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Full Length Research Paper

Metabolic acclimation to heat stress in farm housed Holstein cows with different body condition scores

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The aim of this study was to determine the effects of body condition score to metabolic acclimation in heat stressed Holstein cows. Body condition of cows had no effect on any of the tested parameters during the thermal neutral period, except for the percentage of protein in milk. Heat stress has been demonstrated to have an impact on all the selected parameters, and has been found to be the cause of decreased milk production and quality, increased rectal temperature and respiration rate, decreased glucose, non esterified fatty acids (NEFA) and cholesterol concentration, increased urea and bilirubin concentration, and loss of subcutaneous fat. Obese cows (BCS>4) had less ability of acclimation to heat stress as compared to normal and thin cows (significantly lower production and milk quality, and significantly higher rectal temperature and respiration level in relation to the other groups of cows). Obese cows showed a significantly higher concentration of NEFA and significantly lower glucose during exposure to heat stress. The steady increase in NEFA concentrations and decrease of backfat thickness suggested that fat was used for energy purposes, which significantly increased the heat balance and led to poor acclimation to heat stress in obese cows. Cows with high body condition were at higher risk of developing liver failure and lipidosis (reduced cholesterol and elevated bilirubin concentration) during heat stress, which could be linked with increased concentrations of fatty acids in the blood. This was found with an increased concentration of urea during exposure to heat stress, with significantly higher concentration in obese cows. Using the fat for energy purposes depends on the sensitivity to insulin, which increases during heat stress. Obese cows are naturally less sensitive to insulin and more prone to lipolysis. However, these signs should be the focus of future research.

Key words: Body condition score, heat stress, metabolic status, cow.

INTRODUCTION

Heat stress affects the health and productivity of dairy cows negatively. Homeothermic animals have a thermoneutral zone in which energy expenditure to maintain normal body temperature is minimal, constant and independent of environmental temperature (Youself, 1987). The most sensitive cattle category to high ambient temperature are lactating dairy cows because they produce much more heat than non-lactating ones (Blackshaw and Blackshaw, 1994). Cattle housed in a climatic chamber and exposed to 23 days of heat stress revealed that complete recovery of feed intake was obtained only after 10 days at thermoneutrality. After reestablishment of thermoneutrality, respiration rates returned to normal level, while rectal temperature undershot, possibly indicating adaptation to heat stress (Williams et al., 2009). In analogous conditions, Beatty

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Abbreviations: THI, Temperature-humidity index of heat stress load; **BCS,** body condition score; **NEFA,** non esterified fatty acids; **BHB,** beta hydroxy butirate; **NEBAL,** negative energy balance; **NEM,** nett energy for maintenance.

(2005) found similar results. Farms in our geographic regions during summer (June, July and August) decreases milk production and reproductive efficiency (Cincović et al., 2010a), and returns to the original level until the first days of autumn.

The gist of acclimation to heat stress in dairy cows increased heat loss and reduced heat production in order to maintain body temperature within the range of normality. Acclimation is a temporal biphasic process which includes short-term and long-term heat acclimation process (Horowitz, 2002). In ruminants, short-term heat acclimation is characterized by reduced dry matter intake and milk yield and increased water intake and evaporative heat loss, while long-term acclimation is characterized by a reprogrammed gene expresion (Collier et al., 2008).

Reduced dry matter intake (DMI) leads animal to negative energy balance (NEBAL). NEBAL during heat stress is additionally induced by using an amount of productive energy for the purpose of cold thermoregulation. This means that the nett energy for maintenance (NEM) needs are increasing. Most researches, although not all have demonstrated that DMI decreases with increasing BCS at calving; generally, DMI is greater in cows carrying less condition. Body fat has a negative feedback impact on DMI, with increasing levels of body fat causing a reduction in DMI (Bewley and Schutz, 2008). NEM requirements under hot conditions are dependent on body condition. As a consequence, cattle with greater body condition begin displaying signs of heat stress sooner than those with less body condition (Mader, 2002).

As a result of NEBAL occurrence, the main factor of a series of metabolic adaptations in cows is lipid mobilization (Herdt, 2000). During heat stress, lipid mobilization is usually lowered, although the results are different (Ronchi et al., 1999; Abeni et al., 2007). Metabolic adaptation to NEBAL, induced by heat stress, implies an increased sensitivity to insulin, which results in reduced glucose concentration, lipid mobilization and decreased concentration of non esterified fatty acids (NEFA) (Itoh et al., 1998). It is known that insulin sensitivity depends on the fatty deposits / body condition. Obese cows were demonstrated with decreased sensitivity to insulin (Holtenius and Holtenius, 2007), which could affect the metabolic acclimatization to heat stress. Reduced insulin sensitivity with reduced dry matter intake of food in obese cows lead to negative energy balance and increased lipid mobilization with many metabolic changes. This is in contrast with the acclimation process to heat stress.

We hypothesized that during exposure to heat stress, obese cows showed a higher concentration of NEFA and utilization of fat depots in relation to the normal condition cows. The aim of this study was to determine the effects of body condition score to metabolic acclimation in heat stressed Holstein cows.

