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ARTIGO ORIGINAL

Lipid and glucose profiles of dairy buffaloes during lactation and dry period

Lipidograma e glicemia de búfalas leiteiras durante a lactação e o período seco

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ABSTRACT: The purpose of this study was to analyze the influence of lactation and dry period in the constituents of lipid and glucose metabolism of buffaloes. One hundred forty-seven samples of serum and plasma were collected between November 2009 and July 2010, from properties raising Murrah, Mediterranean and crossbred buffaloes, located in the State of Sao Paulo, Brazil. Biochemical analysis was obtained by determining the contents of serum cholesterol, triglycerides, beta-hydroxybutyrate (β-HBO), non-esterified fatty acids (NEFA) and plasma glucose. Values for arithmetic mean and standard error mean were calculated using the SAS procedure, version 9.2. Tests for normality of residuals and homogeneity of variances were performed using the SAS Guide Data Analysis. Data were analyzed by ANOVA using the SAS procedure Glimmix. The group information (Lactation), Farm and Age were used in the statistical models. Means of groups were compared using Least Square Means (LSMeans) of SAS, where significant difference was observed at $P \leq 0.05$. It was possible to conclude that buffaloes during peak lactation need to metabolize body reserves to supplement the lower amounts of bloodstream lipids, when they remain in negative energy balance. In the dry period, there were significant changes in the lipid profile, characterized by decrease of nutritional requirements, with consequent improvement in the general conditions of the animals.

RESUMO: *Com o objetivo de se analisar a influência da lactação e do período seco nos constituintes do metabolismo lipídico e na glicemia de búfalas leiteiras, foram coletadas amostras de soro e plasma de 147 animais, entre novembro de 2009 e julho de 2010, sendo estes oriundos de propriedades criadoras de búfalos das raças Murrah, Mediterrâneo e Mestiços, localizadas no Estado de São Paulo. A análise bioquímica foi obtida por meio da determinação dos teores séricos de colesterol, triglicérides, ß-hidroxibutirato (ß-HBO), ácidos graxos não esterificados (NEFA) e teores plasmáticos de glicose. Os valores da média aritmética e o erro padrão da média foram calculados utilizando-se o procedimento Means do SAS versão 9.2. Os dados foram analisados por ANOVA, usando o procedimento Glimmix do SAS. As informações de grupo (Lactação), Fazenda e Idade foram utilizadas nos modelos estatísticos. A comparação entre as médias dos grupos foi realizada por meio do teste de médias Least Square Means (LSMeans) do SAS, no qual foi considerada diferença para $P \leq 0,05$. Concluiu-se que em búfalas, durante a fase de pico da lactação, existe a necessidade de o animal metabolizar as reservas corporais para suprir as menores quantidades de lipídios circulantes, estando os animais em balanço energético negativo. No período seco, existem significativas alterações no lipidograma, caracterizadas por diminuição na exigência nutricional, com consequente melhora na condição geral do animal.*

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1 Introduction

Blood lipids play an important role in the synthesis of fatty acids (fat) of milk from dairy animals (KANEKO, 2008). The main sources of fatty acids in milk are triglycerides, phospholipids, cholesterol esters and non-stratified fatty acids (NEFA) originated from the bloodstream (IVERSON; HAMOSH; BOWEN, 1995; TRIPATHI et al., 2010).

In buffaloes, particularly, the understanding of lipid metabolism is very important, once the organoleptic properties of products derived from buffaloes (fattier milk and meat with lower cholesterol) differ from those observed in bovines. While buffaloes produce milk with 8.2% of fat - ranging from 5.5 to 13%, bovine cows produce milk with 3.8% of fat in average (VERRUMA; SALGADO, 1994).

Metabolic blood profiles have been widely utilized to identify and indicate metabolic disturbs and low yield (LEE et al., 1978). Therefore, studies regarding lipid and glucose profiles can assist the understanding of buffaloes' adaptations and peculiarities, enabling to adjust the handling and feeding of these animals (CAMPANILE et al., 1998; HAGAWANE; SHINDE; RAJGURU, 2009).

