# Association of maximum voluntary dietary intake of freeze-dried garlic with Heinz body anemia in horses

Wendy Pearson, MSc; Herman J. Boermans, PhD; William J. Bettger, PhD; Brian W. McBride, PhD; Michael I. Lindinger, PhD

**Objective**—To characterize hematologic and clinical consequences of chronic dietary consumption of freeze-dried garlic at maximum voluntary intake in horses.

**Animals**—4 healthy sex- and age-matched horses.

**Procedure**—An initial garlic dose (0.05 g/kg, twice daily) was fed to 2 horses in a molasses carrier as part of their normal ration and was gradually increased to maximum voluntary intake (0.25 g/kg, twice daily) over 41 days. Dietary supplementation then continued for a total of 71 days. Two control horses were fed molasses with no garlic with their ration. Blood samples were collected weekly and analyzed for hematologic and biochemical changes, including the presence of Heinz bodies. Recovery of affected blood values was followed for 5 weeks after termination of dietary supplementation with garlic.

**Results**—At a daily dose of > 0.2 g/kg, horses fed garlic developed hematologic and biochemical indications of Heinz body anemia, as characterized by increases in Heinz body score (HBS), mean corpuscular volume (MCV), mean corpuscular hemoglobin, platelet count, and serum unconjugated and total bilirubin concentrations and decreases in RBC count, blood hemoglobin concentration, mean corpuscular hemoglobin concentration, and serum haptoglobin concentration. Recovery from anemia was largely complete within 5 weeks after termination of dietary supplementation with garlic. Heinz body score and MCV remained high at the end of the 5-week recovery period.

**Conclusions and Clinical Relevance**—Horses will voluntarily consume sufficient quantities of garlic to cause Heinz body anemia. The potential for garlic toxicosis exists when horses are chronically fed garlic. Further study is required to determine the safe dietary dose of garlic in horses. (*Am J Vet Res* 2005;66:457–465)

Garlic is among the most widely used medicinal plants in the world. It has a historical place in some of the oldest medical texts in human culture and has occupied the thoughts of some of the greatest historical figures of ancient and contemporary literature.[1] Widely divergent primeval cultures have come to similar conclusions regarding the benefits of garlic.[1] More recently, scientific and intellectual curiosity has substantiated many of the historical applications of garlic. The plant and its constituents have well-characterized antibacterial,[2,3] antiviral,[4,5] antiparasitic,[6,7] antifungal,[8,9] antithrombotic,[10,11] anticancer,[12,13] and hypoglycemic[14,15] properties.

The broad application of garlic to human health has led to increasing use of the plant and its constituents in veterinary industries. Its well-characterized antifungal properties make it a popular choice for preserving livestock feed to prevent mycotoxin contamination,[16] and it is a widely used flavoring agent in livestock and pet foods.[17] Currently, no recommendation exists by the Canadian Food Inspection Agency concerning maximum inclusion rate of garlic or garlic oil in livestock feed; products containing these ingredients are exempt from registration if they meet regulatory labeling standards.[17]

In the absence of regulatory control over garlic inclusion rates, there exists a potential for acute or chronic garlic intoxication, which has been described in the literature. Oxidation of RBCs has been reported for dogs[18,19] and sheep[20] consuming garlic and for dogs[21] and horses[22] consuming onions (*Allium cepa*). Other reported adverse effects of *Allium* compounds include gastric irritation when garlic powder is applied directly to gastric mucosa,[23] inhibition of cecal microflora by voluntary consumption of garlic extract,[24] contact dermatitis from topical exposure to fresh cloves,[25] occupational asthma,[26,27] decreased spermatogenesis following high-dose gavage of garlic powder,[28] and interactions with conventional drugs.[29]

It is generally agreed that organosulfur compounds provide the basis for the pharmacologic activity of garlic.[12,30,31] The intact bulb contains a complex mixture of cysteine sulfoxides (alliin, cycloalliin, methiin, and isoalliin, of which alliin is the most abundant) and γ-glutamylcysteines (γ-glutamyl-S-allylcysteine, γ-glutamyl-S-methylcysteine, and γ-glutamyl-S-t-propenylcysteine).[32] Upon mechanical disintegration of the bulb, alliin is cleaved by alliin lyase (alliinase), which is abundant in garlic, to the active thiosulfinate allicin (diallyl thiosulfinate). In vitro studies[11,33,34] have characterized the pharmacologic effects of allicin. However, the applicability of these results to the in vivo situation is unclear, as allicin is a highly unstable compound that is readily degraded by heat, organic solvents,[35] and amino acid residues (particularly cysteine) in blood.[30] Once absorbed into the circulation,

Received December 11, 2003.
Accepted June 19, 2004.
From the Departments of Animal and Poultry Science (Pearson, McBride), Biomedical Sciences (Boermans), and Human Biology and Nutritional Sciences (Bettger, Lindinger), University of Guelph, Guelph, ON, N1G 2W1 Canada.
Supported by the Ontario Horse Racing Industry Association, the Ontario Ministry of Agriculture and Food, and the Natural Sciences and Engineering Research Council of Canada.
Address correspondence to Dr. Lindinger.

allicin is rapidly metabolized to allyl mercaptan through a series of metabolites, including a mixture of monosulfides; diallyl-disulfide, -trisulfide, -tetrasulfide; and S-allylmercaptocysteine.[35,36] Allyl mercaptan appears to be further biotransformed to allyl methyl sulfoxide and allyl methyl sulfone, both of which are excreted via urine.[37]

Healthy horses typically do not have appreciable numbers of circulating RBCs with Heinz bodies.[38] Heinz bodies are a hallmark of oxidative stress on RBCs. During oxidative stress, hemoglobin within the RBC is reversibly oxidized to methemoglobin subsequent to an irreversible denaturation to sulfhemoglobin.[39] The insoluble Heinz bodies precipitate out of the cytosol, forming an extra- or intraerythrocytic projection at the RBC membrane. This projection is effectively removed from the RBC by the spleen, leaving the surviving RBC with a "bite" out of its membrane, which negatively affects its deformability.[40] Its fate, therefore, is to be either phagocytized by macrophages or removed from the circulation by the spleen. The mean life span of an equine RBC is approximately 155 days,[41] and a low incidence of Heinz bodies may have no effect on RBC count. However, as more cells are damaged and removed from circulation, RBC count inevitably decreases, which is concurrent with a decrease in Hct.

The purpose of the study reported here was to evaluate the safety of dietary supplementation with **freeze-dried garlic (FDG)** in horses. It was hypothesized that horses consuming FDG at a maximum amount of voluntary intake would not develop Heinz body anemia or other biochemical indicators of garlic toxicosis.

## Materials and Methods

**Animals**—Four healthy horses (1 Thoroughbred and 3 Standardbreds; 2 females and 2 geldings) participated in the study. Horses ranged in age from 2 to 12 years old and in body weight from 475 to 557 kg.

