Metabolic evaluation of dairy cows submitted to three different strategies to decrease the effects of negative energy balance in early postpartum[1]

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ABSTRACT.- García A.M.B., Cardoso F.C., Campos R., Thedy X.D. & González, F.H.D. 2011. **Metabolic evaluation of dairy cows submitted to three different strategies to diminish the effects of negative energy balance in early postpartum.** *Pesquisa Veterinária Brasileira 31(Supl.1):11-17.* Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: alejandrabarrera.ufrgs@yahoo.com

In early lactation dairy cattle suffer metabolic alterations caused by negative energy balance, which predisposes to fatty liver and ketosis. The aim of this study was to evaluate the metabolic condition of high yielding dairy cows subjected to three treatments for preventing severe lipomobilization and ketosis in early lactation. Fifty four multiparous Holstein cows yielding >30 L/day were divided into four groups: control (CN= no treatment), glucose precursor (PG= propylene-glycol), hepatic protector (Mp= Mercepton®), and energy supplement with salts of linolenic and linoleic faty acids (Mg-E= Megalac-E®). Treatments were administrated randomly at moment of calving until 8 weeks postpartum. Blood samples were collected on days 1, 7, 14, 21, 28, 35, 42 and 49 postpartum. Body condition score (BCS) was evaluated at the same periods and milk yield was recorded at 2nd, 4th, 5th, 6th, 7th, and 8th weeks of lactation. Concentrations of non-esterified fatty acids (NEFA), albumin, AST, ß-hydroxybutyrate (BHBA), cholesterol, glucose, total protein, urea and triglycerides were analyzed in blood samples. Cut-off points for subclinical ketosis were defined when BHBA ≥1.4 mmol/L and NEFA ≥0.7 mmol/L. General occurrence of subclinical ketosis was 24% during the period. An ascendant curve of cholesterol and glucose was observed from the 1st to the 8th week of lactation, while any tendency was observed with BHBA and NEFA, although differences among treatments were detected ($p \leq 0.05$). BCS decreased from a mean of 3.85 at 1st week to 2.53 at 8th week of lactation ($p=0.001$). Milk yield was higher in the Mg-E group compared with the other treatment groups ($p \leq 0.05$) Compared with the CN group, the treatments with Mp and PG did not show significant differences in blood biochemistry and milk yield. Cows receiving PG and Mg-E showed higher values of BHBA and NEFA ($P<0.05$), indicating accentuated lipomobilization. Supplementation with Mg-E also resulted in significant higher concentrations of cholesterol, BHBA, urea, AST and lower values of glycemia. This performance may be explained by the highest milk yield observed with this treatment. Treatments with PG and Mp did not improve milk yield, compared with control cows, but did not show metabolic evidence of ketosis, fat mobilization or fatty liver. These results suggest that treatment with Mg-E improves milk production but induces a higher negative energy balance leading to moderated lipomobilization and ketone bodies production, increasing the risk of fatty liver.

INDEX THERMS: Fatty liver, ketosis, biochemical indicators, early lactation, transition period.