Furthermore, blood values obtained from animals raised in a given region cannot be considered without appropriate evaluation, such as benchmark, for animals raised outside that location; because, under different environmental, climatic and handling conditions, substantial variations in blood constituents can be noticed (POGLIANI; BIRGEL JUNIOR, 2007).

The scant information found in the Brazilian literature regarding lipid metabolism in buffaloes (MARQUES et al., 2004; FECKINGHAUS, 2009; VERDURICO, 2010) and, mainly, the lack, in Brazil, of research appropriately planned to assess the influence of inducing factors in the variation of blood profile in these animals, have motivated the elaboration of the present research, which aims to analyze the influence of lactation and dry period in the serum contents of cholesterol, triglycerides, beta-hydroxybutyrate (β-HBO), non-esterified fatty acids (NEFA) and plasma glucose in buffaloes.

2 Material and Methods

In order to carry out this research, 147 buffaloes were utilized – average of seven years of age and four calving - Murrah, Mediterranean and crossbred; the animals were raised in three dairy farms located in the State of Sao Paulo. Two of these farms are located in the 'Vale do Ribeira' region, one in municipality of Registro and the other in Pariquera-Açu; the third farm lies in the Midwestern region of the state, in the municipality of Sarapui.

The farms located in the 'Vale do Ribeira' region used extensive handling system and unfertilized pastures of brachiara grass (*Brachiaria* sp.). There was water in all pastures, but the mineral mixture was provided only to the dairy buffaloes, which were milked only once a day. In the farm located in the Midwestern region of the state, the buffaloes were raised under rotational grazing of fertilized cultivated forage, with water availability at ease, and supply of feed comprising soybean meal, corn meal, cottonseed, citrus pulp, urea and *Panicum maximum* silage, which was offered before each of the two daily milking.

The animals were clustered according to the yielding period, as shown in Table 1.

One hundred forty-seven samples of serum and blood plasma from the buffaloes were collected between 2 and 6 h after the last feeding, whether it was based on grazing forage or on supplemented feeding in the trough. For the lactating buffaloes, the collection started after 9 AM, after feeding and the first milking of the day.

The buffaloes used in the research were selected according to the necessities for the formation of the experimental groups. Prior to collection, the animals were submitted to clinical examination and those considered unhealthy were excluded from the research. After this selection, blood samples were collected by puncturing the external jugular vein with the use of vacuum multiple collection system. The tubes containing sodium fluoride anticoagulant were used for glucose determination and the ones with no anticoagulant were used for cholesterol, triglycerides, non-stratified fatty acids (NEFA) and beta-hydroxybutyrate (β-HBO) determinations.

The samples for the determination of glucose plasma contents were kept under refrigeration until analysis performance. Those meant for cholesterol, triglycerides, non-stratified fatty acids (NEFA) and beta-hydroxybutyrate (β-HBO) assessment were maintained at room temperature to facilitate clot retraction.

In the laboratory, the samples were centrifuged at 3,300 rpm actual strength of spin for 15 min, so that clot syneresis or blood elements sedimentation could occur; then, serum and blood plasma were separated by aspiration in three aliquots each, totalizing six aliquots of Eppendorf tubes per animal. The samples were kept in freezer at –20 °C until new tests were performed.

Table 1. Experimental group formation to assess the influence of lactation on the lipid profile of buffaloes raised in the State of Sao Paulo.

Groups	Formation	Collected samples		
		Extensive	Semi-intensive	Total
61 - 90	Buffaloes between 61 and 90 days of lactation	27	19	46
91-200	Buffaloes between 91 and 200 days of lactation	25	13	38
>200	Buffaloes with more than 200 days of lactation	13	14	27
Dry	Non-lactating or dry pregnant buffaloes which calved at least once, between 272 and 414 days after delivery at the moment of collection	5	31	36
Total		70	77	147

Serum contents for cholesterol, triglycerides, β-HBO and NEFA were quantified by enzymatic colorimetric methodology (WILLIAMSON; MALLANBY; KREBS, 1962; ELPHICK, 1968; ALLAIN et al., 1974; FOSSATI; PRENCIPE, 1982). The results for the first three were expressed in mmol L^{-1}, and for the last in μmol L^{-1}. Plasma glucose contents were quantified through the method described by Barham e Trinder (1972), and the results were expressed in mmol L^{-1}. All samples were determined in automatic biochemical Labmax 240 analyzer - Tokyo Boeki Medical System, Japan.

