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Effect of protected niacin and chromium-rich yeast in dairy cows under thermal stress - milk production and metabolic parameters. Efeito da niacina protegida e da levedura rica em cromo em vacas leiteiras sob estresse térmico - produção de leite e parâmetros metabólicos.

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Abstract

The purpose of this study was to evaluate the effect of protected niacin and yeast rich in chromium on the productive and metabolic performance of dairy cows under thermal stress. 46 lactating Holstein cows were divided into four treatments: protected niacin (GN), yeast rich in chromium (GCr), niacin+chromium (GNCr) and control (GC). Were measured: milk production, SCC, MUN, NEFA, BHB, glucose, cholesterol, insulin, cortisol, T4 and T3. There was an 8% increase in milk production in the GNCr. The use of these elements is an alternative for producers who need to maintain production during periods of thermal stress.
Keywords: Cattle. High temperatures. High humidity. Supplementation. Production.

Resumo

O objetivo desse trabalho foi avaliar o efeito da niacina protegida e levedura rica em cromo sobre a produção e perfil metabólico de vacas leiteiras em estresse térmico. 46 vacas Holandesas foram distribuídas nos tratamentos: niacina protegida (GN), levedura rica em cromo (GCr), niacina + cromo (GNCr) e controle (GC). Foram mensurados: produção de leite, CCS, NUL, AGNE, BHB, glicose, colesterol, insulina, cortisol, T4 e T3. Houve um incremento de 8% na produção de leite no GNCr. O uso desses elementos é alternativa para os produtores que precisam manter a produção durante os períodos de estresse térmico.
Palavras-chave: Bovinos. Temperaturas elevadas. Alta umidade. Suplementação. Produção.

Introduction

High temperatures combined with high air humidity are factors that interfere with productivity and welfare of dairy cows (Polsky and Von Keyserlingk, 2017). One of the main effects of the stress caused by the climatic conditions is the reduction of milk production. In mild climate, the milk production can decrease up 8.3% (Ominski et al., 2002) while in more severe conditions this decrease can achieve 35% (St Pierre et al., 2003). The decrease observed in milk production in cows under thermal stress occurs because of the direct and indirect effects of the high temperature in thermal regulation, in the balance and the energy distribution, and in endocrine functions (West, 2003).

Niacin is an essential part of the coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (Niehoff et al., 2009), and these are involved in oxidation and reduction in many metabolic reactions of electron transfer. These coenzymes have the function to conserve free energy produced by oxidation of substrates, acting directly on the metabolism of carbohydrates, lipids and amino acids (NRC, 2001). Hence, the niacin supplementation in mild climate conditions reduces the levels non-esterified fatty acids (NEFA) (Karkoodi and Tamizrad, 2009) and increases milk production, mainly in early lactation (Yanxia et al., 2008). In addition, niacin has been related to the superficial vasodilatation and increased peripheral heat loss in animals (Di Costanzo et al., 1997), through higher production of prostaglandin D by epidermal langerhans cells (Benyo et al., 2006).

Chromium (Cr) is an essential micro mineral that play a role in the absorption of glucose across cellular membrane, since optimizes the action of insulin to facilitate its binding to membrane receptors and amplify their intracellular signal (Chen et al., 2006). Neto et al. (2009) observed the positive effect of chromium supplementation on productive parameters in beef cattle.

The combination of niacin and chromium has been studied in experimental models for the treatment of hyperlipidemia and for the prevention of damage caused by this condition (Inceli et al., 2007; Niu et al., 2009) for humans, showing excellent results. However, there is still no consensus on the mechanisms responsible for the effects of chromium and niacin together on energy metabolism. In rats, the supplementation of these two substances was able to reduce the levels of cholesterol and triglycerides, allowing recovery of hepatocytes damaged by a hyper lipemic diet (Bolkent et al. 2004). Niacin and Cr supplementation used separately in thermal stress situations in dairy cows has generated positive results in some studies (Di Costanzo et al. 1997; Zimbelman et al. 2010). However, there are no studies about the effect of protected niacin and chromium-rich yeast on the productive and metabolic performance of dairy cows under thermal stress, being these the objectives of this study.