A minimum of 2 horses was required for the **garlic-treated (GT)** group to demonstrate significant changes in key hematologic and biochemical indicators. This number of horses was statistically determined by use of the power of analysis test to detect relatively large minimum differences between control and treatment means that could be indicative of toxic pathophysiologic effects of garlic consumption on key blood parameters (specifically, **Heinz body score [HBS]**, RBC count, Hct, and corpuscular hemoglobin concentration).[18] This was a requirement of the University of Guelph's Animal Care Committee, and the project was approved with the use of ≤ 2 treatment horses because of the expectation that high quantities of consumed garlic may have toxic effects. Horses were cared for and used with the approval of the University of Guelph's Animal Care Committee in accordance with the Guidelines of the Canadian Council on Animal Care.

Results of an initial blood test indicated that all 4 horses were selenium deficient (range, 0.09 to 0.13 mg/L; lower reference limit, 0.14 mg/L). All horses, GT and control, were injected IM with a vitamin E-selenium composite[a] (potassium selenate [1.5 mg/mL] and dL-α-tocopherol acetate [68 mg/mL] at a volume of 10 mL/horse) on day –27 (ie, 27 days before the beginning of dietary garlic supplementation [day 0]). Upon reanalysis 7 days later, serum selenium concentrations were within reference range.

All horses received a balanced ration that met their nutritional requirements (National Research Council, 1984), which consisted of 1.5-kg concentrate pellets[b] and a third of a bale of hay twice daily. The percent composition of the concentrate pellets was as follows: 41% grain corn, 28% barley, 28% oats, 1.6% premix, 0.5% dry molasses, 0.5% animal-vegetable blend, 0.2% trace mineral salt, 0.1% dicalcium phosphate, 0.05% vitamin E-selenium, and 0.05% magnesium oxide. Concentrate pellets contained added selenium (12 mg/kg) and vitamin E (4,000 U/kg). Trace minerals[c] (Mg, 120 mg/kg; Cu, 330 mg/kg; I, 70 mg/kg; and Co, 40 mg/kg in 95.6% NaCl) and water were provided ad libitum. Horses were turned out daily and housed indoors at night. The project began in October 2002 and terminated in March 2003, with estimated outdoor ambient temperatures ranging from 10° to –40°C. The barn was not heated, and horses were not blanketed while outdoors.

**Experimental design**—For both groups of horses, 3 baseline blood samples were taken by jugular vein puncture on days –41, –7, and 0 (beginning of dietary garlic supplementation). Thereafter, venous blood was collected weekly for the duration of the supplementation period. Blood samples were taken between 3 and 6 hours after providing the morning meal. A baseline value for body weight was calculated from 7 measurements taken during the month period immediately preceding day 0. Thereafter, body weights were recorded twice weekly. Dietary garlic supplementation was terminated on day 71. The recovery period began on day 72 and lasted through day 107.

Garlic-treated horses were fed FDG mixed with molasses into their concentrate pellet ration over a period of 71 days. Feed was provided individually within each horse's stall. Initially, top dressing of FDG (0.1 g/kg) resulted in complete feed refusal for both GT horses. Garlic was removed, and their original feed ration was fed for 1 week. Garlic supplementation then resumed with 0.05 g/kg provided twice daily. Garlic supplementation was increased once or twice weekly, at a rate that allowed for complete consumption of grain ration over a 24-hour period, until a maximum dose was reached that horses would consume (ie, maximum dose not associated with feed refusal). The supplementation schedule for increases in dietary consumption of FDG was as follows: 0.05, 0.075, 0.1, 0.15, 0.2, 0.25, 0.2, and 0.25 g/kg (initial body weight) 2 times daily beginning on days 1, 5, 10, 15, 17, 21, 27, and 41, respectively. Dietary supplementation of FDG continued until an attending veterinary toxicologist recommended termination on the basis of a predetermined minimum Hct of 20%.

**Garlic preparation**—Over a period of approximately 3 months, 230 kg of fresh garlic[d] was peeled, sliced,[e] and stored at –80°C. The frozen product was freeze-dried[f] and immediately grated[e] to a variable particle size (**Figure 1**). The ground FDG was stored in a 25-gallon plastic covered container until use. Garlic selenium content and dry matter were quantified at a commercial laboratory.[g]

**Allicin analysis**—A core sample of FDG and a sample of fresh garlic were analyzed for allicin.[h] Freeze-dried or fresh garlic samples were homogenized in water, centrifuged, and filtered, and the filtrate was analyzed for allicin by use of high-performance liquid chromatography coupled with UV detection. The analysis was performed isocratically with a mobile phase of water and methanol (50:50, vol/vol); absorbance was reported at 254 nm. Allicin standard[i] (3.92 mg/mL) stock solution was stored at –86°C. The peak areas of known amounts of allicin standard were measured, and a calibration curve was plotted against the amount of allicin. The calibration curve and peak areas were then used to determine the amount of allicin in the experimental samples.[35]

**Hematologic evaluation**—Jugular venous blood was collected into a sterile evacuated tube containing EDTA. Complete blood count determination was conducted.[j] Parameters quantified included WBC count; RBC count; hemoglobin; Hct; **mean corpuscular volume (MCV)**; **mean corpuscular hemoglobin (MCH)**; **mean corpuscular hemoglobin concentration (MCHC)**; red cell distribution width; platelet count; mean plasma volume; total serum protein concentration; and segmented neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts. White blood cell differentials were conducted manually, and morphologic data were determined manually from slides stained with a Wright stain. Methemoglobin was measured[k] at absorbances of 535, 560, 577, 622, 636, and 670 nm.

For determination of HBS, blood was collected in an evacuated tube containing EDTA from the jugular vein. A wet mount was prepared from 1 drop of blood from a capillary tube and was air-dried. An equal volume of brilliant cresyl blue stain was then added and allowed to set for 1 minute. Heinz bodies were identified as dark-blue staining structures on the periphery of RBCs under a light microscope at an objective power of 40× magnification. Heinz body prevalence was subjectively scored according to their prevalence (a score of 0 = no visible projections on smear, 1 = 1 or 2 projections/smear, 2 = 1 or 2 projections/ocular view, 3 = up to 10 projections/ocular view, 4 = approximately 75% of all RBCs with visible projections, and 5 = virtually all RBCs with visible projections).

**Biochemical analysis**—Venous blood was collected into a silicone-coated evacuated tube. An equine serum biochemical analysis was conducted.[l] Parameters quantified included calcium, phosphorus, magnesium, sodium, potassium, chloride, albumin, globulin, albumin-to-globulin ratio, urea creatinine, glucose, cholesterol, total bilirubin, conjugated bilirubin, unconjugated bilirubin, alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, creatine kinase, glutamyl dehydrogenase, haptoglobin, sodium-to-potassium ratio, and calculated osmolarity.