Means of milk yield from the buffaloes, for the period concerning blood collections, were obtained by routine milk control performed monthly in the farms.

For the evaluation of the general condition of the animals, the body condition score (BCS), advocated by Houghton et al. (1990) and modified for the buffalo species by Baruselli et al. (2001), was utilized. Scores from 1 to 5, added by half unit to the measurements, were marked (lean 1; fed 5). All assessments were performed by the same person.

The values of arithmetic mean and standard error mean (SEM) were calculated using the procedure *Means* SAS 9.2 (SAS/STAT, SAS Institute Inc., Cary, NC). The normality of the residues and homogeneity of variances tests were conducted using the *Guide Data Analise* SAS. Data that presented abnormal distribution were transformed accordingly. Data were analyzed by ANOVA, *Glimmix* SAS procedure. The group information (Lactation), Age and Farm were used in the statistical models. In the final model, variables were removed by *Backward Elimination,* based on the Wald criterion at $P > 0.20$.

The comparison between groups means was done by the *Least Square Means* (*LSMeans*) SAS test, where difference was considered at $P \leq 0.05$; that is, probable repeatability ≥95%. The correlation between the metabolites analyzed and the milk yield was performed using the *Corr* SAS procedure.

3 Results and Discussion

Maximum cholesterol contents, around 3.50 mmol L^{-1}, were observed between 60 and 200 days of lactation; after 200 days of lactation, cholesterol contents began to drop gradually, reaching the lowest contents, around 2.00 mmol L^{-1}, when the buffaloes were already in the dry period (Table 2; Figure 1).

The cholesterol content reflects the acetyl-CoA available for energy generation, originated basically from food ingestion (KANEKO, 2008). Thus, in cases of food ingestion increase, there is consequent increase of insulin (BAN-TOKUDA et al., 2007), increasing leptin (CHILLIARD; DELAVAUD; BONNET, 2005), and reduction of glucagon and adenosine 3',5'-cyclic monophosphate (cAMP) intracellular; that is, when the organism is in dephosphorylation (smaller hydrolytic breakdown of the ATP due to adenylate cyclase action) cholesterol production is stimulated (KANEKO, 2008).

The serum cholesterol content variations observed in this research can be explained by the reduction in milk yield after 200 days of lactation (Figure 2), because during the reduction of milk yield, a decrease in nutritional demand and a reduced need of food ingestion also occur.

This fact can be pointed out by the correlation between the amounts of cholesterol and milk yield. The milk yield means for the periods assessed were 9.8 liters for buffaloes between 61-90 days of lactation, 8.4 liters for buffaloes between 91-200 days, and 5.7 liters for those with more than 200 days of lactation, where mild correlation was noted ($r = 0.39$) ($P = 0.0010$) between the amounts of cholesterol and milk yield. For confined buffaloes, blood cholesterol contents also followed the amount of food ingested (BAN-TOKUDA et al.,2007).

For buffaloes at several lactation times, cholesterol contents equal to 2.30 mmol L^{-1} were described (HAGAWANE; SHINDE; RAJGURU, 2009), while for buffaloes between 65-85 and 95-175 days of lactation they were equal to 2.98 and 2.78 mmol L^{-1} (GRASSO et al., 2004). In Brazil, buffaloes between 100 and 200 days of lactation - submitted