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RESUMO.- [**Avaliação metabólica de vacas leiteiras submetidas a três estratégias para diminuir os efeitos do balanço energético negativo no pós-parto inicial.**] Durante o início da lactação as vacas leiteiras sofrem transtornos metabólicos causados pelo balanço energético negativo, o que predispõe a infiltração gordurosa hepática e cetose. O objetivo deste estudo foi avaliar o status metabólico de vacas leiteiras de alta produção submetidas a três tratamentos para prevenir severa lipomobilização e cetose no início da lactação. Cinquenta e quatro vacas de raça Holandesa multíparas produzindo >30 L/dia foram divididas em quatro grupos: controle (CN= sem tratamento), precursor de glicose (PG= propileno-glicol), protetor hepático (Mp= Mercepton®) e suplementação com sais de ácidos linolênico e linoléico (Mg-E= Megalac-E®). Amostras de sangue foram coletadas nos dias 1, 7, 14, 21, 28, 35, 42 e 49 do pós-parto. A condição corporal foi avaliada nos mesmos períodos e a produção de leite foi registrada nas semanas 2, 4, 5, 6, 7 e 8 de lactação. As concentrações de ácidos graxos não esterificados (AGNE), albumina, AST, ß-hidroxibutirato (BHB), colesterol, glicose, proteína total, uréia e triglicerídeos foram determinadas nas amostras de sangue. Pontos de corte para diagnosticar cetose subclínica foram definidos quando BHB ≥1,4mmol/L e AGNE ≥0,7mmol/L. A ocorrência geral de cetose subclínica foi de 24% durante o período. Uma curva ascendente de colesterol e de glicose foi observada desde a 1ª até a 8ª semana de lactação, enquanto que nenhuma tendência foi observada com BHB e AGNE, embora diferenças entre os tratamentos foram detectadas (p≤0,05). A condição corporal diminuiu de uma media de 3,85 na 1ª semana até 2,53 na 8ª semana de lactação (p=0,001). A produção de leite foi superior no grupo de Mg-E comparado com os demais tratamentos. Comparado com o grupo CN, os tratamentos de Mp e PG não mostraram diferenças significativas na bioquímica sanguínea nem na produção de leite (p≤0,05) As vacas que receberam PG e Mg-E mostraram maiores valores de AGNE, indicando uma acentuada lipomobilização. A suplementação com Mg-E também resultou em maiores concentrações de colesterol, BHB, uréia, AST e menores valores de glicemia. Este resultado pode ser explicado pela maior produção de leite observada com este tratamento. Os tratamentos com PG e Mp não melhoraram a produção de leite, comparados ao grupo CN, mas também não mostraram evidências metabólicas de cetose, alta lipomobilização nem infiltração gordurosa hepática. Os resultados sugerem que o tratamento com Mg-E melhora a produção de leite, mas induz um balance energético negativo maior levando a moderada lipomobilização e produção de corpos cetônicos, aumentando o risco de fígado gorduroso.

TERMOS DE INDEXAÇÃO: Lipidose hepática, indicadores bioquímicos, início da lactação, período de transição.

INTRODUCTION

During early lactation dairy cows experience a negative energy balance (NEB), which causes lipid mobilization from adipose tissue (Campos et al. 2005). When the lipid mobilization is intense, and persevere for a long time, the glycogen reserves in the liver can be depleted, compromising the gluconeogenesis that lead the animal to a high risk in developing ketosis (Drackley 1999). Fatty liver and ketosis are metabolic disorders that usually develop between the second and seventh week after calving (Herdt 2000). Prevention of metabolic disorders in dairy cattle is necessary to maintain optimal hepatic function (Bertics & Grummer 1999).

Excessive lipid mobilization can be prevented with different strategies such as the reduction of blood levels of non-esterified fatty acids (NEFA), increase in the complete oxidation of NEFA in extra-hepatic tissues, and increment of the liver exportation rate through very low density lipoproteins (VLDL) (Grummer 2008). The prophylactic use of additives that can increase ruminal propionate concentration, such as propylene glycol, have been associated with insulin stimulation, decrease in lipolysis resulting in a better energetic status in dairy cows (Studer et al. 1993, Grummer et al. 1994, Christensen et al. 1997, Duffield 2000).

Feed additives have been studied in order to alleviate the NEB experienced by dairy cattle through increased energy density of the diet. Some of these additives are known as protected fat or "by-pass" fat (Grummer & Carol 1991, Bertics & Grummer 1999, Pickett et al. 2003). This nutritional strategy consists in the utilization of fatty acids, originated from the diet that could positively influence the energetic status in the early post-partum by offering high energy in a period where depression of dry matter intake (DMI) is eminent. The fatty acids originated from the diet are utilized by extra-hepatic tissues, differently than NEFA that are metabolized in the liver (Bertics & Grummer 1999, Pickett et al. 2003). Therefore, the lipid mobilization would be less intense and would have less negative effect on liver metabolism (Drackley 1999, Picket et al. 2003).