Material and methods

Animals studied

The experiment was conducted in the northwest of the state of Rio Grande do Sul, southern Brazil, during the summer (february and march). We used 46 Holstein cows, with an average of 207 days in lactation and an average production of 23.4 liters of milk/day. The cows were housed in a free-stall system. The animals received 41kg of dry matter/cow/day of a diet based on corn silage, Tifton hay, soybean meal, corn bran, barley and oats grain, and mineral mixture (Table 1).

Table 1 – Composition and percentages of inclusion of ingredients and dietary minerals*.

Item	%
Ingredients	
Corn silage	61
Corn bran	16
Soybean meal	8
Barley grain	6
Tifton hay	4
Oat grain	4
Mineral mixture	1
Chemical composition of total diet	
Dry Matter	48,6
Crude protein (% dry matter)	15,5
NDF†	34,9
ADF‡	15,65
EE§	3,42
Calcium	0,81
Phosphorus	0,35

* Based on a diet of total mixture composed of 41 Kg of dry matter

† NDF: Neutral detergent fiber, ‡ ADF: Acid detergent fiber. § EE: Ether extract.

The Ethics Committee on Use of Animals at the Federal University of Santa Maria (64/2010) approved the experimental protocol. The animals were divided into four groups by sampling of random block, related to the days in lactation, in different treatments: niacin (GN, n=12), 12g of protected niacin (BALCHEM, New Hampton, New York); chromium (GCr, n=11), 20g of yeast for each animal (*Saccharomyces cerevisiae* chromium-rich 0.5mg of chromium/Kg DM) (ALLTECH, Brazil); niacin + chromium (GNCr, n=12), 12g of protected niacin + 20g of yeast; and control (GC, n=11) without treatment. The treatments were provided daily for 35 days, mixed to 100g of soybean meal, individually, after the morning milking. The control group received only the soybean meal.

Analysis

The environmental data: dry bulb (DBT) and wet bulb temperatures (WBT) were obtained using black globe thermometer (TGD-200 model, INSTRUTHERM). The measurement was done daily, at 7am, 10am, 13pm, 16pm and 19pm. The index of temperature and humidity (THI) was calculated in accordance with Thom (1959): THI= DBT + 0.36 WBT + 41.2.

Samples of blood (20 ml) were collected by coccygeal vein puncture on days zero (Period 1), 12 (Period 2), 26 (Period 3) and 35 (Period 4) of treatment. We centrifuged the blood for 10 minutes at 5000 x g and serum that we stored at -20ºC until the moment of the analysis. The analyses of NEFA (NEFA, FA 115, RANDOX, England), beta-hydroxybutyrate (BHB) (RANBUT – RB 1007, RANDOX, England), cholesterol (COLESTEROL LIQUIFORM – LABTEST, Brazil) and glucose (GLICOSE PAP LIQUIFORM – LABTEST, Brazil) were performed using commercial kits. Insulin, cortisol, triiodothyronine (T_3) and tetraiodothyronine (T_4) were determined by radioimmunoassay. The cows were milked three times a day (at 4am, 10am and 5pm) and milk production was measured daily. Samples of milk were collected during 1, 2 and 3 for somatic cell counted (SCC) by flow cytometry and analyzed for milk urea nitrogen (MUN) by infrared technique.

Statistical analysis

The experimental period was divided into four separate periods for performing the statistical analysis, according the average periods of THI (Figure 1). The periods 1 and 2 were defined as times in which the THI have higher average and periods 3 and 4 as the times in which the THI was mild or stayed for more days below 74 (Figure 1).

