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Effects of a hepatoprotectant and different feed energy sources on milk composition and nutrient partitioning in lactating cows

Efeitos de um hepatoprotetor e diferentes fontes de energia na dieta sobre a composição do leite e a partição dos nutrientes em vacas lactantes

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ABSTRACT

The present study aimed at assessing the effects of hepatoprotective agents in diets that contain sources of energy on milk production, milk composition, and nutrient partition in lactating cows. Sixteen Holstein x Gir crossbred mid-lactation cows with an average body weight of 553 ± 85 kg were used in this study. These animals were allocated in a 4x4 Latin square design. A 2x2 factorial arrangement was employed in this feeding experiment. In each treatment, cows received diets with or without a hepatoprotective agent and variable in ground corn grain or citrus pulp as energy sources. Evaluated parameters included nutrient intake and digestibility, milk production, milk composition, energy balance, and nitrogen balance. Performance and nutrient balance variables were assessed and no interaction was observed between the hepatoprotective compounds and the dietary sources of energy. Dry matter intake, milk production and net energy for lactation were higher in corn as an energy source whereas milk fat content was higher in citrus pulp diets. There was a reduction in protein and casein contents in the milk of cows that was supplemented with an hepatoprotective agent. In this study, the hepatoprotective agent improved nitrogen balance in dairy cows. The use of the hepatoprotective compounds in the diet of these lactating cows in confinement reduced the milk protein fraction and favored a higher nitrogen balance in these animals. Retention of nitrogen compounds in the metabolism of lactating cows under confinement is influenced by hepatoprotective agents.

Keywords: dairy cows, liver, milk production, nutrients balance

RESUMO

Objetivou-se com este estudo avaliar os efeitos de fontes de energia associadas a um hepatoprotetor sobre a produção, composição do leite e a partição dos nutrientes em vacas lactantes confinadas. Foram utilizadas 16 vacas mestiças Holandês x Gir no terço médio da lactação, com peso corporal médio de 553 ± 85 kg, distribuídas em delineamento experimental quadrado latino 4x4 quádruplo em arranjo fatorial 2x2. Os tratamentos consistiram de dieta com ou sem a inclusão do hepatoprotetor, e variação de milho moído ou polpa cítrica como fontes de energia. Não houve interação (P>0.05) entre as fontes de energia e o hepatoprotetor para as variáveis de desempenho e balanço de nutrientes. O consumo de matéria seca, a produção de leite, o leite corrigido para 4% de gordura foram maiores (P<0.05) nas dietas com milho moído como fonte de energia. O teor de gordura do leite foi maior (P<0.05) nas dietas com a polpa cítrica. Houve redução (P<0.05) no teor de proteína bruta e caseína no leite das vacas que receberam o hepatoprotetor na dieta. O hepatoprotetor favoreceu maior (P<0.05) retenção e balanço de nitrogênio nas vacas. Portanto, o milho moído pode ser utilizado na dieta como fonte de energia para impulsionar a produção de leite. Enquanto, a fonte de energia proveniente da polpa cítrica afeta a composição do leite, através do incremento no teor de gordura. O uso de hepatoprotetores na dieta favoreceu maior retenção de nitrogênio em vacas lactantes confinadas.
Palavras-chave: balanço de nutrientes, fígado, produção de leite

INTRODUCTION

The metabolic demands for milk production as well as the high proportion of metabolizable nutrients in diets given to dairy cows under confinement may cause impairment in the liver function (XU et al., 2017). The inability of the liver to circumvent metabolic overload may trigger subclinical metabolic diseases that may negatively affect milk production throughout the cow's productive lifetime (WANKHADE et al., 2017).

Hepatoprotective agents may be added to the diet of lactating cows in order to optimize hepatic metabolism and prevent and treat metabolic disorders. These compounds protect the liver through a variety of mechanisms as these hepatoprotective agents have antioxidant, antimicrobial, antiinflammatory and antifibrotic properties (GIRISH & PRADHAN, 2017). In this context, hepatoprotective agents which are characterized by their ability to protect the liver against damage may be used as potential growth promoters optimizing liver metabolism as well as preventing and repairing metabolic disorders.