Another strategy used to prevent the lipid mobilization effects is the administration of precursors necessary for very low density lipoproteins (VLDL) synthesis, that what represents a challenge in ruminants because of the low exportation rate of these lipoproteins (Grummer 1995). Relatively little information is available on the effectiveness of lipotropic agents to enhance hepatic VLDL secretion in ruminants (Bertics & Grummer 1999). The utilization of hepatic protectors with components such as methionine and choline are an alternative to increase the efficiency of VLDL secretion in ruminants. In southern Brazil, Rio Grande do Sul, it is a common practice the utilization of liver protectors during the early postpartum in dairy cows. However, the information regarding the advantages and disadvantages of such practice on the metabolic status of dairy cows is limited. Tedesco et al. (2004) have shown the potential use of silimarina; i.e. human liver protector, in dairy cows during the transition period. The effect of this substance is due to the ant-oxidant effects of the flavolignans extracted from the *Silybum marianum* plant. The utilization of silimarina could help in the prevention of liver disorders through the reduction of per oxidation of lipids, and destruction of hepatic cells membrane. Nevertheless, the number of studies evaluating liver protectors are just a few. This present study has the objective to evaluate three different strate-

gies applied during the first thirty days postpartum to prevent NEB in dairy cows. Metabolic indicators were used from plasma and urine in the first eight weeks postpartum, under commercial dairy production characteristics in Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

Animals

Fifty four multiparous Holstein cows yielding more than 30L/cow/day were used. All animals were part of the same herd in a commercial dairy farm in the Taquari region, Teutonia county, Rio Grande do Sul (latitude 29°S, longitude 51°W). The herd consisted of 75 milking Holstein cows managed in a semi-confinement system. One week before the expected calving date, a clinical exam was performed in each cow to check for health problems. In addition, it was recorded each cows health history, register, age, previous lactation milk production, parity and body condition score (BCS). For the total period of experiment the BCS determination followed the methodology proposed by Edmonson et al. (1989) where a scale of 5 points is used (1 - extremely skin; 5 - extremely obese). BCS evaluations were recorded weekly from the first trough the eighth week postpartum. Evaluations were made by the same person in order to reduce variation of this index.

Treatments

Treatments were randomly assigned to each cow at calving in one of the following groups:

Control group (CN, n=15), received the basic farm diet.

Propylene glycol group (PG, n=14), besides the basic farm diet, received 300mL propylene glycol (Nuclear Laboratory), orally, every 48 hours during the first 30 days postpartum. A total of 15 applications were performed always at 19:00 (Grummer et al. 1994, Christensen et al. 1997).

Mercepton group (Mp, n=15), besides the basic farm diet, received 20 mL Mercepton® (Bravet), intramuscular injection (IM), every 48 hours during the first 30 days postpartum. A total of 10 applications were performed always at 19:00. Dose was adjusted following fabricant recommendations.

Megalac-E group (Mg-E, n=10), besides the basic farm diet, received 250 g/cow/day Megalac-E®[6] (Arm & Hammer) mixed in the ration and administered daily at 19:00, during the first 30 days postpartum. Dose was administered following fabricant recommendations.

Besides the treatments, each cow received daily, in a dry matter basis: 0.6 kg of mineral supplement, 0.89 kg of wheat bran, 5.7 kg of ground corn 4 kg of soybean meal, 0.2 kg of sodium bicarbonate (buffer) and 8.05 kg of corn silage. The nutritional values of the diet followed the NRC (National Research Council, 2001) recommendations to Holstein cows with 570 Kg of body weight and 33 L of milk production with 3.5% of milk fat and 3.2% of milk protein. The diet was formulated using the software CPM Dairy Cornell-Penn-Miner Version 3.0.10.

In the beginning of the study cow's average age and previous lactation milking production (L/day) were: 5.53±1.96 years and 33.8±3.84 L; 5.86±1.75 years and 33.43±3.08 L; 5.40±1.30 years and 32.53±4.14 L; 4.50±0.97 years and 33.49±3.13 L for the groups CN, PG, Mp and Mg-E respectively.

Samples

Blood and urine samples were collected on days 1, 7, 14, 21, 28, 35, 42, and 49 after calving, always before the evening milking (16:30), before feeding and treatment administration. Blood samples were collected in two aliquots through puncture of the coccygeal vein or artery in sterile vacuum tubes (Vacutainer, Becton Dickinson) 10mL without coagulant and 4mL with $EDTAK_2$ (7.2mg). Plasma was separated immediately after collection (3mL), never after the period of ten minutes. The samples without coagulant were maintained at room temperature for 2 hours and then centrifuged (980g for 15 minutes). The serum was extracted and fractioned in several aliquots of 600 µL in 1.5mL *Eppendorf* tubs identified and kept under refrigeration until its transport to the laboratory where they were stored at -20°C until the moment of the biochemical analysis.