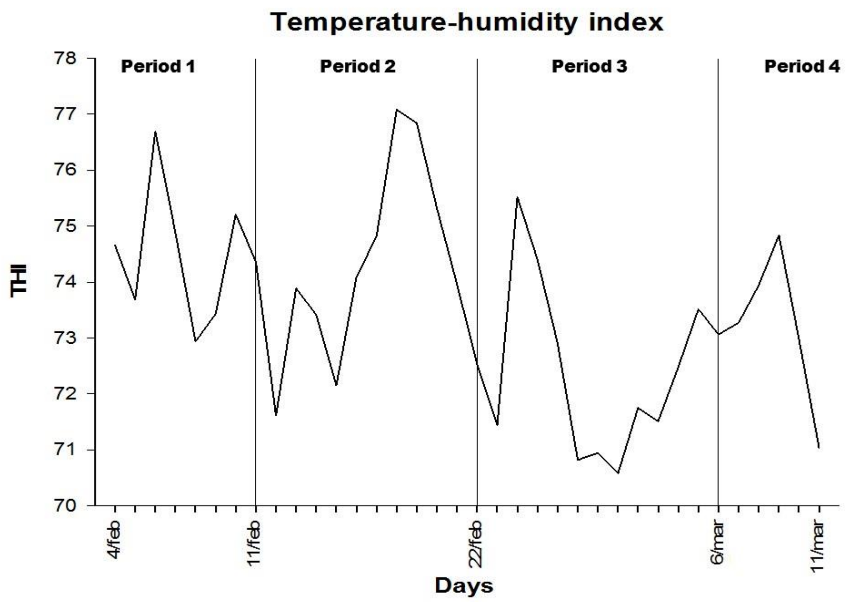

Figure 1 - Temperature and humidity index (THI) of the experimental period and distribution of experimental times. The periods 1 and 2 were defined as the intervals with more days with high THI, above 74, considered the periods with more severe heat stress. The periods 3 and 4 have been determined as the periods with lower peaks of THI or with more days with THI below 74, considered the periods with moderate heat stress.

The effects of different treatments on blood and hormonal parameters, MUN and SCC were analyzed by ANOVA (one-way). The data of SCC, insulin and cortisol have undergone the logarithmic transformations prior (log (10)). The comparison between the averages was performed by Tukey test (5% significance) by statistical program (version 8.02, program SAS, SAS Institute Inc., Cary, NC, EUA).

Results

The thermal stress occurred during the experiment was of moderate to intense, according to the classification of ITU. The index of average temperature and humidity obtained during the periods 1, 2, 3 and 4 was 74.2 ± 1.8, 74.0 ± 1.8, 72.2 ± 1.4 and 73.0 ± 1.4, respectively (Figure 1).

Highest values in milk production were observed in periods 2 and 3 in the group of animals treated with the association of niacin and chromium in contrast to those that received these compounds isolated. The milk production on period 4 in the group that received niacin + chromium was higher in relation to the other groups (P<0.05) (Table 2).

Table 2 - Effect of treatments with niacin, chromium, and niacin + chromium on milk production, biochemical variables, somatic cell counted and milk urea nitrogen of dairy cows under thermal stress (media ± standard error).