**Recovery period**—Beginning on day 72, horses were released to paddocks for 24-hour turnout and provided with free-choice hay and water. Blood was collected by jugular puncture every 7 days until blood parameters were within reference range.

**Statistical analysis**—Values are reported as means (± SE). Data were analyzed with a 2-way repeated-measures ANOVA to assess changes over time and between treatments. When a significant F ratio was obtained, the Bonferroni test was used to compare means. The pretreatment (baseline value) and treatment periods were separately assessed for time effects with a 1-way repeated-measures ANOVA. Data from the recovery period and day 71 of the treatment were also assessed for time effects with a 1-way repeated-measures ANOVA. Significance was accepted at values of $P \leq 0.05$ with a power of 0.8.

## Results

**Allicin analysis**—The content of allicin in fresh garlic was 3,192 µg/g, and the content of allicin in FDG was 9,200 µg/g **(Figure 2)**. The water content of fresh garlic and FDG was 62% and 6.3%, respectively. The selenium content of FDG was below the detection threshold of 60 ppm (µg/g).

**Garlic consumption**—On the basis of the results of FDG allicin analysis, GT horses received 460 µg of allicin/kg on day 1. This amount increased to a maximum of 2,300 µg of allicin/kg on day 21.

**Body weight**—The body weight of GT horses increased significantly from 552 ± 6 kg on day 0 to 574 ± 3 kg on day 49 and 572 ± 6 kg on day 52 **(Figure 3)**. No change in the body weight was found in control horses. Body weight was not measured during the recovery period because horses were out on pasture.

**Hematologic evaluation**—A significant increase in mean HBS was found in GT horses, from a baseline value of 0 ± 0 to 2.0 ± 0 on day 7. Mean HBS increased to 4 ± 0 on day 28, peaked at 5 ± 0 on day 59, and remained high through day 71, compared with the baseline value **(Figure 4)**. In control horses, the HBS varied from 0 to 2 with no significant change over time.

Red blood cell count decreased significantly in GT horses from $8.75 \pm 0.85 \times 10^{12}$ cells/L on day 0 to $6.0 \pm 0.2 \times 10^{12}$ cells/L on day 49 and remained low for the remainder of the supplementation period, reaching a minimum of $5.3 \pm 0.4 \times 10^{12}$ cells/L on day 62 **(Figure 5)**. The RBC count was below the reference limit of $6.9 \times 10^{12}$ cells/L from days 49 to 71. No changes were observed in the RBC count of control horses.

The Hct decreased significantly in GT horses from 36 ± 1% on day 0 to 28 ± 1% on day 49 and approximated the lower reference limit of 28% through day 68. The Hct of control horses did not change over time and was maintained within the reference range. Blood hemoglobin concentration decreased in GT horses from 141.5 ± 2.5 g/L on day 0 to 116.5 ± 13.5 g/L on day 35 and 102.0 ± 14.0 g/L on day 62; blood hemoglobin concentration was below the lower reference limit of 112.0 g/L from days 49 to 68. In control horses, hemoglobin remained unchanged and within the reference range. Methemoglobin concentrations remained unchanged in both groups with no difference between groups (GT horses, 0.204 ± 0.076 g/L; control horses, 0.342 g/L).

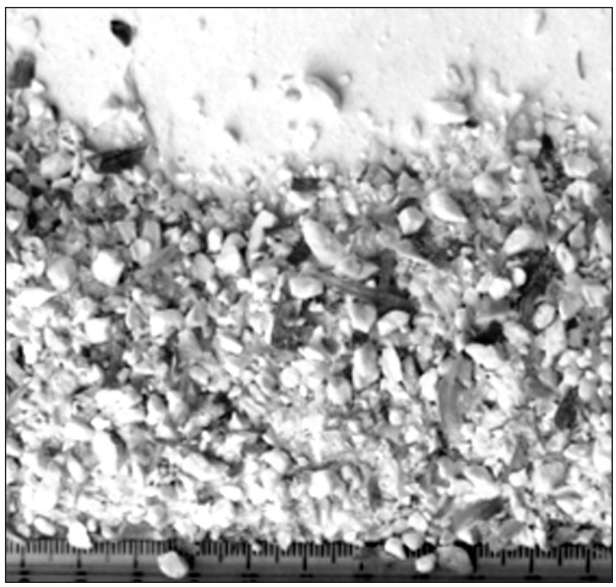

Figure 1—Photograph of a sample of the chopped freeze-dried garlic that was fed to horses after being mixed with molasses and added to the pelleted feed.

The MCH increased significantly in GT horses from 16.0 ± 2.0 pg on day 0 to 19.0 ± 1.0 pg on day 59 and 19.5 ± 0.5 pg on days 68 and 71; the MCH in GT horses was greater than the upper reference limit of 18.0 pg from days 59 to 71. The MCHC of GT horses decreased from 391 ± 0.0 g/L on day 0 to 372 ± 2.0 g/L on day 28. The MCHC of GT horses reached a minimum of 368.5 ± 2.5 g/L on day 49, which approximated the lower reference limit of 369 g/L. In control horses, values of MCH and MCHC remained unchanged and within the reference ranges.

The MCV increased significantly in GT horses from 42.0 ± 5.0 **femtoliters (fL)** on day 0 to 47.0 ± 4.0 fL on day 49 and reached peak values of 51.0 ± 3.0 fL on days 68 and 71; the upper reference limit of 45.0 fL was exceeded from days 42 to 71. In control horses, MCV remained unchanged and within the reference range.

Platelet count increased significantly in GT horses from 172 × $10^9$ platelets/L on day 0 to 216 ± 21 × $10^9$ platelets/L on day 49, peaking at 245 ± 30 × $10^9$ platelets/L on day 55; platelet counts remained within the reference range through day 71. No change in platelet count was found in control horses. The mean plasma volume of GT horses increased significantly from 5.50 ± 0.20 fL on day 0 to 6.25 ± 0.05 fL on day 59 and 6.55 ± 0.25 fL on day 62. In control horses, mean plasma volume was also increased from 5.95 ± 0.14 fL on day 0 to 7.30 ± 0.40 fL on day 62.

In both groups of horses, no differences were found between treatments or changes over time in total WBC count and cell counts of basophils, monocytes, segmented neutrophils, or eosinophils. Lymphocyte count decreased significantly in GT horses from 5.31 ± 0.29 × $10^9$ cells/L on day 0 to 3.10 ± 0.195 × $10^9$ cells/L on day 59 and 3.15 ± 0.23 × $10^9$ cells/L on day 71; day 0 lymphocyte counts for GT horses were greater than the upper reference limit of 4.7 × $10^9$ cells/L. No changes in lymphocyte counts were found in control horses. Morphologic features of RBCs from both GT and control horses included anisocytosis, rouleaux formation, and occasional crenation, with no discernible difference between treatments and over time.

