of a herd and the availability of young animals for future replacement. Reduced growth rate and high mortality in young calves can be prevented by providing a good calf starter concentrate, proper care and good health cover etc. In Pakistan maize grain are used as energy source in animal feed industry due to its huge production. Oats are also cultivated in many parts of country and is used in equine feed. As it contains 1.7% protein and a little higher in Ca & P than corn. So this comparative study was planned to investigate the effects of oat and corn in calf starter concentrate on growth rate in Nili-Ravi buffalo calves.

MATERIAL AND METHODS

Twenty-seven newly born calves of both sexes were procured and randomly divided into three groups A, B & C each having 9 animals. The calves were fed colostrum and whole milk according to the following schedule :-

Age	Rate of Feeding
Birth to 3 days	Colostrum 1/10 of body weight.
4 days to 6 week	Whole milk 1/10 of body weight.
7 week	Whole milk same as on 6 week.
8 to 12 week	Whole milk 1/6 less every week.

Calves were fed colostrum and whole milk by means of nipples in weighed quantity. The daily milk allowance was divided into two equal halves fed in the morning and evening. Three calf starter concentrates I, II and III containing either Maize, or Oats or Maize + Oats as a source of energy having 20% crude protein and 80% of total digestible nutrients were formulated and allotted to the groups-A, B & C, respectively. The composition of different calf starter concentrates is presented in Table 1. In addition to above ration each group was also offered sun dried berseem hay along with ration on feed lot basis. All the calves under study were de-wormed by drenching piperazine to check the Ascaris problem likely to occur at the age of two weeks. Fresh drinking water and salt lick was made available round the clock. Each calf was weighed at birth and at every weeks end after-words till the end of the trial. Record of daily milk consumption, intake of both starter ration and berseem hay was maintained. The chemical analysis of both the ration was done by the method2 and statistical analysis was done by using T. Test4.

RESULTS AND CONCLUSIONS

Milk consumption

Average total milk consumption in a period of 90 days was 297, 333 and 299 litres for the groups A, B & C, respectively. The calves in respective groups consumed on average 3.30, 3.70 and 3.32 litres of milk per day.

Weight gain

he average total weight gain up to 90 days was 52.50, 35.30 and 42.75 kg while the average daily weight gain was 0.583, 0.392 and 0.475 kg in groups A, B and C, respectively. The young calves fed on starter ration containing maize as energy source gained 18.57 and 13.71 percent more weight as compared to the calves fed on concentrates having oats or oats + maize as a source of energy. The analysis of variance showed that weight gain of calves of group A was significantly higher that those of group B or C. Also it reflects a picture that ration containing maize as a energy source yielded the maximum weight gain. Also mixture of maize and oats in ration gave better results (Table-II) than single oats as energy source in ration-II. This is due to ingredients used in the concentrates, as oats are higher in crude fiber (10.6% VS 2.0) than corn and accordingly lower in TDN 66.3% VS 78%). Almost similar daily weight gain (0.404 kg) in Murrah buffalo calves was obtained in a study7 when calf starter was given ad libitum alongwith conventional whole milk. The results1 also reported similar values (0.470 kg) of growth rate in Nili-Ravi buffalo calves when fed on calf starter concentrate ad libitum and green fodder. Growth rate in buffalo calves in the present study are higher. It may be due to composition of concentrate and feeding regime etc.

Table 1. Composition of different calf starter concentrates (%)

Particulars	Concentrate-I	Concentrate-II	Concentrate-III
Maize grain (crushed)	50	--	25
Oat grain (crushed)	--	60	30
Cotton seed meal	14	14	14
Maize gluten meal 60%	15	15	15
Wheat bran	15	--	7
Vegetable oil	--	8	4
Molasses	5	2	4
Mineral mix. + Vitamens A&D	1	1	1
Total :-	100	100	100
Nutrients:			
Dry matter (DM) %	86.70	81.20	84.06
Crude protein (CP) %	20.00	19.80	19.90
Total digestible nutrients(TDN) %	78.50	77.08	79.00

Feed consumption

The average daily intake of starter concentrate and berseem hay was 0.410 & 1.80; 0.320 & 2.25 and 0.372 & 2.00 kg in groups A, B & C, respectively. Analysis of variance revealed that difference between two groups was statistically non-significant. The results3 obtained are in accordance with the present study who reported 0.378 Kg average daily intake when Holstein bull calves were fed on calf replacer containing non-spray dried hydrolyzed red blood cells (SDHRBC) along with calf starter for 28 days only. However, another study1 reported 0.95 Kg feed intake in Nili-Ravi buffalo calves when fed on calf starter concentrate ad lib and whole milk @ 10% of body weight. This may be due to different formulation of concentrate, climatic effect or different feeding regimes.

Feed efficiency

The calves of group-A consumed 5.66 litres of milk, 0.703 kg concentrate and 3.09 kg berseem hay for one kg weight gain (Table 2). The calves of group-B consumed 9.44 litres of milk, 0.816 kg concentrate and 5.74 kg berseem hay and calves of group-C consumed 6.99 litres of milk, 0.783 kg concentrate and 4.21 kg berseem hay for each kg of liveweight gain (Table 2). Analysis of variance revealed that FCR values of group A were significantly higher ($P<0.05$) than those of group B or C. These values are below than earlier reported3 who obtained higher FCR values (3.10) in Holstein calves. Similarly6 observed higher FCR values 2.17 in buffalo calves fed on milk @ 10% of their body weights and calf starter along with green fodder. This may be due to difference/ variation in breed, feeding regime, climate or ration formulation. In this study calf starter containing corn as an energy source had higher feed intake and weight gain than other calf starter concentrates due to low C.F. contents and readily digestible portion of carbohydrates. Also corn had carotene contents that are precursor of Vitamin A. Moreover, Vitamin A is an indispensable factor in the diet of calves5. In short calf starter concentrate containing maize grain as energy source was ideal, in terms of weight gain, feed conversion ratio in buffalo calves.

Table 2. Weight gain, feed intake & feed efficiency of young buffalo calves under different calf starter concentrates

Particulars	Group-A	Group-B	Group-C
No. of animals	9	9	9
Days on experiment	90	90	90
Av. initial body weight (kg)	34.30	35.70	36.25
Av. final body weight (kg)	86.80	71.00	79.00
Total weight gain (kg)	52.50	35.30	42.75
Daily weight gain (kg)	0.583	0.392	0.475
Total milk consumed (Lit.)	297.00	333.00	299.00
Av. daily milk consumed/head (Litre)	3.30	3.70	3.32
Av. total starter concentrate consumed (kg)	332.00	259.00	301.00
Av. daily intake of concentrate/head (kg)	0.410	0.320	0.372
Av. total berseem hay consumed (kg)	162.00	202.50	180.00
Av. daily intake of berseem hay/head (kg)	1.80	2.25	2.00
Feed efficiency			
Milk (Litres)	5.66	9.44	6.99
Concentrates (kg)	0.703	0.816	0.783
Berseem hay (kg)	3.09	5.74	4.21

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Comparative study of voluntary intake, digestibility and production performance of Ryegrass and Berseem fodders in Nili-Ravi buffaloes

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Abstract

Nili-Ravi lactating buffaloes (n=10) were randomly selected and divided into two groups. They were offered either ryegrass or berseem fodder adlibitum. Results revealed significant differences (P<0.05) in voluntary feed intake (63.3±1.82 kg vs 47.46±6.48 kg) and palatability (44.85±6.65 kg vs 27.44±7.43 kg) of ryegrass and berseem, respectively. No significant difference was observed in milk yield of the animals in both the groups. Among digestibility coefficient parameters, dry matter digestibility was higher (P<0.05) in ryegrass (66.82±1.82 %) as compared to berseem fodder (47.46±6.48 %), while crude protein and ether extract digestibility were comparatively higher in berseem (66.55±5.63 %, 45.48±5.02 %) and lower (48.66±4.26 %, 42.06±8.49 %) in ryegrass. Digestibility of crude fiber and nitrogen free extract were similar in both the fodders.

Key words: Rye grass, berseem, intake, digestibility, milk yield, Nili-Ravi buffaloes

INTRODUCTION

In Pakistan, fodder is the main source of livestock feeding and covers about 16% of the total cropped area. More than 51% of animal feed is coming from fodder crops and crop residues1. Berseem (Trifolium alxandricum) is the main fodder during winter season; however, the major constraint is its slow growth under low freezing temperature during winter. Another disadvantage is the presence of saponins that cause bloat in grazing animals. Hence, need is always felt that there should be an alternate fodder which grows well during extreme cold conditions with less anti-nutritional factors. Considering it, ryegrass (Lolium perenn) was imported from Australia in early 1980's and tested at Bahadarnagar Okara. Its performance was good and grew very well during winter season. Due to these properties, it is gaining popularity among the farmers. Present study was planned to compare the performance of these fodders in terms of voluntary intake, palatability and digestibility in lactating buffaloes.

MATERIALS AND METHODS

To determine voluntary intake and nutrients utilization, two groups of Nili-Ravi lactating buffaloes, five in each, were offered chopped green ryegrass (Lolium perenne) and berseem (Trifolium alxandrium) fodder ad libitum on individual basis. Wheat straw was mixed at the rate of 8% in each fodder to increase the dry matter contents of the feed. Animals were given concentrate ration equal to one third of milk production. Weighed quantities of fodder offered and milk production were measured daily. During the last five days of experiment, digestibility was determined by recording the

fodder offered, refused and total weight of faeces voided during 24 hours. Samples were pooled for proximate analysis.2 In order to determine palatability of both fodders, the wooden mangers were partitioned into two equal halves. Chopped ryegrass and berseem fodders mixed with wheat straw were placed separately in each part of manger. Each buffalo had access to both parts of the manger. Weighed quantities of both the fodders were offered and refusal was weighed back daily. A period of one week was given as adjustment phase and then data recording was done for the period of 10 days. The data thus obtained for all the parameters were statistically analyzed using t-test.3

RESULTS AND DISCUSSION

The dry matter, crude protein (CP), ether extract (EE), crude fiber (CF) and total ash percent in ryegrass were 23.25, 10.19, 24.43, 3.99, 11.11 % while for berseem, these were 14.8, 15.66, 25.82, 4.35, 14.24 %, respectively. It was observed that ryegrass had higher dry matter and nitrogen free extract contents than berseem. However, crude protein and ash contents of berseem were higher than ryegrass. Little variations were observed in ether extract and crude fiber contents of the two fodders. It is reported4 that tropical legumes contain more crude protein than tropical grasses, while contents of ether extract are rather similar to that of grasses. Likewise5, in a comparative study of ryegrass and alfalfa silages it was observed that legume silages contained more CP than ryegrass silage.

The average voluntary feed intake, palatability and milk yield on both fodders are given in table-1. Buffaloes consumed more ($P<0.05$) ryegrass than berseem fodder. However, in a comparative study6, no effect of treatment among ryegrass, white clover and red clover on feed intake was observed. Palatability of feed is an important factor in efficient feeding of the stock. In our study, animals preferred ($P<0.05$) ryegrass than berseem. However, no preference of dairy cows was observed when ryegrass, white clover and red clover were offered.6 In milk yield, no significant differences ($P>0.05$) were observed on both fodders. In a comparative study7 of red clover and ryegrass silage, it was concluded that both treatments had similar effect on milk yield. In our study, the comparative digestibility of DM, CF, and NFE was higher ($P<0.05$) in ryegrass where as digestibility of C.P and E.E was higher ($P<0.05$) in berseem. Similarly, 16% greater apparent digestibility for DM on the ryegrass diets as compared to alfalfa is reported5.

It is concluded that ryegrass has good palatability, voluntary intake, and dry matter digestibility and has got good potential to be used as conventional fodder in winter season.

Table 1. Comparative efficiency of Ryegrass and Berseem in voluntary intake, palatability, milk production and digestibility co- efficient in Nili-Ravi buffaloes

Parameter	Voluntary Intake (Kg)	Palatability (Kg)	Milk Prod. (Litre)	Nutrients Digestibility %				
				DM	CP	EE	CF	Ash
Ryegrass	63.30[a] ± 2.9	44.85[a] ± 6.65	8.60[a] ± 3.71	66.82 ± 1.82[a]	48.76 ± 4.26[a]	42.06 ± 8.49[a]	52.37 ± 6.57[a]	53.57 ± 2.50[a]
Berseem	59.64[b] ± 5.2	27.44[b] ± 7.43	8.20[a] ± 1.30	47.46 ± 6.48[b]	66.55 ± 5.63[b]	45.48 ± 5.02[b]	47.79 ± 10.03[a]	50.43 ± 8.25[a]

*Means with different superscripts within column are significantly different ($P<0.05$)

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Distribution of Ca2+-Sensing Receptor (CaSR) and Na-K-ATPase in the Gastrointestinal Tracts of Neonatal Calves after Colostrum Ingestion

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Abstract

Calcium sensing receptor (CaSR) and Na+K+ATPase pump expression in gut tracts from newborn buffaloes after colostrum ingestion were evaluated in order to clarify the mechanisms by which gut undergoes to fast and important functional changes during the first period of life.

Key words: buffalo calf gut, calcium sensing receptor, Na+K+ATPase pump

INTRODUCTION

The CaSR and the Na+K+ATPase pump play an important role in regulating the GIT function in mammals. Various aspects of cellular function and processes modulated by CaSR1. The extracellular calcium is essential for a number of vital processes, including bone mineralization, blood coagulation, regulation of enzymatic activity and the modulation of the permeability and excitability of the plasma membranes. The activity of Na+K+ATP ase and Na+ transport rate in the intestine epithelial cells have been shown to correlate with the basolateral membrane area of these cells2. After birth, the small and large intestine of mammals undergo a series of developmental changes that allow an efficient and effective transition from milk diet to a post weaning period. In particular, Na+ transport and Na+K+ATP activity exhibit considerable ontogenic changes and it reveals an age dependent increase, although Na+ absorption is very high during the suckling period and significantly decreases later3. During the neonatal period the intestine of calves endures morphological and physiological modification and the ability to absorb colostrum components is restricted to a short time. Therefore, gut absorption features in newborn calves are fundamental for survival and for the future productive performance. No data are available concerning CaSR and Na+K+ATPase expression in the gut of newborn ruminants. The elucidation of CaSR and Na+K+ATPase role may be of great interest in order to improve the absorption rate. The aim of this study was to evaluate CaSR and Na+K+ATPase expression in gut tracts from newborn buffaloes after colostrum ingestion in order to clarify the mechanisms by which gut undergoes to fast and important functional changes.

MATERIAL AND METHODS

Twelve healthy neonatal buffalo calves from a single dairy farm in southern Italy were used in the study. They were assigned to 2 groups of six animals each (first group: 6 to 36 hours of life, second group: 36 hours up to one week). The calves were euthanized using a lethal injection of Phenobarbital and than exsanguinated. The abdominal cavity was opened and the gastrointestinal tracts (GIT) were removed. Tissue samples from the abomasum, duodenum, jejunum and

colon were obtained. All procedures were approved by Italian laws regarding animal use in research. In each samples CaSR and Na+K+ATPase expression was evaluated by immunofluorescence1 and real time PCR4.

RESULTS AND DISCUSSION

As depicted in figure 1, the immunofluorescence of CaSR and Na+K+ATP ase has been detected in the abomasum and along the entire small and large intestine from newborn buffaloes. In the abomasum they are expressed both in the mucosa epithelial cells and in the gland cells often showing a colocalization (Fig. 1a), CaSR is mainly located on the basolateral membranes (Fig. 1b) while the higher expression of Na+K+ATP ase was detected in the basal membrane (Fig. 1c).In the duodenum the colocalization is more evident and the two receptors show an homogeneous distribution, the transversal section of the villi (Fig. 1d) show the CaSR and Na+K+ATP ase localised in the basal and lateral membrane of epithelial cells and a similar pattern was detected for Lieberkuhn glands (Fig. 1e). In the jejunum both receptors were detected (Fig. 1f) but in a non-homogenous way since CaSR was much more expressed. CaSR was detectable in all cells of villi showing the highest expression on the luminal surface whereas Na+K+ATP ase was found at the base of villi often showing a colocalization with CaSR. Figures 1H-L show CaSR and Na+K+ATP ase localization in the colon tract. As seen, the colon shows the highest expression of CaSR which was found in surface and crypt cells at the apical and basolateral level. Na+K+ATP ase showed a very high expression in the luminal side of cells and was also detected inside the cells at a lesser extent but in a homogenous way. In contrast with previous tracts the colocalization was not evident in the colon and the two receptors showed distinct sites of expression. As detected in figure 2, Real-time PCR experiments confirmed that CaSR mRNA levels were significantly lower ($P<0,05$) in the group 2 (>36 h). The real-time PCR data were recorded as the expression relative to the Group 1 (<36 h) as the calibrator sample. Group 1 (6-36 h) was used as the calibrator sample. As seen, the relative amount of CaSR was statistically ($P<0.05$) lower in the second group (36-168 h). Results show that CaSR and Na+K+ATPase are involved in gut changes that occur in the newborn calf in particular during the first hours of life. This suggests that they may play a critical role in determining the intestinal features that allow colostrum nutrients absorption in calf.

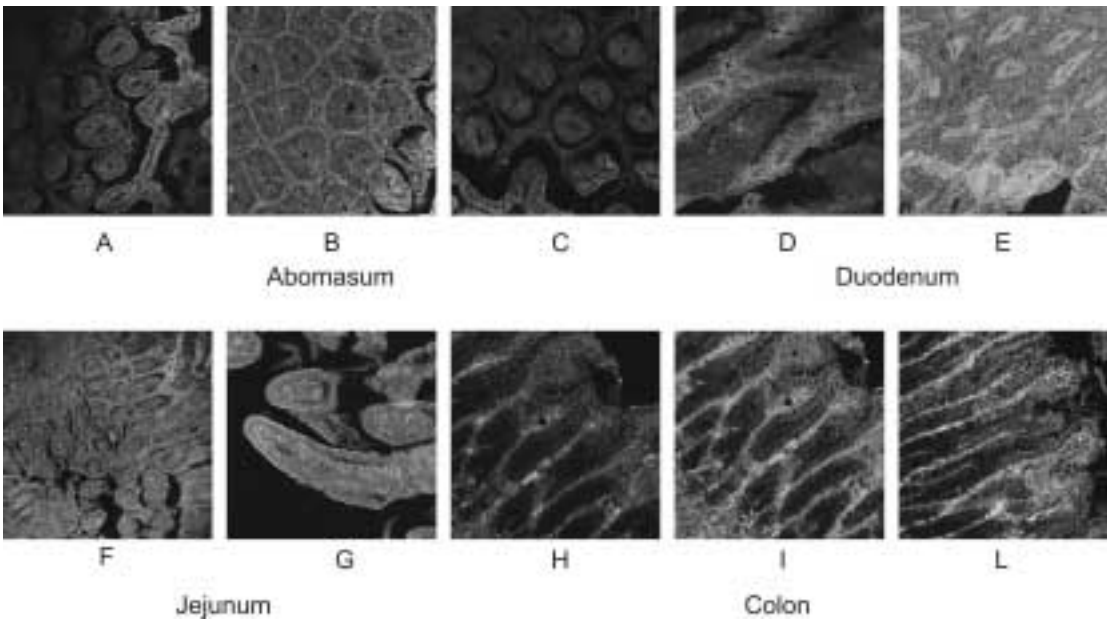

Figure 1. CaSR and Na+K+ATPase expression evaluated by immunofluorescence.

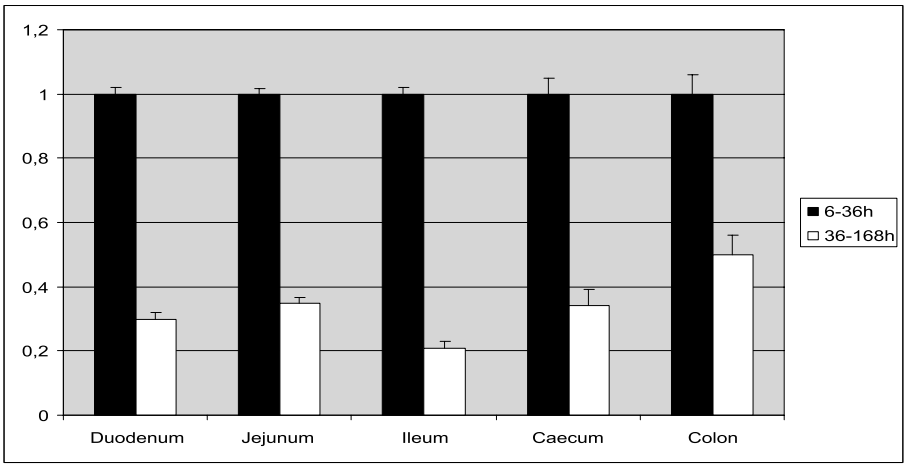

Figure 2. CaSR mRNA levels in the gastrointestinal tracts from neonatal buffalo detected by real-time PCR .

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Effect of Biological, Natural and Chemical Suplements on the Productive Performance of Lactating Buffaloes

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SUMMARY

In-vitro study was conducted to evaluate the effect of dried yeast (Saccharomyces cervisiae) and yeast selenium as a biological additives, sodium acetate and sodium succinate as a chemical additives and chamomile flower, garlic and fenugreek seeds as natural additives on dry matter and organic matter disappearances. Results clearly indicated that combination of yeast and chemical mixture and combination of chamomile and chemical mixture supplementation were recorded the highest rate of IVDMD and IVOMD.

Sixteen lactating buffaloes after two weeks of calving were divided into 4 groups (four animals each) using complete random block design to evaluate the effect of biological, natural and chemical supplements on the productive performance of lactating buffaloes. Treatments were; 1) control (60% concentrate feed mixture "CFM", 20% rice straw and 20% berseem clover); 2) control + chemical mixture (sodium acetate 100 g/head/day + sodium succinate 3.5 g/head/day); 3) control + chemical mixture + dry yeast 10 g/head/day and 4) control + chemical mixture + chamomile flower 10 g/head/day. Dry matter intake was significantly increased for animals fed rations of T1, T2 and T3 compared with control. Apparent nutrients digestibility were significantly ($P<0.05$) improved by treatments. Milk yield and 4% fat corrected milk were significantly ($P<0.05$) higher in animals fed additives than control. Milk protein, fat, lactose, total solids and ash contents were higher ($P<0.05$) in animals fed rations of T1, T2 and T3 compared with control. Feed efficiency (Milk yield/DMI and FCM/DMI) was higher ($P<0.05$) for animals fed experimental additives than animals fed control. Animals fed on T1, T2 and T3 rations have a higher value of serum total protein, albumin, globulin and glucose contents than control animals. The results of the present study suggest that adding the combination of chamomile, sodium acetate and sodium succinate for lactating buffaloes was improved nutrients digestibility, milk production and composition and feed efficiency with no deleterious effect on general health of the treated animals.

Keywords: in-vitro, nutritional additives, digestibility, buffaloes, milk production, blood serum.

INTRODUCTION

One of the most successful attempts accomplished in the last decade is using feed additives which help in improving animal productivity and increasing milk production. From these additives, biological additives (yeast cultures), natural additives (medicinal plants as its seeds) and chemical additives (sodium acetate and sodium succinate). It has been reported that yeast cultures improved animal microbial growth, fiber digestion and feed efficiency of lactating animals (Dawson, 1993). In addition, using Saccharomyces cervisiae improved feed consumption and increasing microbial proteins synthesis in the rumen besides improving fiber digestion, feed efficiency and milk production (Hubert et al.,

1989). Moreover, Abo El-Nor and Kholif (1998) and Kholif et al., (2000) emphasized that adding 10-l5gram Yea Sacc1026 increased significantly milk production by about 20% in addition to improve digestion and milk quality.

In the last decade, natural additives such as (Fenugreek seeds, Carum carvi, Nigella sativa and chamomile) have been increased the central concern of scientists as useful resource for treating diseases and improving animal productivity (Abo El-Nor, 2000 and Kholif and Abd El-Gawad, 2001). Results indicated that using medicinal plant seeds improved the productivity of lactating animals and its hormonal alert effect on animals is resulting from increasing prolactin and growth hormone release (Abo El-Nor, 2000), in addition to activating udder tissues in line with increasing glucose concentration with a reduction in cholesterol concentration in blood.

Chemical additives have been used to improve animal productivity from milk that depends on direct manipulation in rumen environment for increasing milk production. The remarkable effect is increasing propionate level in the rumen with maintaining the level of acetate to propionate constant in order to maintain fat concentration in milk. Several investigators indicated that adding 7mg/kg LBW/d from sodium succinate to lactating buffalo's ration before milking was increased milk production to more than 20% (Abo El-Nor, 2000). Addition of sodium acetate, succinate, propionate and bicarbonate as buffers resulted in improving milk production.

The aim of this study is to evaluate the best nutritional additives for improving lactating buffalo's performance with the best economical cost.

MATERIALS AND METHODS

In-vitro studies:

A total of fourteen additives were evaluated through in-vitro dry matter and organic matter disappearances using two stage technique according to method of Tilley and Terry (1963) modified by Norris (1976).

In first stage ten additives were tested from garlic, fenugreek, chamomile and its mixture, sodium acetate and succinate and its mixture and dried baker's yeast, selenium yeast and concentrate feed mixture.

In second stage four combinations from the previous additives of the first stage (chamomile and yeast), (chamomile, sodium acetate and sodium succinate), (yeast, sodium acetate and sodium succinate) and (chamomile, yeast, sodium acetate and sodium succinate) were tested for dry matter and organic matter disappearances to obtain the proper combinations will be used in lactating buffaloes experiments. Concentrate feed mixture was added to all tested combination additives.

Rumen contents samples were collected after four hours after morning feeding from adult Baladi bucks fed on berseem hay diet and transferred directly to the laboratory in separate warmed oxygen free plastic jugs. Rumen content was strained through two layers of cheese cloth and the obtained liquor was used for the in-vitro studies.

Feeding and management:

Sixteen lactating buffaloes weighting on average of 563 ±20 kg at the 3rd and 4th season of lactation) were used in the present study. The animals were randomly assigned into four groups (four each) in complete random block design. The animals were introduced to treatments starting two weeks after the calving and continued till the 12th week of lactation. Treatments were; 1) control group fed 60% concentrate feed mixture (CFM), 20% rice straw and 20% berseem clover (on dry matter basis), 2) (T1) control ration + chemical mixture (sodium acetate 100g + sodium succinate 3.5 g/head/day, 3) (T2)control ration + chemical mixture + dry yeast 10 g/head/day and 4) (T3) control ration + chemical mixture + chamomile 10 g/head/day. Additives were add at the top of concentrate feed mixture daily.

Chemical composition of concentrate feed mixture, berseem clover and rice straw used in experimental rations are shown in Table (1). Diet was formulated to meet the animal's requirements (Shehata, 1971). Animals were fed individually and concentrates were offered twice daily during milking time at 6.0 a.m. and 4.0 p.m. while, berseem and rice straw were

offered at 8 and 11 a.m., respectively. Fresh water was available to the animals all time.

Digestibility trial:

Four digestibility trials were applied during the last three months of the experimental period using two animals from each experimental group. Grab sample method (according to Gallup et al., 1945 and Forbes and Garrigus, 1948) was used and silica as internal marker was applied for determining the digestibility. Feces grab samples were collected manually at 12.00 a.m. for three successive days from each animal. Solution of 10 % H_2SO_4 was added to the representative samples then dried oven at 70oC for 24 h.

The dried feces samples from each animal were mixed and saved for chemical analysis. The digestibility coefficient of certain nutrient was calculated according to the following formula:

Feed and fecal analysis:

The chemical composition of different feedstuffs and fecal samples were analyzed according to the A.O.A.C. (1995) methods to determine moisture content, DM, OM, ash, CP, CF and EE contents. NFE content was calculated by difference.

Sampling and analysis of milk:

Individually, milk samples were collected every two weeks of the experimental period (90 days). The animals were handily milked (twice/day), milk yield was recorded and pH of milk was determined using a digital pH-meter. Milk samples were also, analyzed for fat, total solids (TS), total protein (TP) and ash (Ling, 1963) lactose (Barnett and Abd El-Tawab, 1957). Solids-not-fat (SNF) was calculated. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines (1928):

FCM = 0.4 M + 15 F.

Where: M= milk yield, F= fat yield.

Blood serum analysis:

Blood samples were collected from the jugular vein of each animal at the last day of each month (4 hr. post morning feeding). The collected blood samples were centrifuged at 4000 r.p.m./20 min. to separate the serum. The obtained serum was stored at –18oC till it was analyzed. Serum total protein was determined as described by Armstrong and Carr (1964), albumin (Doumas et al. 1971), urea (Patton and Crouch,1977), glucose (Siest et al.,1981) and serum GOT and GPT (Reitman and Frankel, 1957). Globulin and albumin/globulin ratio were calculated.

Statistical analysis:

Data obtained from this study were statistically analyzed by SPSS (2002) according to procedures outlined by Snedecor and Cochran (1982). The procedure was complete randomized block design where the model as:

$$Yijk = \mu + Ti + Pj + (T*P)\,ij + Eijk$$

Where:

Y = is the effect of the observation, μ =is the overall mean, T =is the effect of the treatment, P = the effect of the period, T*P = the interaction between treatment and period, E = the experimental error.

