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Original study

The effect of the season on some blood metabolites and haptoglobin in dairy cows during postpartum period

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Abstract

The aim of this study was to evaluate the effect of the season (ambient temperature and relative humidity) on some blood metabolites in dairy cows during postpartum period. Blood samples were collected from 195 clinically healthy dairy cows of 8 Italian dairy herds in spring (April-May 15.5±4 °C and 69.5±0.7 %), summer (June-July 23±1.5 °C and 69±1.3 %) and autumn (September-October 17±2 °C and 72.5±2 %). Total proteins, albumin, globulins, urea, glucose, triglycerides, total cholesterol, non-esterified fatty acids, β-hydroxybutyrate and haptoglobin were analysed. One-way repeated measures analysis of variance (ANOVA) showed a significant effect of the season ($P<0.05$) on all studied parameters, except to the packed cell volume, globulins and β-hydroxybutyrate. A positive significant correlation was found only between albumin and total cholesterol in summer (r=0.39; $P<0.01$) and autumn (r=0.40; $P<0.001$). These results indicated the influence of the season on blood metabolites in dairy cows during postpartum period.

Keywords: dairy cows, haematochemical parameters, haptoglobin, postpartum period

Abbreviations: β-HBA: β-hydroxybutyrate; NEFA: non-esterified fatty acids; PCV: packed cell volume

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Introduction

The postpartum period is a period of great metabolic stress in which dairy cows with more severe and prolonged negative energy balance have an increased risk of disease and culling (Rajala-Schultz & Grohn 1999, Butler 2005). It is not clear how much of the reduction in performance can be attributed or accounted directly (hyperthermia) or indirectly (reduced to feed intake) to heat stress (Bernabucci *et al.* 2010). The performance of animals, in fact, is the result of the interaction between different factors that may exert negative effects on farm animal welfare, performance and health (Nardone *et al.* 2006). Reduction in reproductive performance of lactating cows during hot climate has been well documented (Thatcher *et al.* 2010) and is associated with a decreased thermoregulatory competence of lactating dairy cows, partially due to intensive genetic selection for high milk production (Al-Katanani *et al.* 1999). The influence of the moderate variation of climate conditions on metabolic parameters of dairy cows has been less investigated. Animals react to disturbances of their homeostasis with a set of physiological changes known as acute phase response (Piñeiro *et al.* 2003). This response is associated with changes in lipid and glucose metabolism (Hardordottir *et al.* 1994) and in some serum proteins such as the acute phase proteins (Petersen *et al.* 2004). For example haptoglobin, an acute phase protein released from hepatocytes in response to tissue injury or infection (Murata *et al.* 2004), acts as a potent immunosuppressor of lymphocyte function and as an antioxidant (Sadrzadeh *et al.* 2004). It has been demonstrated that its values increase in dairy cows during postpartum period and its high concentrations have been associated with an increased incidence of endometritis (Sheldon *et al.* 2001, Williams *et al.* 2005) or with other inflammatory problems (Dubuc *et al.* 2010). In dairy cows, the haptoglobin levels have been also evaluated in relation to thermal stress showing the relationship between the changes and the different climatic conditions (Giannetto *et al.* 2011). In dairy cows, impaired glucose production would likely lead to increased adipose tissue mobilisation, elevated plasma non-esterified fatty acids (NEFA) and increased β-hydroxybutyrate (β-HBA) production by the liver. In dairy cows the key metabolic analytes used as indicators of negative energy balance and subclinical ketosis are properly NEFA and β-HBA (Oetzel 2004). In this respect the aim of the present study was to evaluate the concentrations of some blood metabolites, together with haptoglobin, during postpartum period in different seasons.