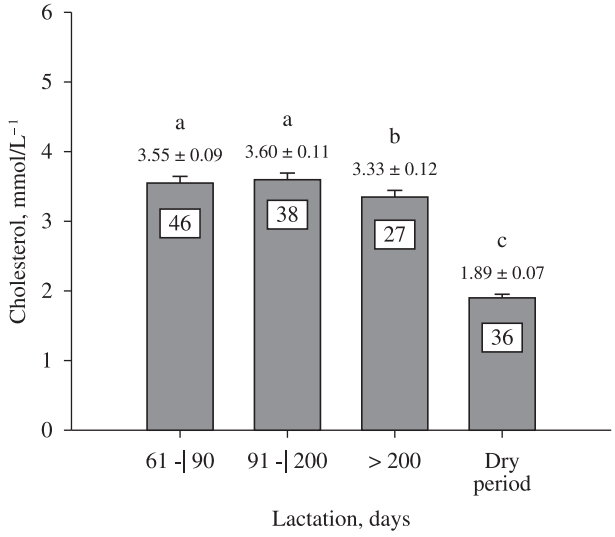

Figure 1. Effect of the different lactation (days) groups and dry period in serum cholesterol content (mmol L^{-1}) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed ($P < 0.0001$).

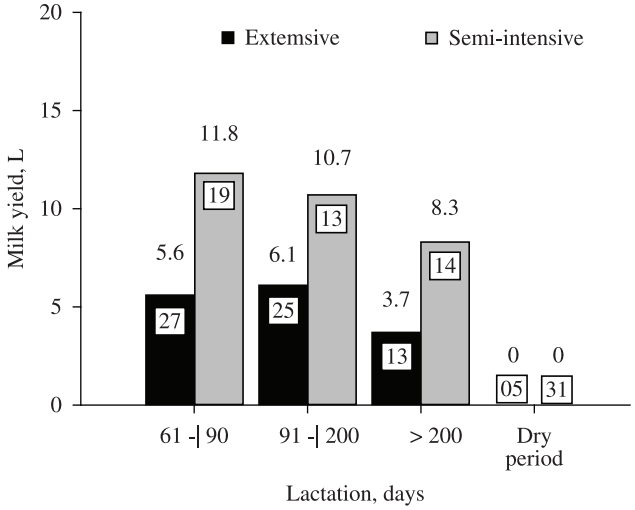

Figure 2. Effect of the different lactation (days) groups and dry period in the milk yield (liters) of buffaloes. Values in the white squares represent the amount of animals per group.

Table 2. Means and standard error mean (mean ± SEM) of the serum contents of cholesterol, triglycerides, β-HBO and NEFA, plasma glucose contents and BCS in the different lactation (days) groups in buffaloes.

Variable	Lactation phase of buffaloes			Dry period[1]	P
	61 -| 90	91 -| 200	>200		
Cholesterol. mmol L^{-1}	3.55 ± 0.09a	3.60 ± 0.11a	3.33 ± 0.12b	1.89 ± 0.07c	<0.0001
Triglic. mmol L^{-1}	0.18 ± 0.01c	0.23 ± 0.01b	0.32 ± 0.02a	0.31 ± 0.02a	<0.0001
β-HBO. mmol L^{-1}	0.85 ± 0.04a	0.70 ± 0.02b	0.67 ± 0.03bc	0.64 ± 0.03c	<0.0001
NEFA. µmol L^{-1}	268.55 ± 18.94a	176.64 ± 19.59b	110.76 ± 12.40b	175.52 ± 15.34b	<0.0001
Glicose. mmol L^{-1}	3.39 ± 0.05ab	3.41 ± 0.06a	3.37 ± 0.07bc	3.45 ± 0.05c	<0.0155
BCS	3.09 ± 0.06b	3.17 ± 0.09b	3.31 ± 0.10b	3.68 ± 0.07a	<0.0001

Different letters (a, b, and c) in the same line indicate statistical difference. [1]Dry period – Pregnant non-lactating animals which calved at least once, between 272 and 414 days after delivery.

or not to treatment with recombinant bovine somatrotopin (rBST) - presented cholesterol means equal to 2.99 and 3.08 mmol L^{-1} (FECKINGHAUS, 2009); while, in Italy, an approximate range of 2.5-3.5 mmol L^{-1} of cholesterol was described by Campanile et al. (1998) for the same period.