The Duncan's multiple range test was used to test the significance between means (Duncan, 1955).

RESULTS AND DISCUSSION

In-vitro study:

The respective values of pH, IVDMD and IVOMD of concentrate feed mixture (as control) were 7.4, 56.3 and 58.36 (Table 2). Results of Table (2) clearly indicated that supplementation of chamomile recorded the highest rate of IVDMD and IVOMD followed by garlic and then fenugreek. This improvement may be due to enhancing the microbial digestion by a certain essential oils included in the medicinal plants. Trivedi and Hotchandani (2004) observed an inhibition in growth of E. coli, Klebsiella, Poteus and Pseudomonas sp. by the essential oil of Eucalyptus globules leaves. The present results are in the line with those of Aboul-Foutouh et al. (1999) and El-Ashry et al. (2006) who observed that IVDMD of tested diets were improved by medicinal plants supplementation compared to the control diet.

In respect of yeasts additives, dried yeast has higher values of IVDMD and IVOMD than yeast selenium. El-Ashry et al. (2003) reported a significant improvement in IVDMD and IVOMD with yeast supplementation to diets compared with control. Regarding to chemical additives, sodium acetate recorded the highest values of IVDMD and IVOMD followed by sodium succinate and then the mixture of chemical additives.

Also, data in Table (2) shows the IVDMD and IVOMD of different combinations of experimental additives. The highest values of IVDMD and IVOMD were observed with combination of yeast and chemical mixture followed by the combination of chamomile and chemical mixture and then the combination of chamomile, yeast and chemical mixture, while, the combination of chamomile and yeast recorded less values. This improvement of IVDMD and IVOMD with combinations may be due to one or more of these reasons; 1) available of essential feed ingredients as vitamins, enzymes and essential amino and fatty acids to microflora from yeasts, 2) improvements of flora environments for higher digestibility with yeasts, 3) available of volatile fatty acids and other salts with chemical additives, 4) available of medicinal oils (essential oils).

Regarding to pH values, the different combinations decreased pH values compared with different individual additives, combination of chamomile and yeast recorded the lowest pH values (being 5.7) followed by combination of chamomile, yeast and chemical mixture (being 6.6) and combination of chamomile and chemical mixture (being 6.7) and then yeast and chemical mixture (being 7.2). In contrast, pH values of individual additives were not differed significantly and ranged between 7.3 and 7.8. The highest values of pH were noted with chemical additives, while, the lowest values were observed with natural additives, however, biological additives recorded moderate pH values.

Lactation trial:

Dry matter intake:

Dry matter intake and nutrient digestibility are shown in (Table 3). Data of Table (3) showed that total dry matter intake (DMI) was not affected by experimental additives compared with control. Values of DMI calculated as proportion from metabolic body size (MBS) (kg/kg W0.75/day) showed a significant increase ($P<0.05$) with experimental additives compared to control. This increase may be due to higher live body weight of animals fed supplemented rations than control animals (Table 3). Robinson and Garrett (1999) and Wohlt et al., (1998) observed an increase in DMI, while, El-Ashry et al., (2001) and Kholif and Khorshed (2006) suggested that DMI were not affected by yeast culture supplementation to animal rations. Abo El-Nor and Kholif (2005) reported that DMI was not affected by sodium succinate or sodium acetate and sodium succinate mixture supplementation to dairy rations. When cows are in negative energy balance (early lactation) the additional energy available due to nutritional supplementation is used to increase DMI, improve animal performance and reduce body reserve losses (Tedeschi et al., 2003). Buffaloes used in the present experiment have a negative energy balance (early lactation).

Nutrients digestibility:

It is well established that, all combinations of biological, natural and chemical additives were increased ($P<0.05$) the values of nutrient digestibility coefficients than that in control (Table, 3). Animals fed T2 and T3 showed higher ($P<0.05$) digestion coefficient values for dry matter, organic matter and crude protein than those fed T1 and control.

Also, all additives showed higher (P<0.05) digestion coefficient values for crude fiber and nitrogen free extract than control. On the other hand, animals in T1 recorded the highest (P<0.05) value of ether extract digestibility compared with T2 and T3, however, all additives showed higher (P<0.05) digestion coefficient value of ether extract than control. Experimental additives were significantly improved nutritive values as TDN (P<0.05) and digestible crude protein of rations compared with control.

Results obtained with chamomile flower might indicate the stimulation of rumen micro-flora activity through one of the following; 1) decreasing number and activity of antagonistic organisms, 2) saving some micro factors to rumen micro-flora as (micro elements, vitamins, hormones, enzymes and unknown factors) which are required to the efficient digestion, absorption and metabolism and available as effective groups or components in medicinal plants, 3) decreasing hazards of some harmful heavy metals and 4) minimizing effectively hazards of mycotoxins by inhibition of fungi growth and aflatoxins production (Allam et al., 1999 and Mohamed et al., 2003).

Similar results were obtained by El-Ashry et al. (2001) and Kholif and Khorshed (2006) with yeast cultures supplementation to goat's or buffalo's rations. Also, Aboul-Foutouh et al. (2000), Ali et al. (2005) and El-Ashry et al. (2006) were observed similar results when they were added chamomile flowers or other medicinal plants to dairy buffaloes or growing lambs. On the other words, El-Bedawy et al. (1999) found that sodium acetate treatment significantly improved CF, EE and NFE digestibilities of lucaena leaves fed to lactating goats.

Milk yield and composition:

The productive performance data and milk analysis are shown in Table 4. Milk yield in the present study was higher in all treated groups and was in agreement with El- El-Bedawy et al. (1999), Ashry et al. (2001), Abo El-Nor and Kholif (2005), Kholif and Khorshed, (2006) and Abo El-Nor et al. (2007). Milk yield and 4% FCM were significantly higher (P<0.05) in animals fed treated groups compared with control. The increase in milk yield with yeast supplementation may be due to one or more of the following reasons; 1) higher DMI and higher nutrients digestibility (Table, 2); 2) slightly increase of milk lactose, which had a positive correlation with milk yield. 3) yeast culture is more effective on manipulation of rumen environment and 4) the positive effect of chamomile on animal health and immunity of animals. In addition, the relative improvement of milk production of T3 (chamomile flower) might be due to the increase in rumen microflora activity and the associated effect between acetate and succinate on rumen microflora, which lead to improve feed efficiency and increase milk production. Data of milk composition showed that milk protein, fat, total solids, ash and lactose contents were higher (P<0.05) in animals fed experimental additives than control. Kholif and Khorshed (2006) found that rations supplemented with yeast significantly increased milk protein and lactose contents compared with control. Moreover, Abo El-Nor and Kholif (2005) fed lactating goats on sodium acetate and sodium succinate supplemented rations and found that treatments significantly increased milk total solids and solids not fat contents. Generally, feed efficiency calculated as milk yield/DMI and 4% FCM/DMI were significantly improved by T3 followed by T2 and T1 and then control (Table 4).

Blood serum parameters:

Data in Table (5) showed that animals fed supplemented rations had higher (P<0.05) serum total protein, albumin and globulin concentrations than control. Blood serum urea nitrogen was decreased (P<0.05) with chemical additives (T1) compared with other treatments. These results may be due to the improvements occurred in metabolic process as a response to the experimental additives. Serum total protein reflects the nutritional status of the animal and it has a positive correlation with dietary protein (Kumar et al., 1980). These results are parallel with values of crude protein and organic mater digestibilities in the experimental ration (Table, 3). Serum glucose values (P<0.05) were higher in animals fed supplemented rations compared with control.

Serum glucose had the same trend of milk yield (Table, 4) which was in accordance with the results of Clark et al., (1977) who claimed a positive correlation between blood glucose and milk yield.

Blood serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) values were significantly affected by treatments. Shehata et al., (2004) and Ali et al., (2005) found that chamomile supplemented goats

ration increased blood proteins and decreased cholesterol, GOT and GPT concentrations which were in normal range for healthy animals. Similar results were reported by Abo El-Nor and Kholif (2005) with chemical additives, El-Ashry et al., (2006) with medicinal plants, and Kholif and Khorshed (2006) with yeast culture. These results indicated that tested additives to lactating buffalo's rations were not affecting on liver activity or animals health.

CONCLUSIONS

It could be concluded that lactating buffalo's rations supplemented with combination of chamomile, sodium acetate and sodium succinate showed the best improvement of nutrients digestibility, milk production and milk composition and feed efficiency followed by combination of yeast, sodium acetate and sodium succinate with no deleterious effect on general health of the treated animals as compared to animals fed the control diet.

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Table (1): Chemical composition of concentrate feed mixture, berseem clover and rice straw (% on dry matter basis) used in experimental rations.

Item	Concentrate feed mixture	Berseem clover	Rice straw
Dry matter	92.30	13.10	91.80
Organic matter	90.60	88.40	85.20
Ash	9.40	11.60	14.80
Crude protein	14.10	13.80	3.50
Ether extract	4.08	2.60	1.40
Crude fiber	15.23	27.40	34.20
Nitrogen free extract	57.19	44.60	46.10

Table (2): Effect of different additives on pH, IVDMD and IVOMD.

Item	pH	IVDMD%	IVOMD%
CFM+ garlic	7.3	60.8	63.51
CFM+ fenugreek	7.3	46.6	49.50
CFM+ chamomile	7.4	60.9	64.25
CFM+ garlic + Fenugreek + chamomile	7.4	55.9	57.89
CFM+ yeast	7.5	58.1	60.35
CFM+ yeast selenium	7.6	56.0	58.52
CFM+ sodium acetate	7.8	66.95	69.25
CFM+ sodium succinate	7.8	62.6	65.31
CFM+sodium acetate+sodium succinate	7.7	61.1	64.58
CFM+ yeast + chamomile	5.7	64.1	68.35
CFM+chamomile + sodium acetate + sodium succinate	6.7	75.07	77.21
CFM+yeast + sodium acetate + sodium succinate	7.2	78.46	79.98
CFM+chamomile + yeast + sodium acetate + sodium succinate	6.6	73.50	75.05
CFM (control)	7.4	56.3	58.36

Each value of means obtained from 12 samples.

Table (3): Live body Weight, dry matter intake (DMI) and nutrient digestibility as affected by treated lactating buffaloes.

Item	Control	T1	T2	T3	±SE
Live body weight (kg)	526.25^{c}	570.0^{b}	593.75^{a}	563.75^{b}	0.512
DMI (kg/h/d)	15.69	15.75	15.86	15.83	0.006
MBS ($w^{0.75}$)(kg)	111.43^{c}	116.45^{b}	120.27^{a}	115.67^{b}	0.512
Nutrients digestibility (%):					
DM	66.41^{b}	69.12^{b}	74.51^{a}	76.11^{a}	0816
OM	70.90^{c}	75.03^{b}	79.10^{a}	80.19^{a}	0.588
CP	70.92^{b}	72.25^{b}	74.12^{a}	75.12^{a}	0.395
EE	71.53^{c}	76.54^{a}	74.69^{b}	74.06^{b}	0.325
CF	53.37^{b}	62.76^{a}	60.52^{a}	61.95^{a}	0.867
NFE	61.56^{b}	67.27^{a}	67.23^{a}	67.23^{a}	0.565
Nutritive value %					
TDN	47.31^{b}	49.49^{a}	49.56^{a}	49.63^{a}	0.958
DCP	8.45	8.61	8.84	8.95	0.421

Table (4): Effect of different additives on milk yield, FCM, milk composition % and feed efficiency.

Item	Control	T_1	T_2	T_3	± SE
Milk yield (kg/h/d)	6.21^{c}	7.33^{b}	7.27^{b}	8.52^{a}	0.246
4% Fat corrected milk (kg/h/d)	8.90^{c}	10.53^{b}	10.55^{b}	12.43^{a}	0.355
Milk composition (%):					
Total protein	3.68^{c}	3.85^{b}	3.86^{b}	4.02^{a}	0.023
Fat	6.89^{c}	6.92^{bc}	6.98^{b}	7.06^{a}	0.023
Lactose	4.54^{c}	4.80^{b}	4.81^{b}	5.09^{a}	0.051
Total solids	16.56^{c}	16.89^{b}	16.97^{ab}	17.17^{a}	0.063
SNF	9.67	9.96	9.61	9.99	0.131
Ash	0.74^{b}	0.75^{b}	0.77^{a}	0.76^{a}	0.023
Feed efficiency:					
Milk yield/DMI	0.396^{c}	0.465^{b}	0.458^{b}	0.538^{a}	0.311
FCM/DMI	0.567^{c}	0.656^{b}	0.665^{b}	0.786^{a}	0.431

Each value represents an average of four animals each group.
a, b and c means at the same row with different superscript are significantly (P<0.05) different.

Table (5): Effect of different additives on some blood parameters of lactating buffaloes.

Item	Control	T_1	T_2	T_3	± SE
Total protein (g/dl)	7.07^{c}	7.16^{b}	7.21^{b}	7.31^{a}	0.040
Albumin (g/dl)	3.60^{b}	3.62^{b}	3.65^{ab}	3.69^{a}	0.041
Globulin (g/dl)	3.47^{c}	3.54^{b}	3.56^{b}	3.62^{a}	0.019
A:G ratio	1.04	1.02	1.03	1.02	0.004
Glucose (mg/dl)	55.43^{c}	59.23^{b}	58.14^{b}	62.15^{a}	0.646
Urea (mg/dl)	23.56^{a}	22.43^{b}	23.89^{a}	24.52^{a}	0.792
GOT (units/ml)	34.33^{b}	34.30^{b}	34.78^{ab}	35.35^{a}	0.714
GPT (units/ml)	15.47^{b}	15.70^{ab}	15.99^{a}	15.68^{ab}	0.419

Each value represents an average of three animals each group.
a, b and c means at the same row with different superscript are significantly (P<0.05) different.

Effect of feeding ammoniated bagasse on growth performance of buffalo and Friesian calves.

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Abstract

Twenty four animals of buffalo (BF) and Friesian (FR) male calves almost at the same age and weight. (Two groups, twelve in each) were used in this study. Animals of each genotype were randomly distributed into three equal groups. Each group was fed according to (ARC, 1980) on one of the three different rations. The Control group fed on concentrate feed mixture (CFM) + 100% clover hay (CH), T1 fed on CFM + 50% CH + 50 % Ammoniated bagasse (AB), while T2 fed on CFM + 25%CH + 75% AB. Results indicated that the digestibility of most of the nutrients in T2 was the best followed by control in both BF and FR males. The higher daily gain (DG) was recorded by the control and T2 for both BF and FR and the higher of daily feed intake was recorded by T2 group followed by T1 then the control in BF, while in FR the higher of daily feed intake was found by T1 followed by T2 then the control. It can be concluded that replacement of CH by AB (75%, T2) led to higher DG and shorter fattening period in both genotypes.

INTRODUCTION

Buffalo provide about 70% of milk and 30% of meat in Egypt (FAO, 1990). Their limited contribution to meat production is mainly due to that males are slaughtered, as veal, at younger ages. Friesian has been introduced to improve primarily milk in Egypt is questionable. Many investigators have attempts to utilize some crop residues and agro-industrial by-products in animal feeding. Sugar can is an important cash crop in tropical and subtropical regions of the world and results in the production of large quantities of by-product, bagasse. The present study was designed to evaluate growth rate, feed conversion and digestibility of Buffalo and Friesian calves fed ammoniated bagasse.

MATERIALS AND METHODS

The present work was designed to evaluate the effect of ammoniated bagasse on growth rate, feed conversion and digestibility of buffalo and Friesian calves.

Animal feeding

Twenty four animals of Buffalo and Friesian male calves (Two groups, twelve in each) were used in this experiment. Animals were almost at the same age. The twelve animals in each group were randomly distributed into three equal groups, (n=4 in each). Each ration contained the same concentrate mixture but differed in the source of roughages as following; 1) 100% clover hay (Control), 2) 50% clover hay plus 50% ammoniated bagasse (T1) and 3) 25 % clover hay plus 75% ammoniated bagasse (T2). Claves were offered rations separately.

Calves were fed according to Agricultural Research Council allowances (ARC, 1980). The concentrate mixture was composed of : 23% cotton seed cake; 30% maize grains; 24% wheat bran; 15% rice bran, 4% molasses; 3% calcium and 1% ordinary Salt. Concentrate were offered twice daily at 8:00 am and 2.00 pm; clover hay and ammoniated bagasse were available all the day round. The means of age and weight of the three feeding groups for Buffalo and Friesian male calves are shown in Table1.

Table 1: Means of age and body weight of Buffalo and Friesian male at the beginning of the experiment.

Classification	N	Initial age (Month)	Initial weight (Kg)
Buffalo (BF)			
Control	4	12.9	167.8
T1	4	12.1	177.0
T2	4	11.0	174.3
Friesian (FR)			
Control	4	10.9	169.5
T1	4	11.4	169.8
T2	4	11.4	17.15

The refused residues of ammoniated bagasse were weighed and the daily intake the both roughage and concentrate was offered daily twice in winter and three times in summer. Complete chemical analysis of concentrate mixture, clover hay and ammoniated bagasse were performed according to the methods of the Association of Official Agriculture Chemists (A.O.A.C., 1990). The chemical analysis of feeds is presents in Table 2.

Table 2: Chemical analysis of feed stuffs which used in the experimental study (on dry mater basis).

Feed Stuff	Chemical analysis (%)				
	CP	EE	CF	NFE	Ash
Concentrate mixture	15.38	2.58	12.50	54.68	14.86
Clover hay	13.79	2.66	31.23	42.86	9.46
Ammoniated bagasse	6.89	1.25	43.68	44.78	3.40

The calves were weighed once every four weeks in the morning before feeding. Absolute body weight gain was calculated as the difference between the final and initial weights. The amounts of concentrates, clover hay and the ammoniated bagasse were recorded every four weeks.

Ammoniated technique

One stock was made from sugar cane bagasse bales. The standard dimension of the stack was 20×2×1.5 m. Ten tons of bagasse were used in the stack. A plastic sheet with a suitable thickness was used under the stack to minimize soil contamination and another fine plastic ropes were used for bailing bagasse. The bales were arranged in the stack similar to building like style. A polyethylene sheet (0.13 mm thickness, 25 m length and 6 m width) was used as cover. It was placed over the stack leaving a free margin of not less than 50 cm from each side of the stack. The stack was lightly covered by fixing the margins with soil. A hydrous ammonia gas (NH3) was transported under high pressure in special tanks fixed on a truck.

Ammonia was injected through perforated metal pipe of three meters length. This pipe was fitted under the polyethylene margin at the middle of the stack and withdrawn after injection had been completed. Ammonia was injected at the rate of 3% (on DM basis) by using 350 Kg NH3 for the stack. The stack was tightly closed by a plastic cover for a reaction period of three weeks. The polyethylene cover was gradually taken off according to the daily needed quantities of treated bagasse and for five days before f

Digestibility trials

Four digestibility trials were carried out to evaluate the digestion coefficients of each ration, using silica as an indicator for determining the digestibility. Four rations were used as following:

1. Clover hay (as a basal ration).

2. Concentrate mixture + clover hay.

3. Concentrate mixture + 50% clover hay + 50% ammoniated bagasse.

4. Concentrate mixture + 25% clover hay + 75% ammoniated bagasse.

Six representative calves (three rams from each group) were used in the digestibility trail. Calves were weighed at the beginning and the end of the trials. Trial consisted of 15 days as a preliminary period followed by seven days as a collection period, the quantities of ration were offered twice daily at 8:00 am and 2:00 pm., according to the maintenance of requirements of (ARC, 1980). Collecting small grab samples of faces obtained directly from the rectum at various times of the day (0, 3 and 6 hrs after feeding). Throughout the whole collection period, sprayed with 10% sulphoric acid and 10 % formaldehyde and dried at 70?? for 24 hrs in a drying oven, then at 105 ?? for 3 hrs. Dried faces were kept in tight plastic bottles for chemical analysis. Digestibility coefficients were calculated by using the following formula:

Digestibility coefficient =100-{100 x ([% of indicator in feed/% of indicator in faces] x [%of nutrient in faces / % nutrient in feed])}, according to Crampton and Harris (1969).

Statistical Analysis

The data were analyzed using least squares method (Harvey, 1960). Duncan Multiple Range test was used to test the differences among means (Duncan, 1955). Using the following model: $Yijk = \mu + Ti + ejk$

Where:

Yijk = the observation on the jth animal in ith the treatment group.

μ = Overall mean.

Ti = The effect due to the ith treatment group (i=1, 2, 3) where

1= Control group (feed 100% clover hay).

2= treated group (fed 50% clover hay + 50% ammoniated bagasse).

3= treated group (fed 25% clover hay + 75% ammoniated bagasse).

eij =The random error term.

RESULTS AND DISCUSSION

1. Digestibility trial

Digestibility coefficients and nutritive values of the different experimental rations are presented in table (3).

The digestibility coefficients of DM, OM, CP, CF and NFE did not differ significantly in both buffalo and Friesian calves fed T1 and T2 rations compared to the control ration. Also, the digestibility coefficient of EE did not differ significantly in Friesian calves fed T1 and T2 rations compared to the control ration. However, the digestibility coefficient of EE was lower significantly in Buffalo calves fed T1 ration compared to the control ration.

Table 3: Digestibility coefficients and nutritive values of Buffalo and Friesian calves fed the experimental rations.

Classification	N	In vivo nutrient digestibility % (on DM basis)						Nutritive values % (on DM basis)	
		DM	OM	CP	CF	EE	NFE	TDN	DCP
Buffalo (BF)									
Control	3	69.1	71.5	73.4	66.8	81.0	72.4	64.9	10.8[a]
T1	3	67..7	69.7	71.8	61.8	61.9	73.3	63.5	9.30[b]
T2	3	70.2	70.4	72.5	67.1	668.6	74.3	66.3	9.00[b]
Friesian (FR)									
Control	3	62.6	64.4	69.9	58.2	72.8	64.9	58.4	10.2[a]
T1	3	62.9	63.8	66.8	53.1	65.5	68.0	58.4	8.64[b]
T2	3	63.4	64.7	72.2	58.4	65.7	65.8	59.5	8.93[b]

Control= concentrate mixture (CM) + 100% clover hay (CH), T1= CM + 50% CH + 50% ammoniated bagasse (AB), T2= CM + 25% CH + 75% (AB)

The reduction of EE digestibility in both Buffalo and Friesian calves fed T1 and T2 rations compared to the control ration might be attributed to the associated balanced effect of the concentrate and roughage (Abou-Raya, 1963).

The present results are in agreement with those of EL-Basiony (1992) who found no significant difference among four groups of Buffalo calves fed on a restricted amount of concentrate feed mixture with one of the experimental roughages; 1) bean straw, 2) soya bean straw, 3) fenugreek straw and 4) berseem hay. Etman et al., (1992) reported no significant differences among three rations (clover hay and rice straw along with concentrate mixture including 0, 10, or 20% cassava meal as a replacer of yellow corn) in digestion coefficients of al nutrients, except CF digestibility which was significantly lower with the last ration. It could be concluded that the digestibility of most of nutrients in T2 was the best which might be due to the ammoniation effect of the added bagasse.

Nguyen (2007) found that there were no differences in DM, OM, NDF, ADF digestibility and nitrogen retention among the treatments. The buffaloes were fed on urea and molasses (UM), cotton seed cake and molasses (CM), urea, sesbania leaves and molasses (USM), urea and sesbania (US), urea, cotton seed cake and molasses (UCM) and urea and cotton seed cake (UC). Usman et al., (2004) reported that the digestibility of dry matter, organic matter and crude protein were Influenced {P<0.01) by diet composition in Friesian calves fed Friesian calves fed four diets were untreated maize stovers {Diet A), untreated maize stovers with MUB {Diet B), ammoniated maize stovers {Diet 'C) and ammoniated maize stovers with MUB {Diet D). Dry matter digestibility was minimum (P< 0.01) on Diet A and remained the same on diets B, C and D {55.82, 58.02 and 58.14%, respectively). Organic matter and crude protein digestibility were higher in the claves receiving ammoniated maize stovers. Supplementation of MUB increased (P< 0.01) the digestibility of all the three nutrients in untreated maize stovers but did not affect the digestibility of ammoniated maize stovers. The results demonstrated that MUB supplementation might not be required when calves are fed ammoniated maize stovers.

2. Nutritive values

The nutritive values as TDN and DCP are presented in Table (3). In general, the values of TDN and DCP for Buffalo calves were higher than the values that by Friesian calve which are mainly due to the higher values of the digestibility of all nutrients in Buffalo rather than Friesian calves.

The nutritive value as TDN did not differ significantly in both Buffalo and Friesian calves fed T1 and T2 rations compared to the control ration. However, the nutritive value as DCP was lower significantly in Buffalo and Friesian calves fed T1 and T2 rations compared to the control ration. The higher values of DCP in the control ration may be due to the high crude protein content.

Average daily gain, feed conversion and daily feed intake

1. Average daily gain

2. Tale (4) shown the average daily gain, dry matter intake and feed conversion in both Buffalo and Friesian calves fed ammoniated bagasse.

In Buffalo and Friesian calves, the average daily gain (ADG) did not differed significantly in T1 and T2 compared to the control calves. In both Buffalo and Friesian calves, average daily gain was slightly higher (P>0.05) in T2 (CH + 25% CH + AB) compared to T1 and the control calves.

Average daily gain of Buffalo in the present study was nearly similar to that obtained by Ghoneim et al., (1956, 0.64 Kg) and lower than those obtained by EL-Ashry et al., (1972, 0.71 Kg) or Awadalla (1987, 0.76 Kg).

Table 4: Average daily gain of Buffalo and Friesian calves.

Classification	N	Average daily gain (Kg)	Dry matter intake (Kg)	Feed conversion (DM/gain)
buffalo (BF)				
Control	4	0.66[a]	2287[a]	09.9[a]
T1	4	0.62[a]	2473[a]	10.9[a]
T2	4	0.67[a]	2470[a]	10.7[a]
Friesian (FR)				
Control	4	0.77[a]	1815[a]	7.86[a]
T1	4	0.75[a]	2040[b]	8.73[a]
T2	4	0.83[a]	1827[a]	7.75[a]

On the other hand it was higher than that reported by EL-Feel et al., (1992) who reported that ADG of Buffalo calves fed 80, 100 and 120% of the feeding requirements were 0.40, 0.45 and 0.53 Kg, respectively. Also, Mehrez et al., (1992) who reported that the daily gain of three groups of Buffalo calves were 0.52, .57 and 0.66 fed one of three dietary levels, being CM at the rate of 1.6, 2.0 or 2.4% of body weight (BW), in addition, rice straw supplemented with urea given at the rate of 1.0% of BW to all group. Sadek et al., (1992, 0.54 Kg).

Average daily gain of Friesian calves was higher than that obtained by Sadek et al., (1992, 0.73 Kg) and those obtained by EL-Ashry et al., (1972, 0.68 Kg) or Talha (1990, 0.70 Kg).

3. Dry matter intake and Feed conversion

Dry matter intake (DMI) and feed conversion (FC) in both Buffalo and Friesian calves fed ammoniated bagasse are presented in Tale (4). In Buffalo calves, the DMI did not differ significantly in T1 and T2 compared to the control Buffalo calves. However, in Friesian calves the DMI was higher (P<0.05) in T1 than the T2 and the control Friesian calves.

Feed conversion in both Buffalo and Friesian calves did not differ significantly in T1 and T2 compared to the control calves. As expected, DMI was higher in Buffalo than that observed in the Friesian calves; this could explain the poor feed conversion in Buffalo than the Friesian calves.

EL-Mounir (1990) showed lower FC expressed as DM/Kg gain (8.40) in Friesian calves than that obtained in the present study. Also, Talha (1990) who found that lower FC in Friesian calves (11.3 DM/Kg gain). Awadalla (1987) reported that one Kg gain in Buffalo calves fed 100% CM, 50% CM + 50% rice hulls, and 100% rice hulls e required 11.1, 11.8 and 13.8 Kg DM, respectively. In Friesian, EL-Mounir (1990) who found that one Kg gain needed 8.49, 8.55 and 8.16 Kg DM for Friesian calves fed 1) 1% ammoniated rice straw 2) 1% ammoniated rice straw + 2.5% CM of BW and 3) 1% ammoniated rice straw + 2.0% CM of BW.

Nguyen (2007) found that there were no significant differences in feed intake among the treatments; The buffaloes were fed on urea and molasses (UM), cotton seed cake and molasses (CM), urea, sesbania leaves and molasses (USM), urea and sesbania (US), urea, cotton seed cake and molasses (UCM) and urea and cotton seed cake (UC).However the rice straw intake was higher for the UC and US treatments. Usman et al., (2004) reported that the daily consumption of maize

stovers and total feed by the Friesian calves were higher {P< 0.01) on the diets containing ammoniated maize stovers than those containing untreated maize stovers. Ammoniation increased the intake of maize stovers by 61 %.

CONCLUSION

It could be concluded that the replacement of concentrate mixture by ammoniated bagasse (75%, T2) was safety and economically and led to higher DG and shorter fattening period in both genotypes.