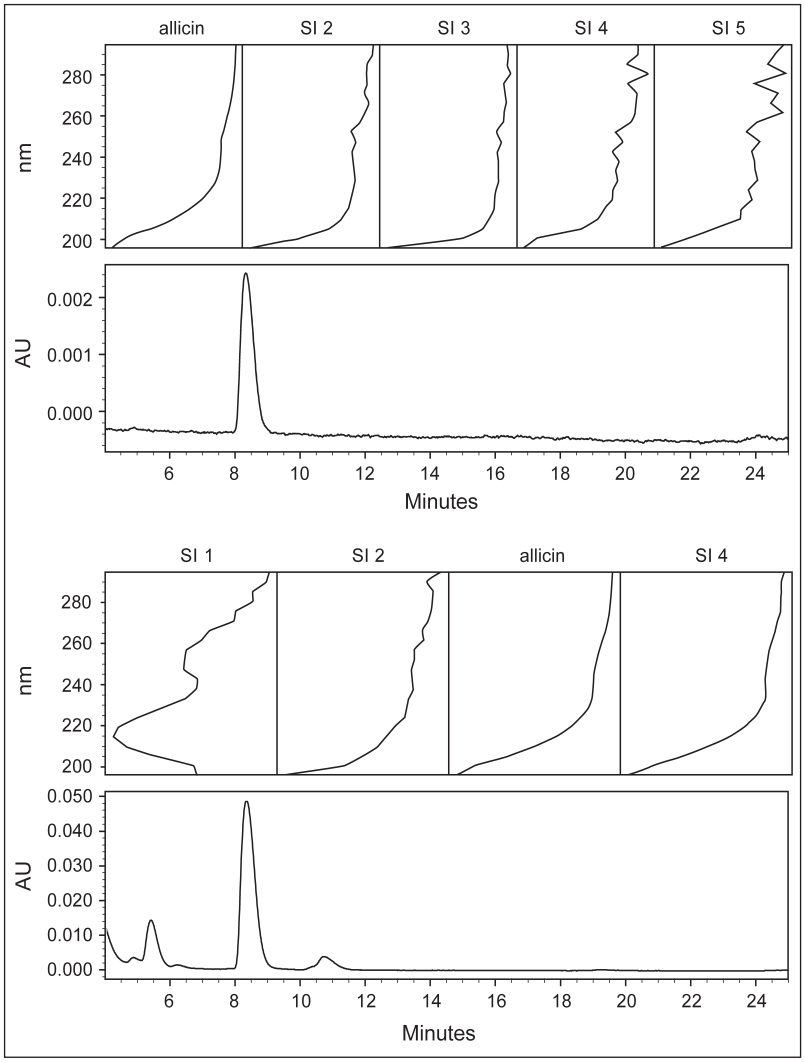


Figure 2—Top panel—Chromatogram of an allicin standard obtained by use of high-performance liquid chromatography coupled with UV detection. Bottom panel—Chromatogram of an allicin peak obtained from a sample of freeze-dried garlic. SI = Spectrum index. nm = Nanometers. AU = Arbitrary units.

**Biochemical analysis**—In GT horses, serum unconjugated bilirubin concentration increased significantly from a baseline value of 16.5 ± 3.5 µmol/L to 60.5 ± 15.5 µmol/L on day 29, peaking at 72.0 ± 28.0 µmol/L on day 55. Serum unconjugated bilirubin concentration remained greater than the upper reference limit of 55 µmol/L from days 29 to 71. Serum total bilirubin concentration increased similarly in GT horses from a baseline value of 19.5 ± 3.5 µmol/L to 63.5 ± 15.5 µmol/L on day 28 and peaked at 76.5 ± 28.5 µmol/L on day 55. Serum total bilirubin concentration was greater than the upper reference limit of 57 µmol/L from days 28 to 71. Serum conjugated bilirubin concentration was not increased and remained unchanged in GT horses, compared with baseline values. In control horses, no changes were found in serum unconjugated, total, and conjugated bilirubin concentrations.

In control horses, serum cholesterol concentration increased significantly from 2.16 ± 0.05 mmol/L on day 0 to 2.60 ± 0.03 mmol/L on day 49, remaining increased (although within the reference range) through day 71. In contrast, serum cholesterol remained unchanged in GT horses.

Serum creatinine concentration decreased significantly in GT horses from 85.0 ± 0.0 µmol/L on day 0 to 67.5 ± 2.5 µmol/L on day 68, with values below the lower reference limit of 80 µmol/L from days 55 to 71. Serum creatinine concentration remained

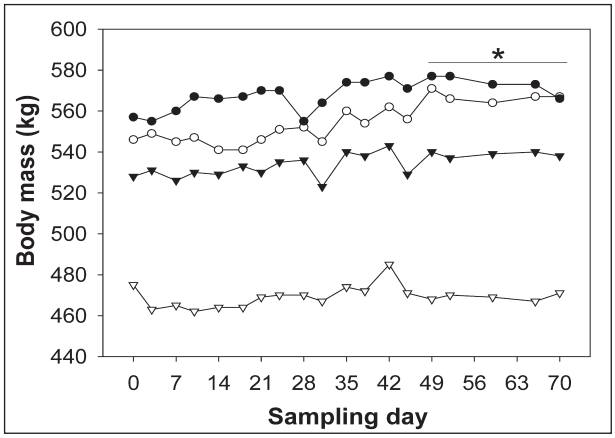


Figure 3—Body weight (mass) versus day of sample collection during dietary supplementation with increasing amounts of garlic (days 0 to 71) in garlic-treated (GT) horses (circles) and control horses (triangles). *Significant ($P < 0.05$) increase in body mass of GT horses, compared with day 0 and with control horses.

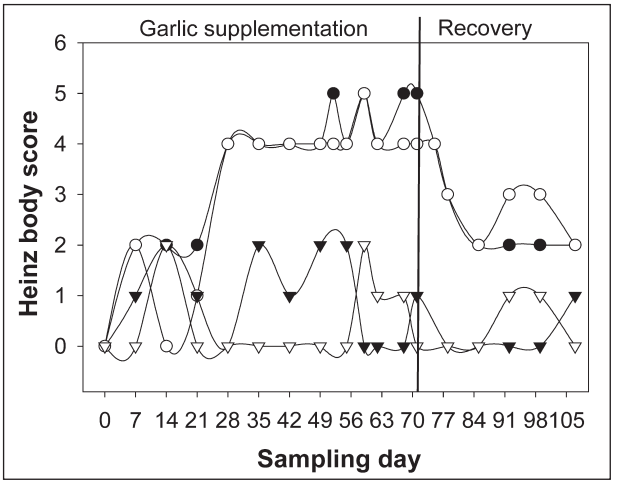


Figure 4—Heinz body score versus day of sample collection during dietary supplementation with increasing amounts of garlic (days 0 to 71) and following termination of garlic supplementation (horizontal line; days 72 to 107) in GT horses (circles) and control horses (triangles).