On the other hand, Tripathi et al. (2010) observed higher cholesterol values in buffaloes that were between 51 and 65 days (3.98 mmol L^{-1}), between 125 and 160 days (5.12 mmol L^{-1}) and between 198 and 262 days of lactation (4.48 mmol L^{-1}). It is believed that these differences are related to the regional availability of foods and the nutritional handling of buffaloes raised in India, where the following products and byproducts are often utilized: rice, wheat, cotton, oak, peanut, and Alexandrian trefoil (*Trifolium alexandrinu*) - a kind of leguminous plant seldom used in Brazil.

The triglycerides detected in serum are those available in the body: both those found in the chylomicrons lipoproteins - result of absorption of fat from the small bowel; as well as those found in very low-density lipoproteins (VLDL) - originated from the triglyceride synthesis by hepatocytes (HOUCQUETTE; BAUCHART, 1999).

During the peak of lactation, between 61 and 90 days, minimum values of triglycerides (0.18 ± 0.01 mmol L^{-1}) were found (Table 2; Figure 3). With the progress of lactation, the amount of triglycerides has increased, raising to levels of 0.23 mmol L^{-1} in buffaloes between 91 and 200 days after delivery, till reaching mean values of 0.32 mmol L^{-1} in buffaloes with more than 200 days of lactation and 0.31 mmol L^{-1} in buffaloes in the dry period. High negative correlation was noted between the amount of triglycerides and milk yield (r = –0.60; P < 0.0001); that is, the serum contents of triglycerides gradually increased according to the decrease in milk yield (Figure 2).

Blood lipids are fundamental for the synthesis of milk fat (KANEKO, 2008). The main sources of this fat are triglycerides, phospholipids, cholesterol esters (part of the total cholesterol) and bloodstream non-stratified fatty acids (NEFA) derived from lipolysis (IVERSON; HAMOSH; BOWEN, 1995; TRIPATHI et al., 2010).

The withdrawal of bloodstream triglycerides for maximum fat yield in buffalo milk justified the minimum contents of this metabolite during peak lactation phase. Furthermore, the limited amounts of VLDL physiologically produced by ruminants (HOUCQUETTE; BAUCHART, 1999; KANEKO,

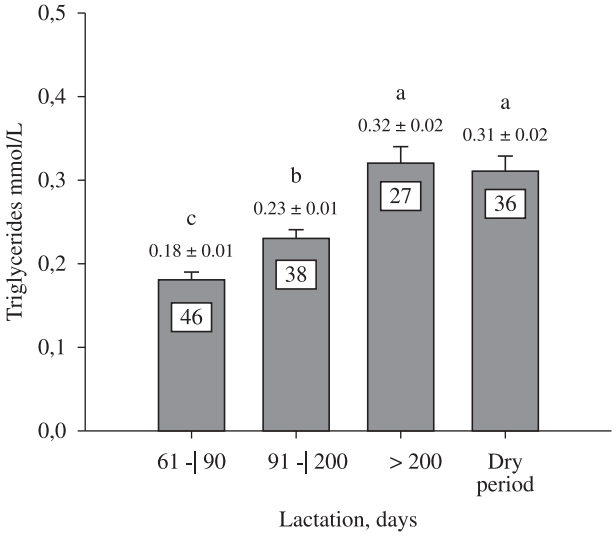

Figure 3. Effect of the different lactation (days) groups and dry period in serum triglycerides content (mmol L^{-1}) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed (P < 0.0001).

2008), plus the presence of negative energy balance (NEB) (BONNET et al., 2004) and the greater presence of bloodstream glucagon (GIBBONS, 1990), as observed in the lactation peak, contribute to the reduction of its serum contents. The negative energy balance has disappeared and the milk yield has lowered with the progress of lactation. There was an improvement in the body condition score (BCS) and an increase in the serum triglycerides contents with the restoration of metabolic balance.