Urine samples were collected through induction by perineal massage. The first jets of urine were despised. A minimum of 200mL was collected in sterilized recipients. The samples were put under refrigeration and were analyzed before 24 hours after the collection. Urine analysis made in the field were pH determination using a digital pHmeter (pHTEK model pH100), and the presence of ketone bodies through reagent strips (Multistix 10SG [MTX], Bayer Corp., USA) sensitive to acetoacetate and ketone.

Laboratorial analysis

The following metabolites were determined by spectrophotometric methods: Non-esterified fatty acids (NEFA), albumin, aspartate-aminotransferase (AST), beta-hydroxybutyrate (BHBA), cholesterol, glucose, total protein, urea and triacylglycerol. All the analysis were performed through automatic equipment (Metrolab D-1600), using diagnostic commercial kits (Randox, for NEFA and BHBA and Labtest for the others). Globulin values were obtained by the difference between total protein concentration and albumin. All the samples were determined in duplicates. Samples with coefficient of variation (CV) greater than 10% were re-analyzed and discrepant values were discarded.

Statistical analysis

The experiment was developed as a complete random design (CRD) with four treatments and repeated measures in time (collection periods). For each one of the variables to be analyzed (metabolites or clinical status of the metabolic disease) the ANOVA method was used with a multivariate model using the option (GLM, general linear model), for different sample size between treatments. When a minimal significant difference between the means was identified, Duncan test used.

The Duncan test is a dynamic test and allows comparisons of treatments in system-all versus all. Previously, descriptive analysis was done by treatment, period and variable. Using the Pearson correlation, possible mathematical correlations were tested among the analyzed metabolites. A probability of 95% ($p \leq 0.05$) was considered significant in the statistical analysis. The software SPSS-PASW 18 for Windows (Chicago) was used for the statistical analysis.

RESULTS

In the present multivariate model, statistical significance was not identified in the principal effects group (treatment), week of collection, and the interaction (treatment by week). The results regarding the determinations of cholesterol, trilglycerides, glucose, BHBA, NEFA, total protein, albumin, globulins, urea, body condition score (BCS) are presented in table 1 by week, and table 2 by treatment and milk yield are presented in table 3. Individual results are not shown due to its non significance in the univariable analysis.

[6] Megalac E®: calcium salts of unsaturated faty acids linolenic 3-4%and linoleic 42-49%.

Table 1. Average, standard deviation, probability values (*p*) for blood metabolites analyzed in each treatment group (average for eight weeks)

Parameter	Treatments				
	CN (n=15)	PG (n=14)	Mp (n=15)	Mg-E (n=10)	p
Cholesterol (mmol/L)	3.37^{a} ± 1.3	3.14^{a} ± 1.06	3.37^{a} ± 1.09	4.41^{b} ± 162	0.001
Triglycerides (mmol/L)	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.03	0.13 ± 0.02	0.259
Glucose (mmol/L)	3.34^{b} ± 0.48	3.13^{a} ± 0.57	3.34^{b} ± 0.43	2.90^{a} ± 0.48	<0.001
BHBA (mmol/L)	0.82^{a} ± 0.33	0.83^{a} ± 0.34	0.90^{ab} ± 0.28	1.00^{b} ± 0.63	0.017
NEFA (mmol/L)	0.30^{a} ± 0.28	0.41^{b} ± 0.28	0.25^{a} ± 0.17	0.50^{c} ± 0.49	0.001
Total protein (g/L)	80.72^{a} ± 8.85	84.07^{b} ±10.17	83.37^{b} ± 8.92	81.87^{a} ± 7.81	0.012
Albumin (g/L)	28.66^{a} ± 3.72	29.10^{ab} ± 3.37	30.04^{b} ± 6.31	31.50^{b} ±2.19	0.001
Globulins (g/L)	52.06^{b} ± 9.99	54.96^{b} ± 11.46	53.76^{b} ± 9.98	50.36^{a} ±8.05	0.063
Urea (mmol/L)	7.03^{b} ± 1.82	6.01^{a} ± 1.34	7.53^{bc} ±1.75	7.61^{c}±1.19	0.001
AST (U/L)	126.5^{b} ± 43.2	103.8^{a} ± 30.5	133.9^{b} ± 62.5	144.8^{c} ± 57.3	<0.001
BCS	3.2^{b} ± 0.48	3.06^{a} ± 0.55	3.03^{a} ± 0.59	3.11^{b} ± 0.60	0.001

CN = control, PG = propylene glycol, Mp = Mercepton, Mg-E = Megalac-E, BCS = Body condition score (1-5). Different values between groups are indicated by different letters (P ≤0.05).