MATERIALS AND METHODS

The experiment included 30 Holstein-frisian cows in the middle of the second lactation. The cows were healthy and were fed a total mixed ration with a double distribution in the morning and in the night. Diets were based on corn silage (~33% DM) (25 kg), lucerne hay (6 kg) and concentrate (~16% DM) (7 kg). It formed three groups of 10 cows, which were characterized as thin (BCS<3), normal (BCS 3.25 to 3.75) and obese (BCS>4) cows. The body condition scoring (using five-point scale with quarter-point divisions) was performed using the system provided by *Elanco Animal Health Buletin Al 8478*. BCS was determined by palpation and careful visual examination.

Collection of data and samples was performed on the thermoneutral (March 10th, April 10th, May10th) and heat loaded part of the year (June 15th, July 15th and August 15th). In each of the six days, the following measurements were carried out: the value of THI, milk production, milk fat and protein, measuring the rectal temperature and the level of respiration, backfat thickness and the taking of a blood sample for biochemical analysis. All indicators were determined for each cow separately (except for THI, which is a general meteorological data).

Ambiental temperature and relative humidity values were obtained from the regional meteorology station, and by direct measurement on the farm. The THI index was calculated by the standard equation: mean THI = 0.8 x mean AT + [mean RH x (mean AT-14.4)]+46.4, where AT is the ambient air temperature, and RH relative humidity was expressed in decimals (McDowel, 1979). Blood was taken by puncturing of the jugular vein and then immediately centrifuged, and the obtained serum was sent to the laboratory for analysis. The serum was transported in a refrigerator at +4°C. Blood collections were accomplished as rapidly as possible between 12:00 and 15:00 h during the day's heat period. Plasma samples were immediately analyzed for NEFA (Randox kit, UK), glucose, total protein, urea, cholesterol and bilirubin (Pointe science kit, USA), after which measurements were performed using commercial photometric assays and Rayto RT-1904 C analyzer.

The rectal temperature and respiration rate were recorded in parallelism with blood sampling. Data on milk production were obtained from the farm's database, while measures of milk ingredients were obtained from Milcoscan analyzer (Foss, Denmark). However, these methodologies were spectrophotometric.

Backfat thickness was used for confirmation of lipolysis and was measured by Falco-vet ultrasound system (Esaote Pie Medical, Netherlands). The examination site was located in the sacral region between the caudal one-quarter and one-fifth connection line going from the dorsal part of the tuber ischia (pins) to the tuber coxae (hooks). This site corresponded to the area between the end of the crista sacralis and the end of the os sacrum. However, backfat was positioned between the skin and the profound fascia.

Statistical analysis

The difference between the parameters of the thermoneutral and stressful period (regardless of the BCS group) was tested by a comparison of the general average values via Z-test. The test was carried out to examine the effect of six different types of treatment on selected parameters of acclimation: 1) thin cows in thermoneutral period (BCS <3 and THI <72); 2) heat stressed thin cows (BCS <3 and THI> 72); 3) normal cows in thermoneutral

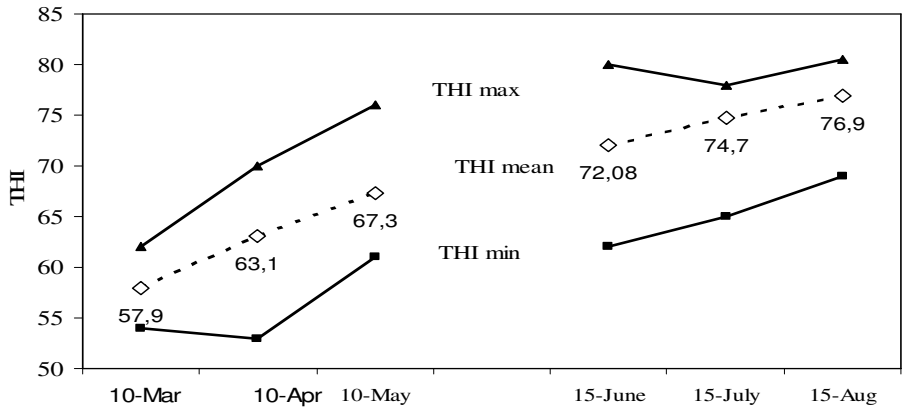

Figure 1. Minimal, maximal and mean THI values on the day of sampling.

period (BCS3.25-3.75 and THI <72); 4) heat stressed normal cows (BCS3.25-3.75 and THI> 72); 5) obese cows in thermoneutral period (BCS> 4 and THI <72); and 6) heat stressed obese cows (BCS> 4 and THI> 72). It was observed that ANOVA analysis was used for this test. When a significant difference was determined between treatments, LSD test was used to accurately determine which treatments were significantly different. In order to determine if BCS, by their nature, affected the value of selected indicators, a test was conducted to compare the values of selected parameters between treatments 1, 3 and 5 (control and thermoneutral period). The emphasis in this paper was on the difference between treatments 2, 4 and 6 (heat stress period). Consequently, the original data were presented at the graphics (mean + SEM). The relationship between THI and mean values of all measured parameters in the three categories of cows that were examined was analyzed by calculating the Pearson moment and parameter b of linear equations, after which statistical analysis was performed using the statistical package Statistica 5 (Stat.Soft.Inc.).