All triglycerides contents found in the literature were close to the ones revealed in this research. For buffaloes between 65-85 and 95-175 days of lactation, Grasso et al. (2004) found 0.10 and 0.10 mmol L^{-1} triglycerides means. For buffaloes between 100 and 200 days of lactation, submitted or not to treatment with recombinant bovine somatrotopin (rBST), means were equal to 0.21 and 0.20 mmol L^{-1} (FECKINGHAUS, 2009).

Triglycerides contents equal to 0.19, 0.32 and 0.25 mmol L^{-1} in buffaloes in the beginning (51-65 days), in the middle (125-160 days) and in the end (198-262 days) of lactation were described by Tripathi et al. (2010). This suggests that

the changes observed in bloodstream triglycerides contents during the lactation curve are physiological.

The highest β-HBO serum contents were found in buffaloes between 61 and 90 days of lactation (Table 2; Figure 4) and gradual reduction was observed as lactation progressed, until reaching minimum contents in buffaloes already in the dry period.

The ketone body β-HBO may originate either from nutritive or metabolic ketogenesis. In nutritive ketogenesis, which is physiological in ruminant animals, the ruminal butyrate is transformed in β-HBO by the paunch cells. In the metabolic ketogenesis, which is related to lipolysis and NEB, large amounts of NEFA reach the liver, exceeding the amount of oxaloacetate available to enter the Krebs cycle, also forming ketone bodies, but this time, through the alternative way of β-Oxidation.

Under normal conditions, about 70% of bloodstream ketone bodies are produced in the paunch – nutritive ketogenesis, while only 30% are produced by the liver – metabolic ketogenesis (HOUCQUETTE; BAUCHART, 1999). Regardless of their origin, ketone bodies can be used as source of energy by most tissues (HOUCQUETTE; BAUCHART, 1999; KANEKO, 2008).

NEFA basically reflect the lipolysis levels of animals, that is, how much is being demanded by the body to feed the energy needs, being indicated to determine the energy balance (BONNET et al., 2004).

Serum NEFA contents (Table 2; Figure 5) in the group of buffaloes between 61-90 days after delivery are significantly higher than those observed in the groups formed by animals with 91 to 200 days of lactation, with more than 200 days of lactation, and in animals in the dry period.

As noted, numerically, BCS values (Figure 7) and, statistically, in triglycerides and β-HBO contents, the amount of NEFA is also altered during the period of greater milk yield of these buffaloes, reaching means of about 250 μmol L^{-1}, confirming the hypothesis of energy deficit at lactation peak, probably due to the smaller availability of nutrients compared to the amount demanded by the buffalo milk.

During lactation peak, animals needed to resort to body reserves in order to supplement the smaller amount of bloodstream lipids, reflecting in the higher contents of β-HBO and NEFA and in the smaller contents of triglycerides available in the blood. This fact was noted in the mild negative correlation $r = -0.35$ ($P < 0.0001$) between the NEFA values and the amounts of triglycerides available.

With the subsequent decrease in the milk yield of the animals evaluated, it was possible to notice the normalization of contents of the referred metabolites, alongside the drop of NEFA contents to 150 μmol L^{-1}. This can be verified in the mild positive correlation between the amount of NEFA and the milk yield ($r = 0.52$; $P < 0.0001$).

For buffaloes between 65-85 and 95-175 days of lactation, Grasso et al. (2004) found means of 370 and 239 μmol L^{-1} of NEFA, while Campanile et al. (1998) reported values between 200 and 300 μmol L^{-1} for buffaloes within 100-200 days of lactation. In contrast, the literature brings experiments with buffaloes where means of 454.33, 417.41 and 460.15 μmol L^{-1} of NEFA are reported for buffaloes in the beginning (51-65 days), middle (125^{-1}60 days) and in the end (198-262 days) of lactation (TRIPATHI et al., 2010), respectively, or average mean value equals to 518.45 μmol L^{-1} of NEFA in buffaloes within 100 and 200 days of lactation (FECKINGHAUS, 2009). These data demonstrate the existence of disturbs in the energy metabolism.