The BCS at calving was similar in all studied population, but during the following seven weeks there was a significant loss ending in the 8th week with a BCS difference of 1.3, parturition affected BCS changes during the experimental period, and statistical differences were found (p=0.001) (Table 2).

During the experimental period data relative to milk production was recorded during the 2nd, 4th, 5th, 6th, 7th, and 8th weeks. For the analyzed period the averages for milk production (L/day) were 32±6.8, 30.3±6.9, 31.2±8.4 and 38.5±4.9 for the CN group, PG, Mp and Mg-E, respectively. Mg-E group showed higher (p≤0.05) milk production when compared with the other groups (Table 3).

The least square mean value for glucose in the population studied was at calving (2.83±0.61mmol/L), with continuous increment in the next 7 weeks. The mean concentration of cholesterol showed a gradual increase with increasing days in milk ranging from 2.29±0.90mmol/L in the first week to 4.43±1.26mmol/L in the eighth week (Table 2). There was no significant difference for TG between treatments (p=0.259) or weeks (p=0.300) (Table 1 and 2).

Table 3. Average and standard deviation for milk yield from dairy cows in each treatment group (average for eight weeks).

Treatments	Milk yield (L/day)
CN	32.00^{a} ± 6.8
Mg-E	38.50^{b} ± 4.9
Mp	31.20^{a} ± 8.4
PG	30.30^{a} ± 6.9

CN = Control group, Mg-E = Megalac-E group, Mp = Mercepton group, PG = propylene-glycol group. Different values between groups are indicated by different letters (P≤0.05).

BHBA values greater than 1.2mmol/L were used as a cut-off value for risk factor of a cow develop ketosis (Duffield et al. 2009). It was established as a diagnostic parameter of subclinical ketosis BHBA values greater than 1.4mmol/L (Oetzel 2004) together with NEFA concentrations greater than 0.7mmol/L (Whitaker 2004). The 2nd week was the period with the greater number of samples with BHBA and NEFA concentrations compatibles with subclinical ketosis (17.9%; n=19). From a total of 428 samples, 196 had high concentration of NEFA (52) and BHBA (54), leading to a 24.76% prevalence of subclinical ketosis. These results are related with the appearance of ketone bodies in urine, since 102 samples were positive, resulting in 23.87% prevalence of subclinical ketosis. It was observed 52 samples (12.1%) with NEFA concentration ≥0.7mmol/L, from which 39 (75%) had trilglycerides concentration (TG) between 0.04 and 0.1mmol/L, being 25 (64.10%) with high AST concentration (ranging from 100 to 322 U/L). NEFA concentration ranged from 0.7 to 2.67mmol/L in the first four weeks of lactation, reducing through the end of the experiment demonstrating the severity of NEB in the beginning of the lactation.

DISCUSSION

The results presented in this study are the reflection of expected physiologic adaptations of the energy metabolism

Table 2. Average, standard deviation, probability values (*p*) for blood metabolites in dairy cows analyzed during the first eight weeks of lactation

