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# Changes in ruminal fermentation and mineral serum level in animals kept in high temperature environments[1]

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**ABSTRACT** - In order to evaluate the effect of environmental temperature on ruminal fermentation and on mineral levels of growing ruminants, it was used 12 male calves (initial average weight 82.9 ± 7.7 kg, 100 days of age), were employed in a randomized block design (by weight) experiment, with repeated weight measurement and two environmental temperatures: thermoneutral (24°C) and heat-stressed (33°C), during 38 days. The animals exposed to 33°C presented lower dry matter ingestion, lower $T_3$ (triiodothyronine) serum level, higher ammoniacal nitrogen ($NH_3$-N) level in the rumen liquid, and higher rectal and body temperatures during all the experimental period when compared to the animals kept in thermoneutral environment (24°C). The animals kept under heat stress environment (33°C) presented higher calcium serum level, which was the highest on 31st day and the lowest on the 38th day of the experiment; phosphorus level was the lowest during all the experimental period; sodium level was lower on the 17th, 31st and 38th experimental days. Potassium and zinc levels were lower after 24 days; copper level was lower until the 24th day; magnesium level was higher until the 17th day, if compared to the ones from the animals kept in thermoneutral environment (24°C). The heat-stressed animals presented higher levels of ammoniacal nitrogen in the ruminal liquid and a decrease in the phosphorus, sodium, potassium and zinc serum levels. These results show the necessity of changes on feed management to ruminants in temperatures over the thermal comfort limits so that performance loss is decreased.

Key Words: ammoniacal nitrogen, dry matter ingestion, heat stress, macromineral, micromineral, volatile fatty acids

# Mudança na fermentação ruminal do alimento e na concentração sérica de minerais em animais mantidos em ambientes de alta temperatura

**RESUMO** - Para verificar o efeito da temperatura ambiente na fermentação ruminal do alimento e nas concentrações de minerais em bovinos em crescimento, foram utilizados 12 bezerros machos (peso médio inicial de 82,9 ± 7,7 kg com 100 dias de idade), durante 38 dias, em delineamento experimental de blocos ao acaso, com medidas repetidas no tempo e duas temperaturas ambiente: ambiente termoneutro (24°C) e ambiente de estresse por calor (33°C). Os animais expostos à temperatura de 33°C tiveram ingestão menor de matéria seca, menor nível sérico de $T_3$ (triiodotironina), maior concentração de nitrogênio amoniacal ($NH_3$-N) no líquido ruminal e maior temperatura retal e corporal em todo o período experimental em comparação àqueles animais mantidos no ambiente termoneutro (24°C). Os animais em ambiente de estresse por calor (33°C) apresentaram maior concentração de cálcio no soro, que foi maior aos 31 dias de experimentação e menor aos 38 dias. A concentração de fósforo nesses animais foi menor durante todo o período experimental e a de sódio, menor aos 17, 31 e 38 dias de experimentação. As concentrações de potássio e zinco foram menores depois de 24 dias; a de cobre menor até 24 dias; e a de magnésio maior até 17 dias de experimentação, todas em comparação à dos animais mantidos em ambiente termoneutro (24°C). Os animais sob estresse por calor apresentaram maiores concentrações de nitrogênio amoniacal no líquido ruminal e diminuição nas concentrações séricas de fósforo, sódio, potásseio e zinco. São necessárias mudanças no manejo alimentar de ruminantes em temperaturas acima do conforto térmico, para diminuir as perdas no desempenho.

Palavras-chave: ácidos graxos voláteis, estresse por calor, ingestão de matéria seca, macromineral, micromineral, nitrogênio amoniacal

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## Introduction

Animals from tropical and subtropical areas are under heat stress during some periods of the year, a condition that impairs performance. The climate can change body temperature, energy and water consumption, and hormone balance, which influences growth, reproduction and milk and egg production (Johnson, 1987).