Yet, in the research carried out by Feckinghaus (2009), NEFA contents were kept around 280 μmol L^{-1} (levels close to those found in this research) until the time of the dramatic decrease in temperature (0 °C) when the study was conducted,

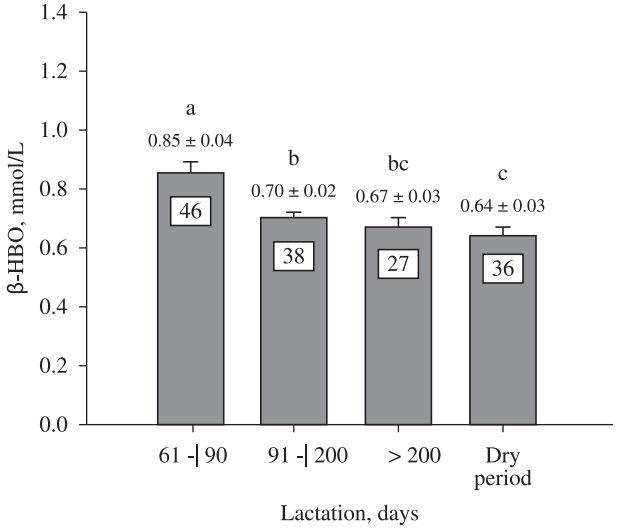

Figure 4. Effect of the different lactation (days) groups and dry period in serum β-HBO content (mmol L^{-1}) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed ($P < 0.0001$).

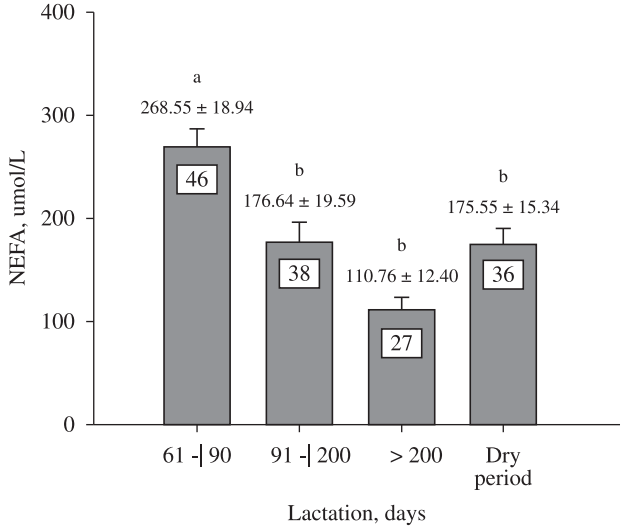

Figure 5. Effect of the different lactation (days) groups and dry period in serum NEFA content (umol L^{-1}) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed ($P < 0.0001$).

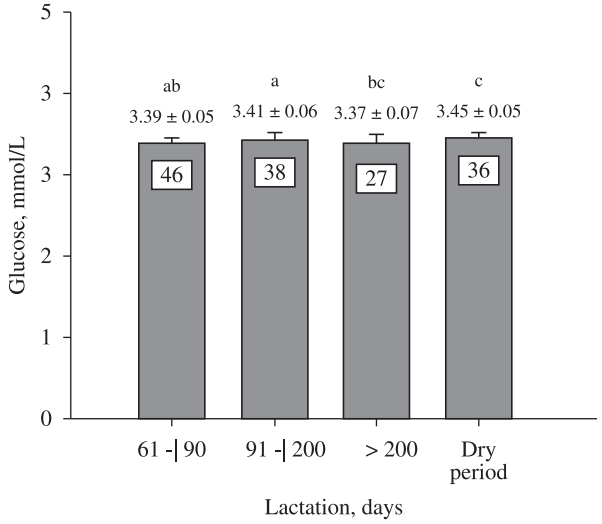

Figure 6. Effect of the different lactation (days) groups and dry period in blood glucose (mmol L^{-1}) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed (P = 0.0155).