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Effect of methionine supplementation on the performance of early lactating Nili Ravi buffaloes

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Abstract

Thirty nine early lactating Nili-Ravi buffaloes, divided into three groups i.e. GROUP-1, GROUP-2 and GROUP-3, were used to evaluate the effect of Metasmart (Isopropyl ester of 2-hydroxy-4-metyhylthiobutanoic acid) and Smartamine (synthetic by-pass methionine). The buffaloes were randomly assigned to one of the three treatments viz; GROUP-1=Control, GROUP-2-Metasmart (15 g/animal/day), GROUP-3=Smartamine (10 g/animal/day). The animals were kept in tie stall and fed green fodder ad libitum and concentrate according to their milk production. Milk production was recorded daily and milk samples were collected on weekly basis for analysis of various milk components. The blood samples were also collected and analyzed for blood bio-chemistry. Highest milk production per day (9.77±0.10 Litres) was recorded in GROUP-2, followed by GROUP-3 (9.64+0.09 kg) and control (9.20+0.07). Milk fat and protein contents were increased significantly with both types of methionine supplementation, while other milk components i.e. lactose, total solids and SNF remained unaffected with methionine supplementation. All the tested serum components like total protein, cholesterol, triglyceride, urea and glucose were non significant among all the groups. Overall, methionine supplementation increased milk production, milk protein and fat contents, while the other parameters remained unaffected.

Key words: methionine supplementation, metasmart, smartamine, Nilli Ravi buffaloes, milk yield, milk composition

INTRODUCTION

Ruminant dietary protein requirements are determined by the needs of the animal and the rumen microorganisms. Ruminal microorganisms may alter the amount and quality of ingested protein before it reaches the small intestine, where it is actually absorbed, thereby making it complex to determine the actual amount and quality of dietary proteins. The AA profiles available for absorption in the small intestine i.e. metabolizable protein (MP), reflect predominantly the combination of microbial protein, ruminally undegradable protein (RUP), and endogenous proteins1. Therefore, the protein available for absorption in the animal's small intestine has little resemblance to the quantity and quality of protein provided through diet2. For the reason, the specific dietary amino acid requirements are of little importance in ruminant animals.

Like other mammalian species, the amino acids absorbed in the small intestine and their supply to the mammary glands can affect milk protein content and milk volume particularly during early lactation3. In addition to providing amino acids for milk production, amino acid profile also affects metabolism and immune function and thus the productivity of the animal4. The resemblance of essential amino acids between MP and the amino acids requirement of the animal, for the combined functions of maintenance and production, is the most important factor for efficient use dietary protein.

Methionine (Met) and Lysine (Lys) have been frequently identified as the two most limiting AA for milk yield and milk protein content1. Therefore, the present study was designed with the objective to increase the methionine content in RUP to the extent needed to increase its absorption.

MATERIALS AND METHODS

The present trial was designed to evaluate the effect of two sources of methionine, "Metasmart" (Isopropyl ester of 2-hydroxy-4-methylthiobutanoic acid) and "Smartamine" (synthetic by-pass methionine) on milk production and composition of Nili-Ravi buffaloes. Thirty-nine Nili Ravi buffaloes were used in this experiment at Buffalo Research Institute, Pattoki, Pakistan. The buffaloes used in this trial were approximately at the same stage of lactation (1-3 months) and milk production level (8-10 litres/day). Buffaloes were housed and managed under intensive tie stall system and were offered roughages ad libitum but the concentrate was given according to the animals' individual production levels at the rate of 1 kg of concentrate for 2.5 kg of milk production after the first three liters of milk. All the animals were given free access to fresh drinking water round the clock. After two weeks of adaption period, the animals were divided into three groups namely group-1, group-2 and group-3 on the basis of milk yield of individual animal in such a way that total daily milk production of all the animals in individual group was almost same among the groups. Group-1 was given control feed (no supplementation), while the buffaloes in the group-2 and group-3 were given metasmart (partially metabolizable methionine in the rumen) @ 10 g/day/animal and smartamine (synthetic bypass methionine) @ 15 g/day/animal, respectively. The inclusion level of metasmart and smartamine was according to the manufacturer's specifications. The total duration of the study was 6 weeks (2 weeks of adaptation and 4 weeks for actual trail and data collection). To give a specific quantity of concentrate ration within each production group, the feeding of the animals was done on individual basis. Metasmart and Smartamine were added individually in ration at the time of feeding. For the purpose, 10 g smartamine and 15 g metasmart were weighed and manually mixed with the portion of each animal's first meal of their daily ration. The concentrate ration consisted of the ingredients given in Table 2, which contained 16% protein, 75% TDN, 0.25% methionine and 0.58% lysine content.

Animals were milked twice a day (morning & evening) and milk yield was recorded on daily basis by adding milk produced in the morning and evening. Milk samples were taken on weekly basis and analyzed for various milk components i.e. protein, fat, lactose, solid not fat (NSF) and total solids by using Milkoscan (Milkoscan TM FT2, FOSS, USA)

The blood samples of the buffalo were also taken on weekly basis to determine the impact of individual methionine supplement on serum biochemical profile. About 10 ml of blood was collected from jugular vein in sterilized disposable syringes adopting all aseptic measures. Blood was allowed to clot in slanting position at room temperature for 8 hours in syringes sealed with wax using Paraffin Embedding apparatus. Serum was separated from blood samples by centrifugation for 5 minutes at 5000 rpm in a laboratory centrifuge machine (Model: Sorvall RC-5B; DuPont Instrument, Newtown, Conn., USA) and the serum was stored in the plastic disposable specimen bottles at -20 °C until used for biochemical analysis i.e. cholesterol5 triglycerides6, glucose7, total protein8 and urea9 in the WTO quality control labs, University of Veterinary and Animal Sciences, Lahore, Pakistan.

The data thus obtained from all the parameters were analyzed through analysis of variance technique10 using using Cohort version 6.1 (Co-stat, 2003) to determine the level of significance. The separation of means or significant difference comparisons were done using DMRt. The statistical significance was defined as P?0.05.

RESULTS AND DISCUSSION

The statistical analysis indicated significant increase in milk production with both types of methionine supplementation in feed of the Nili Ravi buffaloes, however the increase in milk yield was independent of the type of supplementation. The highest milk yield (9.77+0.09 Litres/day) was recorded in buffaloes on metasmart supplements followed by buffaloes in smartamine group (9.64+0.10 Litres/day), that is increase of 6.20% and 4.78%, respectively as compared to control group (Figure 1, 2). The increase in milk yield might be attributed to increased availability of methionine

(limiting amino acid) for milk production. The results of the milk production in current study are in accordance with the findings of many other workers11-14; reported a significant increase in the daily milk production with methionine supplementation. On the other hand, some others15-18 have reported a non significant impact of methionine supplementation on milk production.

Fat and protein are the two most important constituents of milk chemical composition. In many countries the price of milk is primarily determined considering concentration of these wo constituents in milk chemical profile. Among milk composition parameters, fat and protein content indicated significant increase with methionine supplementation, whereas increase in the methionine content in buffalo diets did not influenced solid not fat (SNF), total solids and lactose contents (Table 2). Milk fat content of buffaloes increased significantly (8.06%) with smartamine while, metasmart resulted in slight non significant increase (2.15%) as compared to fat content of control group. These results are similar with the results of various other workers3, 19-20 who reported significant rise in the milk fat percentage with methionine supplementation. While, Kowalski21 reported non significant behavior of milk fat percentage with methionine supplementation.

Methionine supplementation resulted in significant increase in milk protein content (Figure 2) irrespective of the source of methionine. The increased protein content in milk might have been resulted due to increased absorption of limiting amino acid (methionine) from intestine in buffaloes on supplemented diets and thereby, increased availability to mammary glands. Many other workers12-14, 18, 21 have also reported an increased milk protein content with methionine supplementation of bovine diets contrary to the findings of some others22-24; found non significant effect of methionine supplementation on protein content in milk.

All the tested blood serum constituents like serum glucose, cholesterol, total protein, urea and triglycerides of experimental and control group buffaloes were not significantly different, which is also in harmony with the results of some earlier studies. Colin-schoellen22 and Hayirli11 also reported non-significant effect of methionine supplementation on blood glucose in dairy cows, however, Berthiaum17 reported higher concentrations of arterial plasma glucose by feeding ruminally protected methionine. Hayirli11 also reported that chromium-methionine supplementation did not affect the concentrations of blood total protein and other metabolites.

Overall, it is concluded from the results of present investigation that both types of methionine supplementation increased the overall milk production, milk fat and milk protein contents in Nili Ravi buffaloes however, increase in fat content was more pronounced with smartamine supplementation. Increased ruminally undegradable methionine did not show any marked effect on other milk parameters like lactose, SNF and total solids. Similarly, serum biochemical profile also remained unaffected with methionine supplementation.

Table 1: Ingredient used to formulate experimental concentrate ration

Sr.No.	Ingredients	Quantity (%)
1	Maize grains	15
2	Wheat bran	24
3	Rice polishing	10
4	Maize gluten 30	9
5	Canola meal	5
6	Sunflower meal	5
7	Cotton seed cake	15
8	Molases	15
9	Mineral mixture	2

Table 2: Effect of different sources of methionine supplementation on milk composition of experimental buffaloes

Treatment Groups	Fat (%)	SNF (%)	Total Solids (%)	Protein (%)	Lactose (%)
GROUP-1	5.58±0.08b	8.55±0.16a	14.32±0.19a	3.28±0.002b	4.15±0.012a
GROUP-2	5.70±0.09b	8.41±0.18a	14.11±0.15a	3.32±0.001a	4.17±0.014a
GROUP-3	6.03±0.11a	8.20±0.14a	14.40±0.17a	3.34±0.004a	4.15±0.010a

Means sharing the same letter in a column are not significantly different.GROUP-1 = control group with no supplementation; GROUP-2 = Metasmart supplementation @ 15gm/animal GROUP-3 = Smartamine supplementation @ 10gm/animal

Table 3: Effect of different sources of methionine supplementation on various serum biochemical profile parameters of experimental buffaloes

Treatment Groups	Glucose (mg/dL)	Cholesterol (mg/dL)	Total Protein (g/dL)	Triglycerides (mg/dL)	Urea (mg/dL)
GROUP-1	3.96±0.58a	45.45±4.83a	6.37±0.54a	5.58±0.58a	1.40±0.24a
GROUP-2	4.08±0.60a	35.66±4.96a	6.74±0.55a	4.16±0.59a	1.03±0.25a
GROUP-3	4.72±0.63a	48.78±5.17a	5.97±0.58a	4.33±0.62a	1.56±0.26a

Means sharing the same letter in a column are not significantly different. GROUP-1 = control group with no supplementation; GROUP-2 = Metasmart supplementation @ 15gm/animal; GROUP-3 = Smartamine supplementation @ 10gm/animal

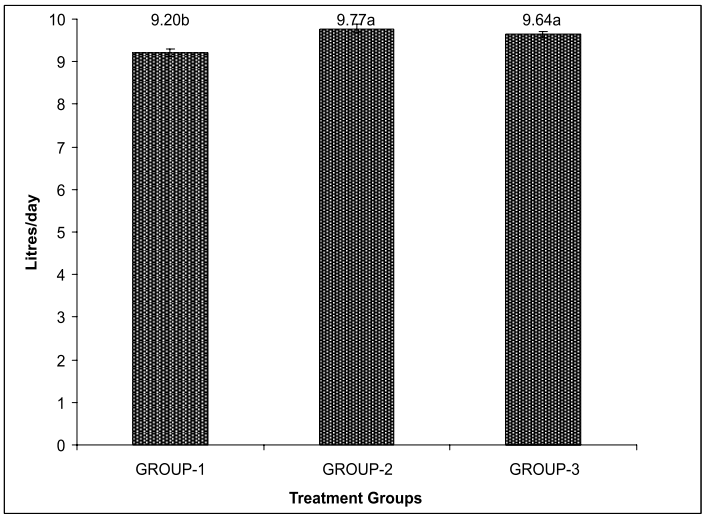

Figure 1. Average daily milk production of various groups of experimental Nili Ravi buffaloes.

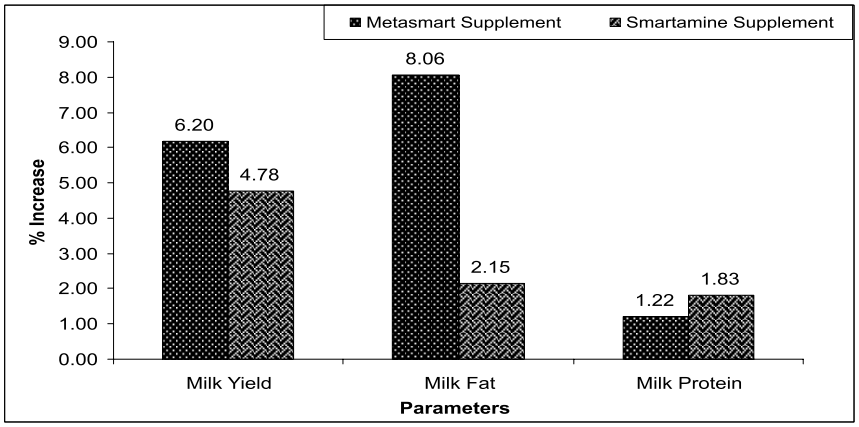

Figure 2. Percent increase in milk yield, milk fat and milk protein content with different types of methionine supplementation in Nili Ravi buffaloes

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Effect of oilpalm solid waste on milk production of Murrah buffalo

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Abstract

A field trial was conducted to study the effect of oilpalm solid waste on milk production of Murrah buffalo raised under oilpalm plantation. Two farmers from different district, each has 80 and 200 heads of buffalo were chosen to be involved in this study. Fourty cows with 7 – 8 month pregnancy from each farm were used; they were divided into 2 groups A, Control: offered supplement of 1 kg copra meal + 1 kg milled cassava + mineral mix. and B, Control + 1 kg oilpalm solid waste. This supplement was offered to the cows for 2 months before and 2 months after calving.The cows grazed forage grow under oilpalm plantation. The addition of oilpalm solid waste in the diet improved milk production (8.5 l/d vs 10.5 l /d), calve weight at birth (19.6 kg vs 22.1 kg) and live weight gain of the calves (0.66 kg/d vs 0.99 kg/d). It was estimated that those offered with additional palmoil solid waste may loose weight of 0.32 kg/d while the control group may gain 0.06 kg/d. Further improvement of milk yield is expected to achieve by higher inclusion of oilpalm solid waste in the diet and that need to be further studied.

Key words: oilpalm waste, Murrah buffalo, milk production.

INTRODUCTION

Indonesia is estimated to have 6 million ha of oilpalm plantation, an enormous potential for the development of large ruminants. The cover crops particularly under 5 years old oilpalm which consist of grasses and pasture legumes are good source of forage (Chen, 1990). Chen and Dahlan (1995) estimated a stocking rate of 0.3 – 3 head/ha and 2.0 – 1.4 head/ha for cattle and sheep/goat respectively. However, the intergration of large ruminants in to oilpalm plantation, should not reduce the production of palmoil but rather gives more economic viable to the plantation. Therefore, it needs a careful management of intergration.

The oilpalm industry generates by-products such as palm kernel cake (PKC), palm press fiber (PPF) and sludge cake or decanter cake (solid waste) which can be used as feedstuff. Palm kernel cake known to have high nutritive value compared to other oilpalm by-products and has been used as ingredient in concentrate feed (Chin, 2002). In Indonesia, PKC is mostly exported to overseas, only solid waste that left untouched by the plantation owner, yet has the potential to be used as a feed. There are only few farmers who live around the palm oil factory use this by-product as supplement for their livestock. The reason is that farmers lack of knowledge of the feed value of this by-product and most farmers live too far a way from the factory so need cost to transport it. In addition to that, since solid waste contains 1.5 % oil and 25 % water, it easily get rancid and contaminated by fungus (Utomo and Wijaya, 2004). Therefore, it has to be offered fresh, anaerobicly stored or dried milled (Batubara et al, 2005).

The production of oilpalm solid waste is approximately 2 % - 3 % (Utomo and Wijaya, 2004) of empty fruit bunch (EFB). It depends on the capacity, facility of the factory and EFB produced, an oilpalm factory can produce solid waste of approximately 8 – 20 ton/day. Indonesia is estimated to produce oilpalm solid waste as much as 460.000 ton/year (BPS, 2002). This solid waste is only produced by palm oil factory which has facility of 3 phase (decanter) that can separate sludge from the solid part.

There have been some studies on the use of oilpalm solid waste as feed for cattle, sheep and goat, duck and chicken. Utomo and Widjaja (2004) found, in cattle, supplement of oilpalm solid waste at the rate of 1.5 % body weight (BW) and ad libitum resulted in live weight gain (LWG) of 0.45 kg/d and 0.77 kg/d compared to control (0.06 kg/d); while in sheep, 1 % BW of oilpalm solid waste offered, resulted in LWG of 0.05 kg/d. A further 0.02 kg/d of LWG can be reached in both sheep and goat if the oilpalm solid waste is fermented. The increased LWG in animal given fermented oilpalm solid waste was caused by increased metabolisable energy (ME) compared to the unfermented one. Increased ME in fermented oilpam solid waste is due to a reduction in fiber and an increase in protein content (Pasaribu et al, 1998). The cost of fermentation however, needs to be considered economically.

Studies in 1 – 2 weeks old ducks offered oilpalm solid waste fermented or unfermented showed that inclusion of oilpalm solid waste in the diet should not exceed 10 %; while in older ducks (5 – 6 weeks old), 15 % inclusion of oilpalm solid waste in the diet is tolerable (Sinurat et al, 2000). Sinurat et al, (2000) also found that in broiler, oilpalm solid waste can be included in the diet as much as 5% to 10 % respectively for the unfermented and the fermented one.

This paper reports a field study on the use of oilpalm solid waste to improve milk production of Murrah buffalo.

MATERIALS AND METHODS

Eighty (80) buffalo cows with 7 – 9 month pregnancy and estimated body weight (BW) of 500 kg were randomly chosen from 2 farmers in North Sumatera. The cows were raised under the existing farm management system, whereby all animals were grazed under oilpalm plantation and fed supplement containing1 kg coconut meal and 2 fresh kg cassava meal every day. Each farm held 40 cows which was divided in to two groups, the control and the treatment diet.

The control diet consists of: 1 kg coconut meal + 2 kg cassava meal (50 % moisture)+ mineral mix. The treatment diet consist of: the control + 1 kg dried milled oilpalm solid waste. These ingredients were mixed thoroughly and offered twice a day in the barn, two months before and 2 month after calving.

Milk production were measured in the morning and the afternoon during the first 2 months of lactation. Body weight of calves were taken at calving and 2 months afterward.

Chemical analysis

Proximate analysis was done on coconut meal, casava meal and oilpalm solid waste according to (AOAC, 1984)

Estimated Metabolizable Energy (ME).

To estimate ME, in vitro organic matter digestibility (IVOMD) was carried out using Tilley and Terry (1963) method. The estimated ME based on MAFF (1975) is 0.15 x IVOMD.

RESULTS AND DISCUSSION

Nutritive value of forage and the supplement

The cover crops under oilpalm plantation consists of various tropical grasses and legumes. Forage is required by ruminants not only as a source of fiber but also as a source of vitamins and minerals. Large ruminants can grow approximately 0.25 kg/d when grazed under oilpalm plantation (Wong and Chin, 1998). However, feeding forage alone for lactating

cows will not meet their nutrient requirements, and so it needs an additional feed supplement. In this case, farmers have been using coconut meal and cassava meal as feed supplement for dairy buffalo. Table 1 shows coconut meal has protein content of 19.3 % and ME of 14 MJ/kg whereas cassava meal has 2.5 % protein and 14.6 MJ/kg ME. Both ingredients are good source of energy. A high ME in cassava meal is due to its high starch content. In addition to high energy content, coconut meal is also a good source of protein as 70 % of its protein is by-pass protein (Deville et al, 1980). Compared to these 2 supplements, oilpalm solid waste only contain 12 MJ/kg ME , but a much higher in protein content (12 % vs 2.5 %) than cassava meal. Thus oilpalm solid waste can be considered as medium type of feed supplement.

Milk production

The addition of 1 kg oilpalm solid waste in the diet of dairy buffalo improved milk yield of approximately 2 L/d (10.5 L vs 8.5 L). The increased milk yield was the result in increased metabolizable energy intake (MEI) of the cows which is estimated to be 5.2 MJ/d (see Table 1). If the oilpalm solid waste is free of charge, then for every L increased in milk yield will increase the farmer income. The average milk production showed in Table 2 was taken during the first 2 months of lactation, the value in which milk production may be at peak. It is anticipated that even after peak lactation (in which milk yield will decline) the cows given an additional of 1 kg oilpalm solid waste (treatment diet) will produce higher milk yield than the controls. Milk production of these buffalos may be further increased if higher inclusion of oilpalm solid waste was given. Dhanda (2006) reported that milk production of Murrah buffalo in India can reach up to 15 L/d. A cow with BW of 500 kg, producing 15 L/d of milk, requires 175.4 MJ/d of ME. This amount of energy can be met by feeding forage ad libitum (under oilpalm plantation) + 6 kg of oilpalm solid waste + 2 kg coconut meal + 2 kg cassava meal.

In the first 2 months of lactation, the treatment diet will result in estimated weight loss of 0.32 kg/d while the control diet will result in estimated weight gain of 0.06 kg/d (Table 2). During the first 12 weeks of lactation, nutritional requirements is usually exceed voluntary intake, so body fat reserves are drawn upon to make up the nutrient deficit. In this experiment however, the values of weight loss and weight gain are only estimation since body weight of the cows are not measured. Furthermore, the standard energy requirement for buffalo has not been made available; therefore all of the estimation here is based on the energy requirements for dairy cattle (see MAFF, 1975).

Supplementation of oilpalm solid waste in the diet of lactating cows may speed up postpartum estrus, thus shorten the calving interval. This needs to be further investigated.

Birth weight and calve growth

Table2 shows calves birth weight from cows offered 1 kg oilpalm solid waste were higher (22.1 kg vs 19.6 kg) than those from cows offered the control diet.

This difference is associated with improved (5.2 MJ/d) metabolizable energy intake of the dam. Calves born from the treated cows also had a better growth rate (0.99 kg/d vs 0.66 kg/d) than those born from the controls. The growth of calve before weaning is dependent upon 2 factors, the high quality colostrum and feeding management to stimulate rumen growth (Moran 2005). These two factors may be responsible to the high growth rate of the calves born from the treated cows. The treated cows may have a better quality of colostrum; since their calves were born with a higher BW, they can consume more colostrum than those with lower BW. In practice, all calves were always with their dam (both the treated and the control); those born from the treated cows may have access to oilpalm solid waste and thus have a better LWG. Further study should be carried out to look at the effect of oilpalml solid waste on fattening of Murrah or/and Swamp buffalo. It is predicted that supplementation of oilpalm solid waste may reduce slaughter age, so that the meat produced will have a better quality and better price. Study by Johnson and Charles (1978) showed that in fattening, buffalo had a lower LWG than cattle, but the percentage of meat produced was higher and fat was lower than cattle. Since buffalo meat contain less fat and less cholesterol than beef (Lemcke, 2004), for human health, buffalo meat is better than beef. Therefore it is expected that buffalo meat can be sold with a higher price.

CONCLUSION

The inclusion of oilpalm solid waste in the diet of Murrah buffalo significantly improves milk yield and calve growth rate. This is caused by a high metabolisable energy content of oilpalm solid waste thus can be used as ingredient in concentrate.

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Table 1. Estimasi konsumsi ME, rata-rata produksi susu, bobot lahir, dan pertambahan bobot badan (PBB) kerbau pada perlakuan A dan B

Parameter	Perlakuan A	Perlakuan B
Estimasi konsumsi ME * (MJ)	124.6	129.8
Produksi susu (l/hari)	8.5 ± 0.75^{a}	10.5 ± 0.61^{b}
Bobot lahir (kg) pedet	19.6 ± 1.43^{a}	22.1 ± 1.92^{b}
PBB (kg/hari)	0.66 ± 0.13^{a}	0.99 ± 0.09^{b}

Konsumsi bahan kering = 0.025 BB + 0.1 produksi susu. BB = 500 kg

Superskrip berbeda pada kolom yang sama menunjukkan berbeda nyata ($P<0.05$)

Table 2. Estimasi energi metabolisme yang dibutuhkan untuk ternak kerbau perlakuan A dan B

ME (MJ)	Perlakuan A	Perlakuan B
Kebutuhan pokok (maintenance)	54	54
Produksi (Pakan – Maint)	70.6	75.8
Susu	68.8	85.0
Selisih	1.8	-9.2
Kehilangan BB (kg/hari)	+ 0.06	-0.32

ME susu = 1.694 (0.0386 BF + 0.0205 SNF – 0.236); BF susu kerbau = 80 g/kg; SNF susu kerbau = 94 g/kg. ME kehilangan 1 kg BB = 28 MJ

Effect of Protein/Energy Balance in Diets for Buffalo Herds on Milk Yield and Nitrogen Excretion

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Abstract

The protein and energy content of diets administered to lactating buffaloes and milk performance traits were analysed in 90 Italian herds to optimize the ingested/excreted nitrogen ratio. The considered parameters were: dry matter (DM) intake, crude protein (CP) and net energy (NE) level of the diets, milk yield and composition (protein and fat). In each herd, the differences (?CP or ?NE) between crude protein or net energy content of the administered diets and the requirements, on the basis of the daily milk production, were assumed as the index of the balance of the diets. So threshold values of CP and NE required for milk production were established and the herds were divided into three groups either according to the estimated ?CP: group 1 (lack of protein), group 2 (balanced), group 3 (excess) or according to the estimated ?NE: group 1 (lack of energy), group 2 (balanced), group 3 (excess). A CP excess in the diet (group 3) did not improve the normalized milk yield (8.09 vs 8.06 kg/d) in respect to the herds of group 2, whereas the normalized milk yield of group 1 (7.36 kg/d) was statistically different ($P<0.10$) from the other two groups. As far as NE balance was concerned, both lack and excess significantly reduced normalized milk yield, with respect to balanced diets (7.02, 7.34 vs 8.72 kg/d, $P<0.05$).

Key words: Protein/energy balance, milk yield, nitrogen excretion.

INTRODUCTION

The volume of buffalo breeding in Italy is in constant expansion but knowledge relating to the environmental impact of breeding milk buffalo is still very scarce. So the first step in order to optimize the utilization of nitrogen is to improve the sustainability of the herds and acquire information on the diets actually administered and on the production levels achieved. The aim of the present study was to monitor the protein and energy levels of the diets administered and to evaluate the effects of the nitrogen/energy balance on production and milk quality and on nitrogen excretion.

MATERIAL AND METHODS

The research was carried out on 90 herds, situated in the region of major interest (central and southern Italy) for the production of buffalo milk destined for transformation into mozzarella cheese. In each herd the following data were collected: the quantity of dry matter administered (DM, kg/head/d), the content of crude protein (CP, g/kg DM) and net energy (NE, MilkFU/kg DM), the quantity of milk produced (kg/head/d) and its protein and fat content (g/kg). By utilizing the quanti-qualitative milk data, the daily normalized milk (NM) yield was estimated:

NM (kg/d)={[Fat (g/kg)-83.0] + [Protein (g/kg)-47.3]} x 0.00687 + 1 x milk yield1, 2.

The following equations 2, permit a calculation of the requirements of the daily intake of dry matter: DM (kg/d) = 0.4643 x NM (kg/d) + 11.4210, net energy: NE (MilkFU/d) = 0.7265 x NM (kg/d) + 6.8944 and crude protein: CP (g/d) = 121.08 x NM (kg/d) +1247.60. The differences (?PG, g/d and ?NE MilkFU/d) between the quantity of crude protein and net energy actually provided to the animal and those estimated on the basis of the milk yield, provide an indication of the protein and energy balance of the administered diet. In this way it is possible to select a value-threshold for the quantity of crude protein of the diet below which there could be both a quantitative and qualitative decrease in the production, and above which a situation of excess protein in the diet could occur with a resultant increase in nitrogen excretion. The same principle was followed for net energy, which regulates the state of fattening of the animals. So threshold values of the CP and NE required for milk production were established and the herds were divided into three groups either according to the estimated ?CP [group 1 (lack of protein) ?CP<-100 g/d, group 2 (balanced protein) ?CP ranging from -100 to +100 g/d, group 3 (excess of protein) ?CP>+100 g/d] or according to the estimated ?NE [group 1, ?NE<-1 MilkFU/d, group 2, ?NE ranging from -1 to +1 MilkFU/d, group 3, ?NE>+1 MilkFU/d]. The differences between groups were tested utilizing the GLM/SAS procedure3 by a monofactorial model: Yij = ? + ?i + ?ij where ?=general mean; ?=group (i=1,.,3); ?ij=error of model.