According to Dukes (1984), animals which are exposed to environmental heat stress undergo metabolic adaptations to reduce its effects. Examples of these adaptations are basal metabolism, acid-base balance, water and electrolyte metabolism, rumen fermentation and endocrine function changes. In environment temperatures in which animal heat production exceeds its loss, there is an increase in body temperature (Johnson, 1987) and the most important effect related to heat exposure is the decrease of food ingestion, according to Phillips & Piggins (1992).

The electrolyte body changes resulted from heat stress can lead to a negative mineral balance as a consequence of the decrease in nutrient ingestion and the increase in mineral excretion (Phillips & Piggins, 1992).

The development of nutritional technologies to many species under heat stress conditions can provide advantages in order to deal with animals which are not placed in comfortable environmental zones, therefore enhancing productivity (Beed & Collier, 1986).

Nutritional management has been made, but there are not enough experimental data sufficiently validated (NRC 2001). Nutritional knowledge in animals under heat stress is an important tool to estimate the nutritional necessities and to suit feed management to animals under these environmental conditions. This experiment meant to study ruminal fermentation and mineral serum level changes in growing ruminants exposed to heat stress in order to enhance nutrition knowledge, which improves the performance of these animals as a consequence.

## Material and Methods

The experiment was held at Faculdade de Zootecnia e Engenharia de Alimentos - USP, Campus de Pirassununga, in the facilities of Departamento de Bioclimatologia, using 12 male Holstein calves (initial average weight 82.9 ± 7.7 kg, final average weight 91.8 ± 11.0 and at 100 days old), in a randomized block design (by weight), with repeated weight measurements, and two environmental temperatures, thermoneutral (24°C) and heat-stressed (33°C).

The animals at high environment temperature were kept in a climatic chamber. Air temperature and humidity were recorded using SATO thermohygrometer (battery powered, graphic display, resolution of 0.1°C and 1% relative air humidity) and for heat stress it was used a Vernom globe thermometer (0.2°C graduation black globe). Circadian temperature variation was simulated by maintaining higher temperatures from 11:00 a.m. to 0:00 a.m., and lower temperatures from midnight to dawn. The chamber was turned off every day around 6:00 p.m. and it was turned on again at 7:00 a.m. on the following day (Table 1). All the animals were kept in individual rubber-covered iron cages. The experimental period lasted 38 days, from which 10 days were for temperature adaptation and 28 days for data collection (17th, 24th, 31st and 38th day from the experimental period), totalizing four collections per animal, used to obtain the individual mean of each animal. It was intented to start data collection when the animals were already heat-stressed.

Diet consisted of total mixed ration and it was offered twice a day (Table 2), according to AOAC (1995). Food

Table 1 – Weekly temperature and relative humidity means inside and outside the climate chamber where the animals were kept

| | Inside the climate chamber | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Temperature (ºC) | | | | Relative air humidity (%) | | | |
| Experimental days | 7:00 | 13:00 | 17:00 | Mean ± SEM | 7:00 | 13:00 | 17:00 | Mean ± SEM |
| 1 to 17 | 28.6 | 34.7 | 35.1 | 32.8 ± 0.1 | 74.8 | 62.7 | 60.3 | 65.9 ± 0.4 |
| 17 to 24 | 29.5 | 36.4 | 34.5 | 33.5 ± 0.2 | 78.7 | 56.6 | 63.6 | 66.3 ± 0.5 |
| 24 to 31 | 30.3 | 36.5 | 33.0 | 33.2 ± 0.1 | 75.1 | 54.7 | 49.3 | 62.8 ± 0.5 |
| 31 to 38 | 29.6 | 36.9 | 32.9 | 33.1 ± 0.2 | 74.7 | 55.0 | 57.9 | 63.0 ± 0.6 |
| | Outside the climate chamber | | | | | | | |
| | Temperature (ºC) | | | | Relative air humidity (%) | | | |
| | 7:00 | 13:00 | 17:00 | Mean ± SEM | 7:00 | 13:00 | 17:00 | Mean ± SEM |
| 1 to 17 | 14.8 | 22.6 | 23.2 | 20.2 ± 0.3 | 82.4 | 58.4 | 59.4 | 66.8 ± 1.0 |
| 17 to 24 | 16.6 | 24.3 | 23.9 | 21.6 ± 0.2 | 90.1 | 60.7 | 58.9 | 69.9 ± 0.9 |
| 24 to 31 | 17.6 | 29.1 | 24.1 | 23.6 ± 0.3 | 73.7 | 42.3 | 39.6 | 51.9 ± 0.8 |
| 31 to 38 | 17.7 | 29.2 | 27.8 | 24.9 ± 0.4 | 72.8 | 40.5 | 37.7 | 50.3 ± 1.2 |