RESULTS

Cows were loaded with heat stress during summer months in our geographical region (Figure 1). The maximum values of THI index (75 to 80) indicate moderate to high intensity of thermal stress. Lack of air conditioning stables with deep litter increases the effect of heat stress.

Influence of heat stress on the value of the selected parameters, regardless of body condition of cows was first tested (Table 1). The mean value of rectal temperature, respiration levels, milk production and serum glucose and cholesterol levels were highly statistically different than the values obtained in a thermal neutral period (P<0.01). Significant difference during heat stress (P<0.05) was found for urea, fat, milk protein, NEFA concentrations and backfat thickness change. The concentration of bilirubin showed a tendency to change during heat stress (P<0.1). Body condition of cows had no effect on any of the tested parameters during the thermal neutral period (Treatments 1, 3 and 5), except for the percentage of protein in milk (Table 2). Significant effect of body condition on the selected parameters was found when cows were exposed to heat stress (Treatments 2, 4 and 6).

Cows under heat stress showed significant increase of rectal temperature and respiration rate. Heat stressed obese cows (BCS>4) had higher rectal temperature than the other two observed groups (p<0.01) (Figure 2). Respiration rate was significantly higher in obese cows at the start of heat stress (p<0.05) (Figure 3), but it was observed that this difference was lost during the time. The correlation between THI and rectal temperature (y = 0.0567 x + 34.969) was positive and significant (r = 0.94, <0.01), while the value of parameter b was approximately 0.056 / THI unit. This finding was identical for all the three categories of cows. A similar finding was found when examining the correlation between THI and respiration levels (r = 0.94, <0.01). The value of the parameter b/THI unit was approximately 1.61, except for BCS<3 group where parameter b was 1.23.

Obese cows showed significant lower milk production during heat stress as compared to thin and normal cows in the same period (p<0.01) (Figure 4). Decline in milk production was followed by the decline in the quality of milk. Obese and thin cows showed the tendency to intensively drop in milk protein and fat as compared to cows with normal BCS (p<0.1) (Figures 5 and 6). THI value (intensity of heat stress) was negatively correlatedwith milk production (r = -0.91, p<0.01), the amount of milk fat (r = -0.57, p<0.05) and protein (r = -0.93, p <0.01), regardless of the BCS group.

Heat stressed cows showed a drop in glucose, NEFA and cholesterol (Figures 7, 8 and 9) concentration, and the difference in cholesterol concentration between groups was not significant (p>0.05). LSD test showed that obese cows had a significant decreased glucose

Table 1. Influence of heat stress on parameters of acclimation.

Parameter	P (Z-test)
Rectal temperature (°C)	P<0.01
Respiration rate (per min)	P<0.01
Milk production (L)	P<0.01
Milk fat (%)	P<0.05
Milk protein (%)	P<0.05
Glucose (mmol/l)	P<0.01
NEFA (mmol/l)	P<0.05
Urea (mmol/l)	P<0.05
Cholesterol (mmol/l)	P<0.01
Bilirubin (µmol/l)	P<0.1
Backfat thickness change (mm/2week)	P<0.05

Table 2. Influence of BCS on the examined parameters of acclimation via LSD test.

Parameter	Difference between treatments 2, 4 and 6 THI>72	Difference between treatments 1, 3 and 5 THI<72
Rectal temperature (°C)	0.0425*	ns
Respiration rate (/min)	0.00021**	ns
Milk production (L)	0.001248**	ns
Milk fat (%)	0.002929**	ns
Milk protein (%)	0.0405*	0.043*
Glucose (mmol/l)	0.00109**	ns
NEFA (mmol/l)	0.000274**	ns
Total protein (g/l)	0.2366	ns
Urea (mmol/l)	0.002098**	ns
Cholesterol (mmol/l)	0.2947	ns
Bilirubin (µmol/l)	0.4505	ns
Backfat thickness change (mm/2week)	0.0000898**	ns

*Significant effects; **high significant effects.

concentration ($p<0.01$) and increased NEFA concentration ($p<0.01$) during heat stress. The equation of linear regression and correlation show that in obese cows, glucose concentrations decreased more with increasing values of the THI index when compared with other groups of cows: $y= -0.0092x + 4.2863$, $r = 0.49$ ($p<0.05$) (BCS<3); $y = -0.0143x + 4.6013$, $r = 0.63$ ($p<0.01$) (BCS3-4); $y = -0.0265x + 5.3281$, $r = 0.86$ ($p<0.01$) (BCS>4). The correlation between the THI and concentration of NEFA was negative, as shown in the following formulas: $y = -0.0115x + 1.1061$, $r = 0.96$ ($p<0.01$) (BCS<3); $y = -0.0092x + 0.9802$, $r = 0.97$ ($p<0.01$) (BCS3-4); $y = -0.0009x + 0.4611$, $r = 0.093$($p>0.05$) (BCS>4). However, this correlation was not statistically significant in obese cows. Positive correlation between NEFA and THI was found during exposure to heat stress (June, July and August) in obese cows ($y = 0.0036x + 0.1107$, $r = 0.93$), while the other two groups retained a negative correlation.