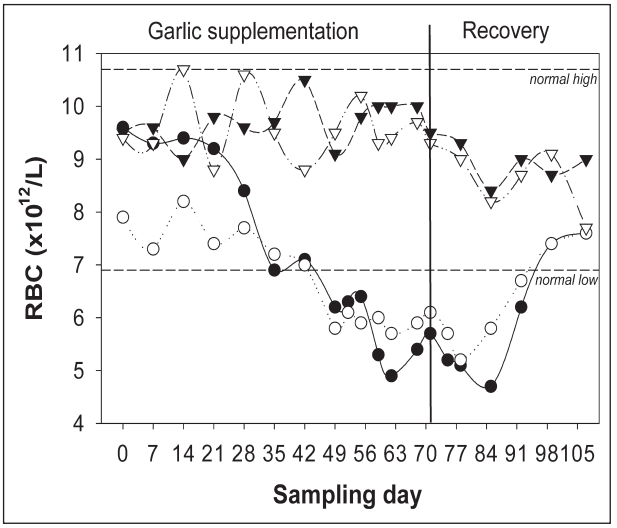


Figure 5—Red blood cell count versus day of sample collection during dietary supplementation with increasing amounts of garlic (days 0 to 71) and following termination of garlic supplementation (horizontal line; days 72 to 107) in GT horses (circles) and control horses (triangles).

unchanged in control horses. Serum creatine kinase activities were unchanged in both groups.

Serum glutamyl dehydrogenase activity decreased significantly in GT horses from 2.0 ± 0.0 U/L on day 0 to undetectable activity by day 68. No change in serum glutamyl dehydrogenase activity occurred in control horses. Serum aspartate aminotransferase, alkaline phosphatase, and γ-glutamyltransferase activities remained unchanged in both groups.

Compared with the baseline value, serum haptoglobin concentration decreased significantly in GT horses from 1.0 ± 0.0 g/L to 0.56 ± 0.07 g/L on day 21 and remained significantly decreased through day 71, with undetectable concentrations on days 49, 55, 68, and 71. In control horses, serum haptoglobin concentration remained unchanged at 1.01 ± 0.04 g/L.

Serum sodium concentration decreased significantly in GT horses from a baseline value of 140.5 ± 0.5 mmol/L to 135.5 ± 1.5 mmol/L on day 21, 130.5 ± 0.5 mmol/L on day 35, and 134.5 ± 0.5 mmol/L on day 42. In GT horses, serum chloride concentration decreased from a baseline value of 101.0 ± 0 mmol/L to 93.5 ± 1.5 mmol/L on day 68. Similar decreases in serum sodium and chloride concentrations occurred in control horses. Serum potassium concentration was not different between groups and remained unchanged over time. Serum osmolarity decreased significantly in GT horses from a baseline value of 281.0 ± 2.0 mmol/L to 271.5 ± 3.5 mmol/L on day 21 and 260.5 ± 2.5 mmol/L on day 35; serum osmolarity partially recovered to 268.0 ± 1.0 mmol/L on day 42. In control horses, serum osmolarity had a similar time course of changes. In both groups, no changes were found in total serum protein, albumin, globulin, or BUN concentrations; no differences in these values were found between groups.

**Sweating**—Both GT horses had a profuse idiopathic diaphoretic response. The first horse began to sweat on day 42, followed 7 days later by the second GT horse. Sweating in both horses persisted until removal of garlic supplements. No sweating was observed in control horses.

**Recovery after supplementation**—Parameters that were significantly increased in GT horses on day 71, compared with baseline values and values of control horses, included HBS, MCV, MCH, platelet count, and serum unconjugated bilirubin and total bilirubin concentrations. Parameters that significantly decreased in GT horses at day 71, compared with baseline values and values of control horses, included RBC count, blood hemoglobin concentration, MCHC, and serum haptoglobin concentration.

Upon removal of dietary garlic (day 72), platelet count and MCHC recovered rapidly and were similar to baseline values by day 78. Most biochemical parameters recovered within 14 days (day 85), including serum-free and total bilirubin, and haptoglobin concentrations. Blood hemoglobin concentration recovered within 21 days (day 92), and RBC count and MCH

recovered within 28 days (day 99). The HBS and MCV remained high within 35 days (day 107).

## Discussion

Our study is the first to report the time course and magnitude of hematologic and serum biochemical responses to chronic consumption of dietary FDG in horses. The primary purpose of our study was to determine whether the amount of garlic that horses would voluntarily consume over the long-term caused toxic effects; the purpose was not to determine whether garlic was safe for horses. A secondary purpose was to determine the maximum daily amount that horses would voluntarily consume. Biochemical and hematologic findings that were indicative of garlic toxicosis were evident 4 days after consuming 0.20 g of FDG/kg twice daily. As a result of the increasing amount consumed over time in our study, a safe dose for dietary consumption of garlic cannot be determined, although it is certainly < 0.4 g of FDG/kg daily. It is unlikely that horses would normally ingest toxic quantities of garlic on a daily basis. However, the potential for garlic toxicosis is present when FDG is fed to horses.

Serum biochemical and hematologic responses to increased consumption of FDG consistent with Heinz body anemia included the presence of Heinz bodies; decreases in Hct, RBC count, and blood hemoglobin concentration; and an increase in MCV. Others have reported a decrease in RBC count[18,42,43] and Hct[20] and the presence of Heinz bodies[18,19,44] in vivo and in cells exposed to garlic in vitro. An increase in MCV is also a consistent finding in anemic horses.[45-47] Increases in MCV reflect increases in the population of larger-volume, newly produced RBCs, which is a response consistent with the assumed increase in degradation of RBCs that had Heinz bodies.

Dogs consuming 5 g of fresh garlic/kg also had an increase in oxidation of hemoglobin within RBCs and a decrease in total hemoglobin concentration,[18] similar to that observed in GT horses of our study. Exposure of RBCs to garlic in vitro had similar responses.[19] Decreases in blood hemoglobin and serum haptoglobin concentrations, as occurred in GT horses, may result in tissue hypoxia. Serum concentrations of haptoglobins (serum proteins that bind and transport oxygen) decrease to undetectable amounts in anemic horses.[46] Ensuing tissue hypoxia is a stimulus for erythropoiesis,[47] initiating repletion of RBCs by large, immature RBCs, as evidenced in our study by the increase in MCV. These large, immature RBCs have increased amounts of hemoglobin,[48] and this contributed to the increase in MCH observed in GT horses. The responses that we observed are not consistent with a simultaneous increase in RBC degradation and a garlic-induced decrease in erythropoiesis. Increases in MCV and MCH support an interpretation of increased erythropoiesis. Therefore, although total hemoglobin concentration decreased as a result of fewer RBCs per volume of blood, the concentration of hemoglobin per cell was increased. Furthermore, Hct did not decrease at the same rate as the decrease in RBC count because the immature RBCs are larger than the aged and damaged RBCs removed by the spleen; this partially compensates for the reduction in absolute cell numbers. The decrease in blood hemoglobin concentration was greater than the decrease in Hct, and this accounted for the observed decrease in MCHC.