There are plant extracts with hepatoprotective properties which are frequently used as homeopathic preparations including *Chelidonium majus, Cardus marianus, China officinalis, Chionantus virginica,* and *Myrica cerifera*. These plant extracts have bioactive compounds that are modulators of the liver function. These substances may participate in amino acid degradation (DRSATA et al., 1996), hepatocyte regeneration, oxidative status, and lipid metabolism (GIRISH & PRADHAN, 2017).

Responses on energy metabolism have been observed with the use of hepatoprotective agents in research

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trials (ULGER et al., 2017; TAJMOHAMMADI et al., 2018). Based on the results of previous studies from other researchers, we may infer that the post-absorptive supply of nutrients in diets with corn or citrus pulp as energy sources which provide glycogenic and ketogenic precursors may influence the activity of hepatoprotectors in metabolism. The scarcity of available information on the use of hepatoprotective compounds for lactating cows led us to study the effect of these compounds on milk production and nutrient partitioning in dairy cows receiving different sources of energy in their diets.

Therefore, the aim of this study was to assess the effects of hepatoprotective agent in diets with different energy sources on the consumption, digestibility, production, milk composition, and nutrient balance in dairy cattle in confinement.

MATERIALS AND METHODS

This research project was approved by the Animal Ethics Committee (AEC) of the Federal University of Paraíba (UFPB, Brazil), reference number 4117200519.

The experiment was carried out at the Regional Technological Development Center for Agribusiness located in Alta Mogiana, Colina, São Paulo, Brazil (20º43'05 "S; 48º32'38" W). The trial lasted 76 days and was divided in 4 periods. Each period of this study consisted of 14 days for the adaptation of animals to the research unit and 5 days for sample collection. Sixteen Holstein x Gir crossbred mid-lactation cows with an average body weight of 553 ± 85 kg were used in this study. These animals were allocated in a 4x4 Latin square experimental design. We employed a 2x2 factorial arrangement in this feeding experiment. These animals were divided into homogeneous groups regarding milk production (23 ± 6 kg milk), lactation phase (lactation stage) (109 ± 57 days of lactation) and parity (primiparous, n = 8; multiparous, n = 8).

Experimental treatments consisted of the administration of diets supplemented with a hepatoprotectant and diets without hepatoprotectant and diets containing varying amounts of two different energy sources - ground corn or citrus pulp - as follows: (1) 0 g hepatoprotectant + ground corn; (2) 50 g hepatoprotectant + ground corn; (3) 0 g hepatoprotectant + citrus pulp; and (4) 50 g hepatoprotectant + citrus pulp. The commercial hepatoprotective (Figotonus→, Real H Nutrição e Saúde Animal Ltda.) consisted of a homeopathic formula compound by: *Chelidonium majus* (10^{-24}), *Carduus marianus* (10^{-24}), *Natrum muriaticum* (10^{-400}), *China officinalis* (10^{-24}), *Phosphorus* (10^{-28}), Carboneum tetrachloricum (10^{-30}), Myrica cerifera (10^{-60}), *Chionanthus virginica* (10^{-30}), and 1000 g of calcium carbonate q.s. as the delivery vehicle.

These diets included corn silage as the source of roughage, and ground corn or citrus pulp, soybean meal, protected fat, mineral mixture, urea, sodium bicarbonate and calcitic limestone were used to formulate the concentrate (Table 1). These diets were formulated and balanced (NRC, 2001) to be isoproteic and isoenergetic in order to meet the nutrient requirements of mid-lactation non-pregnant cows with 550 kg of body weight and initial production of 25 kg milk/day with 3.5% fat.

The diets were fed to the animals as complete rations *ad libitum* and included 10% of leftovers divided into two portions of the same weight after the morning and afternoon milkings.

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The roughage:concentrate ratio of the diets was 55:45. After the morning milking, each cow treated with the hepatoprotector received the daily dose of 50 g of the product as the first meal which was mixed in the same proportion with the concentrate in order to guarantee total feed intake.