intake was registered every morning and both the food and the leftovers were weighed. The animals were weighed at the beginning and at the end of the experiment after a 24-hour period of food and water deprivation.

Ruminal liquid was collected by esophageal catheter always at 4:00 p.m. The pH was measured as soon as the liquid was removed, and the remaining material was filtered through several gaze layers. It was removed 5.0 mL from the collected liquid, and 1.0 mL of formic acid was added to it, stored in a glass container and frozen for further VFA analysis. Another 2.0 mL volume of ruminal liquid was placed in a glass container with 1.0 mL sulfuric acid 1N for ammoniacal nitrogen ($NH_3$-N) analysis. The samples were thawed at room temperature and centrifuged during 15 min. at 4°C and 15,000 rpm. The concentrations of VFA were measured by gas chromatography (Varian Star 3600 CX) and ammoniacal nitrogen ($NH_3$-N) obtained by the colorimetric method proposed by Kulasek (1972) and adapted by Foldager (1977).

Blood was sampled by punction of the jugular vein always at 3:00 p.m. The serum was separated by centrifugation, stored in Eppendorf tubes and frozen at –20°C. Cortisol and $T_3$ hormone levels were analyzed by DSL immunoenzymatic kits (ELISA tests). Concentrations of manganese, zinc, and copper were determined using PerkinElmer atomic absorption spectrophotometer. Calcium was analyzed using the same method, but lanthanum was used in the solubilization solution. Phosphorus was analyzed using the colorimetric molybdenum-blue method (Fentro Spectrophotometer) read at 725 nm according to Malavolta et al. (1989). Sodium and potassium were analyzed using the Analyser flame photometer.

Table 2 – Ingredients ratio and chemical composition of experimental diet

| Ingredients | % |
|---|---|
| Coast-cross[1] hay | 30.0 |
| Soy meal | 20.0 |
| Corn meal | 32.0 |
| Wheat meal | 12.5 |
| Mineral supplementation | 1.5 |
| Kaolin | 4.0 |
| Chemical composition | |
| Dry matter 105°C | 89.10 |
| Mineral matter | 2.5 |
| Ether extract | 4.20 |
| Neutral detergent fiber | 10.46 |
| Crude protein | 14.84 |
| Total digestible nutrients | 60.00 |
| Calcium | 0.39 |
| Phosphorus | 0.38 |
| Manganese | 0.22 |
| Sodium | 0.15 |
| Copper | 0.0021 |
| Zinc | 0.0061 |

[1] Crushed to 1.5 cm long.

The data were analyzed in a randomized block design (calves were assigned to one of two weight blocks) employing the SAS PROC MIXED (SAS, 1985) for repeated measures. The model included the effects of treatments, times and treatment × time interaction. The degrees of freedom were adjusted using the Kenward-Roger method. To each independent variable, the most appropriate covariance structure was chosen as recommended by Akaike and Schwarz (Littell et al., 2006).

The results are presented as mean values and standard error of the means. A probability of $P<0.10$ was accepted as significant.