The toxic effect observed in horses consuming garlic in our study was caused, at least in part, by oxidative damage of RBCs. This contrasts with documented antioxidant properties of garlic.[49,50] However, some garlic-derived phytochemicals have oxidant effects at high concentrations.[49,51,52] Vitamin E, a well-known reducing agent, similarly has an oxidizing effect[53] in high concentrations. The ability of garlic-derived phytochemicals to lower the intracellular concentrations of reduced glutathione[33] may increase RBC susceptibility to oxidative damage. Glutathione stabilizes exposed protein sulfhydryl groups, particularly those of sulfhydryl enzymes and hemoglobin, thereby helping to maintain the functionality of these proteins.

In an oxidative crisis, RBCs have a shortened life span and an accelerated rate of breakdown by the spleen and macrophages,[41] resulting in increases in serum concentrations of iron, globin, and unconjugated bilirubin.[41] Unconjugated bilirubin combines with albumin in plasma and is transported to the liver, where it is conjugated and excreted via the biliary system. This leads to increases in serum unconjugated and total bilirubin concentrations,[41] as observed in our study. These results contrast to those observed in rats fed garlic (50 mg/kg) for 28 days.[54] Decreases in serum creatinine concentration, as observed in GT horses of our study, are also associated with anemia in humans.[55] In GT horses, no evidence was found indicating that chronic consumption of large amounts of FDG caused liver damage because serum liver enzyme activities (glutamyl dehydrogenase and aspartate aminotransferase) did not increase and glutamyl dehydrogenase activity decreased (in contrast to previously reported increase in enzyme activity for anemic horses[56]).

When selenium is abundant in soil, garlic is a major accumulator of organic selenium as a result of its ability to incorporate selenium into plant protein.[57] Selenium is an integral component of several isoforms of glutathione peroxidase, a family of phase II detoxification enzymes that use glutathione as a reducing substrate.[58] A diet high in selenium results in increased activity of these reduction-oxidation cycling enzymes[59,60] that protect cell membranes and tissues from oxidative damage. When in excess, however, selenium may result in chronic selenosis characterized by anemia, emaciation, loss of hair, hoof lesions, and lameness.[61] It is unlikely that chronic selenosis contributed to the erythrocytic oxidative damage observed in our study. The low concentration of selenium found in our garlic sample (< 60 μg/g) is consistent with the paucity of the mineral in soils where the garlic was grown. The maximum amount of selenium that was ingested daily by GT horses was 0.033 mg/kg from the concentrate feed and 0.003 mg/kg from the garlic (on the basis of 60 ppm), for a total daily intake of approximately 0.036 mg/kg. Chronic selenosis occurs at dietary inclusion rates of 5 to 40 mg/kg in horses,[61] which is far in excess of that fed in our study.

Little information exists in the literature regarding the median lethal dose for allicin, so it is difficult to ascertain the proximity of the amount of allicin consumed by GT horses in our study to a median lethal dose for horses. A 4-day median lethal dose of approximately 165 mg of allicin/kg for mice was calculated from the data of Chowdhury et al.[62] In our study, the maximum daily allicin ingestion was 2.3 mg/kg, which is 72 times lower than the 4-day median lethal dose in mice. Because our study was long-term, whereas that of Chowdhury et al[62] was acute, it appears reasonable that continued consumption of 2.3 mg of allicin/kg/d could result in death.

Dietary garlic supplementation is widely used in humans for reduction and prevention of hypercholesterolemia, a major risk factor for atherosclerosis. Increases in dietary garlic have been associated with effects on lipogenic enzyme activities that reduce serum cholesterol concentration and oxidize low-density lipoprotein.[52,63] In our study, control horses had a 20% increase in serum cholesterol concentration, whereas GT horses had no increase. Mechanistically, increases in dietary garlic appear to result in a down-regulation of gene expression and activity of the lipogenic enzyme glucose-6-phosphate dehydrogenase.[64,65] Glucose-6-phosphate dehydrogenase, the rate-limiting pentose phosphate pathway enzyme, produces nicotinamide adenine dinucleotide phosphate for reductive biosynthesis and ribose-5-phosphate for nucleotide synthesis.[66] Within RBCs, nicotinamide adenine dinucleotide phosphate reduces the disulfide form of glutathione to the sulfhydryl form,[38,67] which serves as a sulfhydryl buffer that maintains cysteine residues in hemoglobin and other RBC proteins in the reduced state. In many species, including horses, RBCs lack mitochondria so the pentose phosphate pathway is the RBCs' only source of nicotinamide adenine dinucleotide phosphate.[38,47] When a decrease in glucose-6-phosphate dehydrogenase activity occurs, hemoglobin is increasingly susceptible to oxidation and precipitation into Heinz bodies. This phenomenon is well characterized in congenital glucose-6-phosphate dehydrogenase deficiency, which causes hemolytic anemia in humans,[68] dogs,[69] cattle,[70] and horses.[38] In most species studied, RBCs rely on the pentose phosphate pathway for approximately 5% of the glucose metabolism requirements.[47] Horses, however, are unique in that their reliance on the pentose-phosphate pathway to provide glucose exceeds 12%,[47,71] suggesting that horses may be particularly susceptible to changes in the activity of glucose-6-phosphate dehydrogenase.

The pronounced diaphoretic response of GT horses in our study may be explained by the effect of garlic on catecholamines. Horses express a biphasic sweating response to $\beta_2$-adrenergic stimulation. A positive correlation exists between sweating and plasma epinephrine up to an infusion rate of 0.075 µg of epinephrine•$kg^{-1}$•$min^{-1}$, beyond which the sweating response declines.[72] Allyl-containing sulfides from garlic have been shown to increase plasma epinephrine, norepinephrine, and uncoupling protein, resulting in enhanced thermogenesis[73]; this provides some basis for the sweating response observed in our study.

The increase in body weight observed in GT horses can be explained by the fact that diets in control and GT horses were not isocaloric, and by day 49, GT horses were consuming an additional 0.250 g/kg twice daily in their feed. In addition, one of the GT horses was a 2-year-old female that was in a growth phase of development.

It is unlikely that the changes observed in electrolyte and fluid balance parameters were associated with garlic supplementation, as similar changes were observed in control horses. It is possible that these changes occurred as a result of variations in the time of day that blood samples were taken or environmental conditions that may have caused horses to consume less water.

In our study, recovery from garlic-induced Heinz body anemia was nearly complete within 4 to 5 weeks after removal of dietary garlic supplementation. Key indicators of anemia in GT horses (RBC count and hemoglobin, haptoglobin, and bilirubin concentrations) returned to baseline values within 4 to 5 weeks, and HBS continued to decline. These findings indicate that oxidative damage was not perpetuating and that correction of pathophysiologic events occurred. The presence of some Heinz bodies at 5 weeks of recovery reflects the irreversibility of hemoglobin precipitation reaction and the persistence of damaged RBCs within the circulation for > 4 months.[54] Similarly, the persistence of high values for MCV, although declining upon garlic removal, reflects the long life span of RBCs.

