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PRODUCTIVE PERFORMANCE OF SIMMENTAL DAIRY COWS SUPPLEMENTED WITH RICINOLEIC ACID FROM CASTOR OIL

DESEMPENHO PRODUTIVO DE VACAS LEITEIRAS SIMENTAL SUPLEMENTADAS COM ÁCIDO RICINOLEICO EXTRAÍDO DO ÓLEO DE MAMONA

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ADDITIONAL KEYWORDS

Blood cells. Functional oil. Milk yield. Plasma metabolites.

PALAVRAS CHAVE ADICIONAIS

Células sanguíneas. Óleo funcional. Produção de leite. Metabólitos plasmáticos.

SUMMARY

The aim of this study was to evaluate the performance and blood parameters of Simmental dairy cows, supplemented with 2 g/day of ricinoleic acid (RA) in diet. Forty Simmental dairy cows in mid lactation, individually housed in stable type *tie-stall*, were used. The animals were randomly assigned to two treatments: 0 or 2 g of RA/animal/day. The experimental period consisted of 42 days divided into two 21-day. It was observed reduction in dry matter intake (DMI), increased milk, fat and FCM (fat corrected milk) yield, as well as increased of fat content of milk in cows that received RA in diets. No effects were observed for red and white blood cells and blood metabolites. Supplementation of RA improves performance of dairy cows in mid lactation.

RESUMO

O objetivo deste estudo foi avaliar o desempenho e os parâmetros sanguíneos de vacas leiteiras simental suplementadas com 2 g/dia de ácido ricinoleico (AR) na dieta. Foram usadas 40 vacas leiteiras da raça Simental no meio da lactação, alojados individualmente em *tie-stall*. Os animais foram divididos aleatoriamente em dois tratamentos: 0 ou 2 g de RA/animal/dia, fornecido via concentrados. O período experimental consistiu

de 42 dias, divididos em dois períodos de 21 dias. Foi observada a redução no consumo de matéria seca (CMS), aumento da produção de leite, gordura e produção corrigida 3,5 %, bem como um aumento do teor de gordura do leite em vacas que receberam AR na dietas. Nenhum efeito foi observado para os glóbulos vermelhos e brancos e metabólitos no sangue. Suplementação de AR melhorou o desempenho de vacas leiteiras no terço médio de lactação.

INTRODUCTION

The production of milk solids is gaining role in world of dairy farming, by the fact of dairy industries seeking greater efficiency in the production of dairy products. Within a dairy herd several factors can alter the milk solids (protein, fat and lactose). Factors which affect milk composition include genetics, stage of lactation, and level of milk production age of cow, environment, disease (e.g. mastitis) and nutrition. Of the variation in milk composition, 55 percent is due to heredity and 45 percent is due to environmental factors, such as feeding (Allen, 2000).

The composition of milk solids can be handled by nutritional alternatives, mainly, manipulating the quality and quantity of fiber and addition of additives modulators of rumen fermentation. Among the modulators of rumen fermentation ionophores are disseminated over 50 years, with a history of success in reducing losses metabolic hence provide a better rumen sanity and positive results in the increase in milk protein content of and inconclusive results in milk fat (Gandra *et al.*, 2010). However, under the law, ionophores are classified as antibiotics, which makes their use is increasingly criticized by consumer society.

Due to the possible consequences of prolonged use of antibiotics in animal feed on the development of resistant pathogens and the possible transfer of the waste products of animal origin, the European Union, based on the precautionary principle, banned the use of ionophores since January 2006. This has aroused increasing scientific interest for alternatives that mimic the effects of ionophores in control specific microbial populations to modulate ruminal fermentation. Among the various options, secondary plant compounds have great potential use, highlighting the essential oils.

Essential oils are organic compounds represented by a mixture of aromatic terpenoids, and lipophilic fluids obtained from different plant parts such as leaves, roots, stems, fruits, seeds, or more one part extracted by steam distillation processes or volatile solvents such as methanol and hydroxy-acetone (Coneglian, 2009). The mode of action of essential oils in ruminants is still not been completely elucidated. However some hypotheses that have been lifted: control of pathogens by antimicrobial activity, antioxidant activity, improves digestion by stimulating enzyme activity and morphometry of organs (Coneglian, 2009).