The increase in NEFA concentrations in obese cows during their exposure to heat stress was surprising, because the increase in NEFA concentration did not correspond to the concept of adaptation to heat stress. However, the concentration of NEFA in obese cows was lower in heat stress as compared to the thermoneutral period, which corresponds to the concept of adaptation to heat stress. This finding requires further investigation.

A typical negative energy balance (reducing the

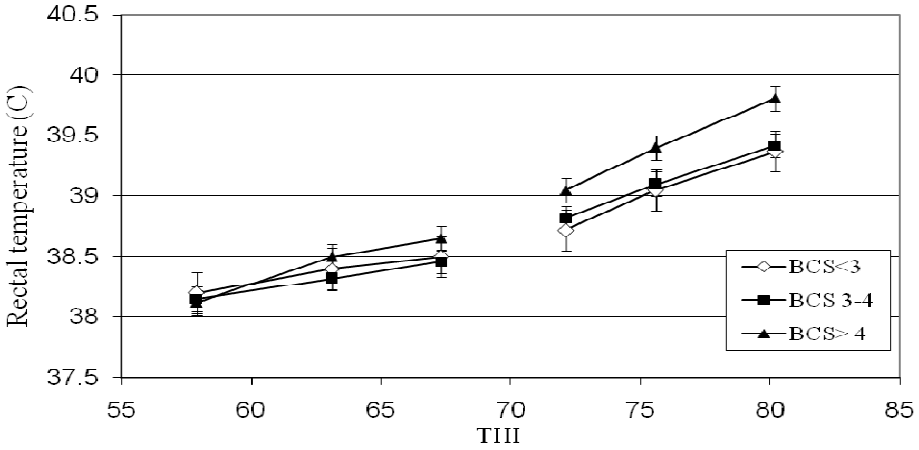

Figure 2. Rectal temperature (°C) as a function of THI in groups with different BCS.

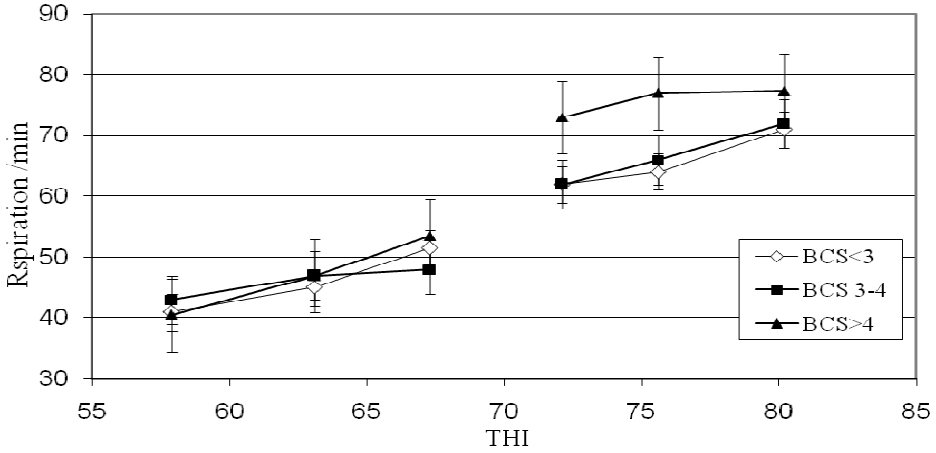

Figure 3. Respiration rate (per min) as a function of THI in groups with different BCS.

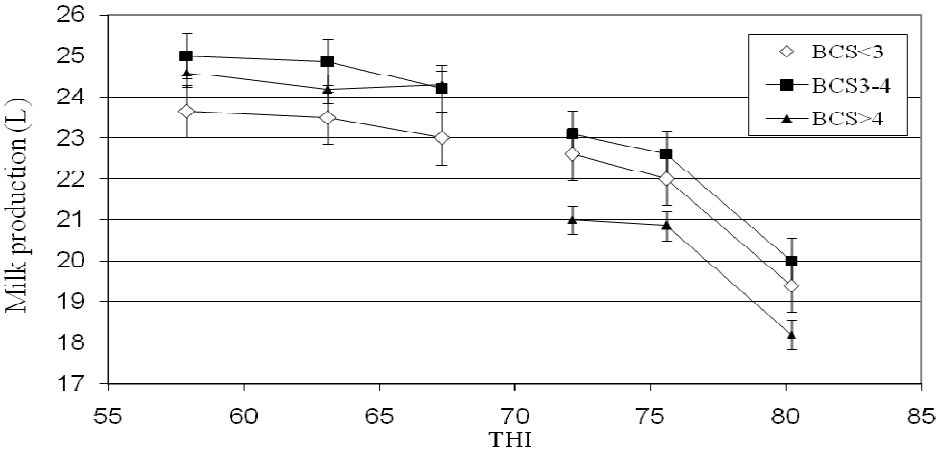

Figure 4. Milk production (l/day) as a function of THI in groups with different BCS.