Table 1 - Proportion of feed ingredients and nutrient content of diets

Ingredients (g/kg)	Energy sources	
	Ground corn	Citrus pulp
Corn silage	550	550
Ground corn	219	-
Citrus pulp	-	229
Soybean meal	185	185
Protected fat[1]	13.5	13.5
Mineral mixture[2]	13.5	13.5
Urea	3.38	4.28
Sodium bicarbonate	5.40	5.40
Calcitic limestone	9.68	-
Nutrient contents (g/kg)		
DM	578	583
MM	70.0	74.9
CP	147	147
EE	34.6	30.9
NDF	430	465
NFC	293	265
NE[3] (Mcal/kg DM)	1.30	1.33

Guarantee levels (kg): [1]Composition of protected fat: 820 g ethereal extract, 65 g calcium, 10 mg NaOH and 5 meq peroxide; [2]Composition of mineral mixture: 190 g calcium, 60 g phosphorus, 20 g sulfur, 20 g magnesium, 35 g potassium, 70 g sodium, 15 mg cobalt, 700 mg copper, 10 mg chromium, 700 mg iron, 40 mg iodine, 1600 mg manganese, 19 mg selenium, 2500 mg zinc, 400000 UI vitamin A, 100000 UI vitamin D3, 2400 UI vitamin E, 1000 mg monensina and 600 mg fluorine; DM: dry matter; MM: mineral matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non-fibrous carbohydrates; NE: net energy.[3] Calculated according to the NRC (2001).

The animals were kept in confinement housing in individual covered stall barns with a total area of 12.5 m² with concrete floors, feed and drinking fountain with free access to water.

Samples of silage, ingredients and leftovers were collected during the 5 days of each sampling period. These samples were dried in a 55 ° C oven for 72 h, ground to 1 mm and homogenized to form a composite sample for each animal per period for further analysis of nutrient contents.

Fecal production for calculating the apparent digestibility coefficients of nutrients was carried out using the concentration of the chromium oxide marker in the feces. Cows received orally 10 g of chromium oxide divided into two meals for 10 consecutive days. Faeces were sampled twice a day after the diet was given to these animals between the 15th and 18th days of the sampling period. After collection, these samples were dried in a 55° C oven for 72h, ground to 1 mm, and homogenized in one specimen per animal per period

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for further analysis of nutrient contents and chromium concentration in the feces.

Milk yield was recorded between the 15th and 19th day of each collection period at 0600 and 1500 hours. Milk production was corrected to 4% fat (FCM) according to the equation described by the NRC (2001). Samples for estimating milk composition were collected between the 15th day and 18th day of the experiment with sampling carried out for 3 days of each experimental period. These samples were analyzed for fat, protein, casein, lactose, total solids, milk urea nitrogen (MUN) and somatic cell count (SCC).

The net energies of the diet, maintenance (NEM), lactation (NEL) and body weight variation (NEBW) for the net energy balance were estimated from equations proposed by the NRC (2001).

Daily urine excretions were estimated from spot samples of spontaneously voided urine collected 4 hours after the morning meal. Samples of 10 mL of urine were collected which were, diluted in 40 mL of H_2SO_4 (0.036 N) and frozen at -20 °C (VALADARES et al., 1999) for further analysis of total nitrogen and creatinine. The total urinary volume was estimated using the correlation between creatinine concentration values in the urine (VALADARES FILHO & VALADARES, 2001) and urinary creatinine excretion per unit of body weight for dairy cattle (CHIZZOTTI et al., 2008).

The daily excretion of nitrogen (N) was estimated from the total N produced in the feces and urinary volume whereas the secretion of N in milk was calculated based on the conversion of the protein using factor 6.38.