## Results and Discussion

There was an interaction effect ($P=0.08$) between environmental temperature and the time on dry matter ingestion ($P=0.04$), on body temperature ($P<0.0001$), on the levels of $NH_3$-N ($P=0.04$) on the rumen liquid and on the serum levels of triiodothyronine, $T_3$ ($P=0.08$), and cortisol (Tables 3 and 4). The animals under heat stress showed lower dry matter ingestion, higher body temperature and higher $NH_3$-N values during all the experimental period in comparison to the animals kept under comfortable temperature. Furthermore, they presented significative lower $T_3$ blood levels until the 24th experimental day and higher cortisol concentration on the 31st day.

The serum levels of calcium ($P=0.04$), sodium ($P<0.0001$), potassium ($P=0.06$), magnesium ($P=0.04$), copper ($P<0.05$) and zinc ($P<0.02$) were also affected by the interaction between the environmental temperature and the experimental time (Table 5). Calcium levels were higher in the heat stressed animals on the 31st day of the experiment and then lower on the 38th day. In these animals, sodium level was lower on the 17th, 31st and 38th experimental days; and the levels of potassium and zinc were lower on the 31st and the 24th, respectively. Magnesium level in animals under heat stress was higher on the 17th day and copper levels were lower until the 24th day of the experiment.

There was an effect of the environmental temperature on the rectal temperature ($P<0.0001$) and on phosphorus levels ($P<0.0001$) in the blood serum (Tables 3 and 5). Heat stressed animals presented higher values of rectal temperature during all the experimental period compared to the ones at comfortable environmental temperature. After 24 days of heat stress, the animals presented lower

phosphorus levels compared to the ones kept at comfortable environmental temperature.

The volatile fatty acid levels, the percentages of propionate, acetate, butyrate, acetate/propionate and the ruminal liquid pH neither presented significative differences ($P \geq 0.10$) at different temperatures nor were changed by temperature × time interaction (Table 4). When the animals were exposed to high temperature, they responded with an increase on rectal temperature and on radiant body heat (Table 3). According to Johnson (1987), at high environmental temperatures, at which heat production exceeds loss, a portion of the heat is stored in the animal body, increasing body temperature and causing a chain of physiological mechanisms so that there is heat loss and the animal gets adapted to the environment. Phillips & Piggins (1992) argue that rectal temperature increase occurs at environmental temperature higher than 26°C in European cattle and higher than 32°C in zebu cattle.

Singh & Newton (1978) exposed male calves adapted to 18.3°C environmental temperature and 50% relative air humidity to 40.5°C and 50% relative humidity. They observed an increase in skin average temperature from 36.6 C to 39.6°C and in rectal temperature from 38.8°C to 40.0°C on the first day of heat exposure. On the following days, they noticed a gradual decrease in the body temperature of all the calves. The rectal temperatures from the 9th to the 14th days of the experiment were significantly lower when compared to the five first days. In this experiment, the animals at both environmental temperatures presented a decrease in the rectal temperature during the experimental period (Table 3). This rectal temperature decrease in the animals to 24°C can be explained by the fact that even under environmental temperature increase during the experimental weeks, the relative air humidity decreased, which made heat loss easier for the animals. Nevertheless, inside the climatic chamber, humidity was relatively stable during the experimental weeks, and an adaptation attempt may have happened in the organism of these animals. In this experiment, even though the difference between the analyzed environmental temperatures (24 × 33°C) was lower than the ones from the experiment conducted by Singh & Newton (1978), which

Table 3 - Dry matter ingestion, rectal temperature, radiant body temperature and triiodothyronine ($T_3$) and cortisol serum levels in experimental animals