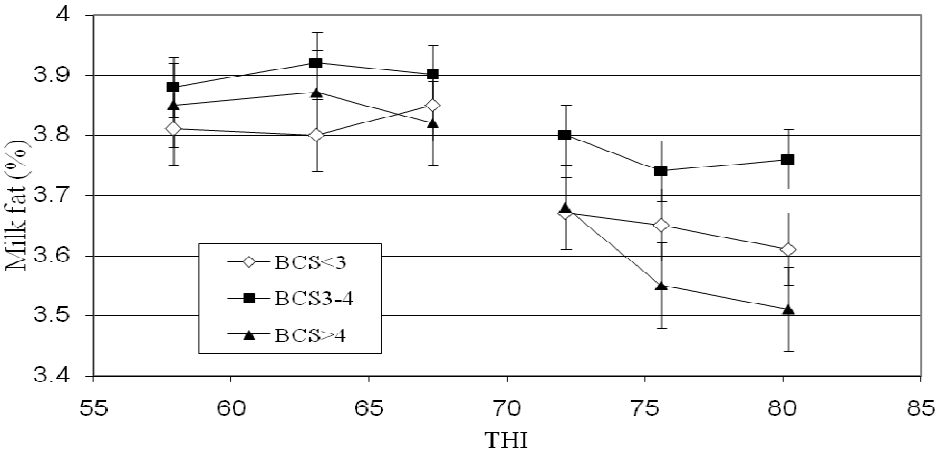

Figure 5. Milk fat (%) as a function of THI in groups with different BCS.

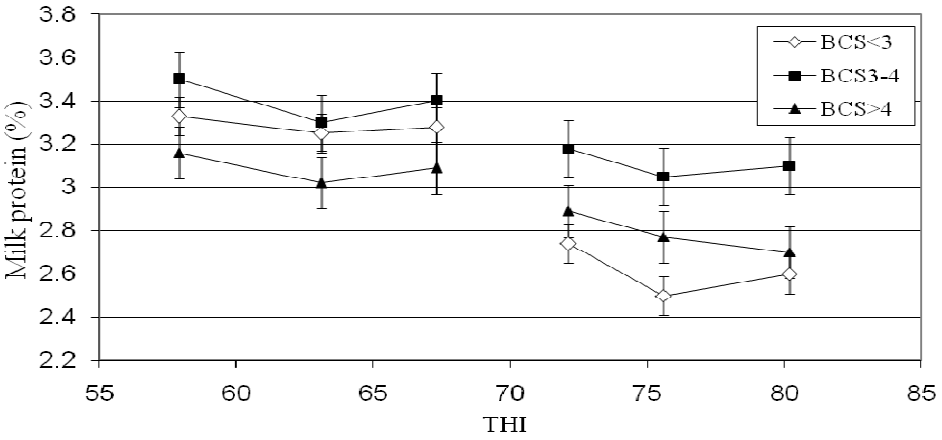

Figure 6. Milk protein (%) as a function of THI in groups with different BCS.

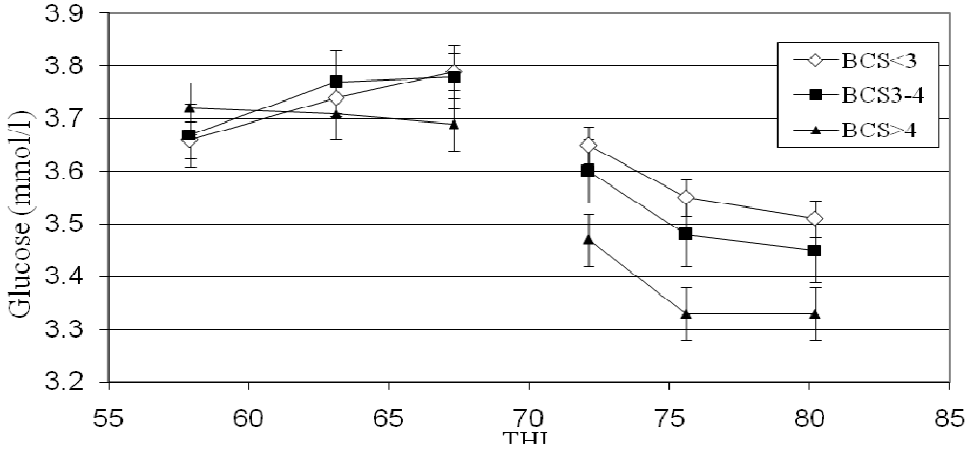

Figure 7. Glucose concentration (mmol/l) as a function of THI in groups with different BCS.