Material and methods

The aim was carried out on 195 multiparous Holstein Frisian dairy cows during postpartum period (n=60 in 3rd-4th week, n=70 in 5th-8th week, n=65 in 9th-10th week), selected from eight Italian intensive dairy herds (45°24'N, 11°48'E). All housing and care conditions were conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals, Directive 2010/63/EU and Directive 1998/58/EU. Thermal and hygrometric records were carried out by means of a data logger (Gemini, Chichester, UK). The average values, minimum and maximum of temperature and relative humidity recorded in the experimental period are reported in Table 1. Table 2 shows the chemical composition of diets used during the experimental period. Blood samples were collected on each animal from the 8th April to the 28th May (spring), from the 11th June to the 26th July (summer) and from 1the 9th

September to the 31st October (autumn). Samples were collected from different subjects for each period as follows: (spring n=60, summer n=65 subjects, autumn n=70). In each period dairy cows were equally distributed for the postpartum week. The body condition score was recorded as the average of two scores assigned independently by two evaluators using a 1 to 5 scale according to Edmonson *et al.* (1989). Dairy herds had a milk production of about 10 000 kg per year. Blood samples were collected by jugular venipuncture into a plastic syringe between 08.00 and 09.00 a.m. The small volume of blood was then transferred into capillary heparin tubes for packed cell volume (PCV) determination by microhematocrit centrifuge (Select-A-Fuge 24, Bio-Dynamics, IA, USA). The remaining whole blood was centrifuged at 1 350 × g for 10 min. The obtained sera were stored, within one hour from withdrawal and stored at −20 °C until analysis. The serum concentration of total proteins (biuret reaction), albumin (bromcresol green method, BCG), urea (UV kinetic with urease and glutamate dehydrogenase, GLDH) glucose (hexokinase, G6PDH reaction), total cholesterol (cholesterol oxidase, CHOD-PAP reaction), triglycerides (enzymatic, glycerol-3-phosphate-oxidase), NEFA (enzymatic colorimetricmethod), β-HBA (enzymatic colorimetric method) were assessed using commercial kits by automated analyser Boehringer Mannheim/HITACHI 911 (Roche, Basel, Switzerland). The determination of haptoglobin concentrations was performed by the method of enzyme linked immunosorbent assay using ELISA kits (Tridelta Development, Ltd., Wicklow, Ireland). The globulin values were calculated by subtracting the values of albumin from the corresponding values of total proteins. One-way repeated measures analysis of variance (ANOVA) was applied to evaluate the statistical differences due to the season. Bonferroni's multiple comparison test was applied for post hoc comparison. *P*-values <0.05 were considered statistically significant.

Table 1
Average values, minimum and maximum values of ambient temperature and relative humidity recorded during experimental period

Season	Environmental conditions					
	Ambient temperature, °C			Relative humidity, %		
	Min	Max	Mean	Min	Max	Mean
Spring	11.50	19.50	15.50	68.00	71.00	69.50
Summer	21.50	24.50	23.00	66.10	72.10	69.10
Autumn	15.00	19.00	17.00	70.50	74.50	72.50

Table 2
Chemical composition (%) of diet used during the experimental period

Chemical composition of diet	
Crude protein	16.59
Ethreal extract	6.01
Ash	7.42
Neutral detergent fibre	30.17
Non fibre carbohydrates	38.81
Dry Metter Degradable	68.48
Acid detergent fibre	20.37
Starch	28.46
Dietary cation-anion balance	49.39

All the data were analysed using Statistica 8 software (StatSoft, Inc., Tulsa, OK, USA). For individual values of all parameters, a linear regression model (y=a+bx) was applied in order to determine the correlation degree between the studied parameters and the correlation coefficient (r) was determined.

Results and discussion

There were no statistical differences among periods on PCV (32±3.38 %) and body condition score (3.07±0.26) in dairy cows. Total proteins and albumin were significantly higher in spring ($P<0.05$) than in summer and autumn ($P<0.05$). Significant lower urea and glucose concentrations were measured in summer respect to spring and autumn ($P<0.05$). Triglycerid concentrations significantly decreased in spring compared to autumn ($P<0.05$), total cholesterol concentrations significantly increased in spring in comparison with summer and autumn ($P<0.05$), NEFA concentrations significantly decreased in autumn compared with spring and summer ($P<0.05$). Haptoglobin values were significantly higher in summer than in autumn ($P<0.05$). Table 3 shows the average values of the studied parameters, expressed in their conventional units of measurement, with standard deviations and statistical significances observed in 195 Holstein Frisian dairy cows during postpartum period and during different seasons. A positive significant correlation was found only between albumin and total cholesterol in summer (r=0.39; $P<0.01$) and autumn (r=0.40; $P<0.001$).