The samples of corn silage, ingredients, leftovers and faeces were quantified in terms of dry matter (DM; 934.01), mineral matter (MM; 942.05), crude protein obtained by determining the total N (CP, N□6.25; 976.06) and ether extract (EE; 945.16) according to the procedures published by the Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fiber (NDF) was evaluated according to the method described by Van Soest et al., (1991). The concentration of non-fibrous carbohydrates (NFC) and total digestible nutrients (TDN) were estimated according to equations proposed by Sniffen et al., (1992) and Weiss (1999), respectively.

The analysis of chromium concentration in the faeces was conducted according to the methods proposed by Detmann et al., (2012).

The content of fat, protein, casein, lactose and total milk solids were estimated by infrared absorption using Bentley 2000® equipment, MUN by the enzymatic spectrophotometric method using a ChemSpeck 150® equipment, and SCC by flow cytometry using Somacount 300® equipment.

The creatinine concentration in urine samples was measured by the colorimetric alkaline picrate method using the Lab Test® kit (Labtest Diagnóstica S/A).

Data were subjected to ANOVA procedures and analyzed using statistical analysis package SAS procedure PROC MIXED (SAS PROC MIXED version 9.1). Analyses were considered different at P≤0.05. The statistical model included the effects of the hepatoprotective agent (H), energy sources (E) and the interaction between these elements (i.e. H×E) according to the following model: $Yijkl = \mu + Li + Cj + Tk + Sl + Q + A(Q) + ijkl$, in which: Y_{ijkl} = general observation; μ = general average; L_i = cows effect (i); C_j = periods effect (j);

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T_k = hepatoprotective agent effect (k); S_l = energy sources effect (l); T□S_{kl} = effect of the interaction between hepatoprotective agent and energy source (kl); Q = Latin square effect (q); A(Q) = effect of animal (a) within each Latin square; ε_{ijkl} = random error associated with each observation.

RESULTS

There was no interaction (P> 0.05) between the hepatoprotective agent and the energy sources for the performance variables and nutrient balance. The intake of DM, CP, EE, and NFC were higher (P<0.05) in diets containing ground corn as an energy source. The citrus pulp had higher (P<0.05) digestibility coefficients for MM and NDF whereas ground corn had greater digestibility for NFC (Table 2).

Milk production, milk corrected for 4% fat, protein yield, casein, lactose and total solids were higher (P<0.05) for ground corn as a source of energy in the diet fed to dairy cows. Fat content of milk was higher (P=0.049) in diets with citrus pulp. There was a decrease in protein (P=0.007) and casein (P=0.016) in the milk of those cows that received the hepatoprotective agent in their diet (Table 3).

The variation in body weight was greater (P=0.001) for cows receiving ground corn. Diets that had corn as the energy source provided greater (P=0.001) net lactation energy (NEL) whereas NE intake, NE maintenance, NE of body weight variation, and the balance and efficiency of NE use were not influenced (P>0.05) by the energy sources added to the hepatoprotective agent (Table 4).

Nitrogen (N) intake of cows and secretion of N in milk were higher (P<0.05) in diets with corn as an energy source. The hepatoprotective agent promoted a higher (P=0.038) N balance in these animals (Table 5).

DISCUSSION

In the present study, the responses of energy sources on the performance variables corroborate with the findings of previous research published by other authors. Corn has a high energy content provided by starch and is therefore the main energy ingredient used in diet formulations for lactating cows. Starch contributes to the optimization of rumen fermentation as it synchronizes with nutrients from forage degradation which in turn stimulate microbial growth and feed consumption (GÓMEZ et al., 2016). As a result, cows that were fed with corn-based diets had the highest nutrient consumption averages.

The composition of diet ingredients influences the use of nutrients by the animal during the performance of its productive functions (BAUMGARD, COLLIER & BAUMAN, 2017). In our study, the chemical characteristics of energy sources in the diets influenced the digestibility of nutrients. The use of citrus pulp in the dairy cows' diet which is an ingredient that has a high fiber and calcium content (BAMPIDIS & ROBINSON, 2006) resulted in higher digestibility coefficients of NDF and MM. In contrast, ground corn which has a high starch content in the grains provided higher digestibility averages of NFC between diets with energy sources.