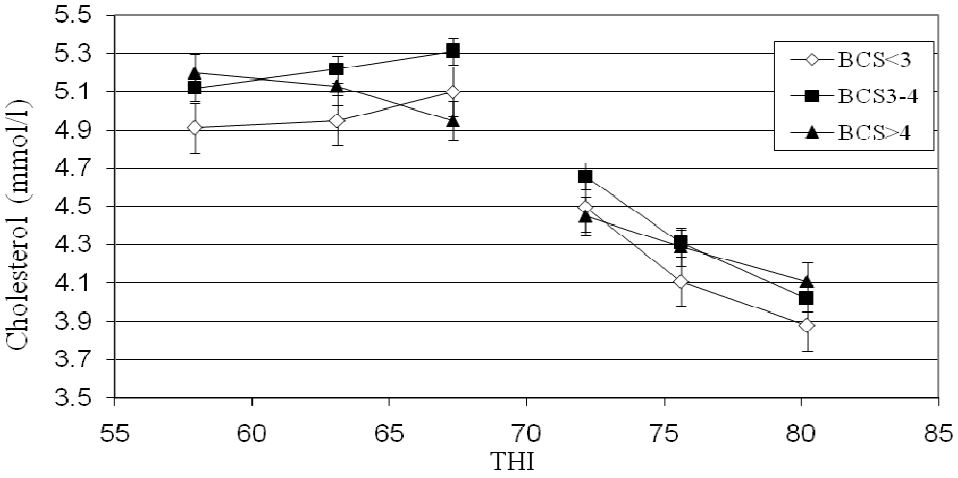

Figure 8. Cholesterol concentration (mmol/l) as a function of THI in groups with different BCS.

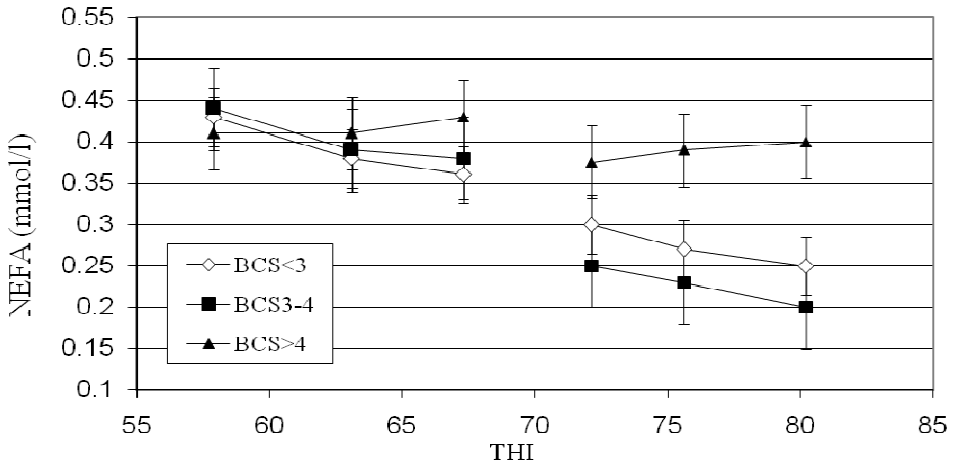

Figure 9. NEFA concentration (mmol/l) as a function of THI in groups with different BCS.

concentration of glucose and NEFA concentrations increase) with increased consumption of its own reserves occurs in obese cows during heat stress. Therefore, a greater reduction in subcutaneous adipose tissue was found in obese cows during heat stress. Backfat thickness decreased by 1.15 mm in obese cows or 0.7/0.1 mm in cows with normal/thin BCS (Figure 10). As such, an increased concentration of urea during exposure to heat stress was found with significantly higher concentration in obese cows ($p<0.05$) (Figure 11). The correlation between THI and the concentration of urea was positive and was statistically significant ($r = 0.91$, $p<0.01$), and so coefficient b varied from 0.038 (BCS<3) to 0.051 (BCS3-4) and even to 0.063 (BCS>4).

In obese cows, increased bilirubin concentration ($p<0.05$) was found as compared to the thermoneutral period (Figure 12), while in contrast, it was found in cows with normal and thin conditions. Heat stressed obese cows had significantly increased bilirubin concentration ($p<0.01$). Analysis of linear equations and Pearson coefficient was revealed with interesting results. Bilirubin concentration and the value of THI were negatively and significantly correlated in the thin ($y = - 0.022x + 4.69$, $r = 0.47$, $p<0.05$) and normal ($y = - 0.037x + 5.98$, $r = 0.76$, $p<0.01$) cows. However, in obese cows, this correlation was positive but not significant ($y = 0.011x + 2.82$, $r = 0.21$, $p>0.05$). This finding is related to the increased concentration of NEFA in obese cows during heat stress.

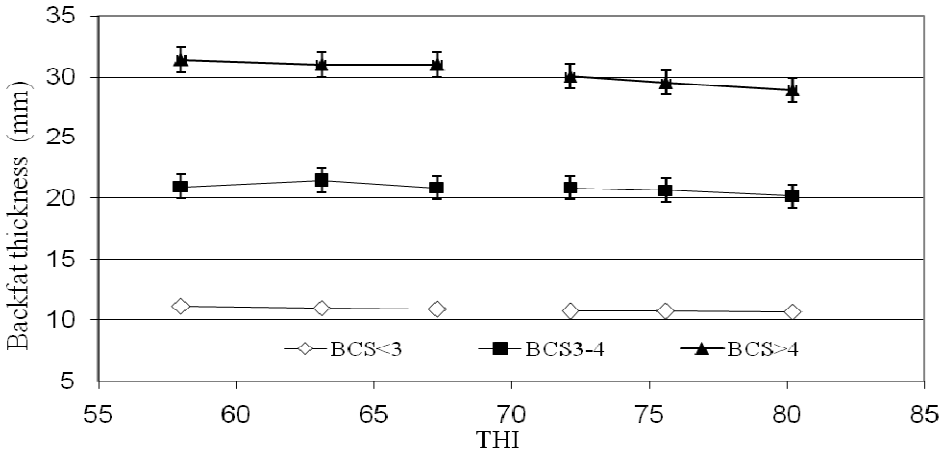

Figure 10. Backfat thickness (mm) as a function of THI in groups with different BCS.