Parameters		**Treatments***			
		GN	**GCr**	**GNCr**	**GC**
Milk production kg/d.	Period 1†	25.7 ± 6.48	23.2 ± 8.34	24.2 ± 7.77	24.9 ± 6.88
	Period 2†	22.9 ± 5.83[ac]	21.9 ± 5.90[ac]	25.0 ± 5.99[b]	24.0 ± 6.38[ab]
	Period 3†	22.0 ± 5.46[ab]	21.2 ± 5.95[a]	24.5 ± 6.50[c]	22.6 ± 6.73[b]
	Period 4†	22.8 ± 7.39[a]	23.2 ± 6.28[a]	25.8 ± 6.24[b]	24.2 ± 8.72[ab]
BHB µmol/L	Period 1	860.1 ± 82.3	774.5 ± 93.3	872.0 ± 80.7	828.3 ± 77.1
	Period 2	880.5 ± 57.4[a]	698.9 ± 91.2[b]	860.2 ± 144[ab]	865.3 ± 50.1[ab]
	Period 3	627.6 ± 46.4	598.8 ± 30.6	659.0 ± 43.6	659.4 ± 35.5
	Period 4	620.9 ± 64.3	574.5 ± 47.7	639.0 ± 43.7	654.3 ± 78.5
NEFA µmol/L.	Period 1	101.6 ± 15.2[ab]	101.8 ± 9.4[b]	149.3 ± 26.9[c]	68.7 ± 8.0[a]
	Period 2	67.3 ± 8.2	86.9 ± 32.7	78.3 ± 9.1	91.9 ± 17.0
	Period 3	116.7 ± 10.1	102.0 ± 11.1	108.1 ± 10.1	124.9 ± 17.6
	Period 4	138.2 ± 53.3[a]	152.1 ± 26.0[ab]	206.9 ± 18.3[b]	159.4 ± 20.1[ab]
Cholesterol mg/dL.	Period 1	241 ± 23.7	218 ± 9.5	257 ± 33.4	241 ± 20.5
	Period 2	272 ± 19.0	255 ± 14.1	299 ± 24.2	261 ± 14.1
	Period 3	272 ± 19.4	229 ± 16.6	283 ± 24.5	240 ± 12.6
	Period 4	327 ± 16.7[a]	209 ± 15.8[b]	307 ± 23.2[a]	298 ± 11.7[a]
Insulin ng/ml.	Period 1	1.04 ± 0.59[a]	1.15 ± 0.43[a]	0.92 ± 0.53[a]	3.96 ± 0.88[b]
	Period 2	1.62 ± 0.39	1.88 ± 0.36	2.36 ± 0.54	2.23 ± 0.53
	Period 3	1.39 ± 0.18	1.83 ± 0.25	2.54 ± 0.24	3.08 ± 0.31
	Period 4	2.77 ± 0.59	3.01 ± 0.43	2.76 ± 0.53	3.97 ± 0.33
T_3. µg/dL.	Period 1	101.6 ± 9.3[b]	142.2 ± 7.8[a]	149.3 ± 7.6[a]	145 ± 6.3[a]
	Period 2	67.3 ± 7.6[a]	146.4 ± 8.1[b]	78.3 ± 12.1[a]	155± 6.4[b]
	Period 3	116.7 ± 10.8[bc]	142.3 ± 8.1[ac]	108.1 ± 8.5[b]	152.8 ± 6.0[a]
	Period 4	138.2 ± 6.3[a]	129.3 ± 8.7[a]	206.9 ± 9.4[b]	148.3 ± 4.7[a]
Cortisol. µg/dL.	Period 1	0.65 ± 0.1	0.69 ± 0.1	0.52 ± 0.05	0.40 ± 0.06
	Period 2	0.87 ± 0.07[a]	0.68 ± 0.11[ab]	0.93 ± 0.09[a]	0.45 ± 0.09[b]
	Period 3	0.31 ± 0.18	0.56 ± 0.15	0.46 ± 0.20	0.31 ± 0.06
	Period 4	0.44 ± 0.13	0.39 ± 0.11	0.28 ± 0.10	0.29 ± 0.16
SCC. x 10^3.	Period 1	1720 ± 360	3478 ± 872	328 ± 189	2146 ± 995
	Period 2	414 ± 206	1983 ± 627	394 ± 119	1474 ± 716
	Period 3	1568 ± 246	2155 ± 646	1047 ± 354	1680 ± 627
MUN. mg/dL.	Period 1	13.58 ± 0.98	13.88 ± 0.86	15.38 ± 1.12	12.58 ± 6.24
	Period 2	13.65 ± 2.46[ac]	10.79 ± 2.32[b]	13.80 ± 2.05[a]	11.84 ± 1.46[bc]
	Period 3	15.67 ± 2.11[a]	12.86 ± 1.92[b]	16.12 ± 3.54[a]	14.09 ± 2.99[ab]

[a, b, c] Difference between groups (P<0.05). *GN: treatment with niacin; GCr: treatment with chromium; GNCr: treatment with niacin + chromium; GC: untreated group. † Milk production data were divided into four experimental periods according to the index of temperature and humidity (THI). Periods 1 and 2 presented higher THI's while 3 and 4 periods showed lower values.