RESULTS AND DISCUSSION

As shown in Table 1, a crude protein excess in the diet (group 3) did not improve the normalized milk yield (8.09 vs 8.06 kg/d) and quality (protein: 45.86 vs 45.56 g/kg; fat: 83.29 vs 83.11 g/kg) with respect to the herds of group 2, whereas the normalized milk yield of group 1 (7.13 kg/d) was statistically different (P<0.10) from the other two groups. As presented in Table 2, group 2, fed with a balanced energy diet, showed a normalized milk production significantly higher than the groups with a lack or excess of energy (8.72 vs 7.02, 7.34; P<0.05). Diversely from the data recorded in the previous table, the milk protein of group 2 was higher (P<0.05) with respect to that of the other two groups while the milk fat was statistically different (P<0.05) only with respect to group 3. As far as the environmental impact is concerned, a substantial percentage of herds (about 40%) received an excess of protein: in group 3 the amount of crude protein exceeding the requirements of lactating buffalo and dispersed into the environment reached about 1 kg/ha/d. This result indicates that, for a high animals/ha ratio, the pollution from the nitrogen excretion could be substantial and that it would be advantageous to achieve an optimization of the crude protein level in the diets.

Table 1. Effect of the protein balance of the diets on the milk quantity/quality.

	Milk yield (kg/d)	Milk protein (g/kg)	Milk fat (g/kg)	Normalized milk (kg/d)	Dry matter daily intake (kg/d)	Net energy daily intake (UFL/d)	Protein daily intake (g/d)
1) Lack	7.36^{β}	44.59	81.13	7.13^{β}	15.04^{b}	12.50^{B}	1792.48^{C}
2) Balanced	$8.13^{\alpha\beta}$	45.56	83.11	8.06^{α}	16.09^{a}	13.87^{A}	2212.00^{B}
3) Excess	8.17^{α}	45.86	83.29	8.09^{α}	16.84^{a}	14.62^{A}	2539.67^{A}
Rmse	1.76	2.93	4.82	1.80	1.67	1.68	281.42

a, b: P<0.05; A, B, C: P<0.01

Table 2. Effect of the energy balance of the diets on the milk quantity-quality

	Milk yield (kg/d)	Milk protein (g/kg)	Milk fat (g/kg)	Normalized milk (kg/d)	Dry matter daily intake (kg/d)	Net energy daily intake (UFL/d)	Protein daily intake (g/d)
1) Lack	7.03^{b}	44.42^{b}	82.47^{ab}	7.02^{b}	13.98^{C}	10.80^{C}	1938.50^{b}
2) Balanced	8.73^{a}	46.41^{a}	83.97^{a}	8.72^{a}	15.55^{B}	13.41^{B}	2239.77^{ab}
3) Excess	7.54^{b}	44.90^{b}	81.86^{b}	7.34^{b}	16.83^{A}	14.54^{A}	2286.59^{a}
Rmse	1.68	2.87	4.80	1.70	1.60	1.59	396.87

a, b: P<0.05; A, B, C: P<0.01

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Effects of diets with increasing level of ground corn grain on the ruminal degradability in buffalo

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Abstract

The rumen degradability of protein and other nutrients has been one of the most significant parameters in systems of feed evaluation for ruminants. A synchronism between the rumen degradation of protein and carbohydrates in the diet is required for optimal microbial growth and protein synthesis in view the need to adequately supply the microbial demand for nitrogen and energy, preventing losses of N as ammonia or energy from methane. Diets containing four levels of ground corn grain (0, 22, 37 and 49% in DM) with the ratio protein:non-structural carbohydrates of 1.01, 0.39, 0.33 and 0.26 (CP:NSC), respectively, were evaluated in four rumen fistulated buffaloes fed on a Latin square 4x4. Grass hay coast-cross was used as roughage and were determined the rumen degradability of dry matter and NDF of hay and dry matter and crude protein of corn. No differences were observed among the treatments in the effective degradability estimated by turnover rate of 2%/h of DM hay (mean 52.54%) and neither with corn (mean 66.10%). The lowest rate of degradation of NDF hay (2.25 %/h) was observed in 37% corn diet and there was higher degradation rate of DM and NDF from corn with the diet with 22% corn (10.37 and 13.18%, respectively). There was no difference in ED2% of NDF hay (mean 53.78%); the ED2% of CP maize was lower in the diet with 37% corn (59.32%) and higher with 49% corn (70.27%). The results indicate that in general the microorganisms in the rumen have good adaptability to promote similar level of degradation of nutrients with different relations of CP:NSC.

Keywords: buffalo, degradability, energy, protein, rumen

INTRODUCTION

The degradability of protein and other nutrients has been one of the most significant parameters in systems of feed evaluation for ruminants, in view of the need to adequately supply the microbial demand for nitrogen and energy, preventing losses of N as ammonia or energy in the form of methane. Rumen degradability studies in buffaloes are still very limited compared to those produced in cattle and sheep. The technique of synchronization of ruminal degradation of protein and starch proposes to increase protein production in the rumen and the efficiency of energy use, because the bacteria in the rumen need these two elements simultaneously available. The study of the best combinations of sources of carbohydrates and protein degradable in the rumen is of great importance in buffalo nutrition since there is an intense microbial activity in the rumen on different diets1,2. The aim of this study was to evaluate the effects of different levels of ground corn grain, with emphasis on protein:non-structural carbohydrates relationship in diets for buffalo on rumen degradability.

MATERIALS AND METHODS

Four buffaloes with rumen fistulae were used in Latin square design (4x4) to evaluate diets based on hay of coast cross (Cynodon dactylon (L.) Pers), formulated with four levels of corn grain (0, 22, 37 and 49% DM) and crude protein

relations: non-fiber carbohydrates (1.01, 0.39, 0.33 and 0.26), calculated from the dry matter intake. In situ degradability of hay (DM and NDF) and corn (DM and CP) were determined in each period by in situ nylon bags technique according to Gives and Huntington (1995)3. The samples of the hay were incubated in the rumen for 3, 6, 12, 24, 48, 72 and 96 hours for determination of DM and NDF disappearance and of the corn for 3, 6, 9, 12 and 24 hours for DM and CP disappearance from the rumen. The data were fitted to models proposed by Ørskov and McDonald (1979)4, PD = a + b (1 – e-ct); ED = a + (b x c)/c + k; where: a= soluble fraction; b= insoluble potential degradable fraction; c = rate of degradation; k = outflow rate; PD = potential degradability; ED = effective degradability.

RESULTS AND DISCUSSION

The effects of levels of corn in the diet of buffaloes on the kinetics of degradability of dry matter (DM) and neutral detergent fiber (NDF) coast-cross hay are showed in the Table 1 and in the Table 2, dry matter (DM) and crude protein (CP) in corn grain. No differences were observed among the treatments in the effective degradability estimated by turnover rate of 2%/h of DM hay (mean 52.54%) and neither with corn (mean 66.10%). The lowest rate of degradation of NDF hay (2.25 %/h) was observed in 37% corn diet and there was higher degradation rate of DM and NDF from corn with the diet with 22% corn (10.37 and 13.18%, respectively). There was no difference in ED2% of NDF hay (mean 53.78%); the ED2% of CP maize was lower in the diet with 37% corn (59.32%) and higher with 49% corn (70.27%). The results indicate that in general the microorganisms in the rumen have good adaptability to promote similar level of degradation of nutrients with different relations of CP:NSC.

Table 1- Kinetics degradability of dry matter and neutral detergent fiber of coast-cross hay in buffaloes fed diets containing increasing levels of corn and ground

	Corn level in the diet (%)				
	0	22	37	49	Mean
	Dry matter				
a	20.01^{ab}	21.39^{ab}	24.42^{a}	18.78^{b}	21.15
b	52.47	58.76	60.51	54.92	56.66
c	0.0351^{a}	0.0233^{ab}	0.0198^{b}	0.0336^{a}	0.0279
PD	72.48	80.16	84.92	73.69	77.81
ED 2%	53.45	51.74	52.46	52.52	52.54
ED 5%	41.71	39.29	40.3	40.42	40.43
ED 8%	36.07	34.05	35.53	34.76	35.10
	Neutral detergent fiber				
a	19.99^{ab}	17.38^{b}	23.48^{a}	21.15^{ab}	20.5
b	55.00	62.18	59.39	57.71	58.57
c	0.0386^{a}	0.0270^{ab}	0.0225^{b}	0.0286^{ab}	0.0291
PD	74.99	79.56	82.87	78.85	79.06
ED 2%	55.22	51.9	53.48	54.54	53.78
ED 5%	43.13	38.35	40.94	41.78	41.05
ED 8%	37.26^{a}	32.47^{b}	35.83^{ab}	36.11^{ab}	35.41

Mean values with different letters in the same row are different (P<0.05)
a= soluble fraction; b= insoluble potential degradable fraction; c = rate of degradation; PD = potential degradability; ED = effective degradability for 2, 5 and 8% of outflow rate.

Table 2 – Kinetics degradability of dry matter and crude protein of ground corn grain in buffaloes fed diets containing increasing levels of ground corn grain

	Corn level in the diet (%)				
	0	22	37	49	Mean
			Dry matter		
a	23.16[a]	15.92[b]	22.83[a]	20.66[a]	20.64
b	62.99	58.75	56.55	69.07	61.84
c	0.0683[b]	0.1037[a]	0.0675[b]	0.0774[b]	0.0792
PD	86.15[a]	74.66[b]	79.38[ab]	85.73[a]	81.48
ED 2%	68.62	63.92	65	66.89	66.10
ED 5%	55.82	53.79	53.56	53.91	54.27
ED 8%	48.83	47.28	47.08	47	47.54
			Crude protein		
a	25.62[a]	19.29[b]	21.56[ab]	22.38[ab]	22.21
b	54.01[b]	74.45[a]	48.25[b]	73.48[a]	62.54
c	0.0561[c]	0.138[a]	0.1029[b]	0.0518[c]	0.0872
PD	79.63b[c]	93.74[ab]	69.81[c]	95.88[a]	84.76
ED 2%	61.98[bc]	67.35[ab]	59.32[c]	70.27[a]	64.73
ED 5%	50.9[b]	52.67[ab]	50.52[b]	54.39[a]	52.12
ED 8%	45.22	45.56	45.23	46.59	45.65

Mean values with different letters in the same row are different (P<0.05)
a= soluble fraction; b= insoluble potential degradable fraction; c = rate of degradation; PD = potential degradability; ED = effective degradability for 2, 5 and 8% of outflow rate.

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Effects of different diets on milk yield and quality of lactating buffaloes: maize versus sorghum silage. Note II

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Abstract

The aim of this trial is to compare the effect of the diet based on forage more adaptable to non irrigated soil on buffalo milk production. Sixteen pluriparous lactating buffaloes were divided into two groups homogeneous for parity, lactation stage and milk yield. The diets assigned to two groups, based on maize silage (group 1) and sorghum silage (group 2), were isoproteic and isoenergetic. Five monthly records of milk yield and quality during lactation were carried out. The physical and chemical characteristics, somatic cell count and coagulation properties were analysed. The results of two diets in term of yield, composition and coagulating properties are not significantly different. However we can observe that the average daily milk yield was higher in group fed sorghum silage (8.78 vs 8.03 kg), whereas fat and protein content were higher in milk of maize silage group (8.74 vs 8.47% and 4.98 vs 4.78%). The results indicated that the sorghum silage diet utilized did not affected the milk yield and quality and could be adopted in lactating buffaloes.

Key words: Buffalo milk, Nutrition, Sorghum silage, Maize silage

INTRODUCTION

The diet of dairy buffaloes was usually based on maize silage but the hot climate recorded during the last years causes increase of irrigation cost. The use of forage more adaptable to non irrigated soil is possible and sorghum is one of these, but poor data are available about buffalo feeding. The trial reported in this paper was included in a research project finalized to reduce the water consumption in forage production and it is the second part of the study direct to test a diet based on sorghum silage in lactating buffaloes. The positive results of previous trial1, where another variety of sorghum was utilized, suggested to verify the effect of different varieties of this forage. In fact the aim of this second trial is to compare the effect of a diet based on silage of a common varieties of sorghum on the yield and quality of buffalo milk.

MATERIALS AND METHODS

The trial was carried out on 16 multiparous buffaloes in the experimental farm of the CRA located near Roma. The animals were divided into two groups homogeneous for parity, lactation stage and milk yield. Two diets with same energy and protein level were administered (0.90 Milk FU/Kg DM and 155 g/Kg DM of crude protein) containing the following composition in percentage as fed: group 1 = maize silage 71.2, alfalfa hay 9.3, concentrate 19 and group 2 = sorghum silage 60.9, alfalfa hay 10.1, concentrate 28.5. The sorghum varieties used in this trial were Nicol-Pioneer + Trudan 8-NK containing a higher dry matter content (31,52%) respect to the variety BMR333-SIS (21,30%), utilized in the previous

trail. The analyses of foodstuffs were carried out according to AOAC methods2 and Milk FU were calculated according to INRA equations3. The first milk recording at an average of 70±16 days in milking (DIM) in group 1 and 63±14 in group 2 was carried out when maize silage diet was given to all animals and milk yield resulted 12.14 kg in both groups. Then the buffaloes were divided and five monthly recordings were performed. The milk yield from evening and morning milking were recorded and individual milk samples from morning milking were carried out. The milk analyses were determined according to ASPA methods4. The lactodynamographic parameters taken into account were the following: clotting time (r) curd firming time (k20), curd firmness at 30 min (A30) and at 2r (A2r). Statistical analysis was performed using a factorial model by GLM procedure5. Only data having SCC lower than 400.000 cells/ml were analysed. This threshold was chosen taking into account that when SCC are higher than 400.000 cells/ml significant changes in milk components were observed6.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition and the net energy of the two diets used. The data of milk production observed in two groups are shown in table 2. The results of two diets in term of yield, composition and coagulating properties are not significantly different. However we can observe that the average daily milk yield was higher in group fed sorghum silage (8.78 kg vs 8.03) whereas fat and protein content were higher in milk of other group (8.74 vs 8.47% and 4.98 vs 4.78%). The urea value of both groups (42.94 vs 42.69 mg/dl) was quite similar to the values found in previous paper7,1 and lower than those observed by Sarubbi et al.8, where high energy (UFL 0,94) and protein (17 and 19%) diets were administered. SCC of samples subjected to statistical analysis (197.000 vs 172.000/ml) were similar and lower than 200.000 cells/ml, threshold that could be used to early identify subclinical mastitis6. The clotting parameters of both groups are comparable to the results of the other Authors7,1. Finally, in accordance with the results of the previous trial1, the diet based on sorghum silage could be adopted in lactating buffaloes without affect milk yield and quality.

Table 1: Chemical composition and net energy of diets utilized.

	Group1 (maize silage)	Group 2 (sorghum silage)
DM(%)	15.9	16.1
Milk FU/kg	0.9	0.9
CP(%)	15.8	15.5
CF(%)	21.1	21.4
NDF(%)	35.4	36.1
EE(%)	4.3	3.8
Ash(%)	5.8	7.3
Starch(%)	23.5	21.3
Ca(%)	0.6	0.6
P(%)	0.4	0.4

Table 2: Yield, physical-chemical characteristics, somatic cell content and clotting properties of buffalo milk.

	Group 1 (maize silage)	Group 2 (sorghum silage)
Daily yield (kg/head/d)	8.03	8.78
Fat (%)	8.74	8.47
Protein (%)	4.98	4.78
Lactose (%)	4.86	4.78
Urea (mg/dl)	42.94	42.69
pH	6.77	6.77
r (min)	22.66	22.78
K20 (min)	2.01	2.15
A30 (mm)	42.46	37.68
A2r (mm)	54.09	54.11
SCC (*10^3/ml)	197	172

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Effects of the enzyme preparation Xybeten-cel on some fermentation processes in the rumen of buffalo calves

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Abstract

We have conducted an experiment aiming to identify the effects of the polyenzyme preparation Xybeten-cel on some fermentation processes in the rumen of buffalo calves.

In the experiment we included five male buffalo calves of the "Bulgarian Murrah" breed, aged fifteen months, with the average liveweight of 320 kg. All experimental animals were fistulated on the dorsal sac of the rumen (according Bassov).

The experiment was carried out by the method of periods – a control and an experimental one. During both periods the buffalo calves were fed individually with portions consisting of 7 kg alfalfa hay and 2,1 kg concentrate mixture. During the experimental period to the concentrate mixture we added the polyenzyme preparation Xybeten-cel as a dose of 3 g per kg mixture.

The results show with proof that the supplement of Xybeten-cel increase the quantity of the ammonia in the rumen before feeding ($p < 0.1$) and 2,5 hours after feeding ($p<0.01$); of the propion acid (C3) 2,5 hours after feeding ($p<0.01$) and 5 hours after feeding ($p<0,001$) and the valerian acid (C5) 5 hours after feeding ($p<0.1$).

Regarding the quantity of vinegar acid (C2) the results show that it is reduced 2,5 hours after feeding ($p<0.01$) and 5 hours after feeding ($p<0.001$).

Xybeten-cel at the dose of 3 g/kg concentrate mixture increases the number of Entodinium sp. infusoria 2,5 hours after feeding ($p<0.001$) and Isotricha sp. infusoria 5 hours after feeding ($p<0.01$).

Key words: exogenous enzyme, rumen infusoria, volatile fatty acids

INTRODUCTION

In recent years, special attention is paid to the possibility of influencing the rumen fermentation processes using biotechnology products and enzyme preparations. Most enzyme products for ruminants contain enzymes that degrade plant cell walls. The mechanism of their action in rations is unclear and may depend on many physiological and technological factors (Hatfield, 1993; McAllister et al., 2000).

The results of studies on the effect of exogenous enzyme preparations on products of fermentation in the rumen are too divergent. Some studies show that after application of enzyme preparations there is no effect on rumen's fermentation - pH, total and molar ratio of volatile fatty acids (Feng et al., 1996; Krause et al., 1998; Kung et al., 2002), while others show little such (Yang et al., 1999; Beauchemin et al., 1999; Beauchemin et al., 2000).

The purpose of this study was to trace the influence of the enzyme preparation Xybeten-cel on some fermentation processes in the rumen of buffalo calves.

MATERIALS AND METHODS

The survey was conducted with five male buffalo calves of the "Bulgarian Murrah" breed, aged fifteen months with an average liveweight of 320 kg. They were fistulated on the dorsal sac of the rumen (according to Bassov), for taking samples from rumen protozoa – in the morning before feeding, 2.5 hours and 5 hours after feeding. The experiment was carried out by the method of periods: a control and an experimental one. During both periods the buffalo calves were fed individually with rations consisting of 7 kg alfalfa hay and 2.1 kg concentrate mixture, which were set twice respectively at 8:00 and 14:00 o'clock.

During the experimental period to the concentrate mixture we added the enzyme preparation Xybeten-cel as a dose of 3 g per kg concentrated mixture. Xybeten-cel is produced by Trichoderma longibrachiatum Rifai No. TW-1 and has mainly cellulase activity, and secondary - glucanase and xylanase. The strainproducer has not been genetically modified.

We determined the total amount and the molar ratio of volatile fatty acids (VFA), the concentration of hydrogen ions (pH) and the quantity of ammonia, the total number of infusoria and their generic composition.

The obtained results were processed with "STATISTICA" – a software application developed by the American company StatSoft, Inc.

RESULTS AND DISCUSSION

In Proventriculus carbohydrates undergoes through complex metabolic changes and as the final products appear VFA.

Table 1 presents data for the influence of the enzyme preparation Xybeten-cel to the overall molar ratio of VFA in the rumen of experimental buffalo calves. The data shows that the addition of 3 g Xybeten-cel per kg of concentrated mixture compared with the control period, decreased (insignificantly) the total amount before feeding and 2.5 hours after feeding, and on the fifth hour after feeding occurred a small increase. According to some authors (Lewis et al., 1996; Colombatto et al., 2003), the addition of enzyme with prevailing cellulolytic activity to the ration leads to a reduction of the total VFA or does not have a significant effect on their level in the rumen. In both periods (control and experimental), we found that the amount of VFA was lowest before feeding and significantly increases in the hours after feeding ($p < 0,001$).

The data for the molar ratio of VFA shows that the inclusion of Xybeten-cel in the ration resulted in a significant ($p < 0,01$) and highly significant ($p < 0,001$) decrease in the amount of acetic acid. Conversely, the amount of propionic acid increases. Most authors state (Michalet-Doreau, 2002; Pinos-Rodriges, 2002) that changes in the quantity of acetic acid greatly depends on the forages used in the ration and less on the imported enzymes. There are no significant changes in the molar percentage of butyric acid, isobutyric acid, valeric acid and isovaleric acid.

For the proper occurring of the fermentation processes in the rumen, an important role plays the concentration of hydrogen ions (pH) in rumen protozoa, which are directly dependent on the level of VFA.

The data for pH in the buffalo calves rumen protozoa (Table 2) shows that the inclusion of the enzyme preparation Xybeten-cel the pH values changed insignificantly compared to those of the control period. They remained insignificantly higher before feeding and 2.5 hours after feeding and decreased on the fifth hour after feeding ($p < 0,05$). This corresponds with minor changes in the values of VFA in those hours determined in this study. Regarding pH values at different feeding times should be noted that in both periods it significantly reduces ($p < 0,001$) in the hours after feeding.

Table 2 also presents data for the level of ammonia concentration the rumen protozoa of the experimental animals. In both periods the highest ammonia concentration remained 2.5 hours after feeding ($p < 0,05$; $p < 0,001$). The inclusion of Xybeten-cel significantly reduces ammonia concentration before feeding ($p < 0,05$) and 2.5 hours after feeding ($p < 0,01$) compared with the control period. This may depend on the number of infusoria during those hours and from the food activity of the animals (the time for feeding and rumination).

Adding Xybeten-cel in the ration had no significant influence on the total number of infusoria (Table 3). Our data coincides with that obtained by Tricarico et al. (1998), who found no change in the number of rumens bacteria and infusoria with the use of cellulolytic enzymes.

As for different infusoria genuses with the inclusion of Xybeten-cel there is a significant increase of Entodinium sp. infusoria ($p < 0,05$) 2.5 hours after feeding ($p < 0,001$) and Isotricha sp. infusoria before feeding ($p < 0,05$) and 5 hours after feeding ($p < 0,01$). Diplodinium sp. infusoria decreased 2.5 hours and 5 hours after feeding.

CONCLUSION

The inclusion of the enzyme preparation Xybeten-cel at a dose of 3 g per kg concentrated mixture under the terms of our experiment has not changed the total amount of VFA and pH values in rumen protozoa.

The tested enzyme decreased the molar percentage of acetic acid ($p < 0,01$; $p < 0,001$), but increased that of propionic acid ($p < 0,01$; $p < 0,001$).

Xybeten-cel reduces the ammonia concentration before feeding ($p < 0,05$) and increases it 2.5 hours after feeding ($p < 0,01$). Xybeten-cel does not affect the total number of infusoria. Generally, it reduced the number of infusoria of the genus Diplodinium 2.5 hours and 5 hours after feeding ($p < 0,01$), but increased the number of Entodinium sp. infusoria ($p < 0,05$) 2,5 hours after feeding ($p < 0,001$) and Isotricha sp. infusoria before feeding ($p < 0,05$) and 5 hours after feeding ($p < 0,01$).

Table 1: Total quantity and molar proportion of VFA in the rumen protozoa.

Period	Time of study					
	Before feeding		2.5 h after feeding		5 h after feeding	
	n	$\overline{x} \pm S_x$	n	$\overline{x} \pm S_x$	n	$\overline{x} \pm S_x$
Total quantity of VFA (mmol/l)						
Control	15	90.08 ± 19.81	15	$127.67^{aaa} \pm 9.51$	15	$119.50^{aaa} \pm 13.35$
Xybeten-cel	15	82.75 ± 20.65	15	$116.50^{aaa} \pm 21.44$	15	$130.75^{aaa} \pm 21.20$
Acetic acid (C_2) - %						
Control	15	75.51 ± 1.40	15	$73.54^{aa} \pm 1.28$	15	74.87 ± 1.21
Xybeten-cel	14	$74.09^{**} \pm 0.61$	15	$71.82^{**aa} \pm 1.18$	14	$72.85^{***aaa} \pm 0.58$
Propionic acid (C_3) - %						
Control	15	11.78 ± 0.62	15	$14.72^{aaa} \pm 0.69$	15	$13.78^{aaa} \pm 0.49$
Xybeten-cel	14	$12.61^{**} \pm 0.53$	15	$15.69^{**aaa} \pm 1.03$	14	$15.00^{***aaa} \pm 0.62$
Butyric acid (C_4) - %						
Control	15	9.42 ± 1.28	15	$8.25^{a} \pm 1.22$	15	8.84 ± 0.99
Xybeten-cel	14	9.95 ± 0.79	15	$8.85^{aa} \pm 1.17$	14	$9.34^{a} \pm 0.57$
Isobutyric acid (C_{4i}) - %						
Control	15	1.05 ± 0.14	15	$0.93^{a} \pm 0.08$	15	$0.72^{aaa} \pm 0.07$
Xybeten-cel	14	1.10 ± 0.15	15	$0.97^{a} \pm 0.09$	14	$0.78^{aaa} \pm 0.09$
Valeric acid (C_5) - %						
Control	15	0.59 ± 0.10	15	$1.41^{aaa} \pm 0.18$	15	$1.06^{aaa} \pm 0.21$
Xybeten-cel	14	0.63 ± 0.10	15	$1.40^{aaa} \pm 0.26$	14	$1.24^{*aaa} \pm 0.20$
Isovaleric acid (C_{5i}) - %						
Control	15	1.65 ± 0.21	15	$1.14^{aaa} \pm 0.12$	15	$0.73^{aaa} \pm 0.11$
Xybeten-cel	14	1.62 ± 0.30	15	$1.25^{aa} \pm 0.22$	14	$0.79^{aaa} \pm 0.13$

Table 2: Hydrogen ions (pH) and ammonia concentrations in the rumen protozoa.

Period	n	Time of study		
		Before feeding	2.5 h after feeding	5 h after feeding
		$\frac{\text{———}}{\text{———}}$ x ± S x	$\frac{\text{———}}{\text{———}}$ x ± S x	$\frac{\text{———}}{\text{———}}$ x ± S x
pH concentration				
Control	15	7.23 ± 0.13	6.72^{aaa} ± 0.19	6.65^{aaa} ± 0.15
Xybeten-cel	15	7.29 ± 0.17	6.76^{aaa} ± 0.19	6.45^{aaa*} ± 0.30
Ammonia concentration				
Control	15	19.17 ± 3.84	23.00^{a} ± 3.12	18.38 ± 4.3
Xybeten-cel	15	15.92^{*} ± 3.67	27.73^{**aaa} ± 3.01	17.35 ± 4.21

Table 3: Total number and generic composition of infuzoria in rumen protozoa.

Period	Time of study					
	Before feeding		2.5 h after feeding		5 h after feeding	
	n	$\frac{\text{———}}{\text{———}}$ x ± S x	n	$\frac{\text{———}}{\text{———}}$ x ± S x	n	$\frac{\text{———}}{\text{———}}$ x ± S x
Total number of infuzoria in rumen protozoa ($10^3/cm^3$)						
Control	15	138.800 ± 23.15	15	95.733^{aa} ± 35.22	15	114.000^{a} ± 30.61
Xybeten-cel	15	135.533 ± 29.32	15	106.400^{a} ± 28.81	15	120.400^{a} ± 38.73
Entodinium sp. infusoria (%)						
Control	15	78.67 ± 5.07	15	83.23^{aa} ± 3.02	15	84.20^{aa} ± 3.05
Xybeten-cel	15	81.40 ± 6.41	15	85.70^{*a} ± 2.17	15	86.33^{a} ± 3.58
Diplodinium sp. infusoria (%)						
Control	15	10.63 ± 5.09	15	9.10 ± 3.95	15	8.07 ± 2.10
Xybeten-cel	15	7.20 ± 4.45	15	5.40^{**} ± 2.60	15	4.93^{**} ± 2.40
Epidinium sp. infusoria (%)						
Control	15	6.13 ± 2.01	15	3.87^{a} ± 2.41	15	4.63 ± 2.29
Xybeten-cel	15	6.83 ± 3.81	15	4.70 ± 1.40	15	4.33^{a} ± 1.32
Isotricha sp. infusoria (%)						
Control	15	2.07 ± 1.59	15	2.90 ± 2.38	15	1.80 ± 1.69
Xybeten-cel	15	3.60^{*} ± 2.19	15	3.87 ± 2.03	15	4.07^{**} ± 1.43
Dasytricha sp. infusoria (%)						
Control	15	2.50 ± 3.43	15	0.90 ± 1.44	15	1.30 ± 1.92
Xybeten-cel	15	0.93 ± 1.31	15	0.33 ± 0.45	15	0.33 ± 0.52

* - statistical significance between experimental and control period

a - statistical significance between before and after feeding

*; a – p<0,05 **; aa – p<0,01 ***; aaa – p<0,001

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Evaluation of diets with increasing corn grain levels on rumen protozoa population and liquid outflow rate in buffalo

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Abstract

In ruminant animals, protozoa population represents around 50% of rumen microbial biomass depending primarily on the diet consumed. The ruminal energy availability favors the protozoa growth while the higher outflow rate can be detrimental to the ciliate fauna. However, the real role of protozoa and their diversity in the rumen are still unclear especially considering the differences existing among the ruminant species. Our objective was to evaluate the effects of corn feeding up to 45% of total diet on the concentration (number) and composition (proportion) of different genus of rumen ciliate protozoa as well as the liquid outflow rate in buffalo. Four rumen fistulated buffaloes, average 328 kg and 14 months old, were fed with diets of different levels of ground grain corn (0, 22, 37 and 49% in the DM) and coast-cross grass hay as roughage in a Latin Square (4x4) design experiment during 112 days. The total number of protozoa increased from 27.1 (without corn) to 66.3 x 104 /mL of rumen content (49% corn diet). The different genus groups were changed according to corn fed level with the diet of 37% corn showing lower proportion of Entodinium and Dasytricha with higher Diplodiniinae. Also, the 37% concentrate diet promoted higher liquid outflow rate (9.86 %/h) and lower rumen volume (36.7 kg).These results indicate that the corn feeding can increase the rumen protozoa concentration with 22% of corn in the same way those 37% and 49% levels of corn in the diet and only a diet with 37% of corn is be able to change the general composition of the rumen protozoa ciliate in buffaloes.