Many essential oils have been studied for this purpose; however, standing out among them is castor oil, which is obtained

by pressing the seeds. It contains 90 % of ricinoleic acid (RA), which gives the oil its unique features, enabling a wide range of industrial use. The cultivation of castor beans is thus important for the economic potential of Brazil. RA is very similar to oleic acid, the only difference being a hydroxyl group present in RA and absent in oleic acid. For this reason, RA is also called hydroxyoleic acid (Gandra *et al.*, 2012).

Although the toxicity of the castor bean has been known since ancient times, castor oil is not toxic because ricin, a toxic protein present in the seeds, is not lipid soluble, so the toxic component is restricted only to the castor bean. Ricin specifically and irreversibly inactivates eukaryotic ribosomes, preventing protein synthesis (Dorman and Deans, 2000). The oil obtained by pressing the seeds is a polymer precursor and a solution of RA esters, and is known as castor oil (Ferreira *et al.*, 2002).

The detergent derived from castor oil, in turn, shows antimicrobial properties in the treatment of necrosis of tooth pulp; its bactericidal activity is similar to sodium hypochlorite (Ferreira *et al.*, 2002).

Given the antimicrobial properties of RA and growing demand for non-antibiotic supplements for ruminant nutrition, it has been proposed to include RA in the diet of dairy cows, in order to observe its effects as a possible modulator of ruminal fermentation on the basis of possible performance improvement without harm to animal health. In order to explore the antimicrobial properties of RA, the objective of this study was to evaluate the dry matter intake, milk yield and composition and blood parameters of Simmental dairy cow supplemented with 2 g/day of RA in the diet, in according with recommendations of (Gandra *et al.*, 2012).

MATERIAL AND METHODS

ANIMALS AND MANAGEMENT

Forty Simmental dairy cows in mid lactation were used, with yield of 25.6 ± 3.4 kg/

PERFORMANCE OF SIMMENTAL DAIRY COWS WITH CASTOR OIL RICINOLEIC ACID

cow/day and weight 563 ± 38 kg, individually housed in stable type *tie-stall*. The animals were randomly assigned to two treatments: 0 g RA/animal/day or 2 g of RA/animal/day directly in the mouth in the morning, just after milking for soft capsules.

The experiment was conducted in a completely randomized design and consisted of 42 days divided into two 21-day. Diets were formulated according to NRC (2001), meeting the nutritional requirements for this stage of lactation, contains roughage:concentrate ratio of 55:45. The roughage used was corn silage. The respective diets, water and mineral salts were provided *ad libitum* throughout the experimental period. The animals were fed twice a day, morning and afternoon.

Ricinoleic acid (RA) is produced from castor oil, which is obtained by pressing the seeds. The oil is then complexed with a polymer to give a solid consistency to the RA.

Before the rations were given to the animals, the orts from the feeder were weighed daily in order to estimate dry matter intake. The animals were fed according to

Table I. *Ingredient composition of the concentrate and the experimental diet.* (Composição dos ingredientes do concentrado e da dieta experimental).

Ingredients (% DM)	Concentrate	Diets
Maize silage	-	55.00
Maize meal	52.14	21.9
Soyabean meal	39.1	16.42
Urea	1.74	0.73
Sulfate of ammonia	0.12	0.05
Sodium bicarbonate	1.48	0.62
Magnesium oxide	0.05	0.02
Mineral mix ¹	4.67	1.96
Limestone	0.28	0.10
Salt	0.48	0.20

¹Composition per kg of mineral mix: Ca: 180 g; P: 90 g; Mg: 20g; S: 20 g; Na: 100 g; Zn: 3000 mg; Cu: 1000 mg; Mn: 1250mg; Fe: 2000 mg; Co: 200 mg; I: 90 mg; Se: 36 mg; F: 900 mg (máx.).

Table II. *Nutritional composition (% dry matter) of the concentrate, maize silage and experimental diet.* (Composição nutricional (% da matéria seca) do concentrado, da silagem de milho e da dieta experimental).