In summary, horses consuming increasing amounts of FDG had hematologic and biochemical findings that were indicative of Heinz body anemia. Increases in HBS, MCV, MCH, platelet count, and serum-free and total bilirubin concentrations and decreases in RBC count, blood hemoglobin concentration, MCHC, and serum haptoglobin concentration characterized the condition. Recovery from the anemic episode was largely complete within 5 weeks after removal of garlic supplementation. The potential for garlic toxicosis is present when horses are chronically fed garlic. Further research is needed to determine the chronic dose at which FDG may be safely consumed by horses and whether this dose is associated with beneficial effects.

a. Dystosel, Intervet, Whitby, ON, Canada.
b. Arkell Feed Mill, Guelph, ON, Canada.
c. Land O'Lakes, Madison, Wis.
d. Freeman Farms, Meaford, ON, Canada.
e. Hobart FP-100C food processor, Hobart Corp, Troy, Ohio.
f. Dura-Dry MP, FTS Systems, Stone Ridge, NY.
g. Purina Laboratories, Strathroy, ON, Canada.
h. Laboratory Services Division, University of Guelph, Guelph, ON, Canada.
i. Gift from Dr. Larry Lawson at Murdock Madaus Schwabe, Springville, Utah.
j. Advia 120, Bayer Corp, Etobicoke, ON, Canada.
k. OSM 3 hemoximeter, Radiometer, Westlake, Ohio.
l. Hitachi 911 biochemical analyzer, Boehringer Mannheim, Laval, QC, Canada.

## References

1. Rivlin RS. Historical perspectives on the use of garlic. *J Nutr* 2001;131:951S–954S.

2. Cellini L, Di Campli E, Masulli M, et al. Inhibition of *Helicobater pylori* by garlic extract (*Allium sativum*). *FEMS Immunol Med Microbiol* 1996;13:273–277.

3. Sivam GP. Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr* 2001;131:1106S–1108S.

4. Weber ND, Andersen DO, North JA, et al. In vitro virucidal effects of *Allium sativum* (Garlic) extract and compunds. *Planta Med* 1992;58:417–423.

5. See D, Gurnee K, LeClair M. An *in vitro* screening study of 196 natural products for toxicity and efficacy. *JANA* 1999;2:25–39.

6. Nok AJ, Williams S, Chidozie Onyenekwe P. *Allium sativum*-induced death of African trypanosomes. *Parasitol Res* 1996;82:634–637.

7. Soffar SA, Mokhtar GM. Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in *Hymenolepiasis nana* and *Giardia*. *J Egypt Soc Parasitol* 1991;21:497–502.

8. Davis LE, Shen J-K, Cai Y. Antifungal activity in human cerebrospinal fluid and plasma after intravenous administration of *Allium sativum*. *Antimicrob Agents Chemother* 1990;34:651–653.

9. Venugopal PV, Venugopal TV. Antidermatophytic activity of garlic (*Allium sativum*) *in vitro*. *Int J Dermatol* 1995;34:278–279.

10. Bordia T, Mohammed N, Thomson M, et al. An evaluation of garlic and onion as antithrombotic agents. *Prostaglandins Leukot Essent Fatty Acids* 1996;54:183–186.

11. Briggs WH, Xiao H, Parkin KL, et al. Differential inhibition of human platelet aggregation by selected *Allium* thiosulfinates. *J Agric Food Chem* 2000;48:5731–5735.

12. Singh SV, Mohan RR, Agarwal R, et al. Novel anti-carcinogenic activity of an organosulfide from garlic: inhibition of H-RAS oncogene transformed tumor growth *in vivo* by diallyl disulfide is associated with inhibition of p21H-ras processing. *Biochem Biophys Res Commun* 1996;225:660–665.

13. Fleischauer AT, Arab L. Garlic and cancer: a critical review of the epidemiological literature. *J Nutr* 2001;131:1032S–1040S.

14. Tahiliani P, Kar A. Mitigation of thyroxine-induced hyperglycemia by two plant extracts. *Phytother Res* 2003;17:294–296.

15. Bever BO, Zahnd GR. Plants with oral hypoglycaemic action. *Q J Crude Drug Res* 1979;17:139–196.

16. Sutabhaha S, Suttajit M, Niyomca P. Studies of aflatoxins in Chiang Mai, Thailand. *Q J Crude Drug Res* 1992;65:45–52.

17. Canadian Food Inspection Agency Web site. Approved feed ingredients. Schedule V, Part 1. Garlic, oil (CAS #8000-78-0). Available at: www.inspection.gc.ca/english/anima/feebet ched5/schvige.shtml. Accessed Jul 17, 2003.

18. Lee K-W, Yamato O, Tajima M, et al. Hematologic changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs. *Am J Vet Res* 2000;61:1446–1450.

19. Hu Q, Yang Q, Yamato O, et al. Isolation and identification of organosulfur compounds oxidizing canine erythrocytes from garlic (*Allium sativum*). *J Agric Food Chem* 2002;50:1059–1062.

20. Stevens H. Suspected wild garlic poisoning in sheep. *Vet Rec* 1984;115:363.

21. Stallbaumer M. Onion poisoning in a dog. *Vet Rec* 1981;108:523–524.

22. Pierce KR, Joyce JR, England RB, et al. Acute hemolytic anemia caused by wild onion poisoning in horses. *J Am Vet Med Assoc* 1972;160:323–327.

23. Hoshino T, Kashimoto N, Kasuga S. Effects of garlic preparations of the gastrointestinal mucosa. *J Nutr* 2001;131:1109S–1113S.

24. Shashikanth DN, Basappa SC, Sreenivasa Murthy V. Effect of feeding raw and boiled garlic (*A. sativum* L) extracts on the growth, caecal microflora and serum proteins of albino rats. *Nutr Rep Int* 1986;33:313–319.

25. Eming SA, Piontek J-O, Hunzelmann N, et al. Severe contact dermatitis caused by garlic. *Br J Dermatol* 1999;141:391–392.

26. Falleroni AE, Zeiss CR, Levitz D. Occupational asthma secondary to inhalation of garlic dust. *J Allergy Clin Immunol* 1981;68:156–160.

27. Lybarger JA, Gallagher JS, Pulver DW, et al. Occupational asthma induced by inhalation and ingestion of garlic. *J Allergy Clin Immunol* 1982;69:448–454.

28. Dixit VP, Joshi S. Effects of chronic administration of garlic (*Allium sativum* Linn) on testicular function. *Indian J Exp Biol* 1982;20:534–536.

29. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: a systematic review. *Drugs* 2001;61:2163–2175.