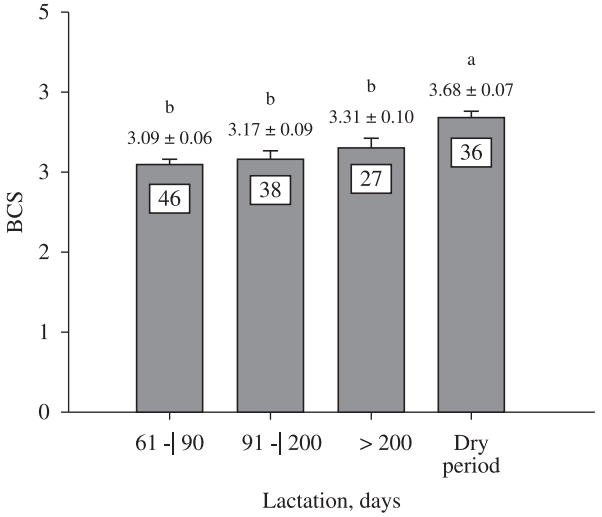

Figure 7. Effect of the different lactation (days) groups and dry period in body condition score (BCS) of buffaloes. Values in the white squares represent the amount of animals per group. Values (mean ± SEM) with one index (a, b and c) have differed (P < 0.0001).

which may have contributed to the significant increase in the average NEFA contents to above 650 µmol L^{-1} just two days after the assessment of the same animals. Another study also reports the possibility that the cold weather stress may have caused inconstant levels of lipolysis (alike nutritional deficits), and consequently, increase of NEFA contents (MARAI; HAEEB, 2010).

For the mean blood glucose contents (Table 2; Figure 6), despite the significant statistical difference between groups, it was not possible to appraise the nature of this influence.

Glucose contents of 2.78 mmol L^{-1} for buffaloes in various lactation phases were described by Hagawane, Shinde and Rajguru (2009). However, considering buffaloes between 65-85 and 95-175 days of lactation, the contents were equal to 3.42 and 3.25 mmol L^{-1}, respectively (GRASSO et al., 2004), and for buffaloes between 100 and 200 days of lactation, the contents were 3.76 and 3.63 mmol L^{-1}, submitted or not to treatment with recombinant bovine somatrotopin (rBST) (FECKINGHAUS, 2009). Buffaloes in period equivalent to 91-200 days of lactation recorded glucose contents between 4.0-4.5 mmol L^{-1} (CAMPANILE et al., 1998). All glucose contents found in buffaloes, though varying in relation to the feeding regime adopted in each study, were close to the contents reported in this research.

The BCS is used to estimate the amount of metabolizable energy stored in fat and muscle (body reserve) of living animals and to quantify the ability of females to use or preserve body reserves, according to the metabolic challenge that they must undergo (BARUSELLI et al., 2001).

Analysis of the results obtained for body condition score (BCS) demonstrates that, in buffaloes in the dry period, there is a significant increase in the scores given to the animals (Table 2; Figure 7). BCS has proven to be a useful and inexpensive tool to estimate energy balance intensity in buffaloes during the lactation and dry period.

The inter-relation between the BCS and the results found for triglycerides, β-HBO and NEFA was verified. During lactation peak, lower contents of triglycerides and higher contents of β-HBO and NEFA were found, with the energy balance deficit corresponding to the lowest absolute values of BCS.

In animals with more than 200 days of lactation, that have already experienced reduction in milk yield, higher contents of triglycerides and basal contents of β-HBO and NEFA were observed during the dry period. This indicates a time of positive energy balance and fat accumulation, which can be noticed by the increase in the BCS found in animals in the dry period. It also confirms the biochemical results found; a mild positive correlation (r = 0.39; P < 0.0001) between the BCS and the amounts of bloodstream cholesterol was observed.

4 Conclusions

Buffaloes during peak lactation phase need to metabolize body reserves to supplement the smaller amounts of bloodstream lipids, when they remain in negative energy balance.

In the dry period, there are significant changes in the lipid profile, characterized by decrease of nutritional requirements, with consequent improvement of the general condition of animals.

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