Nutrients	Concentrate	Silage	Diet
Dry matter, %	89.42	28.96	54.3
Organic matter	89.98	94.47	92.58
Crude protein	27.73	8.82	16.77
Fatty acids	2.9	2.91	2.90
Total carbohydrate	61.34	82.74	72.91
Neutral detergent fiber	9.89	53.2	35.01
Non-fibrous carbohydrate	55.96	29.54	40.64
Acid detergent fiber	7.94	43.69	28.68
Lignin	1.07	5.44	3.61
Ash	10.02	5.53	7.42
Total digestible nutrients ¹	82.48	62.73	71.02

¹Estimated according to NRC (2001).

the dry matter intake of the previous day in order to maintain the percentage of daily orts between 5 % and 10 % of the supplied diets (**tables I and II**).

SAMPLE COLLECTION AND ANALYSIS

On days 14-21 of the experimental periods, orts, silage, and concentrate ingredients were collected for analyses of dry matter (DM), organic matter (OM), ash, crude protein (CP), fatty acids (FA), and lignin in accordance with the methods described by AOAC (2000). Crude protein (CP) was obtained by multiplying the total nitrogen content by 6.25.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were obtained according to the method described by AOAC (2000), using α -amylase without the addition of sodium sulphite in the determination of NDF in an AnkonSystem®.

The total carbohydrates (TC) and total digestible nutrients (TDN) were calculated according to NRC (2001):

$$CT = 100 - (\%CP + \%FA + \%ash);$$

TDN= dCP + dNDF + (dFA x 2.25) + dNFC.

The levels of non-fibrous carbohydrates (NFC) were estimated according to NRC (2001):

$$\text{NFC} = 100 - [(\% \text{CP} - \% \text{CP urea} + \% \text{urea}) + \% \text{FA} + \% \text{NDF} + \% \text{ash}]$$

On days 0 and 21 of each experimental period, before the supply of the diets and after the milking in the morning, the animals were weighed and blood samples were collected by vein or coccygeal artery puncture.

Blood samples were collected (vacutainer) for measurement of blood parameters: total protein, albumin, urea, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) in plasma in addition to the haemogram (red series), that measured erythrocytes, haematocrit, haemoglobin, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), and WBC (white series) that measured leucocytes, neutrophils, lymphocytes, eosinophils, monocytes, basophils, and fibrinogen.

Immediately after collection, the samples were cooled and centrifuged at 2000 g for 15 min to separate the serum or plasma, and then stored at 2°C until the laboratory procedure, using commercially available kits (LABORLAB® and CELM®) for measuring total protein, albumin, urea, AST, GGT, and ALP. Enzymatic colorimetric and end-point methods were used; the readings were performed in an automated blood biochemistry analyzer (CELM-SBA-200®). Blood samples collected for haemograms were analysed fresh on the same day of weighing animals and biochemical analyses.

Cows were mechanically milked twice a day, at 06:30 and at 15:30, being the milk production recorded daily throughout the experimental period. Milk production was corrected for 3.5 % of fat (FCM) according

to formula of NRC (2001):

$$\text{FCM} = (0.432 + 0.1625 * \text{milk fat content}) * \text{kg of milk}$$

and contents of milk fat, protein, lactose were determined in fresh.

Milk samples were collected on days 19, 20, 21 of each experimental period, through the collection cup 50 ml attached to the milking, 60 % during morning milking and 40 % in the afternoon milking. We used the average of three days of collection. The samples were stored in plastic bottles, maintained from 2 to 6°C and sent for analysis to obtain milk composition performed at the Clinic of Milk ESALQ-USP. The total concentration of crude protein, fat, lactose, total solids (TS) and dry extract (DE), somatic cell count (SCC) and total bacterial count (TBC), according to the methodology described by the International Dairy Federation (1996).

STATISTICAL ANALYSIS

The data were analysed by PROC MIXED according to the following model:

$$Y_{ij} = \mu + D_i + T_j + T_j(D_j) + e_{ij}$$

where:

Y_{ij} = dependent variable;

μ = overall mean;

D_i = effect of diet ($i=1-2$);

T_j = effect of days in confinement;

$D_i(T_j)$ = effect of interaction between diet and day of confinement;

e_{ij} = error.

The calculated degrees of freedom were performed according to the Satterthwaitte method (ddfm - satterth). The data were subjected to analysis of variance by the command PROC MIXED of SAS version 9.0 (SAS, 2004). Means were adjusted by LSMEANS and analyzed by Tukey test adjusted PROC MIXED. The data obtained at baseline were used as covariates in the statistical model, with $p < 0.05$. The function pdiff of PROC MIXED was used for ana-

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lysis of interactions, adopting $p < 0.05$.