| | Experimental day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17 | | 24 | | 31 | | 38 | | | | | |
| | Temperature (°C) | | | | | | | | | P value | | |
| Item | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | SEM | Treatment | Time | T × T |
| Dry matter ingestion (kg DM/day) | 2.07 | 1.51 | 3.52 | 2.61 | 2.97 | 2.26 | 3.47 | 2.24 | 0.19 | 0.0045 | <.0001 | 0.0401 |
| Rectal temperature (°C) | 39.00 | 39.85 | 38.84 | 39.78 | 38.73 | 39.66 | 38.50 | 39.06 | 0.17 | 0.0004 | <.0001 | NS |
| Radiant body heat (°C) | 24.90 | 33.93 | 24.56 | 31.44 | 27.29 | 31.55 | 27.22 | 31.20 | 0.34 | <.0001 | <.0001 | <.0001 |
| Triiodothyronine (ng/dL) | 157.77 | 113.66 | 152.66 | 113.50 | 152.77 | 135.00 | 150.58 | 116.00 | 15.16 | NS | NS | 0.0881 |
| Cortisol (mg/dL) | 0.41 | 0.37 | 0.47 | 0.47 | 0.72 | 0.81 | 0.29 | 0.69 | 0.12 | NS | NS | 0.0801 |

P value = probability value; T × T = treatment by time interaction.

Table 4 - Rumen parameters in animals exposed to two environmental temperatures during 38 days of experiment

| | Experimental day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17 | | 24 | | 31 | | 38 | | | | | |
| | Temperature (°C) | | | | | | | | | P value | | |
| Item | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | EPM | Treatment | Time | T × T |
| $N\text{-}NH_3$ (mg%) | 12.57 | 25.32 | 14.18 | 21.62 | 19.55 | 21.93 | 21.85 | 25.44 | 3.55 | 0.0302 | NS | 0.0412 |
| pH | 6.77 | 6.48 | 6.81 | 6.75 | 6.64 | 6.64 | 6.51 | 6.45 | 0.18 | NS | NS | NS |
| Total volatile fatty acids (mM) | | | | | | | | | | | | |
| VFA(por extenso) (mol/100 mol) | 68.19 | 53.00 | 60.92 | 49.09 | 62.99 | 49.58 | 71.13 | 68.44 | 8.14 | NS | NS | NS |
| Acetate | 56.85 | 53.54 | 59.77 | 60.18 | 62.79 | 60.18 | 59.76 | 58.24 | 1.92 | NS | 0.0140 | NS |
| Propionate | 31.63 | 34.89 | 28.00 | 26.95 | 23.92 | 31.01 | 29.74 | 31.05 | 2.56 | NS | 0.0446 | NS |
| Butyrate | 11.52 | 11.57 | 12.22 | 12.87 | 13.29 | 8.81 | 10.49 | 10.70 | 1.46 | NS | NS | NS |
| Acetate/propionate ratio | 1.94 | 1.51 | 2.27 | 2.32 | 2.86 | 1.99 | 2.04 | 1.96 | 0.27 | NS | 0.0386 | NS |

P value = probability value; T × T = treatment by time interaction.

were 18 × 40°C, the difference in the skin average temperature was higher (26 to 32°C, respectively).

Levels of blood $T_3$ decreased and the serum blood cortisol concentration increased in the animals under heat stress (Table 3). The decrease of $T_3$ blood level in heat-stressed animals is corroborated by citation of Encarnação (1986), that as evaluating thermal effects on Holstein calf growth (five months old) during 5 weeks of heat stress (32-34°C) exposure, a rectal temperature increase and a $T_3$ blood concentration decrease was observed. The anterior lobe of the pituitary gland produces thyrotropine hormone which primarily stimulates the thyroid gland to produce thyroxine ($T_4$) and triiodothyronine ($T_3$). These hormones influence many cellular processes, mainly thermogenic activities, which are responsible for approximately 50% of the basal metabolic rate in normal temperature animals. Blood concentrations of $T_3$ and $T_4$ decrease by 25% under stress conditions (Silanikove, 2000). In this experiment, there was an average decrease of 34% during the experimental period.