Keywords: buffalo, corn, energy, protozoa, rumen

INTRODUCTION

Rumen ciliated protozoa represent approximately 50% of microbial biomass in the rumen, however, the true role of the protozoa population is still not clear, since wide differences occur among ruminant species, feeding systems and environmental conditions around the world. Buffalos have presented a different composition of the protozoa population in comparison with other ruminant species on the same feeding condition3. Concentrate diet has a great influence on rumen fermentation metabolism and is an important factor in the establishment and maintenance of species of protozoa in the rumen1.

The ruminal energy availability favors the protozoa growth in some feeding conditions while the higher outflow rate can be detrimental to the ciliate fauna. Franzolin et al. (2010)3 observed no effect of energy (corn or citrus pulp) or nitrogen (urea or soybean meal) sources in buffalo and cattle on rumen protozoa counting. The aim of this study was to evaluate the effects of different levels of ground corn grain diets for buffalo on rumen protozoa population.

MATERIALS AND METHODS

Four male buffaloes with rumen fistulae with average of 14 months old and 328 kg body weight were used to evaluate diets based on hay of coast cross (Cynodon dactylon (L.) Pers), formulated with four levels of corn grain (0, 22, 37 and 49% DM) on rumen protozoa population. The experiment was designed in Latin square (4 x 4) in four periods of 28 days each. Total counts and generic distribution of rumen protozoa population were made in optical microscopic at a magnification of 100x2. Ciliate protozoa belonging to the subfamily Diplodiniinae (e.g. Diplodinium, Eudiplodinium, Ostracodinium, Metadinium, Enoploplastron and Polyplastron) were counted together.

Rumen liquid outflow rate was measured using o polyethyleneglycol 4000 as marker add 100 g at the last day of each period with sampling at 0 (before PEG addition), 1, 2,4,8 and 24 hours after feeding. The data obtained from number/ mL of ruminal content and composition (% of total) were analyzed by Visual GLM module with main effects ANOVA of Statistica program using Fisher LSD test as post-hoc comparisons of observed means with significant differences less than 5%.

RESULTS AND DISCUSSION

The counting values of rumen protozoa concentration (number/ mL rumen content) and the fauna composition (% of total) of buffalo receiving the four treatments can be seen in the Table 1 and liquid outflow rate as well. The total number of protozoa increased from 27.1 (without corn) to 66.3 x 104 /mL of rumen content (49% corn diet), but there were no differences among the three levels of corn diets. The starch present in corn favored the availability of energy used for the growth of species of protozoa, except for the holotrichs (Isotrich and Dasytrich).

The different genus groups were changed according to corn fed level with the diet of 37% corn showing lower proportion of Entodinium and Dasytricha with higher Diplodiniinae. The 37% concentrate diet promoted higher liquid outflow rate (9.86 %/h) and lower rumen volume (36.7 kg). These findings indicate that the corn feeding can increase the rumen protozoa concentration with 22% of corn in the same way those 37% and 49% levels of corn in the diet and only a diet with 37% of corn is be able to change the general composition of the rumen protozoa ciliate in buffaloes, promoting higher percentage of Diplodiniinae in the rumen fauna, i.e. Entodinium:Diplodiniinae less than one. The population of holotrichs is reduced in the total fauna by addition of corn ground grain as replacement of coast-cross grass hay diet.

Table 1- Concentration and composition of rumen protozoa ciliate in buffaloes fed increasing levels of ground corn grain diets

	Corn level in diet (%)			
	0	22	37	49
Concentration (number x 10^4 /mL rumen content)				
Entodinium	11.67^{b}	28.18^{a}	24.64^{a}	30.16^{a}
Diplodiniinae	9.50^{b}	23.91^{a}	27.22^{a}	24.40^{a}
Epidinium	0.63^{b}	2.64^{a}	3.32^{a}	4.35^{a}
Isotricha	1.11^{ab}	0.70^{b}	1.54^{a}	0.65^{b}
Dasytricha	4.15^{b}	5.03^{ab}	3.32^{b}	6.71^{a}
Total	27.05^{b}	60.46^{a}	60.04^{a}	66.27^{a}
Composition (% of total)				
Entodinium	41.74^{ab}	46.03^{a}	37.42^{b}	46.31^{a}
Diplodiniinae	37.39^{b}	38.95^{b}	48.85^{a}	36.31^{b}
Epidinium	2.39^{c}	4.33^{bc}	5.93^{ab}	6.82^{a}
Isotricha	5.02^{a}	1.16^{c}	3.02^{b}	1.26^{c}
Dasytricha	13.45^{a}	9.47^{b}	4.77^{c}	9.30^{b}
Entodinium:Diplodiniinae	1.17^{a}	1.23^{a}	0.87^{b}	1.33^{a}
Liquid outflow rate (% / h)	8.04^{b}	8.35^{a}	9.86^{a}	8.12^{b}

Mean values with different letters in the same row are different (P<0.05)

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In Sacco protein degradability of feed ingredients in Izakhali buffao

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Abstract

Two Izakhali buffalo steer of almost similar age and body weight (400 kg), fitted with permanent rumen cannula were used to determine the in sacco protein degradability of common feed ingredients. The feed ingredients were cottonseed cake; wheat bran, untreated mustard seed cake (UMSC) and formaldehyde treated mustard seed cake (FMSC). Results revealed that in sacco protein degradability at 12 hrs was maximum (88.75%, $P<0.05$) in wheat bran and lowest in FMSC (8.96, $P<0.05$). Protein degradability of UMSC and cottonseed cake was intermediate and recorded as 81.47 and 83.99 %, respectively. Significant differences ($P<0.001$) in sacco protein degradability of UMSC and TMSC at 24 hrs incubation was also observed and the results were 85.28 and 22.63 %, respectively. It was concluded that among these common ingredients wheat bran has highest ruminal protein degradability which could help in microbial protein synthesis while formaldehyde treatment proved beneficial in escaping protein from ruminal degradability.

Key words: In sacco protein degradability, cottonseed cake, wheat bran, mustard seedcake, treated mustard seed cake, Izakhali buffalo

INTRODUCTION

The major constraint which depresses productivity in ruminants is inadequate feeding. Among nutrition, protein plays a unique role in exploiting the production potential of ruminant animals. However, feeding a diet containing more protein is not a satisfactory solution because the breakdown of dietary protein in the rumen by microflora plays crucial role in ruminant nutrition. Hence, supplementation strategy for ruminants feeding required to be based on rumen degradable and undegradable (by pass) part of protein. Various physical and chemical treatments are used to increase the by pass value of the feedstuff. Formaldehyde treatment is most effect effective in this respect .1, 2 By-pass quality of feed for ruminants available in Pakistan is not known, and still ruminant feeds are evaluated on the basis of digestible crude protein. Keeping in view these facts, the present study was planned to determine the ruminal degradable and undegradable part of conventionally used feed ingredients.

MATERIALS AND METHODS

Two Izakhali fistlated buffalo steers of almost similar age and body weight (400 kg), were used to measure the in situ protein degradability3. The animals were fed maize fodder at 90 % of their voluntary intake through out the study. Samples of three commonly used feed ingredients i.e., cottonseed cake (CSC), mustard seed cake (MSC), wheat bran (WB) of ruminant ration were collected, while fourth samples was prepared by treating mustardseed cake (TMSC) with formaldehyde at the rate of 3g/ 100 gram crude protein.4 For this purpose a 32 % solution was prepared by dissolving 47 ml formalin (40 % formaldehyde solution) in 100 mil water. 25 ml of the formalin solution was used for treating 1 kg mustard seed cake. Dacron bags (pore size 50 µm) containing 5 gram samples in triplicate of CSC, MSC, WB and TMSC were incubated for 12 hours. Samples of formaldehyde treated and untreated mustard seed cake were also incubated for 24 hours. Dry matter, crude protein and degraded protein in feedstuff was measured according to the standard methods of

chemical analysis.5 Data were analyzed with the standard procedure of analysis of variance.6 Means were compared for significance of difference with the Duncan's Multiple Range Test.7

RESULTS AND DISCUSSION

Results of in sacco protein degradability of supplements at 12 hour incubation are presented in table-1. Based on in sacco protein degradability, the four supplements.

Table 1. Dry matter, crude protein and in sacco protein degradability of different feed ingredients could be ranked as WB>UMSC>CSC>TMSC, suggesting that rumen degradability of protein in WB was maximum (P<0.05) and that of TMSC remained the lowest (P<0.05).

Feed Ingredients	Dry matter %	Crude protein %	***ISPD % (12 hr)	**Degradation rate (% / hr)
UMSC	90.58	29.87	83.99^{b}	15.15^{b}
TMSC	83.49	30.75	8.96^{d}	5.32^{d}
CSC	87.92	26.44	81.43^{c}	10.53^{c}
WB	86.60	15.50	88.75^{a}	25.65^{a}

Means with different superscripts within the column are significantly (P< 0.05) different.
**Habibi (1995)
***ISPD: In sacco protein degradability

The in sacco protein degradability results at 12 hour incubation compared well with the rate of protein degradation (%/ hr) reported in earlier study4 for the same feed. Formaldehyde treatment was effective in reducing protein degradability in TMSC at 12 hour incubation. Significant differences (P<0.001) in sacco protein degradability of UMSC and TMSC at 24 hrs incubation was also observed and the results were 85.28 and 22.63 %, respectively. Similar response to formaldehyde treatment of protein supplements has been reported in the literature 8, 9 which explained that formaldehyde treatment decreased the degradability of protein due to formation of irreversible cross linkages between amino acids. It was concluded that among these common ingredients wheat bran has highest ruminal protein degradability which could help in microbial protein synthesis while formaldehyde treatment proved beneficial in escaping protein from ruminal degradability.

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In vitro degradability of aflatoxin in rumen liquid of buffalo*

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Abstract:

Aim of this study was to verify if buffalo ruminal microflora was capable to metabolize aflatoxin B1 and B2 in other metabolites. Mixed rumen fluid from two rumen-fistulated buffalos was used. Samples of AFB1 and AFB2 contaminated maize grain were incubated in vitro for 1, 4, 8 and 24 hours and analyzed for aflatoxin AFB1, AFB2, AFM1, AFM2 and aflatoxicol (AT) by HPLC. The aflatoxins B1, B2, M1, M2 and aflatoxicol (AT) were found both in the degraded maize and in the rumen fluid. The mean AFM1 concentration in degraded maize at 8 h was 25.7 ppb, and progressively increased till about 30 ppb at 24 h. Otherwise the AFM2 was detected only in the 2 samples at 24 h. The AT contamination in degraded maize samples was similar in each incubation time (4.2-4.4 ppb). The results showed that rumen microbes were capable to convert AFB1 and AFB2 in other toxin metabolites, besides a different efficiency in degradation between AFB1 and AFB2.

Keywords: aflatoxin, buffalo, in vitro degradability, rumen fluid, maize

INTRODUCTION

Liver is considered the main organ involved in the aflatoxin biotransformation and detoxification1. Cytocrome P450 oxidative enzymatic system was considered the principal pathway of bio-activation of AFB1 in other different metabolites, among them, AFM1 and aflatoxicol 2-3. Likewise, AFB2 is bio-converted in AFM2. These hydroxylated metabolites are found in milk and urine after aflatoxins ingestion. Some authors4;5 reported that aflatoxins are generally poorly bio-converted in the rumen because many rumen bacteria are completely inhibited by concentration of AFB1 below 10 ?g/ml 6. In contrast, other authors7 observed a capability of cow rumen liquid to degrade in vitro more than 42% of AFB1 contained in samples. During in vivo trials, AFM1 and Aflatoxicol were found after AFB1 oral administration to animal8. However, an in vitro study9 showed only aflatoxicol production in rumen fluid. Aim of this study was to evaluate the capability of rumen fluid to bio-transform AFB1 and AFB2 in different metabolites, by in vitro degradability of contaminated maize samples.

MATERIAL AND METHODS

The rumen liquid from 2 rumen-fistulated buffaloes in dry period was mixed and the in vitro degradability trial was carried out10 in quadruplicate. Samples of 20 g of 1 mm-milled maize, contaminated with AFB1 and AFB2 (Table 1), were

* Research carried out within the project AFLARID “Research for the reduction of aflatoxin contamination in milk and by-products” supported by the Italian Ministry of Agriculture, MD n° 290/7303/2004, Coordinator prof. Gianfranco Piva.

incubated for 1, 4, 8 and 24 h in thermal bath at 38-40°C. After incubation, the liquid and residual degraded maize were analysed for aflatoxins. The rumen liquid was filtered and prepared for the analysis following the method used for milk (modified UNI-EN-ISO 14501/1998). The degraded maize was prepared following method for feed analyses11. Aflatoxins were determined by immunoaffinity column (R-Biopharm Rhone) and HPLC (Beckman Gold), using a Microsorb 100 C18 VARIAN column and a fluorescent detector Pro Star Varian, after bromide derivation (Kobracell). The effect of incubation time was statistically processed by GLM ANOVA procedure and Duncan's multiple range test12.

RESULTS AND DISCUSSION

The aflatoxins B1, B2, M1, M2 and aflatoxicol (AT) were found both in degraded maize and rumen fluid (Table 1 and 2). The mean AFB1 disappearance (Dis. B1%) during 24 h of incubation was 91. 26%, but AFB1 disappeared above all at 1 h. Similar trend was observed for AFB2 (Table1).

Time (h)	0	1	4	8	24	Effect
AFB1	1015±21.5 ^{a}	105.5±11.9 ^{b}	112.7±24.5 ^{b}	115.9±15.8 ^{b}	109.3±68.1 ^{b}	***
AFB2	91.3±10.3 ^{a}	9.0±1.0 ^{c}	10.6±2.5^{bc}	10.5±1.5^{bc}	12.4±2.6 ^{b}	***
AFM1	0 ^{d}	22.6±0.6 ^{c}	24.1±1.5^{bc}	25.8±1.4^{b}	29.2±1.3 ^{a}	***
AFM2	0 ^{b}	0 ^{b}	0 ^{b}	0 ^{b}	10.1±12.1^{a}	**
AT	0 ^{c}	4.2±0.1 ^{b}	4.2±0.1 ^{b}	4.4±0.2 ^{a}	0 ^{c}	***
Dis. B1%		90.7±0.7	90.7±2.1	91.1±1.1	92.6±0.4	Ns
Dis. B2%		91.1±0.9	90.2±2.5	90.0±1.2	90.7±1.8	Ns
M1/B1 %		21.6±1.9^{b}	22.1±4.3^{b}	22.4±2.0 ^{b}	26.7±0.6 ^{a}	*

a, b, c, d = means with same letter are not significantly different. * P?0.05; **P?0.01;***P ?0.001

The disappearance of AFB1 and AFB2 was due to two different factors: the simple solubilisation of both aflatoxins into the rumen fluid, and the bio-conversion in the other metabolites by rumen microbes. In fact, in the degraded maize samples were found an average of 25.42 ppb of AFM1 and 3.21 of AT; AFM2 was found only in 24 h samples. These results showed the capability of rumen microbe to bio-converted AFB1 and AFB2, as observed in a previous in vitro study7. This capability was evaluated by AFM1/AFB1 ratio. The data (Table 1) showed that this capability increased during incubation from time 4 h to 24 h, reflecting AFM1 content in the degraded maize samples. In rumen fluid the same aflatoxins of the maize samples have been found, but in a lower concentration (Table 2). The maximum contamination was observed at 8 h for AFB1, at 24 h for AFB2 and at 4 h for AFM1, AFM2 and AT. Also the aflatoxin ratio M1/B1 and M2/B2 reached the maximum value at 4 h.

Table 2. Aflatoxin content in rumen liquid after incubation (ppb, mean ± sd)

Time (h)	1	4	8	24	Effect
AFB1	3.66±1.1	3.78±1.6	3.86±0.9	3.67±0.6	NS
AFB2	0.43±0.2	0.30±0.1	0.46±0.1	0.57±0.2	NS
AFM1	0.52±0.1 ^{b}	0.95±0.2 ^{a}	0.62±0.1 ^{b}	0.53±0.1 ^{b}	*
AFM2	0.11±0.1	0.24±0.1	0.21±0.1	0.20±0.0	NS
AT	0.48±0.1	0.61±0.2	0.59±0.1	0.52±0.1	NS
M1/B1%	14.7±4.0 ^{b}	26.6±4.8 ^{a}	16.5±2.6 ^{b}	14.7±1.8 ^{b}	**
M2/B2%	31.1±24.5 ^{b}	73.0±14.1 ^{a}	48.2±23.3 ^{ab}	37.7±11.1 ^{b}	*

a, b, = means with same letter are not significantly different. * P?0.05; **P?0.01;***P ?0.001

These results in vitro condition showed that besides the Cytocrome P450 oxidative enzymatic system in liver, a rumen system able to bio-convert the fed aflatoxin exists in the buffalo. Further study in situ could explain better the mechanism of this bio-conversion.

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Lysozyme Activity in Buffalo and Bovine Abomasum

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Abstract

Basing on the hypothesis that the lower purine derivatives excretion detected in buffaloes respect to bovines may be due to a lower lyses of rumen bacteria, the lysozyme activity in the abomasums of the two species was studied. Six buffalo and six dairy cows fed a standard diet (NDF 43% DM; CP12% DM) for three month before slaughtering were included in the trial. Samples from three areas of abomasum mucosa (fundus, pylorus and cardias) were dissected, washed and stored at -20°C. After 24 h, the samples were homogenised, centrifuged and the supernatants were analysed by using lyophilized Micrococcns luteus and lyophilized lysozyme from egg. The transmittance of the suspensions was analysed by spectrophotometer, repeating the reading each minute for 10 minutes. Lysozyme activity unity was estimated as 1% of transmittance increase/min. No differences were detected between the species. The lysozyme activity was affected by the site of sampling: close to 600 unit/g of mucosa in the samples from fundus, 235 u/g in pylorum and only 7.0 u/g in cardias. In conclusion, the differences in purine excretion between buffalo and bovine should not be ascribed to abomasum lysozyme activity.

Key words: lysozyme, purine, abomasum.

INTRODUCTION

The urinary excretion of purine derivatives (PD: allantoin, uric acid, hypoxanthine, and xanthine) appears to be a reliable method to estimate the microbial N flow to the duodenum1. The principle is that the duodenal flow of nucleic acids and their derivatives is mainly of microbial origin, which are to a large extent digested and absorbed in the small intestine, purine bases are catabolized to their derivatves and excreted in the urine2. Therefore, microbial N flow can be estimated from the quantitative excretion of PD in urine. Prediction equations for microbial protein supply based on the relationship between duodenal input and urinary output of purine compounds were developed for cattle3,4 and sheep5,6. Despite that, a problem occurred using this method for prediction of microbial protein production in buffaloes since the excretion of PD in such species is less than 50% respect to other ruminants7,8. Different hypothesis were formulated to justify such lower excretion: either to the lower glomerular filtration rate in buffaloes respect to cattle, therefore, more time in the blood and more time for recycling to the rumen and metabolism by bacteria, or to the greater permeability from the blood to the rumen in buffaloes than in cattle9; higher xanthine oxidase activities in buffaloes than cattle10; lower capacity of lyses of bacterial wall in buffalo than in other species.

Lysozyme lyses bacterial wall by degrading the chitin11. In several ruminat species lysozyme activity is particularly high into the abomasum mucosa, even if there are significant differences among the species12. This paper aims to compare the lysozyme activity in abomasums mucosa from buffaloes and bovines.

MATERIALS AND METHODS

Six dairy buffaloes and six dairy cows were included in the trial. Animals were fed for 3 months a standard diet (NDF 43% DM; CP 12% DM), after they were slaughtered and the abomasum from each one was collected and immediately stored (-20°C). After 24 h abomasums were washed with saline solution in order to evaluate the enzymatic activity in the different areas: pylorus and fundus (a square - 10 x 10 cm - of mucosa) and cardias (10 cm around the cardias). After other 24 h, the samples were weighed, homogenised with acetic acid 2% (1g/2ml), centrifuged (27.000 g for 15 min) and the supernatants were collected and frozen. Lysozyme activity was evaluated by adding to 1.5 ml of a solution contained Micrococcus luteus (0.25 mg/ml of sodium acetate 0.022 M and sodium chloride 0.11 M solution, pH 5) to 10 µl of each samples13. A solution (0.1 mg/ml) of lysozyme from egg albumen were utilised as positive control. The activity was measured by spectrometry, repeating the read each minute for 10 minutes. The unity of lysozyme activity was estimated as 1% of transmittance increase/min13. Differences were tested by the Student t-test.

RESULTS AND DISCUSSION

1. The mean values of lysozyme activity registered in buffalo and bovine in the 3 tested areas are depicted in table

Table1. Lysozyme activity (U*/g of mucosa) in buffalo and dairy cows in 3 abomasum areas

Area	Buffalo	Cow
Fundus	606 ± 170	645 ± 199
Pylorus	239 ± 92	232 ± 45
Cardias	6.724 ± 1.324	7.412 ± 1.962

Lysozyme activity U was estimated as 1% of transmittance increase/min.

The fundus values from both species agree to that reported by other authors13, which on the contrary found lower values for the lysozyme activity from the pylorus (on average 236 U/g in present trial vs 48 U/g). This last difference is probably due to the different sampling method. Concerning the comparison between buffalo and bovine species, the activity of lysozyme was not different in all the tested areas. However, as depicted in figure 1, evaluating the only "sampling area" effect, significant differences ($P<0.01$) were registered: fundus showed the highest lysozyme activity (625 U/g) while the cardias the lowest one (7.068 U/g). This results should confirm the hypothesys that lysozyme in ruminant species play only a digestive rule14. In conclusion, our results indicate that the lysozyme activity in abomasum mucosa is similar in buffaloes and bovines and, consequently, the reason for the lower urinary PD excretion cannot be ascribed to a lower lyses of rumen bacteria in the post-rumen districts.

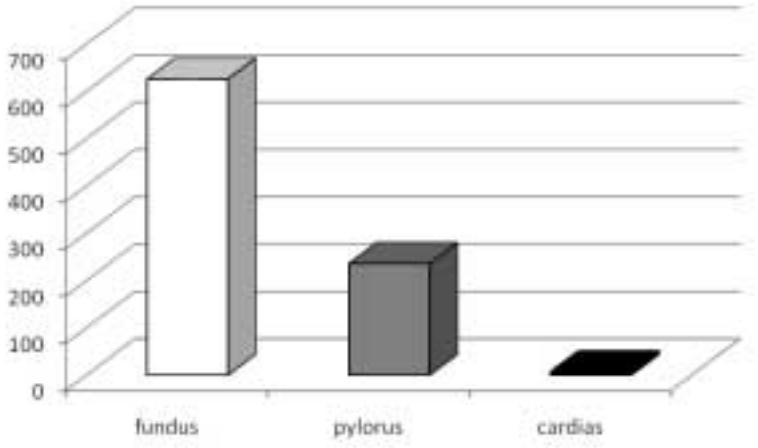

Figure 1. Lysozyme activity values in the tested areas of abomasum mucosa.

Acknowledgements.

The authors wish to thank the Barchiesi Farm, (Cassino, FR – Italy) where the trial was performed.

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Nutritional Evaluation of Several Forage in Guangxi, China

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Abstract

The in vitro gas production method (Menke and Steingass ,1988) was used to evaluate the nutritional valuation of elephant grass, cassava pulp, corn silage and corn bract and cob. Rumen fluid used for determination of gas production (GP) was collected from two rumen-fistulated sheep. The gas production on 0, 3, 6, 9, 12, 24, 36, 72, 96 h were recorded. And after incubation 96 h, the fermentation products were used to determine the content of acetate, propionate, butyrate and ammonia-N.

The GP in different time of cassava pulp was higher than the other forages. The curve of corn silage and elephant grass was similar. The potential GP of corn bract and cob was the highest, following by cassava pulp, corn silage and elephant grass. The total VFA of the elephant grass was the lowest and the three other were similar. The propionate of cassavas pulp was the highest in 4 forages and the ammonia-N of cassava pulp was the lowest among 4 forages. Based on the relative feed value (RFV) calculation, the RFV of those forage were as follows, cassava pulp (149.5)> corn silage(83.3)> elephant grass(68.3)> corn bract and cob(61.5). The arrange of digestible organic matter (DOM) and metabolizable energy (ME) in those four forages were cassavas pulp (734.7 g/kg, 10.16 MJ/kg DM), corn bract and cob (651.4 g/kg, 8.98 MJ/kg DM), corn silage (578.7 g/kg?7.96 MJ/kg DM), elephant grass (575.6 g/kg?7.92 MJ/kg DM)

Key words: animal science, feed nutritional evaluation, in vitro gas production method, metabolizable energy, digestible organic matter

INTRODUCTION

Elephant grass, cassava pulp, corn silage and corn bract and cob were common forage for ruminant feeding in Guangxi, China, and completely evaluation for those forage could give more information to water buffalo and the other ruminant feeding. In vitro gas production methods have many advantage i.e. easily operating and having good repeat in forage evaluation. On the other hand, the organism digestible rate and metabolizable energy for forage could be estimated from combining cumulative gas production after 24 h incubation and chemical composition (Menke and Salewski, 1979?Menke and Steingass ,1988). The objectives of current research were to evaluate the nutritional evaluation of common forage in Guangxi, China.

MATERIALS AND METHODS

The sources of forage

Elephant grass: the samples of fresh elephant grass was the whole plant on the above of earth, collected from breeding farm of Buffalo Research Institute in Guangxi, China. Collection date: the August of 2006.

Fermented cassava pulp: the fresh cassava pulp was collected from Starch factory in Wuming, Guangxi. The fermented cassava pulp samples were fermented for 3-4 days. Collection date: the August of 2006.

corn silage: the samples of corn silage were collected from breeding farm of Buffalo Research Institute in Guangxi, China. And those silage had been fermented for 70 days. Collection date: the August of 2006.

Corn bract and cob: the fresh corn bract and cob were collected from Heng country, Guangxi, the samples of corn bract and cob were short cut then sampled.

All samples were immediately dried in an air-forced oven at 60 oC for 48 h and stored in sealed plastic containers at room temperature until analyzed. In preparation for analyses, dried forages and concentrates were ground first through a 2 mm screen (Thomas-Wiley Laboratory Mill), then through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Dry matter (DM) was determined by drying a subsample at 100 oC for 24h.

Rumen fluid donor

Rumen fluid used for determination of GP was collected from two rumen-fistulated sheep (BW=30±5kg) fed on a diet (700 g/d Chinese wild rye plus 300 g/d concentrate mixture) at 1.3 times maintenance.

Chemical composition determination

All samples were analyzed for crude protein (Yang SHeng., 1993), crude fiber, neutral detergent fiber and acid detergent fiber (Van Soest et al., 1991).

Gas production determination

The gas was determined according to Menke and Steingass (1988). The GP value recorded at 0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation, were fitted to the equation: GP = a + b (1?ec t), where a the intercept of the gas production curve, a+b the potential gas production, c the rate of gas production. Measurement of the in vitro fermentation parameters, ammonia-N and VFAs were also performed after 96 h incubation.

Measurement of in vitro fermentation parameters

Concentrations of the ammonia-N were determined with colorimetry, and the details are as described by Feng et al. (1993). Gas chromatography was used to determine VFAs, and the details are as described by Hu et al. (2005).

Digestible organic matter calculation

The digestible organic matter (DOM) were calculated according to the gas production amount after 24 h incubation by the following equation: DOM=7.65?±0.062?×GP24h+353?±0.59?(Menke and Salewski, 1979), where, the unit of DOM is g/kg and the unit of GP24h is ml.

Relative feed value calculation

The relative feed value were calculated according to the following equation: RFV=DMI(%BW)×DDM(%DM)/1.29, where DMI is Dry Matter Intake and its unit is %BW, DDM is Digestible Dry Matter and its unit is % DM. The DMIand DDM predicted by the following equation: DMI(%BW)=120/NDF(%DM) and DDM(%DM)= 88.9-0.779*ADF(%DM).

Metabolizable energy calculation

The Metabolizable energy calculation (ME) were calculated according to the following equation: ME(MJ/kg DM)=-0.20+0.1410*DO, where DO is Digestibility of the organic matter and its unit is%(Menke and Steingass ,1988).