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Table 2 - Intake and nutrient digestibility in lactating cows given a hepatoprotectant and different sources of energy in the diet

	Hepatoprotectant		Energy sources		SEM	*P* value		
	0 g	50 g	Ground corn	Citrus pulp		H	E	HxE
Dry matter intake								
kg/day	20.4	20.0	20.9[a]	19.5[b]	0.39	0.383	0.001	0.155
g kg$^{0.75}$	17.1	16.8	17.5[a]	16.4[b]	0.26	0.245	0.001	0.137
Nutrient intake (kg/day)								
MM	1.58	1.55	1.56	1.57	0.03	0.402	0.785	0.189
CP	3.27	3.24	3.34[a]	3.17[b]	0.06	0.703	0.002	0.071
EE	0.72	0.71	0.79[a]	0.65[b]	0.02	0.549	0.001	0.110
NDF	8.56	8.52	8.44[b]	8.65[a]	0.16	0.571	0.092	0.061
NFC	6.15	5.98	6.73[a]	5.40[b]	0.15	0.177	0.001	0.679
TND	11.8	11.8	12.1	11.6	0.30	0.859	0.187	0.816
Apparent digestibility (%)								
DM	55.7	56.6	55.1	57.3	1.00	0.707	0.073	0.259
MM	23.8	23.3	21.3[b]	25.9[a]	1.64	0.573	0.033	0.556
CP	62.5	63.6	62.4	63.8	0.89	0.503	0.243	0.452
EE	87.0	87.3	87.4	86.9	0.45	0.752	0.590	0.354
NDF	41.0	42.1	37.2[b]	45.9[a]	1.49	0.768	0.001	0.147
NFC	75.0	76.1	78.7[a]	72.4[b]	1.24	0.754	0.001	0.645

H: hepatoprotectant; E: energy sources; SEM: standard error of mean; DM: dry matter; MM: mineral matter; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; NFC: non-fibrous carbohydrates; TDN: total digestible nutrients; [ab] Means followed by different letters on the same line differ by Tukey (P<0.05).

Positive responses in the production and yield of milk components were observed as a reflection of the increase in feed intake. Therefore, a larger amount of nutrients became available to the mammary gland for milk production resulting in the increase of the net energy for lactation in diets that had corn as the energy source.

The productive responses of lactating cows depend on the partitioning of assimilated nutrients which are coordinated by a physiological system (BAUMGARD, COLLIER & BAUMAN, 2017). Thus, the intake of dry matter from lactating cows allows the supply of energy necessary to initially meet the maintenance requirement and the mammary gland for milk production and subsequently the demand for gain through tissue deposition (JENSEN et al., 2015).

In the present study, starch-rich diets which increased feed intake promoted greater nutrient partitioning for weight gain. However, the sources of energy associated with the hepatoprotective agent in the diet did not induce any changes in the body reserves and in the energy balance of the cows. These results indicate that, despite the composition of the different ingredients, the diets provided similar amounts of energy to animals.

Despite the fact that corn and citrus pulp provide energy in diets fed to dairy cows, these dietary energy sources have some particularities regarding the fermentation pattern which is capable of influencing both milk production and

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milk composition (HALL et al., 2010). Starch from corn kernels is rapidly fermented to propionate by ruminal microorganisms. Propionate produced in the rumen is used as a precursor of glucogenic nutrients in the liver. Proprionate influences and regulates the amount of glucose available in the bovine mammary gland for the synthesis of lactose which determines the volume of milk production (FERRARRETO et al., 2017).

In contrast, citrus pulp is rich in pectin and fiber of high degradability and stimulates the production of acetate which is the main precursor of milk fat (BAMPIDIS & ROBINSON, 2006). Therefore, diets containing ground corn provided greater milk production, whereas the highest fat content in milk occurred in those diets with citrus pulp as an energy source.