Parameter	Weeks postpartum								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	***p***
Triglycerides (mmol/L)	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.03	0.14 ± 0.07	0.13 ± 0.03	0.14 ± 0.02	0.13 ± 0.03	0.300
Cholesterol (mmol/L)	2.29^{a} ± 0.90	2.71^{b} ± 0.88	3.20^{c} ± 1.08	3.54^{cd} ± 1.12	3.78^{de} ± 1.20	3.95^{de} ± 1.15	4.19^{ef} ± 1.03	4.43^{f} ± 1.26	0.001
Glucose (mmol/L)	2.83^{a} ± 0.61	3.09^{b} ± 0.53	3.21^{b} ± 0.55	3.24^{b} ± 0.54	3.15^{b} ± 0.48	3.30^{bc} ± 0.40	3.47^{c} ± 0.34	3.29^{bc} ± 0.62	<0.001
BHBA (mmol/L)	0.82 ± 0.26	0.96 ± 0.54	0.95 ± 0.74	0.89 ± 0.30	0.85 ± 0.28	0.87 ± 0.29	0.83 ± 0.25	0.90 ± 0.29	0.533
NEFA (mmol/L)	0.39 ± 0.29	0.43 ± 0.44	0.38 ± 0.36	0.34 ± 0.41	0.33 ± 0.24	0.34 ± 0.25	0.36 ± 0.36	0.31 ± 0.29	0.597
AST U/L	116.45 ±54.2	124.70 ±53.09	121.02 ± 6.45	118.49 ±47.64	140.77 ±77.42	131.16 ±41.23	128.72 ±35.75	120.17 ±42.74	0.234
Total protein (g/L)	75.22^{a} ± 6.94	78.28^{a} ± 6.60	81.79^{b} ± 6.94	83.85^{bc} ± 8.37	85.27^{d} ± 9.46	84.79^{bc} ± 8.62	85.57^{d} ± 10.6	85.51^{d} ± 9.21	0.001
Albumin (g/L)	30.72 ± 8.08	28.85 ± 3.36	28.94 ± 3.23	29.58 ± 3.55	30.24 ± 3.52	29.51 ± 3.87	20.73 ± 3.77	29.8 ± 3.76	0.5361
Globulins (g/L)	45.34^{a} ± 8.26	49.43^{b} ± 6.87	52.85^{bc} ± 8.13	54.27^{c} ± 9.85	55.03^{c} ± 0.52	55.27^{c} ± 0.05	55.84^{c} ±11.71	55.64^{c} ±10.83	0.001
Urea (mmol/L)	6.58 ± 1.58	6.89 ± 1.84	6.91 ± 1.84	6.82 ± 1.66	7.04 ± 1.68	7.18 ± 1.53	7.38 ± 1.41	7.29 ± 1.75	0.187
BCS	3.85^{f} ± 0.25	3.65^{e} ± 0.28	3.33^{cd} ± 0.27	3.13^{c} ± 0.30	2.96^{c} ± 0.39	2.71^{ab} ± 0.34	2.60^{ab} ± 0.39	2.53^{a} ± 0.37	0.001

BCS = Body condition score (1-5). Different values between means are indicated by different letters in the Duncan test (p≤0.001).

of dairy cows in the early postpartum. In this period, there is an energy deficit that propitiates lipid mobilization from body adipose tissue and, consequently increases in blood concentration of NEFA and ketone bodies (Ingvarsten & Andersen 2000). High concentration of NEFA and ketone bodies can result in subclinical ketosis or clinical ketosis (Duffield 2000, Duffield et al. 2009).

Body condition scores provide an indication of the energy status of dairy cattle (Smith et al. 1997). Treatments did not influence BCS, which decreased through the lactation, as expected (Wang et al. 2009). The CN group had the least decrease in BCS, which can be associated with the stress level that each group experienced. That group received less intervention due to simpler feeding management, what could be responsible for a smaller negative effect in dry matter intake (DMI). There are controversial information about the relationship between BCS and ketosis susceptibility. Busato et al. (2002) stated that higher BCS before parturition and the maintenance of this condition in conjunction with optimal feed intake in the early post-partum period by cows can decrease their risk of ketosis. Therefore, cows would be better metabolically adjusted to increased energy requirements early postpartum. On the other hand, Edmonson et al. (1989) reported that higher BCS can increase the risk of ketosis. The increased risk could be explained by the excessive mobilization of body energy reserves, reflecting the longer negative energy balance in these animals and the consequently development of fatty liver and ketosis.

Urine pH values were in between the reference values for the bovine specie (Kaneko et al. 2008). The Pearson correlation test was highly significant between BHBA serum levels and urine ketone bodies ($p<0.001$). However, did not have any correlation with urine pH. This result is in agree with data shown by Campos et al. (2005) where no statistical relationship was found between serum levels of BHBA and urine ketone bodies with urine pH. Therefore, one could suggest that in the process of subclinical ketosis urine pH can be dismissed.

The cholesterol mean concentration had a gradual increase as the lactation developed, with some variation within groups (Table 2). The present study agrees with the results found by Souza & Junior (2009) that stated a continuous increase as days from calving increased. The crescent serum cholesterol concentration levels can be physiologic during the lactation (Cavestany et al. 2005) as a result of mobilization of fatty acids in consequence of glucagon secretion and increase of plasma lipoproteins concentration. Higher serum cholesterol concentration values in the Mg-E group can be a result from the supplementation of essential fatty acids from the diet (Table 1). Cholesterol concentration is in between of the reference values for Holstein cows in Rio Grande do Sul (González 2000). Lower serum cholesterol concentrations in the first weeks postpartum have been related with fatty liver (Steen 2001, Van den Top et al. 2005).