According to Guyton (1986), any kind of stress, whether physical or emotional, causes an immediate and accentuated increase of ACTH secretion followed by an immediate suprarenal cortisol increased secretion. Glucocorticoids cause fast amino acid and cellular fat mobilization making them available for both energy and other composite synthesis, glucose is included, necessary for different organism tissues. In this research, there was a cortisol concentration increase after 31 experimental days with no plausible explanation. Literature reports conflicting results about the effects of cortisol levels in the plasma of cattle from hot climates regions. There are many studies reporting the increase of glicocorticoids because of heat stress whereas there are others that report a significant glicocorticoid decrease. Other authors suggest that there is a glicocorticoid increase of approximately 38% after 1 hour, and 62% after two hours of exposition to heat conditions, reaching a peak of 120% in four hours, and then gradually decreasing to values not different from the regular ones in 48 hours, and maintaining the same or a little bit lower level until the end of the heat exposition (Phillips & Piggins, 1992).

The animals submitted to high temperature decreased feed ingestion (Table 3). Dry matter intake decrease, according to Dukes (1984), is a consequence of heat stress, which is a practical problem, especially to cattle raising in tropical areas, because it can change animal performance and production. The effects of high temperature on growth reported by Phillips & Piggins (1992) resulted from anabolic activity decrease and tissue catabolism increase. Anabolism decrease is caused by voluntary decrease of nutrient intake, especially metabolized energy used to maintain and gain weight causing production loss by feed unit under heat stress.

In this experiment, dry matter intake by animals under heat stress was lower during all the experimental period, with an average reduction of 28% of dry matter intake compared to the animals kept in comfortable environment. Adans & Ishiar (1996) reported that there is a production decrease of 3% up to 20% in animals under heat stress, and there is a decrease of 8% up to 12% on feed ingestion. When the animal is panting, the maintainance necessities may increase by 20%. According to Phillips & Piggins (1992), the most important reaction to heat exposure is the decrease of feed ingestion. High environmental temperatures stimulate peripherical thermal receptors to transmit nervous impulses to the appetite center in the hypothalamus causing a feed ingestion decrease.

The results mentioned above were all measured to assess and to ratify the stress caused by heat on the animal organism. The results ratify knowledge found in literature and they should offer data to verify the heat stress intensity

Table 5 - Mineral levels in the blood serum collected from animals exposed to two environmental temperatures during 38 days of experiment

| | Experimental day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17 | | 24 | | 31 | | 38 | | | | | |
| | Temperature (°C) | | | | | | | | | P value | | |
| Mineral | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | 24.3 | 33.2 | SEM | Treatment | Time | T × T |
| Calcium (mg/100 mL) | 9.03 | 9.02 | 8.20 | 8.00 | 7.73 | 8.18 | 9.57 | 9.03 | 0.25 | NS | <.0001 | 0.0429 |
| Phosphorus (mg/100 mL) | 6.84 | 5.58 | 8.26 | 6.92 | 7.13 | 4.51 | 7.05 | 5.27 | 0.35 | 0.0003 | <.0001 | NS |
| Sodium (mg/100 mL) | 276.25 | 263.13 | 283.13 | 283.96 | 339.38 | 281.67 | 288.33 | 278.75 | 2.68 | <.0001 | <.0001 | <.0001 |
| Potassium (mg/100 mL) | 11.23 | 11.52 | 13.42 | 12.73 | 14.12 | 12.64 | 14.45 | 12.57 | 0.40 | 0.0094 | <.0001 | 0.0679 |
| Manganese (mg/100 mL) | 1.93 | 2.39 | 2.70 | 2.78 | 2.74 | 2.91 | 2.77 | 2.80 | 0.09 | 0.0487 | <.0001 | 0.0681 |
| Copper (mg/mL) | 0.55 | 0.47 | 0.71 | 0.56 | 0.40 | 0.40 | 0.55 | 0.57 | 0.02 | 0.0188 | <.0001 | 0.0550 |
| Zinc (mg/100 mL) | 0.91 | 0.92 | 2.14 | 1.66 | 1.21 | 0.87 | 1.86 | 1.31 | 0.11 | 0.0042 | <.0001 | 0.0283 |

P value = probability value; T × T = treatment by time interaction.

caused to these animals so that we can clearly discuss the following results.