Related to the levels of MUN, the GNCr group exhibited higher values than the Cr (P=0.04) and GC (P=0.021) groups in the period 1. The GN group also showed higher levels of MUN when compared with the Cr group (P=0.009), in this period. On period 4 animals treated with niacin, in association or not with chromium, showed higher MUN values respect to the animals that received only chromium (Tab. 2). The SCC was lower in the group of animals treated with the association of niacin to chromium than in those that received only the chromium on period 1 (P=0.015).

On period 1, the average values of NEFA were greater in the GNCr when compared to those obtained in the other groups (P<0.05); and in the Cr compared to the GC group. On period 4, plasma NEFA concentration in the GNCr group were greater than those in the GN group (P=0.016) (Tab. 2). On periods 1, 3 and 4, no differences have been observed between the groups (P>0.05) for the levels of BHB. On period 2, the animals that received Cr presented less concentrations of plasma BHB compared to group N (P=0.021). Concerning cholesterol levels there was no difference in treatments on periods 1, 2 and 3. Animals treated with chromium showed, on period 4, less cholesterol values in comparison to other groups (P<0.05) (Tab. 2).

T_3 levels were less in the niacin group compared to other groups on period 1. On period 2, the animals that received niacin or niacin + chromium showed less levels of T_3 respect to other groups (P<0.05). On period 3, the animals that received niacin associated or not to chromium showed less T_3 values comparing to the control group (Tab. 2). The Cr group presented greater T_3 respect to NCr group (P=0.013), on the same day. On period 4, the GNCr presented the greater levels of T_3 than the other treated groups (P<0.05).

Differences in the plasma cortisol concentrations was observed only on period 2, when the animals that received niacin, in association or not with chromium, showed the greatest values of this hormone than the control group (p<0.05) (Tab. 2). Serum insulin levels were greater in the control group compared to other groups on period 1. There were no differences between treatments in levels of T4 and glucose (P>0.05) (data not provided).

Discussion

The supplementation of niacin for dairy cows under thermal stress has been the subject of several studies, though its effect on performance of dairy with the goal of enhancing the response of animals to this substance, some authors evaluated the niacin together with other nutraceuticals with positive results. In a study by Xin-Jian et al. (2006) the use of chelated chromium together with non-protected niacin in cows under thermal stress resulted in a 12% increase in milk production. In the present study, the use of protected niacin together with organic chromium was associated with an 8% increase in milk production. A study with feedlot calves treated with chromium chloride and niacin, showed that this combination resulted in greater weight gain and better feed conversion than animals in the control group or those that received any of the supplements in an isolated form (Chang et al. 1995). Yuan *et al*. (2011), Zimbelman et al. (2010) and Rungruang et al. (2014) using only protected niacin, during the summer, observed no difference in milk production. In our study, increase of production was observed only in animals supplemented with niacin and chromium together, suggesting a possible synergistic action of these two substances.

Blood and milk urea nitrogen (BUN and MUN) are important tools for evaluation of the levels of nitrogen metabolized by the animal and for the balance between the protein use and intake. The use of chromium and niacin in beef cattle did not influence on levels of BUN (Chang et al. 1995). However, in dairy cows under thermal stress the use of niacin reduced the levels of BUN (Belibasakis and Tsirgogianni 1996). In our study, the greatest concentration of MUN occurred in the GNCr, coinciding with the largest milk production presented by the same group (Tab. 1). In this way, is possible that the MUN value found for the animals treated with niacin and chromium is a direct effect of greater production and greatest need for alternative energy sources. The lowest concentration of MUN occurred on period 1, one of the hottest periods of the experiment and these results agree with those found by Srikandakumar and Johnson (2004), who observed lower nitrogen levels in the blood of cows under thermal stress.