Table 3 - Milk production and milk composition in lactating cows given a hepatoprotectant and different sources of energy in the diet

	Hepatoprotectant		Energy sources		SEM	*P* value		
	0 g	50 g	Ground corn	Citrus pulp		H	E	HxE
Milk (kg/day)	25.7	25.9	27.1[a]	24.5[b]	0.67	0.981	0.001	0.399
4% FCM (kg/day)	20.6	22.2	21.6[a]	21.2[b]	0.62	0.482	0.001	0.824
Milk composition (%)								
Fat	3.00	3.03	2.96[b]	3.07[a]	0.05	0.591	0.049	0.087
Protein	3.03[a]	2.93[b]	3.02[a]	2.94[b]	0.03	0.007	0.051	0.993
Casein	2.28[a]	2.19[b]	2.26	2.21	0.02	0.016	0.089	0.874
Lactose	4.23	4.28	4.28	4.23	0.03	0.552	0.552	0.402
Total solids	9.53	9.57	9.56	9.55	0.39	0.948	0.800	0.951
Milk constituents yields (g/day)								
Fat	769	775	786	758	22.1	0.746	0.136	0.316
Protein	778	758	816[a]	720[b]	20.4	0.075	0.001	0.432
Casein	586	567	612[a]	539[b]	15.9	0.087	0.001	0.449
Lactose	1092	1111	1164[a]	1039[b]	31.9	0.809	0.001	0.382
Total solids	2431	2506	2609[a]	2328[b]	121	0.358	0.001	0.175
MUN (mg/dL)	17.8	17.4	17.6	17.5	0.29	0.984	0.350	0.646
SCS log	4.41	3.72	3.98	4.16	0.25	0.154	0.702	0.826

H: hepatoprotectant; E: energy sources; SEM: standard error of mean; FCM: fat-corrected milk; MUN: milk urea nitrogen; SCS: somatic cell score, estimated according to Shook (1982); [ab] Means followed by different letters on the same line differ by Tukey ($P<0.05$).

Carmo et al. (2015) evaluated the levels of starch in the diet of lactating cows and found that the inclusion of starch in the diet of these animals as an energy source provided positive responses on feed intake, milk production, corrected milk for fat, solids in milk and net lactation energy. Similar results were obtained in the present study.

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Table 4 - Body weight variation and energy balance in lactating cows given a hepatoprotectant and different sources of energy in the diet

	Hepatoprotectant		Energy sources		SEM	*P* value		
	0 g	50 g	Ground corn	Citrus pulp		H	E	HxE
Weight (kg)	589	591	589	591	10.6	0.907	0.847	0.906
BW change (kg)	21.6	20.2	24.2[a]	17.6[b]	1.67	0.782	0.001	0.191
NEI (Mcal/day)	26.5	26.5	27.1	25.9	0.67	0.979	0.195	0.744
NE_M (Mcal/day)	9.56	9.56	9.55	9.57	0.13	0.885	0.875	0.838
NE_L (Mcal/day)	15.7	15.8	16.5[a]	15.1[b]	0.42	0.843	0.001	0.908
NE_{BW} (Mcal/day)	0.94	0.94	0.96	0.94	0.01	0.783	0.783	0.173
EB (Mcal/day)	0.32	0.34	0.33	0.33	0.63	0.988	0.991	0.967
NE efficiency (%)	60.7	61.8	62.5	59.9	1.87	0.705	0.295	0.692

H: hepatoprotectant; E: energy sources; SEM**:** standard error of mean; BW: body weight; NEI: net energy intake; NE_M: net energy maintenance; NE_L: net energy for lactation; NE_{BW}: net energy body weight; EB: energy balance; [ab]Means followed by different letters on the same line differ by Tukey (P<0.05).

In addition, the composition of milk was influenced by the inclusion of hepatoprotective agent in the diet which provided a reduction in the levels of protein and casein in the milk. It is assumed that the presence of sanguinarine alkaloids from the extract of the hepatoprotective agent *Chelidonium majus* may have induced a decrease in the protein fraction of milk. This hepatoprotective agent has the ability to inhibit the activity of the aromatic amino acid enzyme decarboxylase (DRŠATA et al., 1996).
The inhibition of the decarboxylation of aromatic amino acids by sanguinarine suppresses the biosynthesis of its biogenic amines increasing the concentration of amino acids in a number of metabolic processes. Tryptophan is one of the main essential aromatic amino acids; it is the precursor of serotonin which a neurotransmitter involved for the cellular transmission of electrical signals. Tryptophan also plays a role in the regulation of the bovine mammary gland (HORSEMAN & COLLIER, 2014).