Statistical analysis

All data in this paper were presented as mean of three repeat.

RESULTS

The GP value reveal digestible rate of forage. The cumulative GP curves are presented in Figure 1. The GP at each time of cassava pulp was higher than the other forage. The curve of corn silage and elephant grass was similar. The potential GP of corn stalk was the highest, following by cassava pulp, corn silage and elephant grass (Table 1). The total VFA of the elephant grass was the lowest and the three other were similar. The propionate of cassavas pulp was the highest in 4 forages and the ammonia-N of cassava pulp was the lowest among 4 forages.

The VFAs have close relationship with energy metabolism, enhance the content of VFAs reveal the digestible rate of substrate. The highest potential gas production was corn bract and cob, the following were cassava pulp, corn silage and elephant grass. The lowest total VFAs was elephant grass, the other three had close value. In the term of chemical composition, based on DM, the crude protein, NDF. ADF and CF of cassava pulp were lower than that of the others three forage (Table 2). Whether in the term of ME which calculated based on the results of in vitro gas production or in the term of RFV which based on the results of chemical composition, the ME and RFV of cassava pulp were higher than that of the other three forages.

DISCUSSION

In the term of forage evaluation, the common evaluation index have Relative Feed Value (RFV), Quality Index (QI) and Relative Forage Quality (RFQ) etc.. In China, Lu De-xun proposed the GI (Grading Indexs) used for forages evaluation. The equation of GI was showed in the following: GI(MJ/kg DM)= ME(MJ/kg DM)×DMI(Kg/d)×CP (%DM)/NDF(%DM) (Wang et al., 2005), the advantage of this equation is that it involving the metabolizable energy and crude protein parameters, but it need the determination Dry matter intake value to calculate the GI. Owing to this research lack the determination dry matter intake for water buffalo, the RFV was used to evaluation the forages in our research.

Acknowledgements.
This work was supported in part by Guangxi Science Foundation, PR China (Grant no. 0832071; 0832072; 0718005-4A).

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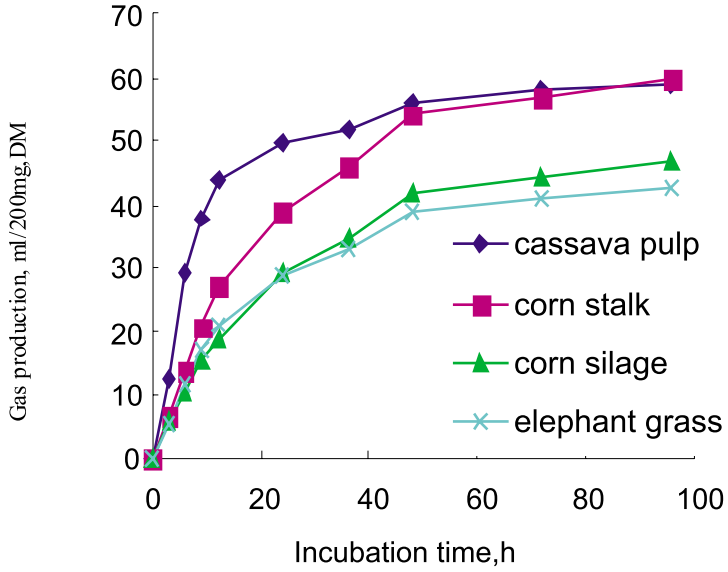

Figure 1. Gas production curve for cassava pulp, corn bract and cob, corn silage and elephant grass, respectively

Table 1.Gas production and fermentation parameters in elephant grass, cassava pulp, corn silage and corn bract and cob.

	Elephant grass	Cassava pulp	Corn silage	Corn bract and cob
Gas production parameters				
GP_{24h} (ml)	29.1	49.9	29.5	39.0
a (ml)	0.6	- 1.1	0.6	0.1
b (ml)	41.6	57.29	47.0	59.6
a +b (ml)	42.1	56.2	47.6	59.7
c (ml/h)	0.05	0.11	0.04	0.05
DOM (g/kg)	575.6	734.7	578.7	651.4
ME (MJ/kg DM)	7.92	10.16	7.96	8.98
Fermentation parameters afer 96 h incubation				
Total VFAs (mmol)	61.4	69.50	70.1	69.2
Acetate (%)	72.9	68.4	71.7	72.0
Propionate (%)	18.0	23.2	19.2	18.3
Butyrate (%)	9.1	8.4	9.1	9.7
A/P	4.1	3.0	3.8	3.9
Ammonia-N (mg/100 ml)	6.9	5.4	7.6	7.9

Notes: a is the very rapidly disappearing fraction;; b is that portion of the feed which will be degraded in time, a+b is the potential gas production, c is the rate of gas production , VFA = volatile fatty acid, A/P = ratio of acetate to propionate.

Table 2. The chemical composition of experimental forage.

Composition (%)	Elephant grass	Cassava pulp	Corn silage	Corn bract and cob
Dry matter	19.1	7.8	18.8	20.0
Crud protein	5.5	2.95	9.41	8.04
NDF	71.40	42.84	64.84	73.10
ADF	46.78	25.74	39.61	35.16
CF	35.20	15.92	27.70	23.51
RFV	68.3	149.5	83.3	78.2

Notes: the dry matter content were based on the fresh sample and other chemical composition were based on the dry matter content.

Nutritive Value of Silages Utilized in Buffalo Nutrition

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Abstract

The trial was carried out on corn, oats, sorghum, triticale and barley silages in order to estimate their nutritive values (net energy for lactation; NEl) using several equations including parameters of chemical composition and of in vitro gas production technique (IVGPT). The equation: NEl (MJ/kg DM) = 0.54 + 0.0959 GP + 0.0038 CP + 0.0001733 CP2 (where GP is the gas produced after 24 h of incubation and CP the crude protein content of substrate) was the most adequate for all the silages, even if this methods always underestimated the NEl compared to the I.N.R.A. method.

Key words: nutritive value, IVGPT, silages.

INTRODUCTION

Corn silage, the main forage in the diets of buffalo cows in South of Italy, shows some weak aspects (i.e. high contamination with aflatoxins, corn parasites and high cost of irrigation). Thus, there is an interest for studying the total or partial replacing of corn silage with other silages1. However, the quality of silages, particularly their nutritive values, is strongly affected by several factors like water availability and stage of maturity. In vivo evaluation is the best estimation method of feed's nutritional value, but in vitro methods are less time consuming and allow more control of experimental conditions. The in vitro gas production (IVGPT) technique has been proposed for estimate the energy value of feeds2 based on the assumption that the gas produced at 24 h by a substrate incubated with rumen liquor is proportional to the digestible carbohydrates. Aim of the present trial was to estimate the nutritive values of silages utilized in buffalo nutrition by using equations where parameters of chemical composition and of IVGPT are included.

MATERIALS AND METHODS

The trial was carried out on five forages: corn (Zea mais L.) class FAO 400 (CS), oats (Avena sativa L.) harvested at milky maturation of grain (OS), sorghum (Sorghum vulgare L.) variety BMR 333 (SS), triticale (var. Mizar) harvested at milky-dough maturity (TS) and whole-crop barley (Hordeum vulgare L.) harvested at milky-waxy maturation of grain (BS). The forages collected at 40% DM approximately, were chopped to a 2-cm length and well pressed in 5 litres polyvinyl micro-silos. After 40 days, the silages were dried at 65°C and ground to pass 1 mm screen; dry matter (DM), ash, crude protein (CP), ether extract (EE) and crude fibre (CF) were determined3 as well as Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and lignin (ADL)4. For the in vitro trial, 1.0113 ± 0.0126 g of each silage was incubated in triplicate at 39°C under anaerobiosis for 24 h with buffered rumen fluid collected from three buffaloes at slaughter5. The nutritive value of the silages (net energy for lactation; NEl, MJ/kg DM) was estimated using several equations, however only the following three were considered adequate:

n. 13a) NEl = -3.49 + 0.0663 GP + 0.0095 CP + 0.0228 EE + 0.0079 NDF
n. 14f) NEl = 1.68 + 0.1418 GP + 0.0049 CP + 0.0130 EE + 0.0010 ash
n. 16e) NEl = 0.54 + 0.0959 GP + 0.0038 CP + 0.0001733 CP2
where GP is the gas produced after 24 h of incubation (ml/200 mg DM) and CP, EE, NDF and ash are the nutrient content in the silages (g/kg DM). The NEl was also estimated from chemical composition according to the French method6. In order to identify the most suitable equation, the correlation between the two methods were also studied and the differences among the nutritive value of the silages were statistically assessed7.

RESULTS AND DISCUSSION

As expected, the proteins of the five silages (table 1) were quite low; the highest value was found in BS (CP: 9.62 % DM). The cell wall carbohydrates were consistent with the average contents commonly found in the silages produced in Southern Italy; lignin in oats and barley silages was particularly high (ADL: 5.70 and 5.99 % DM, respectively). The nutritive value of the five silages showed in each case a not linear trend (figure 1). The equations n. 13a, 14f and 16e showed significant correlation (P<0.01) with the French method (correlation coefficient: -0.724, 0.7068 and 0.9522, respectively). The gas produced at 24 h of incubation and the nutritive value of the five silages are reported in table 2. According to Eq. n. 13a the NEl was statistically (P<0.01) lower for corn and barley silages (3.93 and 4.15 MJ/kg DM, respectively) while NEl from Eq. n. 14f was statistically (P<0.01) lower only in the case of barley (4.15 MJ/kg DM) compared to the other silages. According to Eq. n. 16e, the NEl was statistically (P<0.01) higher in corn and triticale silages (4.50 and 4.83 MJ/kg DM, respectively). This last equation has been used also by other authors1 and emerged as the most adequate in estimating the NEl of the five silage, even if always underestimated the nutritive values compared to the French method.

Table 1. Chemical composition (% DM) of the tested silage

Silage	DM	Ash	CP	EE	CF	NDF	ADF	ADL
CS	22.60	8.79	8.60	3.41	39.25	53.09	36.18	4.32
OS	54.90	8.89	5.24	2.69	33.70	62.80	44.20	5.70
SS	28.00	9.75	7.66	3.29	35.06	63.01	40.72	3.61
TS	35.60	5.95	8.60	1.18	31.85	59.59	38.22	3.81
BS	26.50	7.73	9.62	2.92	34.70	65.71	40.24	5.99

CS: corn silage, OS: oats silage, SS: sorghum silage, TS: triticale silage, BS: barley silage. DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin.

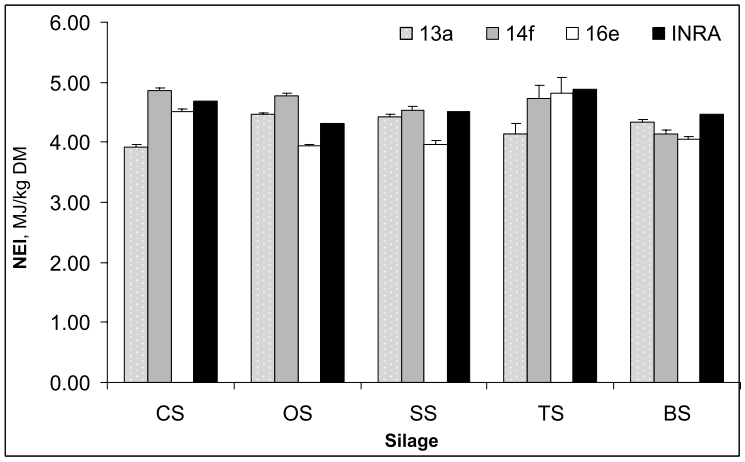

Figure 1. Net energy for lactation for the five silages

CS: corn silage, OS: oats silage, SS: sorghum silage, TS: triticale silage, BS: barley silage. 13a, 14f, 16e: equations reported by (Menke and Steingass, 1988).

INRA: Net energy for lactation estimated according to I.N.R.A. (1987).

Table 2. Gas production and net energy for lactation for the five silages

		NEl			
Silage	GP 24 h	13a†	14f†	16e†	INRA
	ml/200 mg DM	*MJ/kg DM*			
CS	24.57	3.93^{B}	4.87^{A}	4.50^{A}	4.68
OS	28.33	4.46^{A}	4.78^{A}	3.93^{B}	4.31
SS	21.95	4.42^{A}	4.53^{A}	3.95^{B}	4.51
TS	27.75	4.15^{B}	4.73^{A}	4.83^{A}	4.89
BS	16.04	4.34^{A}	4.15^{B}	4.05^{B}	4.46
MSE	-	0.0092	0.0144	0.019	-

CS: corn silage, OS: oats silage, SS: sorghum silage, TS: triticale silage, BS: barley silage. †equation to estimate NEl. GP 24 h: gas produced at 24 h of incubation. A,B: $P<0.01$. MSE: mean square error.

Acknowledgements.
The authors wish to thank the Barchiesi Farm, (Cassino, FR – Italy) where the trial was performed.

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Production and microbial protein synthesis in bubaline fed with diets based on Cynondon hay and products based on propolis

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Abstract

Four crossbred buffaloes (½ Murrah x ½ jafarabadi) castrated, with initial corporal weight of 519,0 ± 13,0 kg and cannulated on rumen were used to evaluate the microbial production in rumen. The animals were allocated according to a 4X4 latin square design, with four animals, four periods, four treatments. The tratament s were differentiated by inclusion of propolis based additives (three different propolis concentrations) or not (control). The addiction of product based on propolis does not have effect over synthesis and efficiency on microbial synthesis in bubaline fed with diets based on roughage.

Key words: additive, purine derivatives, ruminal fermentation, urine “spot” collect

INTRODUCTION

The microbial production is an information systems applied in ruminant nutrition, since the flow of microbial nitrogen to the small intestine is quantitatively the main source of protein and this is of high biological value for the host. The production and efficiency of microbial synthesis may be altered by manipulating rumen, through the use of antimicrobial additives. Propolis as nutritional additive has shown significant features. Increase the flow of microbial nitrogen to the intestines, reduction of protists in buffaloes and reduction in deamination of amino acids were observed with the use of propolis6, 7, 9. The excretion of purine derivatives (PD) has been used as index to predict the microbial protein production on rumen3. The objective in this work was to evaluate the effect of products based on propolis extract on the production and the efficiency of microbial synthesis in the buffalo fed with diet with 70% of cynondon hay and 30% concentrate.

MATERIAL AND METHODS

Four crossbred buffalo (½ Murrah x ½ Jafarabadi) castrated, with initial corporal weight of 519,0 ± 13,0 kg and canulados in rumen has been used in design experimental, 4X4 latin square design, with four animals, four periods, four treatments.

The animals were fed with a diet based on roughage (70% Cynondon hay and 30% concentrate) with 66% of TDN and 11% of crude protein. The products based on propolis were prepared in three different concentrations of propolis (LLOSB3+, LLOSC1, LLOSC1+)4 wich are patented as intellectual assets under number PI 0605768-3. The experimental diets differed

by the inclusion or not of additives based on propolis: LLOSC1, LLOSC1+, LLOSB3+ and control diet (additive free). The products were provided in two intra ruminal doses of 5 grams daily (8:00 at morning and 16:00 at afternoon) at the same time as the feeding.

The four experimental periods lasted 28 days, been 14 days of animal adaptation and 8 collect days and it was adopted a brake of 7 days with no use of additives at the experimental period.

To determinate the microbial production, a urine "spot" collect was done by the sixth day at each collect period, about four hours after fed, during spontaneous urinary excretion. Each 15mL of urine it was added 135mL of sulfuric acid (0,036N). The urine samples were assayed for creatinine and uric acid (in specialized laboratory: Center of Diagnostically laboratorial – CEDLAB) and allantoin2. The urinary volume was determined by the obtained data from the buffaloes1. The microbial nitrogen (N) production was calculated from the quantity of absorbed purine (X, mmol/day), which was estimated from urinary excretion of purine derivatives (PD) (Y, mmol/day)3 through the following equation: : Y = 0,74X + (0,117 LW0,75); where the value of 0,74 represents the recovery of absorbed purine as PD in urine and the constant value of 0,117 mmol/kg of LW0,75/day represents the net endogenous contribution of PD in bubaline. The microbial nitrogen synthesis at rumen (Y, gN/dia) were calculated according to absorbed purine (X, mmol/dia)2.

RESULTS AND DISCUSSION

There was no difference for intake among treatments, which averaged value of the 8.33 kg of dry matter/day (1.56% BW). Similarly the microbial protein synthesis (MPS) (g/day) and the efficiency of microbial protein synthesis (EMPS) (g/100g of total digestible nutrient) were not influenced by the different treatment (P>0,05) (Table 1). The values found are higher than those obtained for bubaline (2,91g/100g of TDN)3 with less weight (297Kg) and eating 95% of voluntary intake (4,75 kg of DM/day and 1,60% LW) with a diet based on wheat straw and concentrate 40:60.

The diet used presented a reason of 70:30 roughage:concentrate considered ideal for the higher microbial growing, due the better conditions of pH, passage tax and condition of colonization8, still presented values considered suboptimal (12,8 g/100g of TDN) for diets contained more than 40% of roughage provided to cattle5. The obtained values could be explained by the low intake of the animals, as the excretion of purine derivates has a positive and direct correlation with the food intake levels, resulting in greater or lesser efficiency of microbial protein synthesis3. Thus it was concluded that, there is no difference observed in the presence of based propolis additive on synthesis and efficiency of microbial synthesis in buffaloes fed with diets based on roughage.

Table 1. Synthesis and synthesis efficiency of microbial protein (g micCP1/100 g of TDN2) in buffaloes fed with 70:30 roughage: concentrate and different additives based on propolis (LLOS)3

Variable	Diets				Mean	P[4]	SE[5]
	Controle	LLOSC1	LLOSC1+	LLOOSB3+			
Urine (L)	21,03	20,37	18,97	24,87	21,31	0,338	1,114
	Purine derivaties in urine (mmol/day)						
Allantoine	69,68	68,60	75,79	71,45	71,38	0,675	3,154
Uric acid	0,57	0,68	0,70	0,64	0,65	0,981	0,108
Total PD[4]	70,25	69,29	76,49	72,09	72,03	0,689	3,216
	Absorbed microbial purine (mmd/day)						
	77,32	76,13	85,76	79,70	79,73	0,700	4,341
	Microbial nitrogen compounds (g/day)						
	56,21	55,35	62,34	57,94	52,96	0,700	3,156
	Microbial protein synthesis (g/day)						
	351,31	345,92	389,65	362,15	362,26	0,700	19,73
	Microbial synthesis efficiency (g CPmic[1]/100g de TDN)						
	8,76	8,05	8,57	8,87	8,56	0,700	5,300

Averages at same line, following by different words, differ statistically by Tuckey test 5%; 1 grams of microbial crude protein;2TDN: Total Digestible Nutrient; 3LLOS: product based on propolis extract presenting different concentrations of propolis (LLOSC1, LLOSC1+ e LLOSB3+); 4probability; 5standart error; PD: derivates purine (mmol/day); 4 Total PD: total purine derivatives.

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Profile of Selected Trace Elements in Soil, Forage and Growing Buffalo Calves in Rice Grown Region of Punjab, Pakistan

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Abstract

Selected trace elements (Zn, Cu and Fe) were analyzed in two age groups (0-6 months and 6-12 months of age) of growing buffalo calves along with their concentrations in soils and dietary sources during winter and summer seasons. Samples were collected from livestock farms of five different locations, which were randomly selected from rice grown region of Punjab, Pakistan. Results depicted that season showed non-significant ($P>0.05$) effect on the concentrations of trace elements in soils and blood plasma of both age groups except zinc and ferrous which illustrate the seasonal effect ($P<0.05$) in soils. Zinc and copper concentrations in soils were significantly ($P<0.05$) affected by location except copper which showed no effect of location in soil. Blood plasma analysis indicated significant ($P<0.05$) effect of age groups on mineral concentrations of zinc, copper and ferrous while no effect of location on both of the age groups except ferrous which showed significant ($P<0.05$) effect of location in age group of 6-12 months. Zinc was found deficient for normal plant growth in the soils of all the locations while copper and ferrous concentrations were within the normal range. Concentrations of ferrous were below the critical limit in blood plasma of both age groups but zinc and copper were found normal. Zinc and copper concentrations in feed resources were marginal to deficient in most of the feed resources to fulfill the animal's requirement while ferrous was found higher than requirement in all the feed resources. On the basis on these results, it is concluded that soils of the studied area was deficient in zinc while feed resources were deficient in all studied elements and need supplementation.

Key words: Trace elements, calves, copper, season, feed resources, soil

INTRODUCTION

Minerals are important for growth and reproduction and are involved in a large number of digestive, physiological and biosynthetic processes within the body1. The trace minerals are present in body tissues in very low concentrations and often serve as components of metallo-enzymes and enzyme cofactors or as components of hormones of the endocrine system. Therefore they affect the production as well as the reproduction of the animal.

Zinc is a component of many metallo-enzymes such as copper-zinc superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase, which affect metabolism of carbohydrates, proteins, lipids, and nucleic acids. Iron primarily functions as a component of heme found in hemoglobin and myoglobin. Enzymes of the electron transport chain, cytochrome oxidase, ferredoxin, myeloperoxidase, catalase, and the cytochrome P-450 enzymes also require iron as cofactors. Another important aspect of iron deficiency is greater morbidity and mortality associated with depressed immune responses2. With the exception of P, Cu is the most common mineral deficiency for grazing ruminants in the world4. A wide variety of disorders in ruminants are associated with a simple or

induced (high Mo and S) Cu deficiency, including anemia, severe diarrhea, depressed growth, change of hair color, neonatal ataxis, temporary infertility, heart failure, and weak, fragile long bones that break easily3. Evaluation of Cu status in livestock, by determination of dietary Cu, has limited diagnostic value unless other elements with which Cu interacts, particularly Mo, S, Fe, and Zn are also determined.

The climates in Pakistan are mostly of arid or semi-arid type. Therefore, except a few instances, the majority of the soils are deficient in macro- and micronutrients necessary for plant and animal growth4. Only fragmentary data on mineral concentrations in soils of rice grown region of Punjab, Pakistan are available and indicates zinc and copper deficiencies in the area. Therefore the present study was planned to evaluate the selected trace elements in soils and blood plasma of growing buffalo calves along with their availability through forages and feed resources during the two main seasons of the country.

MATERIALS AND METHODS

Five locations were selected randomly on the basis of topography, soil type and availability of livestock from the rice grown region of Punjab, Pakistan. Samples of soil, feeds, forages and blood of buffalo calves were taken from each location in two periods, once in summer season and once in winter season while the forages and feed resources samples were collected four times a year according to their availability on a specific stage of maturity.

Samples of each available forages, concentrates and dried roughages were taken and dried at 65 0C in a hot air oven until constant weight was obtained. All samples were grinded in Wiley mill with No. 20 stainless steel sieve. These samples were mixed well and stored in tightly stoppard bottles. About 0.5g sample was wet digested with nitric acid and perchloric acid following the procedure outlined by Sandel5. After wet digestion, further dilution was made accordingly.

Three representative soil samples were taken from each location. A stainless steel soil auger was used for soil sampling. Minerals were extracted from soil samples with diethylenetriaminepentaacetic acid (DTPA) adjusted to pH 7.36.

Buffalo calves were divided into two categories, 0-6 months of age and 6-12 months of age and 10ml blood from the Jugulars vein was collected in a heparin coated vacutainer tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) from 10 animals of each category from each location during winter and summer seasons. Therefore a total of 200 samples were collected from the studied area. Blood was kept refrigerated during transportation to the laboratory. Then the blood was centrifuged at 3000rpm for 10 minutes and plasma was harvested and frozen (? 20 ºC) until analysis. Plasma was diluted as such for mineral analysis.

The samples of forages, feed resources, soil and blood plasma were analyzed for zinc, copper and ferrous by Atomic Absorption Spectrometer (Perkin-Elmer, AA400). The data were analyzed using a split-plot design7. Differences among means were ranked using Duncan's New Multiple Range Test8.

RESULTS AND DISCUSSION

Mineral concentrations in soils

Results depicted that season and locations significantly ($P<0.05$) affected the zinc and ferrous concentrations (Table 1) of soils while copper concentrations were not affected ($P>0.05$). Concentrations of copper and ferrous in soils were far above the critical levels9,10 of deficiencies for the normal plant growth while zinc was found deficient11 during summer season but normal in winter season. Results also indicated that copper and zinc concentrations in soils were high in winter and low in summer but ferrous concentrations were vice versa to it. Khan et al12 showed the similar results in southern Punjab, Pakistan where he found significant ($P<0.05$) effect of season and location on zinc and copper concentrations of soils and reported higher values in winter than summer season. Soil texture was reported to have a significant ($p<0.05$) effect on zinc distribution in Pakistani soils, particularly in semi-arid region, with heavier textured soils having more zinc than the lighter textured sandy soils have already been studied in this semi-arid region of Pakistan by Khan et al12-14.

Mineral concentrations in blood plasma

Results of the study illustrated that season was not affected (P>0.05) the concentrations of all the studied minerals while between the two age groups of buffalo calves and between the locations, only ferrous was significantly (P<0.05) differed. Results also depicted that concentrations of zinc and copper in the blood plasma were found above the critical level15 for normal physiological functions of the body while ferrous was found deficient12 in both of the age groups during both seasons. Zinc and copper concentrations in blood plasma were slightly higher in winter season than summer season while ferrous was found high in summer season. Among the different age groups of the buffalo calves, the age group of 6-12 months of age showed higher concentrations of ferrous and zinc. Nedebele et al16 found the normal values of plasma zinc and copper concentrations in semi-arid region of Zimbabwe and indicated the non- significant (p>0.05) effect of season on plasma zinc concentrations. Ferrous levels in forage and feed resources were high but it's found deficient in buffalo calves. This was probably due to the possible antagonism between the zinc, copper and ferrous with some other elements like sulfur and molybdenum.

Mineral concentrations in forages and feed resources

Forages and feed resources were analyzed for minerals concentrations and results showed that ferrous concentrations were much higher than the animals' needs but below the toxic level15. While zinc and copper were found deficient15 in most of the forages and feed resources. Zinc concentrations in barseem and toria among forages and cotton seed cake and wheat bran among the other supplements were found normal to fulfill the animals' requirements while the remaining resources were found deficient. Copper concentrations in cotton seed cake and barseem forage were found normal. The results of the present study are in line with the findings of Khan et al12 who found deficient levels of zinc and copper in the forages of southern Punjab, Pakistan. The mineral concentration ranges were found to be similar to those reported by different earlier researchers17, 18. The results of this investigation, therefore, confirm that sub-tropical and tropical forages rarely provide all the mineral requirements of grazing ruminants19.

The present study indicated the need of zinc, copper and ferrous supplementation in rice grown region of Punjab, Pakistan to ensure their supply to buffalo calves. Although the ferrous concentrations in the forages and feed resources were far above the requirements but still the blood plasma concentrations of ferrous in growing buffalo calves were found below the normal range. That may be due to the possible interactions between the copper, zinc and ferrous metabolisms. Further studies are also required to evaluate the other mineral elements especially the molybdenum and sulfur to recommend the specific mineral mixture for buffalo calves.

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Table 1. Mineral concentrations in soils and blood plasma of buffalo calves of different age groups

S = between seasons, L=between location, AG = between age group, * = significant at level 0.05, ns = non significant

Locations	Soil (ppm)		Blood (ug/ml)			
			0-6 months of age		6-12 months of age	
	Winter	Summer	Winter	Summer	Winter	Summer
Zn	3.08±1.02	1.68±0.81	0.751±0.041	0.713±0.032	0.784±0.062	0.753±0.037
Significance			S^{ns}, L^{ns}, AG^{ns}			
Cu	4.02±1.23	3.34±0.41	0.637±0.022	0.615±0.051	0.619±0.031	0.577±0.062
Significance			S^{ns}, L^{ns}, AG^{ns}			
Fe	28.5±11.1	44.7±14.7	0.367±0.056	0.474±0.021	0.417±0.036	0.383±0.041
Significance			S^{*}, L^{ns}, AG^{ns}			

Table 2. Mineral concentrations (mean ± SE) in forages and feed resources of the studied area

Sample	Fe	Zn	Cu
Wheat Straw	289.2 ± 76.2	17.5 ± 9.2	14.4 ± 4.3
Cotton Seed Cake	82.9 ± 13.7	92.5 ± 17.4	19.2 ± 7.2
Barseem Fodder	624.8 ± 122.7	55.7 ± 11.4	24.2 ± 8.2
Sorghum Fodder	221.9 ± 56.4	19.1 ± 6.2	8.38 ± 3.1
Rice Straw	236.4 ± 24.4	1.97 ± 0.69	7.51 ± 3.1
Wheat Bran	281.4 ± 64.3	141.2 ± 34.6	16.8 ± 6.4
Oat Fodder	266.5 ± 55.8	22.4 ± 7.3	7.68 ± 2.11
Maize Fodder	221.4 ± 41.3	20.6 ± 6.1	12.9 ± 1.7
Sugarcane Tops	89.2 ± 21.7	11.0 ± 3.3	1.21 ± 0.32
Wheat Grains	124.9 ± 36.4	34.1 ± 11.3	9.46 ± 1.91
Toria Fodder	510.7 ± 167.2	66.1 ± 19.7	12.7 ± 5.1
Millet Fodder	221.5 ± 80.5	38.1 ± 11.9	5.52 ± 1.12
Corn Grains	38.2 ± 9.3	13.7 ± 2.1	2.61 ± 0.56

Rumen microbial counts in buffalo fed different silage based diets

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Abstract

Sorghum could be a potential substitute to maize in Mediterranean buffaloes feeding to improve sustainability of buffalo-based agriculture due to its reduced water and nitrogen requirements compared with maize, which is currently fed primarily. At this purpose in two following years four cannulated buffalo milking cows were fed ad libitum two different iso-energetic and iso-proteic diets, differing only for sorghum silage vs maize silage. Animal daily weight gain was very similar in the two groups. Cellulolytic bacteria values showed the tendency of being higher in S diet compared to M diet in both years, on the contrary xylanolytic bacteria were higher in maize but only in the second year. As no difference was observed in animal weight and milk yield sorghum silage appeared to be a valid substitute to maize silage in dry area.