RESULTS

PERFORMANCE

With supplementation with RA, it was observed reduction in dry matter intake (DMI), increased milk, fat and FCM yields, and reduced DE ($p < 0.05$). The reduction in DMI was 11.70 %, increase in milk yield was 7.52 % and 11.26 % for FCM when comparing the diet supplemented with RA with diet control (**table III**).

The experimental period (time) influenced ($p < 0.05$) dry matter intake, milk yield, fat correct milk, total solids, dry extract, total bacteria count, yield and levels of protein and fat, except SCC. Interaction was observed ($p < 0.05$) between the RA and time for milk and protein yield, as well as levels of protein and fat. Milk yield increased with the over time when if using the RA in the diet. For the control diet was observed

reduction in milk yield, starting 21st day of supplementation, resulting in a significant difference ($p < 0.05$) between treatments (**figure 1A**).

It was observed an increase protein content during trial for the control diet. This increase was not observed in the RA diet, so that the difference between the levels of milk protein between diets were significant ($p < 0.05$) starting 21^o day of supplementation (**figure 1B**). For protein yield, however, no difference was observed ($p > 0.05$) between diets, only effect of time.

Associated with increased protein content there was a reduction of the milk fat content from cows fed the control diet, not observed for diet RA. This reduction caused significant difference ($p < 0.05$) between the diets starting 21^o day of supplementation (**figure 1C**).

HAEMOGRAM AND LEUCOGRAM

Among the hematological parameters

Table III. Productive performance. (Desempenho produtivo).

Indices	Diet ¹		SEM	RA	p value Time	RA*Time
	Control	RA				
kg/day						
Dry matter intake	18.38	16.23	0.18	0.034	<0.001	0.563
Milk yield	22.47	24.16	0.64	0.006	<0.001	0.002
Fat corrected milk, 3.5%	23.44	26.08	0.75	0.020	<0.001	0.841
Fat	0.82	0.97	0.03	0.008	<0.001	0.142
Protein	0.76	0.72	0.01	0.458	<0.001	0.007
Lactose	1.02	1.10	0.04	0.674	<0.001	0.765
Fat, %	3.85	3.91	0.10	0.665	<0.001	<0.001
Protein, %	3.23	3.15	0.01	0.458	0.007	<0.001
Lactose, %	4.56	4.55	0.06	0.897	0.759	0.645
T S, %	13.07	13.07	0.09	0.997	<0.001	0.002
DE, %	9.31	9.09	0.04	0.006	0.002	0.051
10 ³ CFU/mL						
SCC	1140	998	-	0.676	0.125	0.283
TBC	416	521	-	0.598	0.046	0.868

¹Supplement of 2 g/day of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet; TS= Total solids; DE= dry extract; CFU= Colony forming units; SCC= somatic cell count; TBC= total bacteria count; RA= Ricinoleic acid.

evaluated were observed decrease in the concentration of MCHC and absolute values of eosinophil ($p < 0.05$) for RA compared to control diet. The absolute values of eosinophil were similar at baseline between diets and it was observed an increase

starting the 21st days of supplementation ($p < 0.05$) for the control diet which remained until the 42nd day of supplementation (**table IV**).

The time RA supplementation influenced ($p < 0.05$) hemoglobin concentration, MCV, the absolute values of lymphocytes, monocytes and basophils, fibrinogen concentration and neutrophil / lymphocyte ratio.

BLOOD METABOLITES

It was observed interaction ($p < 0.05$) time effects of supplementation with RA on the concentration of urea, blood urea nitrogen (BUN), and alkaline phosphatase (AP). The concentration of GGT and total protein were affected ($p < 0.05$) only for the time of supply of RA (**table V**).

Dosages of urea and blood urea nitrogen were initially higher in animals with diet RA were similar at 21 days lower at 42 days when compared to control diet, characterizing a reduction over time (**figure 2A** and **2B**). Alkaline phosphatase had the highest concentration over time for control diet differed significantly from RA diet for 42 days (**figure 2C**).