In the mammary gland of cows, essential amino acids and their precursors not only serve as substrates for protein biosynthesis but also act as signals regulating protein synthesis (YODER et al., 2020). Based on this information, we hypothesize that in our study the decrease in milk protein and casein occurred due to the inhibition of the tryptophan decarboxylation which resulted in the accumulation of 5-hydroxytritophane. This metabolite is a serotonin precursor and has been linked to suppression of the expression of the α-lactalbumin gene and β-casein in milk (HERNADEZ et al. 2009).
The content and type of protein provided in the diet are factors that influence the supply of nitrogen compounds for the biosynthesis of milk components by the mammary gland (NICHOLS et al., 2019). Diets with the source of energy from corn showed higher N intake as well as higher N secretion in milk. The increase in starch in the diets provided greater availability of NFC which may have favored better

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timing of nutrients for the production of microbial protein.

The greater availability of metabolizable protein in the small intestine provides increased protein yield in milk (CARMO et al., 2015). In contrast, pectin fermentation results in a lower proportion of propionate for hepatic gluconeogenesis favoring a decrease in milk protein due to the stimulus to capture glucogenic amino acids for glucose synthesis (LEIVA et al., 2000).

The N balance was higher in the diets in which the hepatoprotective agent was added to the formulation. It is suggested that the presence of *Chelidonium majus* extract in the hepatoprotector may have influenced the reduction of amino acid degradation. This effect is due to the similarity between the chemical structure of the sanguinarine alkaloids and the aromatic amino acids which inhibit the decarboxylation of the aromatic amino acids contributing to greater nitrogen retention in animal tissues (DRŠATA et al., 1996). Aguilar-Hernández et al. (2016) found increased digestibility of nitrogen compounds as well as lower fecal excretion of N by steers receiving levels of plant extract containing sanguinarine alkaloids. Our findings corroborate the findings of the study previously published by these authors.

Table 5 - Nitrogen balance in lactating cows given a hepatoprotectant and different sources of energy in the diet

	Hepatoprotectant		Energy sources		SEM	*P* value		
	0 g	50 g	Ground corn	Citrus pulp		H	E	HxE
N intake (g/day)	523	518	534^{a}	507^{b}	9.45	0.654	0.002	0.093
Milk N (g/day)	122	119	128^{a}	113^{b}	3.21	0.122	0.001	0.393
Fecal N (g/day)	197^{a}	184^{b}	196^{a}	185^{b}	5.76	0.037	0.009	0.159
Urinary N (g/day)	159	151	161	149	6.57	0.445	0.284	0.262
N balance (g/day)	53^{b}	77^{a}	58	73	8.76	0.038	0.215	0.318

H: hepatoprotectant; E: energy sources; SEM: standard error of mean; N: nitrogen; ^{ab}Means followed by different letters on the same line differ by Tukey (P<0.05).

Our results provide evidence that hepatoprotective agent alter the synthesis of protein components in milk and also provides a greater N balance in lactating cows. In the present study, the use of hepatoprotectant for mid-lactation cows in the dose recommended by the manufacturer did not shed any light on the performance of these animals. Further research is needed in order to establish the mechanisms of action of hepatoprotective substances in the metabolism of cows and assess the implications for the productive response. There is little information available in the literature about the use of hepatoprotective agents in the diets in lactating dairy cows.

CONCLUSION

Therefore, ground corn as a source of energy in the diet boosted milk production whereas citrus pulp as a source of energy increased fat content in milk. The use of hepatoprotective agent in the diet decreased milk protein

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fraction and favoured a higher nitrogen balance in lactating dairy cows under confinement.

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