Key words: Buffalo nutrition, rumen microbiology, sorghum silage

INTRODUCTION

Sorghum could represent an alternative to maize silage in improving sustainability of buffalo-based agriculture due to its reduced water and nitrogen compared with maize requirements, which is currently fed primarily. This work was part of a major project concerning a comparison between sorghum and maize silage in buffalo nutrition1.We scored the ruminal microbiota present following feeding of sorghum or maize based silage in two trials during subsequent years.

MATERIALS AND METHODS

Four cannulated buffalo milking cows were fed two different diets based on maize silage (M1, M2) and sorghum silage (S1, S2) for 120 days according to a Latin square design in two following years (1° year: M1 vs S1 ; 2° year: M2 vs S2). The composition of the administered diets is reported in Tab.1 and 2. The analysis were Diets had the same energetic and proteic content and differed only for sorghum variety (S1: BMR 333-SIS; S2: Nicol-PIONEER plus Trudan 8-NK). The animals were fed ad libitum and milked twice a day. Concerning the microbial counts, samples of whole rumen content, from each animal, were withdrawn at 8 am, for three consecutive days, before animal feeding. Part of each sample was used for pH determination and the protozoa (P) counts performed in a Fuchs Rosenthal chamber according to the Warner procedure (1). The other half was treated with a stomacher to detach the microorganisms from particle of food, than introduced in an anaerobic glove box (atmosphere 95%CO2- 5%H2) where it was diluted and incubated under anaerobic condition at 39°C. Total viable bacteria liquid and solid (TVBL and TVBS) and xylanolyticbacteria (XB) were grown in

Leedle and Hespell solid medium (2). Cellulolytic bacteria (CB) in Hungate medium (3), Fungi (F) in Joblin medium (4), TVBL, used only as reference counts for liquid medium cultures, were counted according to MPN procedure . The data obtained were analyzed separately per year according to the SAS GLM procedure (5).

Table 1: Diet composition

%	1° year		2° year	
	M1	**S1**	**M2**	**S2**
Maize silage	64.4		71.2	-
Sorghum silage		54.9	-	60.9
Alpha-alpha hay	13.9	17	9.3	10.1
Concentrate	21.7	27.4	19.5	29

Table 2: Chemical composition of diets

		DM	MU	CP	CF	ADF	NDF	ADL	L	ASH	STARCH	C	H
1° year	**M1**	16.8	0.9	15.5	18.9	22,4	36,8	3.4	5.4	6.1	21.3	19.4	14.4
	S1	16.8	0.9	15.5	21.6	15,6	32,4	3	5.6	7.8	22.1	12.6	16.8
2° year	**M2**	15.9	0.9	15.8	21.1	22.1	35.4	3.4	4.3	5.8	23.5	18.7	13.3
	S2	16.1	0.9	15.5	21.4	21.9	36.1	3,5	3.8	7.3	21.3	18.4	14

Legend: DM, dry matter; MU, milking Unit, CP, crude protein; CF, crude fibre ADF, acid detergent fibre;; NDF, neutral detergent fibre; ADL, acid detergent lignin; L, lipids; C cellulose; H hemicelluloses

RESULTS AND DISCUSSION

Animal daily weight gain was very similar in the two groups in both years (M1:0.7 vs. S1:0.6 and M2:0.12 vs. S2:0.04 kg/ d) so did milk yields (M1: 9.29 vs S1:9.55 and M2: 8.03 vs S2: 8.78 kg/animal/day). As shown in table 3 no significant difference were revealed in Fungi number (expressed as n° cell/ g dry rumen) and pH values (6.6 vs. 6.7). On the contrary the CB values showed the tendency of being higher in S diet compared to M diets (S1: 3,2.109 vs M1: 1,8 .109 P=0,1) (S2: 4,4.109 vs M2: 1,9 .109 P=0,5). Considering that the two diets had the same energy and protein content and the microflora had the same quantity of available nutrients, probably the cellulolytic bacteria were positively affected by a lower ADF content in S2 diet as shown in table 3, confirming what the authors previously found when the buffaloes were fed with more digestible fiber (6,7). On the contrary XB higher values in M diet in the second year (M2: 3,2 .109 and S2: 1,3.109 P=0,5) could be ascribed to higher slowly degradable fiber fraction and higher content of lignin of M2 diet (data not shown) but more investigation are to be done about. In conclusion as no difference was observed in animal weight and milk yield sorghum silage resulted to be a valid substitute to maize silage in dry area.

Table 3: Microbial counts (n° cells/g dry rumen) and pH values according to diets

Diets	pH	P	F	TVBL	CB	TVBS	XB
M1	6,7	$4{,}6.10^{8}$	$1{,}7.10^{5}$	$1{,}9.10^{11}$	$1{,}8\ .10^{9}$ b	$1{,}4\ .10^{10}$	$1{,}2\ .10^{9}$
S1	6,6	$6{,}3.10^{8}$	$2{,}6.10^{5}$	$6,\ 1.10^{11}$	$3{,}2\ .10^{9}$ a	$1{,}7\ .10^{10}$	$1{,}3\ .10^{9}$
M2	6,6	$3{,}3.10^{8}$	$4{,}4.10^{5}$	$1{,}9.10^{11}$	$1{,}9\ .10^{9}$ B	$1{,}4\ .10^{10}$ B	$3{,}2\ .10^{9}$ a
S2	6,4	$2{,}0.10^{8}$	$4{,}1.10^{5}$	$3{,}2.10^{11}$	$4{,}4\ .10^{9}$ A	$6{,}3\ .10^{10}$ A	$1{,}3\ .10^{9}$ b

Legend: P, protozoa; F, fungi; TVBL/S, total viable liquid and solid; CB, cellulolytic bacteria; XB, bacteria. Upper case A,B P=0.05; lowercase a,b P=0,1

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Rumen protozoa population in river steers buffalo (Bbubalus bubalis) fed different levels of palm kernel cake*

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Abstract

Rumen protozoa evaluations were made in the rumen of crossbred river steers buffalo (Bubalus bubalis) fed increasing levels of Palm Kernel Cake (PKC). PKC (average 8,23% Ether Extract) was provided in levels of 0, 20, 40 and 60% plus grass silage (Pennisetum purpureum). Three steers crossbred buffalos cannulated in the rumen were used. The experimental design was a split-plot in time, with the main plot distributed in a completely randomized design, four post hour feeding (0, 2, 4 and 6 hours) and four treatments (0, 20, 40 or 60% of PKC). The final average protozoa count for 0; 20; 40 and 60 % TAD were 6,20; 2,08; 0,75 and 0,47 (Protozoax104mL-1). No effect upon time post feeding was found. From the results it can be concluded that rumen protozoa in buffalos decrease as palm kernel cake increases.

Keywords: Palm kernel cake, Protozoa, Buffalo, Rumen Microbiology, Buffalo Nutrition, Rumen Microorganism.

INTRODUCTION

The role of protozoa in ruminal fermentation is still not clear. They seem to be both advantageous and detrimental for the host1,2. Protozoa have a direct effect on the wellbeing, productivity and environmental impact in the host animal, as also in their metabolic activities3. When protozoa are presented in the rumen they change the environmental conditions and microbial population effecting rate, extend and site of digestive processes3. Also it is important to state that protozoal protein has a higher biological value than bacterial protein, but a few part of it leaves the rumen1. Under certain situations the decrease in protozoa can result in increased animal growth and productivity4. This study aimed to evaluate total protozoa in buffalo rumen fed increasing level of palm kernel cake (PKC), an important brazilian biofuel byproduct.

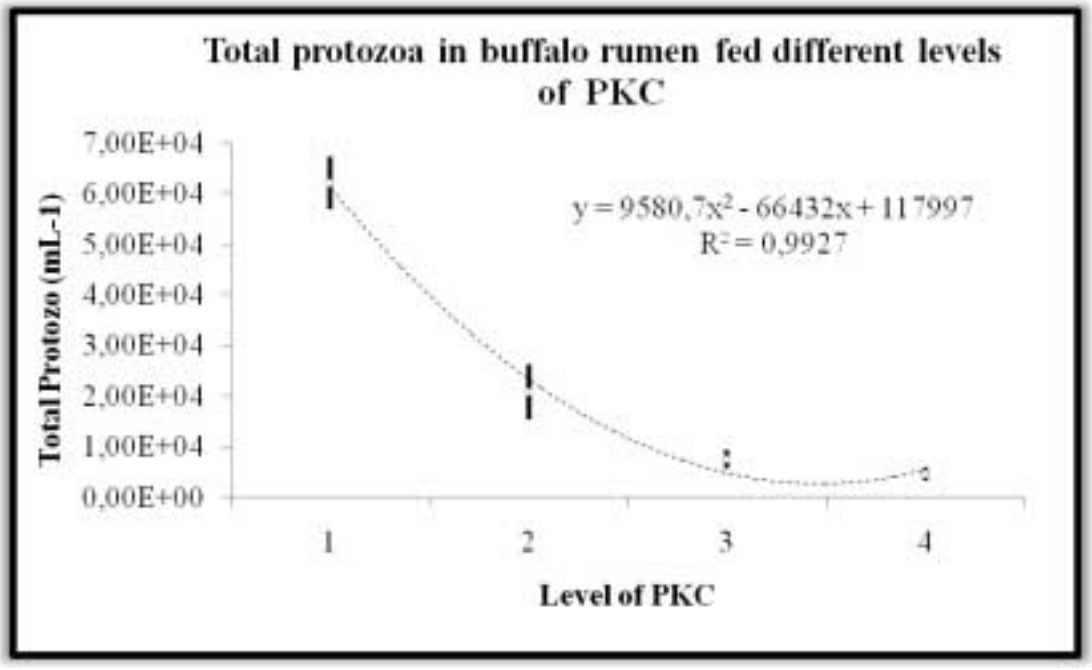
MATERIALS AND METHODS

Three crossbred river steers buffalo (Bubalus bubalis) cannulated in the rumen were fed with increasing levels of Palm Kernel Cake (PKC). The PKC had an average of 8,23% Ether Extract (EE) and was provided in levels of 0, 20, 40 and 60% plus 100, 80, 60 or 40% of grass silage (Pennisetum purpureum) made of 120 days age grass with 5% of corn meal. The animals´ initial live weight was 380.10 ± 27.21 Kg. Animals were kept under individual feed and water systems and were fed twice a day. 40% of the total feed were given in the morning and 60% at the end of the afternoon, in order to respect animals´ feeding behavior. The experimental design was a split-plot in time, with the main plot distributed in a complete-

ly randomized design, with three replications (cannulated buffalos), four feeding hours (0-fasting; 2; 4 and 6 hours after feeding), and 4 levels of PKC (0, 20, 40 and 60%). There were four periods that lasted 21 days each, being 14 days used to adjust intake and seven alternate days for samples collection. As animals were being emptied for another study, sampling for protozoa was made after all material was withdrew from the rumen, proportionally measured in solid and liquid phases, representatively removed and mixtured during 60 seconds. Collections were done as follow: Day one collection -time 0 (fasting); day three - 2 hours; day five - 4 hours and day seven - six hours post feeding. Sample was filtered in a double layer cloth and 1 mL preserved by diluting 1:2 with a 50% formalin5. Samples were then conducted to the laboratory of Federal University of Pará at Castanhal and read in Fuchs-Rosental Chamber as postulated by professor Dehority5. Data was analyzed by PASW Statistics 18, a new version of SPSS, and compared by DMS test, at 5% of significance.

RESULTS AND DISCUSSION

Results can be observed in graph 1 and table 1. Statistically there were no differences between hour after feeding (P>0,05). Differences were found between level of PKC (P<0,05) being lower counts of protozoa found in higher levels of PKC. Prior studies found much higher counts for total protozoa in buffalo rumen6,7 and that can be explained by the type of feed and the type of sampling, whereas the present work used total sampling from emptied animals while others used 3 to 5 points of sampling. From the results it can be concluded that Rumen Protozoa decreases as Palm Kernel Cake increases.



Graph 1: Total protozoa (x104mL-1) and pattern deviation in buffalo rumen fed different levels of PKC (Palm kernel cake).

Table 1: Average rumen total protozoa concentration (x104mL-1) according to sampling time in buffalos fed different levels of Palm Kernel Cake (PKC).

PKC Level*	Total Protozoa ($x10^4$ mL^{-1})			
	Hours after feeding			
	0	2	4	6
1	5,43aA	6,46aA	6,35aA	6,56aA
2	2,39aA	1,98aB	1,35aB	2,60aA
3	0,52aB	0,83aB	0,94aB	0,73aB
4	0,52aB	0,62aB	0,31aB	0,42aB

* Treatments. Levels of PKC: 1(0%); 2 (20%); 3(40%) and 4(60%).

Values in the same row with different capital letters differ (P<0,05) between levels of PKC. Values in the same line with equal lower case letters do not differ (P>0,05) between times after feeding.

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Seasonal Dynamics of Minerals in Soil, Forages, Feed, water and lactating Nili Ravi Buffalo in Muzaffargarh District of Punjab, Pakistan

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Abstract

The objective of this study was to estimate the seasonal dynamics of sodium, potassium and magnesium in soil, forage, feed, water and blood plasma of Nili Ravi buffalos at three different stages of lactation (early, mid and late) in Muzaffargarh district of Punjab, Pakistan. Samples were collected from five randomly selected sites of the studied area during winter and summer seasons over a period of one year. Results indicated that season significantly ($P<0.05$) affected the sodium and magnesium concentrations of soil and potassium and sodium value of blood plasma at different stages of lactation. Stage of lactation showed significant ($P<0.05$) effect on the concentrations of potassium and sodium in blood plasma except magnesium, which showed non-significant ($P>0.05$) effect. Locations also significantly ($P<0.05$) affected the concentrations of all the minerals in soils and buffalo's blood plasma at different stages of lactation during both seasons while sodium, potassium and magnesium concentrations of water showed non-significant ($P>0.05$) effect of season and location. Potassium and magnesium concentrations in blood plasma were found within the normal range during all the stages of lactation except sodium which was found below the critical limit for normal physiological functions during both summer and winter seasons. Sodium and potassium were deficient in soil for normal plant growth in both seasons and in most of the feed resources to fulfill animals' need except magnesium, which was found above the critical level in soil but deficient in feed resources. Study indicated severe deficiencies of sodium and potassium in soil, sodium in animal's blood plasma and all the studied minerals in most of the feed resources, which needs proper supplementation.

Key words: Seasonal, season, feed resources, soil, water

INTRODUCTION

Minerals are inorganic compounds important for production and reproduction performance of livestock. They are the essential nutrients bearing significant effect in animal nutrition because both their excess and deficiency cause detrimental effects on the health and production of livestock. Sodium and potassium are the main intra and extracellular cations and responsible for maintaining osmotic pressure, acid-base regulation, water balance and muscle contraction. Additionally, heart function, nerve impulse conduction and transmission are dependent on the proper balance of sodium and potassium. The Na-K pump is essential for all eukaryotic cells, enabling transport of glucose, amino acids and phosphate into cells, and hydrogen, calcium, bicarbonate, potassium, and chloride ions out of cells1. Magnesium is a major intracellular cation that is a necessary cofactor for enzymatic reactions, vital to every major metabolic pathway. Extra-cellular magnesium is vital to normal nerve conduction, muscle function, and bone mineral formation. Sodium and potassium affect the magnesium uptake by the animal as increased oral intake of potassium reduces the apparent

availability of magnesium in ruminants while adding sodium to the diet can improve transport of magnesium across the ruminal wall when dietary sodium is low2.

Concentrations of minerals in both concentrate and forage feedstuffs vary greatly3. The quality and quantity of nutrients of forages mainly depend upon the factors like type of soil, level of fertilization used and source of irrigation4. Only rarely, however, can pasture forages completely satisfy all minerals requirements4,5.

The Nili Ravi buffalo is the main dairy breed of Pakistan. Therefore, the buffalo occupies a key position in the rural economy of Pakistan. The country's ruminant production depends largely on the use of natural pastures throughout the year without any proper supplementation. There is a need to evaluate the seasonal availability of minerals through forages and feed resources to the Nili Ravi buffalo in the Muzaffargarh District of Punjab, Pakistan before recommendations for specially formulated mineral supplement can be made. The use of mineral supplements without regard to local conditions can cause mineral imbalances and is therefore, likely to impede rather than to promote the improvement in livestock production6. In this view, the present study was planned to evaluate the mineral status of soils and their seasonal availability to the Nili Ravi buffalo through available forages and feed resources along with the water.

MATERIALS AND METHODS

Investigation Area

Muzzafargarh district of the Punjab, Pakistan was selected for this work, which was located in sub-tropical and monsoon region. This district holds the Nili Ravi buffalo as the major milk animal. There are two main seasons, summer (May-July) and winter (Nov-Jan). Five locations were selected randomly on the basis of topography, soil type and availability of livestock. Samples of soil, water and blood of farm livestock was taken from each location in two periods, once in summer and once in winter season while the samples of forages and feeds were collected four times the year according to their availability on a specific stage of maturity.

Sampling and Analysis

Samples of each available forage, concentrates and dried roughages were taken and dried at 65 0C in a hot air oven until constant weight was obtained. All samples were grinded in Wiley mill with No. 20 stainless steel sieve. Then, these samples were mixed well and stored in tightly stoppard bottles. About 0.5g sample was wet digested with nitric acid and perchloric acid following the procedure outlined by Sandel7. After wet digestion, further dilution was made accordingly.

Three representative soil samples were taken from each location. A stainless steel soil auger was used for soil sampling. Minerals were extracted from soil samples with diethylenetriaminepentaacetic acid (DTPA) adjusted to pH 7.38. The water being offered to the animals for drinking as well as used for irrigation of the forages at the selected sites was sampled and used for analysis. Three representative samples of 500ml each were taken from each location and analyzed as such and if there is any turbidity that was removed by centrifugation or by appropriate filtration procedure.

Lactating buffaloes were divided into three categories, early, mid and late lactation and 10ml blood from the Jugulars vein was collected in a heparin coated vacutainer tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) from 10 animals of each category from each location during winter and summer seasons. Therefore, a total of 300 samples were collected from the studied area. Blood samples were kept refrigerated during transportation to the laboratory. Then the blood was centrifuged at 3000rpm for 10 minutes and plasma was harvested and frozen (? 20 ºC) until analysis. Plasma was diluted as such for mineral analysis.

The samples of forages, feed resources, soil, water and blood were analyzed for sodium, potassium and magnesium by Atomic Absorption Spectrometer (Perkin-Elmer, AA400).

Analysis of Data

The data were analyzed using a split-plot design9. Differences among means were ranked using Duncan's New Multiple Range Test10.

RESULTS AND DISCUSSION

Mineral concentrations in soil

Results from this study indicated that the effect of season and location significantly ($P < 0.05$) affect the mineral concentrations of soils except potassium which showed non-significant ($P > 0.05$) effect of season (Table 1). Na and K concentrations in soils were found deficient for normal plant growth but Mg was found above the critical level suggested by Hanlon11, and Sanchez12. Khan et al13 indicated the same results as deficient levels of Na and K in soils of Southern Punjab while found Mg within the normal range with the seasonal effect on mineral concentrations of soil. Water resources showed non-significant ($P > 0.05$) effect of season while Mg concentrations in both of the water sources were significantly affected by season (Table 2).

Mineral concentrations in blood plasma

Mineral concentrations in blood plasma of lactating buffaloes during early, mid and late lactation in both summer and winter seasons are presented in Table 3. Results indicated that season and location showed significant ($P<0.05$) effect on all the studied minerals except Mg which showed non-significant ($P>0.05$) effect of season as well as stage of lactation significantly affect the Na concentration of blood plasma. Results also depicted that K and Mg concentrations were above the critical limits14 for normal physiological functions of the animals' body while Na was found deficient during all the stages of lactation. All the studied minerals were found slightly high in winter season than summer season except Na while buffaloes during early lactation had the higher mineral concentrations than during the later stages. Only Na was found higher in late lactation than early and mid lactational stages. Khan et al15 showed the same results in central region of Punjab, Pakistan.

Mineral Concentration in Forages and feed resources

Mineral concentrations in most common feed resources of the studied area are presented in Table 4. Results illustrated that Na and Mg were deficient in most of the forages and feed resources except barseem (Trifolium alexandrium), toria (Brassica rapa) and oat (Avena Stiva) forages for Na and barseem (Trifolium alexandrium) for Mg, which were found above the critical level to fulfill the animal's requirements while K was found marginal to deficient in some feed resources. In common feeding practices of the studied area during summer and winter seasons, Na and K were found above the critical level but Mg was marginally deficient in winter season while all the studied minerals were found deficient in summer's feeding practices.

The soil of the studied area was found deficient in Na and K while feed resources were marginal to deficient in all the studied minerals. Ultimately, Na was found below the marginal level in blood plasma of lactating buffalo during all the lactational stages which needs specific supplementations. Further studies are required in the area to evaluate the other macro and micro minerals to formulate an area specific mineral supplementation.

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Table 1. Soil mineral concentrations (mg/kg, Mean ± SE) of five locations in summer and winter seasons

Constituent	Season	DM	Significance
Na (ppm)	Winter Summer	24.4 ± 7.4 41.0 ± 11.9	S*, L*
K (ppm)	Winter Summer	43.9 ± 8.6 38.1 ± 9.7	S^{ns}, L*
Mg (ppm)	Winter Summer	94.6 ± 16.3 80.5 ± 11.2	S*, L*

DM = District mean, S= effect of season, L= effect of location
* = significant at 0.05 level, ns = non significa

Table 2. Concentrations of minerals (Mean ± SE) in different water sources

Constituent ppm	Season	Water source		Significance
		Tube well	motor pump	
Na	Winter Summer	0.1466±0.032 0.1147±0.076	0.1640±0.067 0.2800±0.092	Sn^{ns},Sr *
K	Winter Summer	0.1456±0.019 0.0707±0.021	0.0465±0.013 0.0743±0.007	Sn^{ns}, Sr^{ns}
Mg	Winter Summer	2.468±0.932 1.803±0.776	2.103±0.731 3.540±1.174	Sn*, Sr^{ns}

DM = District mean, Sn= effect of season, Sr= effect of water source
* = significant at 0.05 level, ns = non significant

Table: 3. Plasma mineral concentration (mg/100ml, Mean ± SE) of buffalo at different lactational stages

Constituents	Season	Stage of lactation			Significance		
		Early	Mid	Late	BLn	BSn	BLs
Na	Winter	270.8±19.42	284.9±38.40	295.8±28.19	*	*	*
	Summer	300.4±16.21	315.1±26.38	319.2±17.59			
K	Winter	30.39±7.54	29.49±6.09	29.72±5.67	*	*	ns
	Summer	22.49±3.65	23.20±4.84	25.99±7.50			
Mg	Winter	2.506±0.01	2.447±0.49	2.431±0.15	*	ns	ns
	Summer	2.514±0.41	2.210±0.29	2.368±0.36			

W = winter S = summer BLn = between location BSn = between season
BLs = between lactational stage * = significant at 0.05 level ns= non significant

Table 4. Mineral concentrations (% dry matter, mean ± SE) of the common feed resources

Feed source	Na	K	Mg
Wheat Straw	0.0713±0.0142	0.813±0.091	0.053±0.011
Cotton Seed Cake	0.079±0.013	1.37±0.73	0.124±0.073
Barseem Forage	1.349±0.462	3.21±1.07	0.576±0.066
Sorghum Forage	0.063±0.014	1.26±0.27	0.047±0.017
Rice Straw	0.069±0.023	0.89±0.22	0.092±0.008
Wheat Bran	0.074±0.016	1.77±0.56	0.081±0.020
Oat Forage	0.389±0.051	1.52±0.98	0.047±0.037
Maize Forage	0.032±0.018	1.28±1.07	0.051±0.026
Sugarcane Tops	0.0087±0.004	0.372±0.113	0.029±0.013
Wheat Grains	0.0214±0.009	0.570±0.087	0.043±0.011
Toria Forage	0.447±0.078	3.37±1.42	0.087±0.051
Millet Forage	0.161±0.057	2.93±1.21	0.091±0.023
Corn Grains	0.009±0.002	0.0867±0.016	0.073±0.008

Seasonal Variations in the Zinc Status of Soil, Forage and Nili Ravi buffalo of Thal Canal Irrigated Zone of Punjab, Pakistan

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Abstract

A comprehensive, one year planed study was conducted to uncover the deviation of the zinc status in soil, forages, feed resources and blood plasma of lactating and non-lactating Nili Ravi buffaloes through the changing seasons of Thal Irrigated Canal Zone (Khushab district) of Punjab, Pakistan. According to preparation, the zone was divided into five randomly selected sites and representative samples of soil, forages, feed resources and blood plasma were taken from each site and analyzed for zinc concentration. In soil, Non-significant ($p>0.05$) difference was found between the seasons and sites while overall concentration of zinc was found deficient for the normal plant growth. Results also indicated no significant effect of physiological stage (lactating and non lactating) on blood plasma concentration of zinc and they were found above the critical limit for normal physiological functions in both lactating and non lactating animals. Among the forages and feed resources, concentrations of zinc in rice straw, sugar cane tops and corn grains were found deficient, millet, maize, oat and sorghum forages marginally deficient but cotton seed cake, barseem and rapeseed forages were found within the normal range to fulfill the animal's need. Non-Significant ($p>0.05$) difference was found in blood Plasma for Zinc among the sites during the summer and winter season and between the both seasons.

Key words: Zinc, lactating and non-lactating buffaloes, soil, water

INTRODUCTION

The level of nutrition and mineral utilization are known to effect productive and reproductive efficiency of livestock. Deficiency or imbalance of certain minerals of the forages in tropical countries have been reported by several researchers and suggested as one of the limiting factors for improvement of animals' productivity in this region1. In small holder farming systems, native forages and agricultural by-products are the main mineral sources for ruminant feeds in many regions of the world. Similarly in Pakistan, livestock are mostly raised by the small farmers in rural areas under native system on forages and crop residues without any proper feed and mineral supplements. According to McDowell,2 the animals receiving forages only as a main source of their feeds are usually deficient in certain minerals due to the forages rarely contain all the minerals required by the animals. Availability and concentration of minerals in crops and forages are influenced by environmental factors such as climate and seasonal conditions during growth, amount of rainfall, the type and soil fertility on which the forages grow stage of maturity, genus and species or strain (variety) of the forages3. Clinical signs of mineral deficiencies along with soil, plant, and animal tissue analyses have all been used with varying degrees of success to establish mineral deficiencies and toxicities3,4.

The Nili-Ravi buffalo is the main dairy breed of Pakistan and occupies a key position in the rural economy of the country. Zinc is a component of many metallo-enzymes such as copper-zinc superoxide dismutase, carbonic anhydrase, alcohol

dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase, which affects metabolism of carbohydrates, proteins, lipids, and nucleic acids.

Only fragmentary data are available about the mineral status of soil, forages and buffaloes of Thal Canal Irrigated Zone of Punjab especially, the trace elements. The use of mineral supplements without regard to local conditions can cause mineral imbalances and is therefore, likely to impede rather than to promote the improvement in livestock production5. Therefore, the present study was conducted to evaluate the zinc status of the mentioned area to overcome the problem.

MATERIALS AND METHODS

Investigation Area

Five locations were selected randomly on the basis of topography, soil type and availability of livestock from the Thal Canal Irrigated Zone of Punjab, Pakistan. Samples of soil, feeds, forages and blood of Nili Ravi buffalo were taken from each location in two periods, once in summer season and once in winter season while the forages and feed resources samples were collected four times a year according to their availability on a specific stage of maturity.

Sampling and Analysis

Samples of each available forages, concentrates and dried roughages were taken and dried at 65 0C in a hot air oven until constant weight was obtained. All samples were grinded in Wiley mill with No. 20 stainless steel sieve. These samples were mixed well and stored in tightly stoppard bottles. About 0.5g sample was wet digested with nitric acid and perchloric acid following the procedure outlined by Sandel6. After wet digestion, further dilution was made accordingly.

Three representative soil samples were taken from each location. A stainless steel soil auger was used for soil sampling. Minerals were extracted from soil samples with diethylenetriaminepentaacetic acid (DTPA) adjusted to pH 7.37.