DISCUSSION

PERFORMANCE

The reduction in DMI observed for cows fed RA may be related to the antimicrobial ability of the additive and the possible selection of propionate-producing bacteria in the rumen. Propionate can be produced and absorbed at very high rates and very rapidly taken up by the liver, where it is a major fuel used to produce glucose. However, when propionate is absorbed faster than it can be utilized to produce glucose in the liver, it will likely be oxidized, generating ATP and a satiety signal to the brain (Allen, 2000). The reduction of DMI observed contrasts with most of the effects reported in the literature for the supplementation of essential oils and is similar to

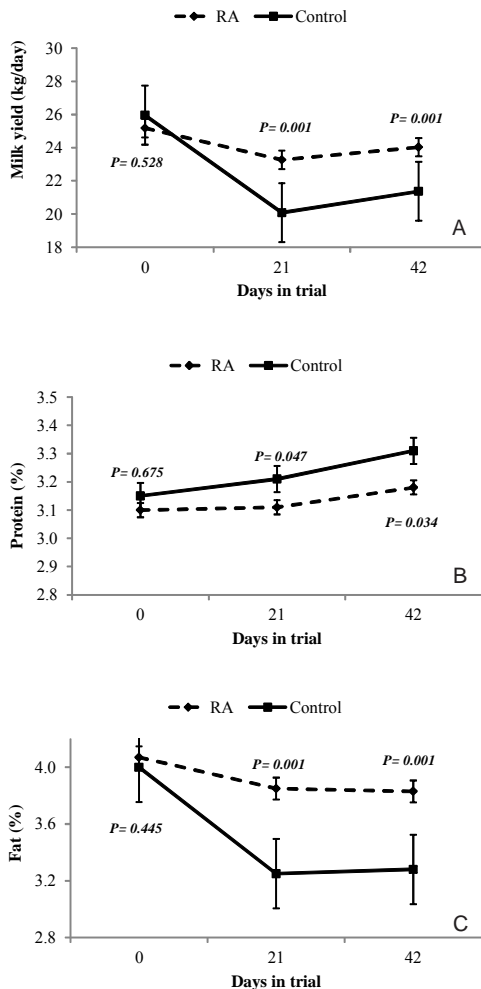


Figure 1. Milk yield (A), protein content (B), and fat content (C), in function of time according with experimental diets. (Produção de leite (A), teor de proteína (B), e teor de gordura (C) em função do tempo de acordo com as dietas experimentais).

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Table IV. Red blood cells and white blood cells counts. (Contagem sanguíneas de células vermelhas e brancas).

	Diet ¹		SEM	RA	p value Time	RA*Time
	Control	RA				
Erythrocytes, 10 ⁶ /mm ³	6.07	6.24	0.10	0.135	0.437	0.402
Haematocrit, %	27.55	28.02	0.44	0.395	0.164	0.564
Haemoglobin, g/dL	8.39	8.40	0.13	0.972	0.012	0.719
MCV,	44.96	45.04	0.33	0.939	<0.001	0.998
MCH, pg	13.907	13.61	0.12	0.889	0.419	0.235
MCHC, g/dL	29.97	29.91	0.32	0.030	<0.001	0.452
Leucocytes/mm ³	16622	14333	-	0.454	0.374	0.103
Neutrophils/mm ³	3636	3938	-	0.453	0.099	0.373
Lymphocytes/mm ³	7279	7184	-	0.793	<0.001	0.683
Eosinophils/mm ³	1540	1066	-	0.024	0.059	0.044
Monocytes/mm ³	473	554	-	0.517	<0.001	0.073
Basophils/mm ³	62	44	-	0.452	0.002	0.589
Platelets (x1000)/mm ³	462877	439256	-	0.437	0.063	0.059
Fibrinogen, mg/dL	49.66	43.00	-	0.198	0.005	0.718
Neut/Lymph	0.66	0.68	0.05	0.786	<0.001	0.410

¹Supplement of 2 g/day of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet; MCV= corpuscular volume; RA= Ricinoleic acid; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration.

effect reported for the use of ionophores.

Tassoul e Shaver (2009) observed that cows in early lactation, supplemented with commercial mixture of essential oils

(CRINA[®]), also presented a reduction of DMI. The authors attributed this reduction to the low palatability of the diet given by CRINA[®]. Benchaar *et al.* (2007) reported,

Table V. Blood metabolites. (Metabólitos sanguíneos).