The action of niacin and chromium in SCC in cows is still controversial in the literature; and there are few studies about the effect of these elements at SCC. An-Qiang et al. (2009) did not observed an effect of chromium on the SCC of dairy cows under high temperatures. However, Zimbelman et al. (2010) detected a lower SCC in dairy cattle fed a protected niacin dietary supplement and undergoing a thermal stress. Treatment with niacin alone or in association with chromium did not influence on the somatic cells count of milk (Tab. 1).

As fat mobilization is a thermogenic process, cows under thermal stress use other sources to obtain energy with low heat production in these conditions (Wheelock et al. 2010), reducing the NEFA production. Under moderate thermal stress, the animals that received niacin + chromium, managed to mobilize more NEFA on period 4 than others, coinciding with the largest milk production in this group (Tab. 1). This finding indicates that the use of chromium + niacin may possibly contribute to the use of this source of energy even during periods of heat stress.

Dietary supplementation with Cr-methionine has been reported to reduce plasma concentrations of BHB in weaned calves during the summer (Yari et al., 2010). Yang et al. (1996) also detected lower concentrations of BHB in lactating cows fed a chromium-amino acid supplement. The plasma concentrations of BHB in animals treated with chromium were numerically lower those in other groups at all periods during the experiment (Tab. 2), indicating a beneficial effect this mineral in the reduction of ketone bodies in dairy cows under heat stress.

In a study carried out on cows in thermal stress, Al-Saiady et al. (2004) reported that dietary supplementation with Cr yeast increased plasma concentrations of cholesterol and dry matter intake (DMI). In the present study, the plasma cholesterol concentrations were lower in the group supplemented with chromium than in the control group, a result similar to that described by Pechová et al. (2002). Belibasakis and Tsirgogianni (1996) observed that feeding a dietary supplement of niacin to dairy cows under thermal stress, did not change their plasma concentrations of cholesterol.

The scientific literature is equivocal as to the effects of niacin and chromium on plasma glucose concentration. Belibasakis and Tsirgogianni (1996) observed no effects on plasma glucose concentrations in cows subjected to thermal stress and treated with niacin. However, cows in thermal stress that received greater amounts of non-protected niacin (14g) (Karkoodi and Tamizrad 2009), when compared to the dose used in this study (12g), exhibited an increase in plasma glucose. The chromium and niacin together did not alter the plasma glucose concentration in heifers subjected to thermal stress (Chang et al. 1995) and immune challenge (Kegley and Spears 1995).

An-Qiang et al. (2009), using chromium in cows in early lactation also did not detect changes in plasma insulin concentrations. In the study of Kegley and Spears (1995) the insulin was greater in animals treated with chromium + nicotinic acid for up to 30 min after being challenged with glucose. However, there was no difference in the levels of this hormone in animals not challenged with glucose.

The thyroid gland is sensitive to thermal stress, because your hormones act to the mechanism of thermogenesis (Magdub et al. 1982). There are no studies that describe the effects of niacin on the

T3. However, we note that niacin in combination or not with chromium produced lower levels of T3 in the hottest periods of the experiment, indicating a possible direct action of niacin on the mechanisms of endogenous heat producers. It is important to note that the low levels of T3 in these groups did not influence the milk production.

According to Chang et al. (1995) calves in food stress situations that receive dietary chromium present lower blood cortisol levels. Similar to the results obtained in this study (Tab. 2). Kegley and Spears (1995) observed no change in cortisol levels in animals subjected to stressful situations and treated with chromium in conjunction with niacin.

Conclusion

The use of niacin in combination with chromium was able to maintain and increase milk production in animals under moderate thermal stress, probably due to increased NEFA mobilization of fat reserves. The 8% increase in milk production during the experimental period, observed in the group supplemented with chromium and niacin, provides a significant increase in profitability of dairy farming.

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