30. Lawson LD, Wang ZJ. Pre-hepatic fate of the organosulfur compounds derived from garlic (*Allium sativum*). *Planta Med* 1993;59:A688–A689.

31. Munday R, Munday CM. Relative activities of organosulfur compounds derived from onions and garlic in increasing tissue activities of quinone reductase and glutathione transferase in rat tissues. *Nutr Cancer* 2001;40:205–210.

32. Lawson LD. In: Koch HP, Lawson LD, eds. *Garlic: the science and therapeutic application of* Allium sativum *L. and related species*. Baltimore: The Williams & Wilkins Co, 1996.

33. Hirsch K, Danilenko M, Giat J, et al. Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutr Cancer* 2000;38:245–254.

34. O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. *Appl Environ Microbiol* 2000;66:2269–2273.

35. Lawson LD, Wang ZJ, Hughes BG. Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfinates in commercial garlic products. *Planta Med* 1991;57:363–370.

36. Egen-Schwind C, Eckard R, Kemper FH. Metabolism of garlic constituents in the isolated perfused rat liver. *Planta Med* 1992;58:301–305.

37. Germain E, Auger J, Ginies C, et al. In vivo metabolism of diallyl disulfide in the rat: identification of two new metabolites. *Xenobiotica* 2002;32:1127–1138.

38. Stockham SL, Harvey JW, Kinden DA. Equine glucose-6-phosphate dehydrogenase deficiency. *Vet Pathol* 1994;31:518–527.

39. Ruwende C, Hill A. Glucose-6-phosphate dehydrogenase deficiency and malaria. *J Mol Med* 1998;76:581–588.

40. Klaausen CD. Toxic responses of the blood. In: Klaassen CD, ed. *Casarett and Doull's toxicology: the basic science of poisons*. 6th ed. New York: McGraw-Hill Book Co, 2001;397.

41. Lumsden JH, Valli VE, McSherry BJ, et al. The kinetics of hematopoiesis in the light horse. III. The hematological response to hemolytic anemia. *Can J Comp Med* 1975;39:332–339.

42. Al-Berkairi A, Sah AH, Qureshi S. Effect of *Allium sativum* on epididymal spermatozoa, estradiol-treated mice and general toxicity. *J Ethnopharmacol* 1990;29:117–125.

43. Allen B, Archer RK. Haptoglobins in the horse. *Vet Rec* 1971;89:106–109.

44. Nakagawa S, Masamoto K, Sumiyoshi H, et al. Effect of raw and extracted-aged garlic juice on growth of young rats and their organs after peroral administration. *J Toxicol Sci* 1980;5:91–112.

45. Mair TS, Taylor FG, Hillyer MH. Autoimmune haemolytic anaemia in eight horses. *Vet Rec* 1990;126:51–53.

46. Easley JR. Erythrogram and erythrocyte distribution width of Equidae with experimentally induced anemia. *Am J Vet Res* 1985;46:2378–2384.

47. Kaneko JJ. Comparative erythrocyte metabolism. *Adv Vet Sci Comp Med* 1974;18:117–153.

48. Simmons A. *Hematology: a combined rheoretical and technical approach*. Philadelphia: WB Saunders Co, 1989.

49. Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. *Phytother Res* 2003;17:97–106.

50. Hwang S-W, Chan K-C. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. *J Agric Food Chem* 2002;50:6143–6147.

51. Gallwitz H, Bonse S, Martinez-Cruz A, et al. Ajoene is an inhibitor and subversive substrate of human glutathione reductase and *Trypanosoma cruzi* trypanothione reductase: crystallographic, kinetic, and spectroscopic studies. *J Med Chem* 1999;42:364–372.

52. Lau BHS. Suppression of LDL oxidation by garlic. *J Nutr* 2001;131:958S–988S.

53. Eder K, Flader D, Hirsche F, et al. Excess dietary vitamin E lowers the activities of antioxidative enzymes in erythrocytes of rats fed salmon oil. *J Nutr* 2002;132:3400–3404.

54. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci Rep* 2001;21:19–24.

55. Zadrazil J, Bachleda P, Krejci K. Hematologic findings and value of value of endogenous creatinine in patients following

kidney transplantation. *Acta Univ Palacki Olomuc Fac Med* 1999; 142:103–106.

56. Palomba E, Martone F, Meduri A, et al. Biochemical studies on equine infectious anaemia. *Folia Vet Lat* 1976; 6:275–288.

57. Whanger PD. Selenocompounds in plants and animals and their biological significance. *J Am Coll Nutr* 2002;21:223–232.

58. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2000;57:1825–1835.

59. Kim YY, Mahan DC. Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing-finishing pigs. *J Anim Sci* 2001;79:942–948.

60. Hoffman DJ. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat Toxicol* 2002;157:11–26.

61. Mihajlovic M. Selenium toxicity in domestic animals. *Glas Srp Akad Nauka (Med)* 1992;42:131–144.

62. Chowdhury AK, Ahsan M, Islam SN, et al. Efficacy of aqueous extract of garlic & allicin in experimental shigellosis in rabbits. Indian *J Med Res* 1991;93:33-36.

63. Liu L, Yeh Y-Y. Water soluble organosulfur compounds of garlic inhibit fatty acid and triglyceride synthesis in cultured rat hepatocytes. *Lipids* 2001;36:395-400.

64. Ide T, Ashakumary L, Takahashi Y, et al. Sesamin, a sesame lignan, decreases fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1. Biochim Biophys Acta 2001;1534:1–13.

65. Fields M, Lewis CG, Lure MD. Garlic oil extract ameliorates the severity of copper deficiency. *J Am Coll Nutr* 1992;11:334–339.

66. Wood T. Distribution of the pentose phosphate pathway in living organisms. *Cell Biochem Funct* 1986;4:235–240.

67. Coetzer T, Zail S. Membrane protein complexes in GSH-depleted erythrocytes. *Blood* 1980;56:159–167.

68. Shannon K, Buchanan R. Severe hemolytic anemia in black children with glucose-6-phosphate dehydrogenase deficiency. *Pediatrics* 1982;70:364–369.

69. Smith JE, Ryer K, Wallace L. Glucose-6-phosphate dehydrogenase deficiency in a dog. *Enzyme* 1976;21:379–382.

70. Naik SN, Anderson DE. Glucose-6-phosphate dehydrogenase and hemoglobin types in cattle. *J Anim Sci* 1971;32:132–136.

71. Harvey JW, Kaneko JJ. Glucose metabolism of mammalian erythrocytes. *J Cell Physiol* 1976;89:219–224.

72. Scott CM, Marlin DJ, Schroter RC. Quantification of the response of equine apocrine sweat glands to beta2-adrenergic stimulation. *Equine Vet J* 2001;33:605–612.

73. Oi Y, Kawada T, Shishido C, et al. Allyl-containing sulfides in garlic increase uncoupling protein content in brown adipose tissue, and noradrenaline and adrenaline secretion in rats. *J Nutr* 1999;129:336–342.