	Diet ¹		SEM	RA	p value Time	RA*Time
	Control	RA				
Urea, mg/dL	38.05	35.48	0.99	0.230	<0.001	<0.001
Blood urea nitrogen, mg/dL	20.54	19.16	0.53	0.230	<0.001	<0.001
Total protein, g/dL	8.77	8.82	0.10	0.793	<0.001	0.605
AST, UI/L	84.17	89.14	2.56	0.263	0.057	0.615
ALP, UI/L	87.23	61.00	9.21	0.098	<0.001	0.001
GGT·UI/L	30.63	32.73	1.45	0.455	0.015	0.950

¹Supplement of 2 g/day of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet. RA= Ricinoleic acid; AST= Aspartate aminotransferase; ALP= Alkaline phosphatase; GGT= Gamma glutamyl transferase.

however, that adding CRINA® does not influence DMI in cows in later stages of lactation.

Benchaar *et al.* (2006) evaluated a factorial experiment, the effect of monensin,

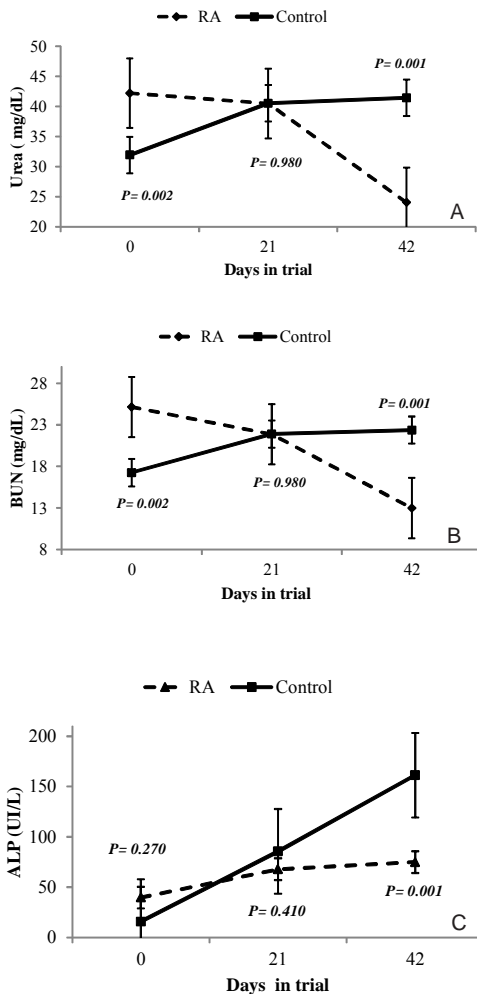


Figure 2. Urea (A), blood urea nitrogen (B), and alkaline phosphatase (C), in function of time according with experimental diets. (Uréia plasmática (A), nitrogênio ureico plasmático (B) e fosfatase alcalina (C), em função do tempo de acordo com as dietas experimentais).

and a blend of essential oils. The results suggest an interaction between treatments for dry matter intake. The essential oil increased dry matter intake when supplemented with monensin and reduced when supplemented alone.

The results described in the literature on the influence of essential oils on the DMI, are inconclusive and unsafe due to the fact that commercial products are composed of mixtures of various plant extracts, moreover the data presented by RA are more satisfactory and concrete due the fact that the final product is composed of only the fatty acids of castor oil and its reducing effect on DMI, has already been reported by (Gandra *et al.*, 2012).

The increase in milk yield showed by to diet supplemented with RA may be related to the change in ruminal fermentation profile caused by antimicrobial properties of the supplement. Kung *et al.* (2008) also reported that supplementation CRINA® increases milk yield. The decrease in DMI associated with an increase in FCM suggests an increased productive efficiency, as observed by (Tassoul e Shaver, 2009).

There was an increase in FCM and fat yield, without influencing the milk fat content. These data corroborate to Kung *et al.* (2008) that obtained fat yields of 1.24 and 1.13 kg / day and FCM 38.2 and 35.5 kg/day for the control and essential oil, respectively.

Kung *et al.* (2008) observed in in vitro assays, adding that CRINA® in normal doses (1g/animal/day) decreased the molar proportion of acetate, butyrate and valerate, with increased propionate. With higher doses (3g/animal/day) there was a decrease in the molar ratio of propionate, associated with an increase of acetate, butyrate and valerate. The milk fat yield observed in this study suggests the same mechanism associated with increased of milk fat yield reported by Kung *et al.* (2008) at high doses CRINA®. However, when analyzing the data DMI and milk yield the mechanism of

increased fat yield which best explains the data shown is the increase in milk fat content associated with decreased in partial biohydrogenation and inhibition of the production of C18: 1 trans-10.