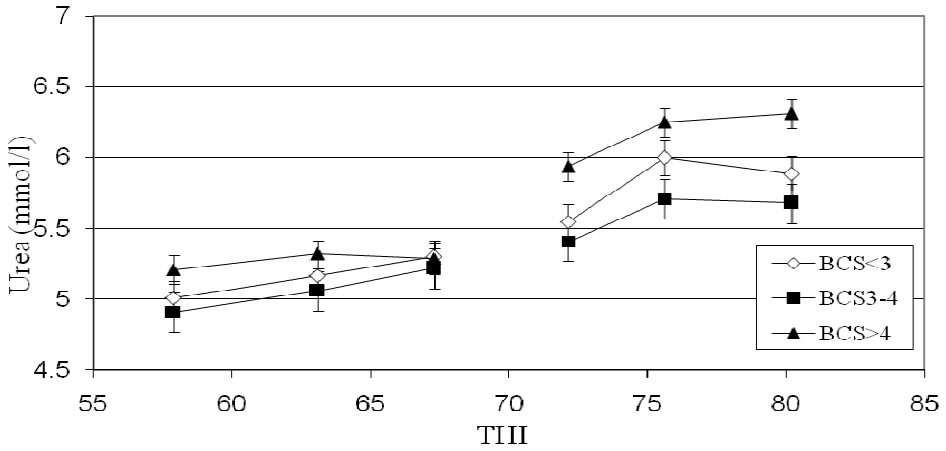

Figure 11. Urea concentration (mmol/l) as a function of THI in groups with different BCS.

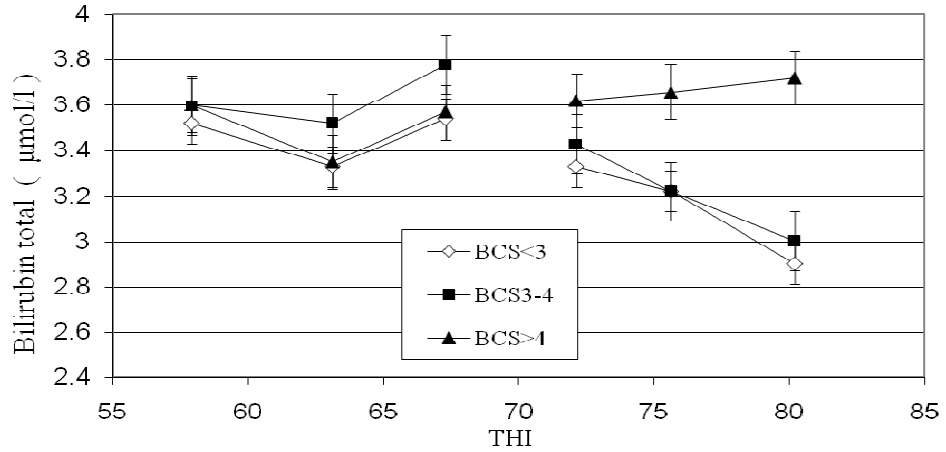

Figure 12. Total bilirubin concentration (µmol/l) as a function of THI in groups with different BCS.

DISCUSSION

The minimal, maximal and average values of THI are given in Figure 1. Minimal THI (07:00 AM) was less than 72 because of the night temperature which was in the thermoneutral zone. Maximal THI in summer was between 76 and 81 (03:00 PM) and the level of THI indicates mild to moderate heat stress. Episodic periods of heat stress may present the cow with greater challenges in the short-term because physiological homeothermic adaptations take weeks rather than days to occur (Collier et al., 2006). THI values of 70 or less are considered comfortable, 75 to 78 as stressful, and values greater than 78 as the cause of extreme distress with lactating cows being unable to maintain thermoregulatory mechanisms or normal body temperatures (Kadzere et al., 2002).

Rectal temperature and respiration rate are in high correlation (Belić et al., 2010), but these findings in obese cows are not in accordance with those conclusions. The skin and respiratory tract of cattle have great capacity to dissipate heat by evaporation, through sweating and panting, respectively. Increased respiration results from a tendency of the organism to deal with the excess heat by evaporation through the lungs (Gaughan et al., 2000). Lemerle and Goddard (1986) reported that although rectal temperature only increased when THI was greater than 80, the respiration rate would begin to increase above a THI value of about 73 and would probably increase steeply at THI values >80. In a hot environment, a small animal has a thermoregulatory advantage over a large but otherwise similar animal, because of its greater surface area per unit of body mass (Bohmanova, 2006). This could explain higher body temperature in obese cows. Body temperature and respiration rate were not strongly correlated in obese cows probably because the intercostal muscles in dairy cows were permeated by fat, abdominal press was loaded with body weight and these cows showed lower respiratory activity than normal condition cows. Brown-Brandl et al. (2006) noted that cows with higher body condition score had higher respiration rate and higher panting score as compared to cows with thinner conditions.