The ruminal parameters volatile fatty acids; acetate; propionate and butyrate; acetate/butyrate; and pH) were not influenced by environmental temperature ratifying that even though the rumen volatile fatty acids concentration values in this study did not show significant difference between the treatments (P=0.11), the heat-stressed animals presented lower levels during all the experimental period if compared to the animals kept in comfortable environment (Table 4). Adans & Ishiar (1996) reported that volatile fatty acids production in the rumen decreases in animals submitted to heat stress, and Beed & Collier (1986) reported that heat stress decreases volatile fatty acids produced in the rumen probably due to the decrease in feed intake.

The animals under heat stress presented higher ruminal liquid $NH_3$-N concentration during all the experimental period compared to the animals kept in comfortable environment (Table 4). According to Nocek & Russell (1988), the interaction between the carbohydrates and the protein in the rumen metabolism is particularly intense, and if there is any kind of deficiency or inefficiency of the feed protein use in the rumen, the carbohydrate digestibility may decrease. If the diet carbohydrate is insufficient to support the microbial growth, the nitrogen will be lost as rumen $NH_3$ and its concentration will increase in the rumen. As there was a decrease on feed ingestion by the animals under heat stress, carbohydrate in the rumen decreased to support microbial growth causing a ruminal liquid $NH_3$-N increase.

Another explanation would be a possible increase in the retention time of protein fraction in the rumen leading to a higher soluble protein degradation therefore increasing $NH_3$-N concentration in these animals. Because of the decrease on ruminal concentrations in animals submitted to high environmental temperatures (Yousef, 1985), there is an excessive concentration of ammonia in the rumen when the protein degradation exceeds the amino acid and ammonia assimilation rates to the microbial protein synthesis (NRC, 2001).

The animals submitted to heat stress presented low concentrations of phosphorus, sodium, potassium and zinc, and higher concentrations of magnesium in the blood serum compared to the animals kept in comfortable environment. The concentration changes of minerals in the blood took place at different times during the experimental period. The concentrations of calcium changed very little in the blood serum of animals exposed to both different environmental temperatures. Copper concentration in the blood serum was lower in heat-stressed animals until the 31st day of exposition, keeping concentrations similar to those of the control animals until the end of the experimental period. On the 17th day of heat exposure, the animals had already presented different concentration values in the blood serum of phosphorus, magnesium and copper and different values of potassium and zinc after 24 days of heat exposure.

During the acute heat exposure, according to Tsuda et al. (1991), the plasma volume and the blood volume increase. The concentration of solid particles in the plasma, which is consisted mainly of protein and glucose, increases significantly when the animals are exposed to acute heat. Different data have been obtained on animals submitted to heat stress and can be explained by the differences in its intensity. Tsuda et al. (1991) did not find differences in sodium, calcium and chlorine concentrations in the plasma of buffalos submitted to acute heat exposure, but the concentrations of potassium and phosphorus decreased after five hours of heat exposure.

Heat stress induces electrolyte changes which can lead to a negative mineral balance. Animals submitted to high environmental temperatures (from 32 to 39°C) decrease the ingestion of most minerals. It has been observed a decrease of 4% of sodium, 10% of potassium, 7% of calcium and 12% of zinc in the blood serum of milking Holstein cows at 38°C, and in the blood serum of Holstein calves at 36ºC a decrease of 3% of sodium, 10% of potassium, 7% of calcium, 13% of phosphorus, 13% of magnesium and 11% of zinc was observed (Phillips & Piggins, 1992). The decrease of aldosterone, parathyroid hormone secretion, feed ingestion and catabolic process in bovines under heat stress contribute to the reduction of electrolytes in the blood (Phillips & Piggins, 1992).

In this experiment, the animals under heat stress presented a mean decrease of 24% of phosphorus, 7% of sodium, 7% of potassium, 9% of copper, 22% of zinc and an increase of 7% of magnesium compared to the animals in comfortable environment.