Nili Ravi buffaloes were divided into two categories, lactating and non lactating, according to their physiological stage and 10ml blood from the Jugulars vain was collected in a heparin coated vacutainer tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) from 10 animals of each category from each location during winter and summer seasons. Therefore a total of 300 samples were collected from the studied area. Blood was kept refrigerated during transportation to the laboratory. Then the blood was centrifuged at 3000rpm for 10 minutes and plasma was harvested and frozen (? 20 ºC) until analysis. Plasma was diluted as such for mineral analysis.

The samples of forages, feed resources, soil and blood plasma were analyzed for zinc by Atomic Absorption Spectrometer (Perkin-Elmer, AA400).

Analysis of Data

The data were analyzed using a split-plot design8. Differences among means were ranked using Duncan's New Multiple Range Test9.

RESULTS AND DISCUSSION

Soil

Zinc concentrations in the soils of Thal Canal Irrigated Zone during summer and winter seasons are presented in Table 1. Results depicted that season was not effect ($P > 0.05$) the zinc concentration while these concentrations were significantly ($P > 0.05$) differed among the locations. Zinc concentrations were found below the critical level10 for the normal plant growth during each season but during winter season, the concentrations were slightly high than summer season. Ndeble11 indicated the same results in Matabeleland region and arid western area of Zimbabwe where non significant ($P>0.05$) effect of season was observed in zinc concentration of soils and highlighted zinc deficiencies in soils. Some zinc deficiencies were also indicated12 in the soils of semi arid region of Pakistan but no effect of location was found.

Blood

Zinc concentrations in blood plasma of lactating and non-lactating buffaloes during summer and winter seasons are presented in Table 1. No significant ($P>0.05$) effect of season and location was found in plasma zinc concentrations while statistically significant ($P > 0.05$) difference was found between the lactating and non lactating buffaloes. Zinc

was found above the critical level13 for normal physiological functions in blood plasma of lactating and non-lactating buffaloes during both seasons except lactating buffalos which were near to margins of deficiency in summer season. Higher zinc values were found in blood plasma of non-lactating buffaloes during winter season than summer season. This may be due to availability of good quality leguminous forages in winter season. Abdelrahman14 specified the effect of season and breeds on plasma zinc concentration in western Sudan.

Forages

Zinc concentrations in most common forage species and feed resources in the studied area are shown in Table 2. Results indicated that most of the forages were marginal to deficient except barseem (Trifolium alexandrium) and toria (Brassica rapa) forages, which were found above the critical limit13 to fulfill the animals requirements. Location significantly ($P>0.05$) affects the zinc concentrations in sorghum (Sorghum bicolor) forage, millet (Pennisetum amricanum) forage and sugarcane tops while all the other forages showed non-significant effect of location on zinc concentration. Among the feed resources, zinc concentrations in cotton seed cake and wheat bran were found above the critical level while the remaining feed resources were deficient to fulfill the animal's requirement. Espinoza15 et al indicated the deficient levels of zinc concentrations in forages of highlands (Altiplano) of Bolivia to fulfill the animals' requirements. Similar results were also depicted in the forages of semi arid region of Zimbabwe11. While Abdelrahman14 showed adequate levels of zinc in forages of western Sudan.

The results of the present study indicated the zinc deficiency in the soils of Thal canal irrigated Zone of Punjab, Pakistan. Most of the forages and feed resources were also not able to fulfill the animals' requirements so this area required supplementations to ensure the supply of zinc to the Nili Ravi Buffalo. Further study is also required to evaluate the other macro and micro elements in the area.

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Table 1. Zinc concentrations in soils and blood plasma of lactating and non lactational buffalos in different locations of the studied area

Locations	Soil (ppm)		Blood (mg/100ml)			
			Lactating		Non-lactating	
	Winter	Summer	Winter	Summer	Winter	Summer
S1	2.63±0.92	2.41±1.03	1.08±0.09	0.65±0.12	0.98 ± 0.12	0.91 ± 0.12
S2	0.73±0.13	0.96±0.22	0.89±0.12	0.69±.18	0.82 ± 0.09	0.76 ± 0.10
S3	1.31±0.76	0.97±0.09	0.95±0.18	0.84±0.18	1.12 ± 0.09	0.79 ± 0.09
S4	2.23±1.02	1.67±0.87	0.79±0.09	0.71±0.18	0.87 ± 0.10	0.69 ± 0.10
S5	0.97±0.16	1.01±0.44	0.92±0.12	0.80±0.23	0.91 ± 0.04	0.81 ± 0.04
OM	1.57	1.40	0.92	0.74	1.09	0.95
Significance	S^{ns}, L*		S^{ns}, L^{ns}, PS*			

S1-S5= locations, OM = overall mean, S = between seasons, L=between location, PS = between physiological stage, * = significant at level 0.05, ns = non significant

Table 2. Zinc concentrations (mean ± SE) in the common available forages in the studied area

Feed source		Zn (mg/kg DM)	Significance
Barseem Forage	(Trifolium alexandrium)	67.12 ± 13.7	L^{ns}
Sorghum Forage	(Sorghum bicolor)	29.26 ± 8.74	L^{*}
Oat Forage	(Avena Stiva)	27.8 ± 6.91	L^{ns}
Maize Forage	(Zea Mays)	38.66 ± 14.2	L^{ns}
Millet Forage	(Pennisetum amricanum)	57.12 ± 16.7	L^{*}
Toria forage	(Brassica rapa)	75.6 ± 11.3	L^{ns}
Sugarcane Tops		18.08 ± 4.36	L^{*}
Wheat straw		26.25 ± 9.21	L^{ns}
Cotton seed cake		104.9 ± 23.19	L^{ns}
Rice straw		2.74 ± 0.89	L^{*}
Wheat bran		127.4 ± 21.92	L^{ns}
Wheat grains		53.67 ± 13.4	L^{ns}
Corn grains		17.52 ± 4.77	L^{*}

L = between locations, * = significant at level 0.05, ns = non significant

The Effect of Nitrogent Level on the Energy Metabolism of Growing Water Buf

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Abstract

Twelve water buffalo heifers aged 12-13 months with body weight of 220 kg, were used in a randomized complete block design to study energy metabolism. Animals were divided into 3 groups based on body weight and breed, each group including 1 Nili-Ravi, 1 Murrah and 2 crossbred (Nili-Ravi × Murrah × local) water buffaloes. Three isocaloric concentrate were formulated to contain three levels of crude protein (CP) (19.9, 17.6 and 15.3 %). Each animal was offered 3 kg concentrate per day. The elephant grass was the main ingredients of forage. The whole experiment consisted of two feeding trials (12-13 and 14-15 months) with 30 d in-between interval, each including a 7-d digestion-metabolism trial. Apparent digestibility and metabolisability of energy, ME/DE, methane loss and UE/GEI were not significantly different among three groups?P >0.05?. The mean apparent digestibility, metabolisability of energy and ME/DE were 68.1, 57.1 and 83.9 %, respectively. Methane loss and UE were 8.9 % and 2.1 %, respectively.

Key words: Energy digestibility, Energy metabolizability, Buffalo heifer

INTRODUCTION

Guangxi is a sub-tropical area which is rich in feed resource, with the biggest population of water buffalo (Bubalus, bubalis.) in China. The increasing interest in water buffalo farming in China is attributed to the increased demand of water buffalo meat and milk for human consumption. With genetic improvement for milk production, dairy water buffaloes are now the major source in milk supply in intensive population area of the Southern China. Chinese government has paid much attention to promoting production of dairy water buffaloes. However, across the world, there have been only a few studies on evaluation of energy metabolism of growing water buffalo (Tiwari, 2000; Khan, 1988a, Zou, 2007). Due to the absence of information in feeding standards for water buffaloes, farmers in China have to rely on personal experience in determining the feeding allowances or use the feeding standards of other breeds of cattle. This would result in considerable errors in quantifying animal feed intake, because there are lots of difference between the physical parameters of water buffalo and other breeds of cattle. Therefore, it is necessary to develop feeding allowances for water buffalo in China to improve production efficiency and animal welfare and reduce the environmental pollution. The aim of current research was to study the energy digestibility and metabolisability in growing water buffalo feeding different crude protein levels.

MATERIALS AND METHODS

Animals and diets

Twelve dairy buffalo heifers aged 12 months (body weight=220 kg, SD=20) were used in a randomized complete block design. Animals were divided into 3 groups based on body weight and breed, each group including 1 Chinese Nili-Ravi, 1 Chinese Murrah and 2 crossbred (Nili-Ravi × Murrah × local) buffaloes.

Experimental buffaloes were fed individually (twice daily, 08.00 and 14.00) and all animals had free access to the drinking water. Three isoenergy diets (I, II and III) were formulated to contain 3 levels of crude protein for lactating (19.80, 17.40 and 15.00 %). The elephant grass and pineapple were the main ingredients as forage. The ingredients and composition of the experimental diets are presented in table 1. Each animal was offered 3 kg concentrate per day.

The whole experiment consisted of 2 feeding trials with 20 days in-between interval, each including a digestion-metabolism trial. Each feeding trial lasted for 30 days, following 15 days for adaptation to the diet. And a 7-day digestion and 3-d respiratory gas metabolism trials were conducted on the 24th and 28th day of feeding trial, respectively. The oxygen and carbon dioxide content were determined for 4 times per day per head (06.00, 12.00, 18.00, 24.00 respectively) during the gas metabolism trial, and the temperature and pressure of atmosphere in experimental fields were also recorded at the beginning and end of gas determination (Guo, 1983).

Samplings, measurements and analysis

Live body weights were recorded for two consecutive days before morning feeding at the beginning and end of feeding/digestion-metabolism trial, respectively. Feeds offered (0800, 1400) and refused (1300, 1700) by individual buffaloes were weighed for each intaking time throughout the trial. Forage and concentrates were sampled weekly. All samples were immediately dried in an air-forced oven at 65ºC for 48 h and stored in sealed plastic containers at room temperature until analysed. In preparation for analysis, dried forages and concentrates were ground firstly through a 2 mm screen, then through a 1 mm screen in a cycloter mill. All samples were analysed for dry matter (DM), crude protein (CP) and gross energy (GE) (PARR 6200).

During digestion-metabolism trial, the total feces and urine were collected and recorded at 0800 before morning feeding and urine/feces samples were acidified immediately after collection by diluting 1 volume of urine/feces with 10% volumes of 10% H_2SO_4 and stored at -20ºC. Later, these samples were thawed at room temperature and analysed for urinary (UE) and fecal energy (FE). Oxygen, carbon dioxide concentration and total exchange gas amount were recorded during gas metabolism trial. Heat production (HP) was calculated from oxygen consumption (O_2), carbon dioxide production (CO_2) and urinary nitrogen loss (Un) using the following formula (Brouwer, 1965): $HP\ (KJ) = 16.1753O_2 + 5.0208CO_2 - 5.9873\ Un$. Estimation of methane was based on Brouwer (1965).

Statistical analyses

Statistical analyses of the obtained data in the experiment were performed using Excel 2003 and the data were presented as means. Data in fasting metabolism trial and feeding trial were analyzed by one-way ANOVA procedure of SAS (1996).

Table 1. Composition and nutrient levels of diets (different crude protein level)

Item			
Diets provided per day (kg/d)			
Concentrate	3	3	3
Fresh elephant grass	16	16	16
Concentrate composition (air-dry basis, %)			
Ground corn grain	34	40	50
Wheat bran	22	23	21
Rapeseed meal	22	20	17
Soybean meal	14	9	5
Limestone	1	1	1
Dicalcium phosphate	2	2	2
Salt	2	2	2
premix [1)]	3	3	3
total	100	100	100
Nutritient level of concentrate (air-dry basis,)			
NE_L (MJ/kg)	6.24	6.28	6.26
Crude protein (%)	19.90	17.60	15.30

Premix composition:?Ca 40%; P 6%; NaCl 21%; Cu 300 mg/kg; ?Zn 1 600 mg/kg; Mn 10 mg/kg; Fe 600 mg/kg; I 10 mg/kg; Se 10 mg/kg; Co 6 mg/kg; VA 12 000 IU; VB 30 000 IU; VE 135 IU; Niacin 300 mg/kg

Table 2. Nutrition value of forage in experimental water buffaloes (determined value)

	Forage	DM, %	Caloric, MJ/kg	CP, %	CF, %	EE, %	Ca, %	P, %
First period	Elephant grass	23.0	16.40	6.4	31.6	2.4	0.32	0.22
Second period	Elephant grass	26.4	16.57	6.3	32.6	2.2	0.34	0.20

Notes: Expext dry matter, the other was on the air-dry basis.

RESULTS

Growing performance

The average air temperature were 26.2? and 23.0? in the first and the second period. The growing

performance of the experimental water buffaloes were presented in Table 3. According to the data of average daily gain in Breeding Buffalo farm of Guangxi Buffalo Research Institue, the ADG of growing Buffalo were 0.49 kg/d to 0.53 kg/d during normal feeding situation. Therefore, the ADG in current experiment were 0.52 kg/d to 0.70 kg/d, which higher than the average level. So, the present data can be used as energy metabolism value during normal feeding situation.

Table 3. The growing performance of the experimental water buffaloes

Group	First Period (42d)			Second Period (30d)		
	I	II	III	I	II	III
IBW	220	229	217	259	267	265
FBW	245	251	240	280	286	284
ADG Mean±MSE	0.59±0.100	0.52±0.107	0.55±0.069	0.70±0.078	0.63±0.057	0.63±0.052

From the list presented in Table 4, the average dry matter intake (DMI) for 12-13 months buffalo in the feeding trial in the first period was 5.05 kg/d, accounting to 2.1 % of their live body weight. And from the data in Table 5, the average dry matter intake (DMI) for 14-15 months buffalo in the feeding trial in the first period was 5.74 kg/d, accounting to 2.1 % of their live body weight. Therefore, the presented data indicated that the effect of crude protein in concentrate DMI/ BW on ADG was small, and the value of DMI/BW was stable, i.e. about 2.0 %.

Energy digestibility and energy metabolizability

Results of energy metabolism of experimental buffaloes are summarized in Tables 4 and 5. It was similar in energy digestibility and energy metabolizability of growing buffalo between the first and the second period digest-metabolizable trial. The energy digestibility and energy metabolizability of growing buffalo in the two periods were 66.8 and 69.5 %.

The value of MEI/DEI of growing buffalo in the two periods were 83.4 and 84.3 %. The energy lose in feces, methane and urine of growing buffalo in the two periods were 33.2 and 30.5 %, 2.2 and 2.0 %, 8.9 and 8.9 %, respectively. Therefore, the total energy lose in feces, methane and urine was about 42 %?45 %.

Table 4. Dry matter intake (DMI), intake of gross (GE), digestible (DE) and metabolizable energy (ME), losses of energy in urine (UE) and methane (CH4), and digestibility and metabolizability of energy in experimental water buffaloes (first period)

12-13 months	I	II	III	Mean±SE
BW (kg)	235	240	237	238±12.63
DMI (kg/d)	5.11	4.90	5.15	5.05±0.230
DMI/ BW(%)	2.2	2.0	2.2	2.1±0.08
N intake(g/d)	126.0	113.6	106.8	115.4±4.37
Energy metabolism (MJ)				
GE intake	91.61	88.02	92.51	90.72±4.586
DE intake	60.36	58.5	62.81	60.56±2.160
ME intake	49.96	48.69	52.86	50.51±2.135
FE	31.25	29.52	29.70	30.16±0.950
UE	2.26	2.00	1.74	2.00±0.260
CH_4E	8.14	7.81	8.21	8.05±0.367
	----------% ----------			
DE/GE	65.9	66.5	67.9	66.8±1.03
ME/GE	54.5	55.3	57.1	55.7±1.33
ME/DE	82.8	83.2	84.2	83.4±0.71
FE/GE	34.1	33.5	32.1	33.2±1.03
UE/GE	2.5	2.3	1.9	2.2±0.30
CH_4E/GE	8.9	8.9	8.9	8.9±0.01

Table 5. Dry matter intake (DMI), intake of gross (GE), digestible (DE) and metabolizable energy (ME), losses of energy in urine (UE) and methane (CH4), and digestibility and metabolizability of energy in experimental water buffaloes (second period)

14-15 months	I	II	III	Mean±SE
BW (kg)	266	273	271	270±11.7
DMI (kg/d)	5.45	5.87	5.9	5.74±0.352
DMI/ BW(%)	2.0	2.2	2.2	2.13±0.086
N intake(g/d)	120.98	119.30	108.03	116.11±7.040
Energy metabolism (MJ)				
GE intake	97.44	105.11	105.93	102.83±4.683
DE intake	64.56	73.47	76.59	71.54±6.242
ME intake	53.62	62.12	65.40	60.38±6.079
FE	32.88	31.64	29.34	31.29±1.797
UE	2.26	2	1.74	2.00±0.260
CH_4E	8.68	9.35	9.45	9.16±0.419
	----------% ----------			
DE/GE	66.3	69.9	72.3	69.5±3.04
ME/GE	55.0	59.1	61.7	58.6±3.38
ME/DE	83.1	84.6	85.4	84.3±1.18
FE/GE	33.7	30.1	27.7	30.5±3.04
UE/GE	2.3	1.9	1.6	2.0±0.34
CH_4E/GE	8.9	8.9	8.9	8.9±0.03

Dry matter intake

Current trial indicated that the DMI/BW was stable, and the value was about 2 %. The value of DMI can reflect the animal healthy condition, production situation, and the DMI index also can used as a target for dietary formulation (LU De-Xun, 2001 and HU Jian,1991). The animal pieces, animal management, environment, feed quality and dietary composition were the main factors for influencing ruminant dry matter intake. Normally, the value of DMI/BW was 2-4%. For the same species and same sex bovine, the dry matter intake was mainly depending on animal live body weight, or more precisely depending on animal metablic body size. If the live body weight less than 350 kg, the dry matter intake will increase significantly, and if it was more than 350 kg, the dry matter intake increase slowly (WU Qiu-Yu, 2006). LU.De-Xun (2001) had proposed the goal of decision-making of improvement of DMI in dairy cow was also a critic foundation for animal nutrition and feed technology. It can be seen that the DMI index was a key decisions in ruminant nutrition.

Energy digestibility and energy metabolizability

In ruminants, 76-86% of the DE is converted to ME, and only 30-65% of the ME is converted to net energy (China Ministry of Agriculture, 2004).

The average energy digestibility in the two periods was 67.9 %, which lower than that of crossbred meat sheep (YANG Zai-Bing et al, 2004), finished beef cattle in tie-condition (ZHANG Yu-Zhi et al, 2002), and Hereford steers (BW=286±5 kg) (Chizzotti et al, 2007). But, higher than that of 7-10 months cross-bred (Luxi×Limousin) beef cattle (MU A-Li, 2007) and 18 months cross-bred cattle (Bos indicus×Bos taurus) (Giri et al, 2000). However, similar to that of early lactating buffalo reported by WEN Qiu-Yan et al (2003). Maybe, the dietary factors play a main role on the effect of energy digestibility.

The average energy metabolizability in the two periods was 56.5%, which lower than that of crossbred meat sheep (YANG Zai-Bing et al, 2004), finished beef cattle in tie-condition (ZHANG Yu-Zhi et al, 2002), Hereford steers (BW=286±5 kg) (Chizzotti et al, 2007), and yak (DONG Quan-ming et al, 2008). But, similar to that of early lactating buffalo reported by WEN Qiu-Yan et al (2003), 7-10 months cross-bred (Luxi×Limousin) beef cattle (MU A-Li, 2007). However, higher than that of 18 months cross-bred cattle (Bos indicus×Bos taurus) (Giri et al, 2000). Similar to the energy digestibility, the energy metabolizability also relate to animal breed. And expect for animal species, the dietary and environment also play some role on the energy metabolizability. In current experiment, the ratio of forage to concentration was same; maybe this was why the energy metabolizability was similar.

The average ratio of ME to DE in the two periods was 83.2 %, lower than that of 8 months cross-bred cattle (Bos indicus×Bos taurus) (Giri et al, 2000), 7-10 months cross-bred (Luxi×Limousin) beef cattle (MU A-Li, 2007). But, which similar to the ratio of ME to DE (0.84) described in the Chinese Feeding Standard of dairy cattle (2000). However, higher than that of early lactating buffalo reported by WEN Qiu-Yan et al (2003). Generally speaking, the ratio of ME to DE was stable, could be used to express the energy metabolism characterization.

The percentage of energy loss

In present experiment, the total energy loss in feces, urine and methane add up to 42-45 %. The average methane energy loss was amount to 9.3 % of GEI., which higher than that of 7 % of GEI reported by Giri (2000). During rumen metabolism process, the dietary play a big role on the effect of methane energy loss, according to nutrition level, dietary composition and digestibility difference, the average methane energy loss add up to 2-15 % (Johnson,1995; Jentsch, 2007).

The average urinary energy was amount to 2.2 % of GEI. The results of Yang Zai-Bing (2000) showed that urinary energy had significant positive relation to nitrogen content in urine. So, maybe the nitrogen content in current urinary sample is constant, which make the average urinary energy was amount to 2.2 % of GEI.

Acknowledgements.
This work was supported by Guangxi Science Foundation (GuiKeQing 0640033, GuiKeQing 0832071, GuiKeQing 0832072, GuiKeZhuan 0718005-4A and GuiKeJi 0991018).

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Utilization of sugar beet-top ensiled with sugar cane bagass and sugar cane molasses in the diet of lactating buffalo

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Abstract

Sugar beet tops harvested and chopped into 3-6 cm, then mixed with sugar cane pulp and sugar cane molasses to provide a mixture contained 30-35 percent dry mater, then it was ensiled in common silo. In a latin square experiment with change over design, the prepared silage was included in the diet of lactating buffaloes where a control diet was compared with treatment diets contained 25 and 50% silage respectively. Results showed that the production of milk as crude milk yield, 3.5 percent fat corrected milk and 4% fat corrected milk were significantly ($p<0.05$) different between the treatments. The percentage of crude fat and the average amount of daily fat yield per animal were significantly ($p<0.05$) affected by the treatments. An increasing trend of milk and fat yield was observed when the animals received treatment diets but this increasing trend was statistically significant by inclusion of the silage (beet top-sugar cane pulp-sugar cane molasses silage) in amount of 50% of the diet dry mater basis.

Key words : Sugar beet top, silage, milking buffalo

INTRODUCTION

|Residues from plants and crops processing constitute a vast amount of organic material that is potentially energy rich sources and could be used as animal feed. The residues from sugar beet crop and sugar cane processing are such materials obtained in a considerable amount in Khuzestan province (southern Iran) where the agroecological conditions, are appropriate for cultivation of these crops. Anuualy about 70 and 10 thousands hectare are under farming of sugar cane and sugar beet crop respectively. Beside the main crop yield, the arail parts of sugar beet including leaves and crown are the by product yield about 20 to 40 tones (fresh basis) per hectare with conseederable amount of nutrients content (Pimlott., 1991; Raesyanzadeh et al., 1999).

Some works have been done in orderr to collect and preserve the sugar beet leaves and crowns (SBLC) and sugar cane pulp to find an appropriate techniques to use of these by-products as aimal feed. Using of moisture absorbents such as wheat straw and sugar cane pulp to reduce the moisture content of SBLC and providing an optimize mixture of roughage and beet tops, for ensiling have been reported (Fazaeli et al., 2006; Raesyanzadeh et al., 1999). These by products should be used to fed the animals that are able to utilize and digest them properly. Buffaloe is the most popular large ruminant adapted in Khuzestan area where could be a capable convertor of by the products to milk and meat.

Buffaloes are known to be more efficient in utilizing fiber component of the coarse feed and they thrive well on crop residues and agricultural by-products (Pannu et al., 2002). This makes buffaloes easy to maintain using locally available

roughages and crop residues. Punia and Sharma (1990) reported that feed degradability in the rumen was higher in buffaloes than in cattle. Singh et al. (2003) reported that digestibility and nitrogen balance were significantly higher in buffalo calves when compared with cattle calves. The ability of buffaloe to use the poor roughages and by products is due to the larger size of rumen, longer retention time of feed in the rumen and specific charachteristics of digestive physiology (Liang and Samiyah, 1988(.

In a study, Kordnegad et al. (2006) studied the silage characteristics of sugar beet top ensiled with different levels of sugar cane pulp and molasses and introduced an optimum mixture ratio as good quality silage.

The objective of this work was to study the milking response of Khouzestan regional buffalo (southern Iran) to the different levels of silage made from a mixture of sugarcane pulp and sugar beet top added with sugar cane molasses.

MATERIAL AND METHODS

Sugar beet top were collected, chopped and mixed with 5% sugar cane molasses and about 40% sugar cane baggase to provide a 35% DM content, then it was ensiled in to a deep pit silo. After two months of ensiling, the silo was oppened and used in the experimental ration of milking buffaloes.

In a 3×3 latin squre experiment with change over design, 15 milking buffloes were used in which three diets including : 1) control diet without silage, 2) diet contained 25% silage, 3) diet contained 50% silage were used in three periods of time, each with 20 days of adaptation and 10 days of recording and data collection.

Basal ration was consisted of wheat straw and alfalfa hay as roughage components, whereas barley grain, wheat bran and soya bean meal as concentrate part in an amount of 70% rouphage and 30% concentrate ratio, dry mater basis (table 1).

Table 1:Fees ingredients and composition of the experimental diets (DM basis)

Items	Diets #		
	I	II	III
Wheat straw	45	25	12
Alfalfa hay	25	17	8
Silage	0.0	25	50
Wheat bran	16.4	16.2	16
Ground barley	11	11	11
Soya been meal	2	2	2
Calcium carbonate	0.0	0.2	0.4
Supplement	0.6	0.6	0.6
Total %	**100**	**100**	**100**
Nutrient contents			
Metabolizable Energy (Mcal/kg)	2	2	2
Crude protein %	9.6	9.6	9.5
Calcium %	0.46	0.47	0.45
Phosphorous %	0.33	0.32	0.31

Diet I = Cotrol without silage II= 25% silage III= 50% silage

Concentrate ingredients were prepared and combined, then mixed with roughage and offered to the animals as total mixed ration (TMR) three times daily. After three weeks of adaptaion, milk yield was recorded for one week and sampled for analyses, during each change over periods of the experiment. Analysis of variance procedure of SAS (2001) was used to determine the effect of treatments on milk yield and composition, as well as the fat corrected milk and feed conversion ratio (FCR). The differences of means were performed using Duncan multiple range test.

RESULTS AND CONCLUSIONS

Results are presented in table 2. As it is shown in the table, the rough milk yield, based on the kg per animal per day, ranged 7.52 to 7.89 that were significantly different among the treatments ($p<0.05$). The fat corrected milk, based on the 4% fat (4FCM) were 8.96, 8.88 and 9.76 kg for treatments I, II and III respectively that was significantly ($p<0.05$) the highest in treatment III. In addition, the 3.5% fat corrected milk (3.5FCM) were 9.73, 9.63 and 10.59 kg respectively for treatments I, II and III respectivley that were significantly ($p<0.05$) different between the treatment III with the other treatments. In all parameters of milk yield recorded and estimated milk corrected, the highest amounts ($p<0.05$) were obtained when the buffaloes received diet contained 50 percent of experimental silage, but when the animals received diet contained 25% silage, showed similar performance with the control diet. In addition, the fat content percentage was significantly affected by the diets. The feed conversion ratio, based on the kg of dry mater intake per kg of 4FCM were significantly ($p<0.05$) reduced when the animals received 50% silage in diet.

Table 2: Effect of diets on the milk production performance

Items	Diets #			SEM ##
	I	II	III	
Milk Yield (kg/animal/day)	7.77 ±0.10 [ab]	7.52 ±0.13 [b]	7.89 ±0.4 [a]	0.10
4% FCM	8.96 ±0.20 [b]	8.88 ±0.17 [b]	9.76 ±0.20 [a]	0.17
3.5% FCM	9.73 ±0.21 [b]	9.63 ±0.18 [b]	10.59 ±0.21 [a]	0.16
Milk Fat %	5.02 ±0.3 [b]	5.24 ±0.15 [ab]	5.63 ±019 [a]	0.13
Fat (g/animal/day)	390.4 ± 11.7 [b]	391.4 ± 9.9 [b]	440.1 ± 12.8 [a]	10
FCR (based on 4FCM)	1.25 ±0.048 [a]	1.26 ±0.054 [a]	1.14 ±0.053 [b]	0.029

Diet I = Cotrol without silage II= 25% silage III= 50% silage
Standard error of mean
Means with different supperscriptions are significantly different ($p <0.05$)

In general, silage made from a mixture of suggar beet tops and sugar cane pulp with 5% molasses could be used as roughage portion of the diet in milking buffaloes. It provides more nutrients to improve the milk performance when it costitutes the major part of the roughage or 50% of the total diet dry mater comparing with the straw–alfalfa mixture as roughage source in buffaloe nutrition.

Acknowledgments.
This research was supported by Animal Science Research Institute of Iran. Th assistance of Agricultural Research Center of Khoozestan and Animal Husbandry Research Station of Dezfool is kindly acknowledged.

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