The decreased in milk protein yield from animals supplemented associated with the effects observed for urea and blood urea nitrogen indicated, unlike suggested by McIntosh *et al.* (2003) for a commercial mixture of essential oils. Supplementation with RA may have increased the rate of deamination of amino acids, because there was no increase in milk protein content. According Gandra *et al.* (2009), monensin in diets for lactating cows can increase levels of blood urea nitrogen. This increase is a result of the higher concentration of undegraded protein in the rumen it reaches the small intestine. These non-essential amino acids absorbed may be used as a substrate for gluconeogenesis, so that the deaminations lead to higher concentrations of BUN.

HAEMOGRAM AND LEUCOGRAM

For erythrocyte parameters, there was no significant difference between diets, regardless of the time of supplementation. The means obtained are in agreement with the reference values established by Morris (2006). Hematocrit between 24 - 46 %, erythrocytes from $5 \text{ to } 10 \times 10^6/\text{L}$, hemoglobin between 8 - 15 g/dL, MCV fL 40 - 60, MCH pg 11 - 17 and MCHC, 30 - 36 g/dL are considered reference values for erythrocyte for dairy cows by (Morris, 2006). Just for MCHC the means obtained in this study were discreetly outside the reference values.

Morris (2006) considered as reference values for white blood cell count in dairy cows (in $\times 10^3 / \mu\text{L}$): 4 to 12 leukocytes, neutrophils 0.6 to 4; lymphocytes between 2.5 and 7.5; monocytes from 0.025 to 0.84, eosinophils to 2.4; 0.2 to basophils and neutrophils / lymphocytes ratio between 0.3 and 0.6. Considering these values, with the exception of total leukocytes and

neutrophils /lymphocytes ratio, all means of the results obtained in this study are in agreement with the reference values. It was observed a discrete leukocytosis relative increase of neutrophils in relation to leukocytes. Morris (2006), however, reports that a transient leukocytosis associated with neutrophilia and lymphocytosis are common and the resulting temporary mobilization compartment neutrophilic marginal, due to the physiological release of adrenaline under stress, excitement, anxiety or exercise, which can be explained by the act to blood sampling in animals.

The results obtained for the counts of red and white blood cells this trial clearly show that RA is safe for use in dairy cattle herds without compromising the health and welfare of animals acting efficiently and evident only modulates rumen fermentation.

BLOOD METABOLITES

The urea and BUN values observed in this study are within the reference values cited by (Rebhun e Guard, 200). For urea and blood urea nitrogen, values were observed for diets with RA, suggesting increased protein deamination in rumen. This decrease in the concentrations of urea and BUN supplemented with RA is consistent with the results obtained by (Gandra *et al.* 2012).

For total protein, Cozzi *et al.* (2011) recommends values between 7.0 to 9.4 g / dL, depending on season and parity order of cow. In this study, it was observed values of 8.77 and 8.82 for the animals that received control and RA diets, respectively. These values are above the reference values cited by (Rebhun e Guard, 200), but justified by the production of animals.

Carlson (2006), considered reference values of aspartate aminotransferase and α -glutamyltransferase 43 to 127 IU / L and 15 to 39 IU / L, respectively. The means obtained in this study are in agreement with the reference values established. For alkaline phosphatase the values obtained in this study are close to the values cited by

(Rebhun e Guard, 200), which can range from 0 to 400 IU / L.

According Benchaar *et al.* (2008), several studies have been conducted to evaluate the effects of essential oils on ruminal fermentation. Various essential oils, different doses in different diets resulting in inconsistent data. The response variability is associated with chemical differences that will influence the biological activity of the blend.

Considering the data obtained in this experiment and Gandra *et al.* (2012), supported in the literature it can be stated that the RA has a promising future in rumi-

nant nutrition, as a modulator of ruminal fermentation promoting increased performance, but more study should be conducted in order to elucidate the precise metabolism ruminal involving deamination rates and production of methane and short chain fatty acid.

CONCLUSION

The inclusion of 2 g/day of ricinoleic acid (RA) in diets of dairy cows improved the performance, without any risk to health and welfare of animals, being a promising additive in ruminant nutrition.

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