Milk production decreased with the increasing value of THI which is over 72 (West, 2003; Bohmanova et al., 2007; Cincović and Belić, 2009). Obese cows showed significant lower milk production during heat stress as compared to thin and normal cows in the same period. Decline in milk production was followed by the decline in the quality of milk. This can be explained by reduced feed intake and efficiency in fat cows (Bewley and Schutz, 2008). Although these findings could be in correlation with increased rectal temperature in obese cows, it is important to mention that Ravagnolo and Misztal (2000) reported that cows' DMI and milk yield were most affected by climatic variables, and not the body temperature of cows. However, decrease in milk production and quality can be explained by metabolic change during heat stress (Cincović et al., 2010b).

Heat stress (especially acute exposure) causes the release of catecholamines and glucocorticoids hormones that typically promote adipocyte lipolysis and NEFA mobilization (Beede and Collier, 1986). Glucose and NEFA concentration showed diurnal variation in dairy exposed to direct solar radiation so that glucose concentration decreases, but NEFA concentration increases in the hottest period of the day (Cincović et al., 2010c). The drop of glucose concentration is the result of reduced food intake, altered gluconeogenesis and increased insulin concentration in the body (Itoh et al., 1998; O'Brien et al., 2010; Rhoads et al., 2010). The metabolic profile of a heat-stressed cow is primarily characterized by low NEFA levels (Rhoads et al., 2009, Wheelock et al., 2010); hence, heat stressed cows showed a drop in glucose, NEFA and cholesterol concentrations. LSD test showed that obese cows had significant decreased glucose concentration and increased NEFA concentration during heat stress. The higher level of NEFA concentration in this study is related to the intense subcutaneous lipid mobilization measured by ultrasound. Cows with good body condition have a significantly higher concentration of NEFA and lower concentration of glucose in the periparturient period. NEFA concentration was higher during intensive lost of fat deposits and changing of body condition (Bernabucci et al., 2005; Busato et al., 2002).

The steady increase in NEFA concentrations and decrease of backfat thickness suggest that fat was used for energy purposes, which significantly increased heat balance and led to poor acclimation to heat stress in obese cows. Decreased glucose concentration and increased NEFA concentration in cows are a classic mechanism that animals on a low plane of nutrition implement to maximize milk synthesis. However, milk production was significantly lower in obese cows despite the apparently greater activation of adipose tissue and glucose consumption. This finding supports the direct effects of heat stress on the body of cows. Nevertheless, milk production is in inverse proportion to the concentration of NEFA (Ospina et al., 2010), which is related to our result in obese cows under heat stress.

An increased concentration of urea was found during exposure to heat stress, with significantly higher concentration in obese cows. Skeletal muscle is also mobilized during the periods of inadequate nutrient intake (in thermal neutral conditions) to support lactation. We have shown that cows have increased plasma urea nitrogen levels in the hottest period of the day when compared with the thermal neutral period (Cincović et al., 2010c). Plasma urea nitrogen can originate from at least two sources: inefficient rumen ammonia incorporation into microbial proteins or from hepatic deamination of

amino acids mobilized from the skeletal muscle. Cows spend more time standing during exposure to heat stress (Tucker et al., 2008), which is related to increased muscle loading (that is, it is higher in massive cows) and their catabolism.

Liver function, which is like a sign of acclimation to heat stress, was also changed. In obese cows, increased bilirubin concentration was found as compared to the thermoneutral period, while in contrast, it was found in cows with normal and thin condition. This clearly indicated the decreased excretory capacity of the liver (Đoković et al., 2010). Health problems in heat-stressed ruminants may also be a consequence of nutritional and metabolic acclimation. In particular, due to increased maintenance requirements for thermoregulation and lower feed intake, summer transition dairy cows were under higher risk of liver lipidosis (Basirico et al., 2009). Increased liver lipidosis probably compromises liver function; as such, authors have reported that heat-stressed cattle have reduced albumin secretion and liver enzyme activities (Ronchi et al., 1999). Increased NEFA concentration and decreased cholesterol concentration could predict cows having liver lipidosis (Holtenius and Hjort, 1990). In this study, all of these signs were found in the obese cows.

Using the fat for energy purposes depends on the sensitivity to insulin, which increases during heat stress. Obese cows are naturally less sensitive to insulin and more prone to lipolysis. As such, future research should focus on these signs.

Conclusions

Obese cows (BCS>4) have less acclimation ability to heat stress. During exposure to heat stress, they significantly increase rectal temperature, their respiratory tract quickly gets weak in the thermoregulatory process, and they significantly decrease the amount and quality of produced milk as compared to normal and thin cows. In obese cows, signs that were similar to the classic negative energy balance were developed (lower concen-tration of glucose increased the concentration of NEFA with significant mobilization of subcutaneous fat). Normal and thin cows showed decreased glucose and NEFA concentration, which is a characteristic of negative energy balance in heat stress, while cows with high body condition were at higher risk of developing liver failure and lipidosis (reduced cholesterol and liver enzymes, and elevated bilirubin concentration) during heat stress. Thus, an increased concentration of urea during exposure to heat stress was found with significantly higher concen-tration in obese cows.

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