Table 3
Average values (±SD) of all studied parameters in dairy cows during postpartum and obtained during different seasons

Parameters	Experimental period		
	Spring	Summer	Autumn
Total proteins, g/L	85.44±6.30[a]	80.54±7.49	80.96±8.28
Albumin, g/L	37.67±2.74[a]	34.76± 3.63	35.01±3.23
Globulins, g/L	47.77±7.90	45.78±8.31	45.99±8.70
Urea, mg/dL	25.74±6.48	22.92±5.82[c]	25.80±6.18
Glucose, mmol/L	3.04±0.50[b]	2.89±0.35[c]	3.15±0.44
Triglycerides, mmol/L	0.12±0.04[c]	0.13±0.03	0.14±0.03
Total cholesterol, mmol/L	4.69±1.04[a]	4.11±1.20	4.11±1.11
NEFA, mmol/L	0.30±0.16[c]	0.31±0.17[c]	0.24±0.11
β-HBA, mmol/L	0.67±0.29	0.68±0.49	0.60±0.22
Haptoglobin, g/L	0.12±0.072	0.14±0.091[c]	0.10±0.059

[a]vs summer and autumn $P<0.05$, [b]vs summer $P<0.05$, [c]vs autumn $P<0.05$

These results showed that metabolic profiles in dairy cows during postpartum period could be influenced by climate conditions. Because PCV was unaffected by periods, the differences observed for total proteins and albumin concentrations were not a consequence of dehydration or plasma volume expansion. The increase of total proteins and albumin in spring is probably correlated to the vegetation available inthis period. In fact, a previous study showed a direct relationship between protein intake and serum albumin in cows (Hoffman *et al.* 2001). So, probably the lower concentrations of total proteins, albumin and urea in summer could be caused by a decrease of nitrogen intake by feed. In addition, the

concentrations of total cholesterol were significantly higher in spring than in summer and autumn in relation to higher concentrations of albumin found in this period. In human a positive correlation was found between serum albumin and serum cholesterol (Gillum & Makuc 1992) and albumin plays a significant role in cholesterol transfer between cells and lipoproteins (Zhao & Marcel 1996). The correlation found in our study in summer and autumn could confirm that also bovine albumin is involved in cholesterol metabolism. Serum glucose in dairy cows is probably derived almost exclusively from gluconeogenesis and lower glucose concentrations in summer suggest that gluconeogenesis is less effective to increase blood glucose than in other periods. Haptoglobin was also influenced by the experimental period with significantly higher concentrations in summer. The effect of climatic conditions on haptoglobin in dairy cows is controversial: some authors did not find a season influence on its concentrations (Chan *et al.* 2004), while other authors found higher concentrations in summer than in winter (Wenz *et al.* 2010). It is believed that serum haptoglobin in cows could be involved in the regulation of lipid metabolism (Kanno & Katoh 2001). In our study the patterns of haptoglobin and NEFA were similar among periods. Haptoglobin is also an effective marker of fatty liver (Ametaj *et al.* 2002). Fat infiltration into the liver may also decreases glucose, total proteins, albumin and urea concentrations (West 1990). These last blood biochemical components were significantly lower in summer. The high concentrations of serum triglycerides observed in autumn, together with lower concentrations of NEFA, could be due to a lower lipid utilisation by peripheral tissues in this period. Moreover, the higher concentrations of glucose in autumn could be related to lower concentrations of NEFA and β-HBA. High concentrations of β-HBA are a consequence of an increased gluconeogenesis pathway that does not permit the full metabolisation of acetyl-coenzyme A by Krebs cycle. These findings could indicate that also moderate environmental conditions influence some blood biochemical components in dairy cows during postpartum period. Further investigation should confirm whether different environmental factors such as altitude, latitude and photoperiod could influence metabolism in different breeds and lead to different predispositions for the development of metabolic diseases and culling risk.

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