Nutritionists should pay more attention to mineral supplementation of animals under heat stress. In the literature, there is recommendation to increase the liquid energy of maintenance for animals at higher temperatures than thermal comfort because of the energy spent during the surplus heat dissipation process (NRC, 2001), but there is little information about mineral supplementation. It is recommended by NRC (2001) a quantity increase only of sodium and potassium in the diet of animals, mainly milking cows, exposed to temperatures over 30ºC. There is a need for more nutritional information about animals under heat stress conditions (NRC, 2001).

The animals which presented a decrease of phosphorus concentration in the blood still presented values which were expected, being the lowest value found in the animals at 33°C on the 31st experimental day (4.51 mg/100 mL). According to MacDowell (1992), phosphorus average concentrations are between 4-9 mg/100 mL, depending on the species and the age of the animals, and phosphorus plasma concentrations are more likely to be influenced by the diet than by the calcium levels. Phosphorus is an important mineral related to the acid-base buffering system in the blood and cellular differentiation, it is a cell wall component and a cell content as phospholipids, phosphoproteins and nucleic acids (NRC, 2001). It is also a necessary mineral to the ruminal microorganisms and to the cellulose digestion in ruminants (NRC, 2001).

The zinc concentration in the blood serum of calves under heat stress decreased with mean values of 0.46 mg/100 mL after 24 days of experiment compared to the calves in comfortable environment. The animals have a limited capacity to store zinc in the organism in a way that it can be rapidly mobilized to prevent deficiency. When there is a deficiency of this mineral in the diet, in only a 24-hour period, the blood serum concentrations of zinc decrease and according to MacDowell (1992), zinc necessities can change for animals in excessive sweating.

There is a linear relation between magnesium blood serum concentrations and the intake of the mineral. Its metabolism is not influenced by a specific hormone. It is indirectly influenced by hormones that regulate calcium metabolism – calcitonine and parathyroid hormones (Underwood, 1999). Thus, the increase of the mineral in the plasma was not caused by an increase of its ingestion because that was reduced in animals under heat stress. A hypothesis for this increase in serum magnesium concentrations is the decrease in the potassium levels in organisms of calves under heat stress. According to Underwood (1999), potassium infusion in the rumen decreases the absorption of magnesium, which is absorbed in the rumen by two active transport processes against an electrochemical gradient, a process which is inhibited by potassium. Even though they increased if compared to the control animals, the values of magnesium in the serum of calves under heat stress were maintained within the expected concentration range of 2-4 mg/100 mL (McDowell, 1992).

A nutritional management concerning mineral supplementation that can be considered to these animals submitted to high temperatures is the inclusion of available sources of feed high in minerals in the diet. In a study on the use of organic and inorganic sources of zinc and copper in animals under heat stress, Nockels et al. (1993) concluded that the animals fed organic sources supplementation presented a less negative mineral balance than the ones fed inorganic sources due to the decrease of urine mineral excretion.

Because of the voluntary intake reduction that occured during heat stress and the metabolism changes which happened in the organism of these animals, it becomes evident that feed management has to be changed for ruminants under these environmental conditions, especially for non-housed animals. This performance of the animals can improve if we provide them with energetic feed that is readily fermentable in the rumen. However, the source of protein should present low ruminal degradability. Special attention should be paid to non protein nitrogen supply, such as urea, because these animals have a higher susceptibility to intoxication. More importance should be given to phosphorus, potassium and zinc supplement formulation with more available sources or, maybe, even increase the quantity of these minerals in the ration formulation.

## Conclusions

Heat-stressed animals present higher concentrations of ammoniacal nitrogen in the ruminal liquid and a decrease in blood serum concentrations of phosphorus, sodium, potassium and zinc. These results indicate the need of a change on ruminant feed management at temperatures over the thermal comfort limits to reduce performance loss of these animals.

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