There was no significant difference in serum TG concentrations between groups. The values were in the same range reported for this region (González 2000, González et al. 2009). Data reported by Van den Top et al. (2005), suggested a continuous plasma TG concentration decrease postpartum, being lower concentration values related to cows with fatty liver. In the present study, it was not observed this tendency. González et al. (2009) reported a lower serum TG concentration in cows with high concentrations of BHBA and NEFA. This founding can be related to the fatty acids excess that is mobilized to the liver to be used as energy source. As a result of the limited liver capacity to export TG as VLDL, the liver storage of TG in the interior of the hepatocytes ends up decreasing the serum concentrations of TG.

Glucose values reported in this study are in agreement with the findings of other Brazilian researchers (González 2000, Campos et al. 2007, Cardoso et al. 2008, González et al. 2009, Souza & Junior 2009). The lowest concentration of serum glucose was at calving. Glucose serum concentration increased in the following seven weeks after calving. Mg-E group presented the lowest glucose serum concentration (Table 1). This result can be explained by the probable severe NEB experienced by the cows in this group that produced more milk during the studied period. The PG treatment seems not to have an effect in the gluconeogenesis when compared to the CN group. This data is in disagreement with the data presented by Studer et al. (1993), Grummer et al. (1994) and Christensen et al. (1997), where the administration of propylene glycol induced higher glycemic levels. The Mp group had glycemic levels similar to the CN group (Table 1).

A 24% prevalence of subclinical ketosis was found in the present study. As a cut off point for subclinical ketosis BHBA serum concentrations ≥1.4 mmol/L were considered (Duffield 2000, Oetzel 2004, Duffield et al. 2009). BHBA concentrations between 1.2-1.39mmol/L were considered by Duffield et al. (2009) as indicators of high ketone levels associated with the risk to develop ketosis. According to Oetzel (2004), BHBA concentrations ≥2.6mmol/L and NEFA ≥0.7mmol/L can be used to define subclinical ketosis. In the present study, only two animals were found in this condition, however, since they did not have clinical signs they were classified as with subclinical ketosis. The highest BHBA value and lowest glycemic value were observed in the Mg-E group. This result is in agreement with the suggestion that this group experienced the most severe NEB and also was more prone to develop ketosis, when compared with the other groups.

NEFA serum concentration is an indicator of the lipid mobilization degree from reserve adipose tissue and, in conclusion, of the NEB in ruminants. Canfield & Butler (1991) showed the importance of NEFA as a lipid mobilization indicator when the serum concentration of this metabolite was evaluated in cows experiencing NEB in the beginning of the lactation. NEFA values ≥0.7mmol/L are considered to be an indicative of severe NEB (Whitaker 2004). In the present study 52 samples (12.1%) were greater than this value. NEFA serum concentration had a tendency to decrease as the lactation progressed, in agreement with Adewuyi et al. (2006). Mg-E group was an exception to this observation, having an increase in serum NEFA concentration through the lactation. This result was expected since this group experience a severe NEB.

The sensibility of the fatty liver diagnose by the AST index is 94% (Bruss 2008). Grummer (1993) and Stojević et al. (2005) found that higher concentrations of AST in dairy cattle are associated with fatty liver syndrome, lower dry matter intake and ketosis signs. In the present study, Mg-E group presented higher serum concentration of AST when compared with the other groups. A tendency for increasing concentration of AST trough the lactation was observed for all groups. This result is in agreement with the ones reported by Dann et al. (2005), Stojević et al. (2005), and González et al. (2009). The highest serum AST concentration values can be an indicative of liver lesions mainly found in the Mg-E and Mp groups. The result also suggests that the supplementation with propylene glycol can have a liver protection action against lipid mobilization.

In conclusion, the results from this study suggest that the supplementation with protected fatty acids lead to a higher milk production, therefore increasing the NEB negative effect in dairy cows. In addition, the supplementation with protected fatty acids can result in liver dysfunction and predisposition to metabolic disorders such as ketosis. Propylene glycol supplementation may have a liver protection effect.

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