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Addition of increasing doses of ricinoleic acid from castor oil (*Ricinus communis* L.) in diets of Nellore steers in feedlots

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ABSTRACT

The aim of this study was to evaluate the performance and blood parameters of feedlot Nellore cattle fed increasing doses of ricinoleic acid (RA) in the diet. Ninety-six Nellore steers divided into 12 groups of 8 animals were used. The animals were randomly assigned to four treatments: 0, 1, 2, or 4 g of RA/animal/day, with three replicates per treatment. The experimental period consisted of 84 days divided into three 28-day periods preceded by three step-up diets. A quadratic effect was found for average daily gain and final body weight, as well as for leukocyte and lymphocyte counts, and for urea and blood urea nitrogen. A linear effect was observed for albumin, alkaline phosphatase, and gamma glutamyl transferase. The inclusion of 2 g of RA daily improved the performance of feedlot Nellore steers.

KEY WORDS: blood cells, castor oil, ricinoleic acid, plasma metabolites, performance, Nellore steers

INTRODUCTION

With the largest commercial herd in the world numbering 176 million heads, Brazil has been undergoing significant changes in the system of livestock beef cattle production. It has been observed that in the feedlot system, more concentrates are

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fed in relation to forage, and that in most Brazilian feedlots, more than 60% of the diet is concentrates.

The use of large amounts of concentrates in beef cattle diets can lead to metabolic disorders such as acidosis, laminitis, hyperparakeratosis, and liver abscesses, resulting in a decrease in dry matter intake and performance (Nagaraja and Chengappa, 1998). Thus, it is common to include additives of rumen fermentation modulators in the diet in order to prevent these disorders when feeding diets with a higher proportion of concentrates. The main additives used are ionophores, such as monensin and lasalocid, and salinomycin antibiotics that are widely employed to modulate rumen fermentation (Gandra et al., 2009). The use of antibiotics in animal feed has been rejected by consumers in some countries, since they believe that residues of these additives may be present in the meat of beef cattle and may cause problems for human health.

Essential oils are secondary metabolites of some plants, responsible for odour and colour, and can obtained by evaporation or distillation. In the rumen they act on the structure of the bacterial cell wall, altering the permeability of the cytoplasmic membrane for hydrogen and potassium ions. The alteration of ion gradients results in deterioration of essential cell processes such as electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions. The resulting loss of control over chemiosmosis by the affected cell consequently leads to bacterial death (Dorman and Deans, 2000).

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% 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.

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 animal performance improvers, it has been proposed to include RA in the diet of feedlot Nellore steers 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 performance and blood parameters of feedlot Nellore steers fed increasing doses of RA in the diet.

MATERIAL AND METHODS

Animals and management

The trial was conducted in the experimental center of OuroFinoAgribusiness Ltd., located in the city of Olimpia, Sao Paulo (Brazil). Ninety-six Nellore steers, initial weight 385 ± 14 kg, were divided into 12 groups of 8 animals. The animals were randomly assigned to four treatments: 0 g RA/animal/day, 1 g of RA/animal/day, 2 g of RA/animal/day, or 4 g of RA/animal/day, with three replicates per treatment.

The diets were formulated according to the Cornell Net Carbohydrate Protein System 5.0, with a forage: concentrate ratio of 45:55 in order to meet the requirements of Nellore steers in the growing and finishing period. The forage used was maize silage. All animals received the same diet differing only in the amount of RA per day and were fed once daily in the morning. The composition and concentrate ingredients of the diet and the nutritional composition of the concentrate and the silage are presented in Tables 1 and 2.

Table 1. Ingredient composition of the concentrate and the experimental diet

Ingredients, % DM	Concentrate	Diet
Maize silage	-	44.94
Maize meal	52.91	28.48
Soyabean meal	42.58	23.85
Mineral Mix[1]	4.51	2.73

[1] composition per kg, g: Ca 187, P 70, Mg 20, K 30, S 50, Na 70, Zn 3.000; mg: Cu 75, Mn 2.000, Fe 2.800, Co 1000, I 90, Se 30, F 663 (max.); IU: vit. A 1400.000; vit. D 28.000; vit. E 800

Table 2. Nutritional composition of the concentrate, maize silage and experimental diet

Nutrient	Concentrate	Silage	Diet
Dry matter[1], %	89.6	28.9	62.3
%DM			
organic matter	91.4	94.5	92.8
crude protein	23.4	6.8	15.9
fatty acid	3.0	2.9	3.0
ash	8.6	5.5	7.2
neutral detergent fibre	14.9	57.4	34.0
acid detergent fibre	7.6	44.0	23.9
lignin	1.3	5.5	3.2
non fibre carbohydrate	51.7	25.4	39.9
total carbohydrate	64.3	82.7	72.6
Total digestible nutrients[2], %	78.7	62.7	71.5

[1] percentage of natural matter; DM - dry matter; [2] estimate according to NRC (2001)

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.

The experimental period was composed of 84 days divided into three periods of 28 days preceded by three step-up diets of 15 days. They were: 5 days at a forage:concentrate ratio of 70:30, 5 days at a ratio of 60:40, and 5 days at a ratio of 50:50.

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

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) were calculated according to NRC (2001) in which: CT = 100 - (%CP + %FA + %ash). The levels of non-fibrous carbohydrates (NFC) were estimated according to Hall (1998), where: NFC = 100 - [(%CP - %CP urea + %urea) + %FA + %NDF + %ash]. Total digestible nutrients, TDN = dCP + dNDF + (dFA x 2.25) + dNFC were calculated according to NRC (2001).

On days 28, 56, and 84 of the trial period, before the supply of the diets in the morning, the animals were weighed and blood samples were collected by vein or coccygeal artery puncture.

After 84 days of the experiment, the animals were transported to a commercial packing plant under federal inspection, where they were slaughtered by stunning and later by the pneumatic bleeding method. 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 (CELMSBA-200®). Blood samples collected for haemograms were analysed fresh on the same day of weighing animals and biochemical analyses.

Statistical analysis

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

$$Yij = \mu + Di + Tj + Tj(Dj) + eij$$

where: Yij - dependent variable; μ - overall mean; Di - effect of diet (i=1-4); Tj - effect of days in confinement; Di (Tj) - effect of interaction between diet and day of confinement; eij - error.

The calculated degrees of freedom were performed according to the Satterth waite method (ddfm - satterth). The data were subjected to analysis of variance and polynomial regression by the command PROCMIXED of SAS version 9.0 (SAS, 2004), adopting a significance level of 5%.

RESULTS

Performance

No linear or quadratic effect was observed (P>0.05) for dry matter intake. The difference between the control diet and inclusion of dietary RA was +0.41; -0.04; +0.74 kg/day, for 1, 2, and 4 g of RA/day, respectively (Table 3). The mean dry matter intake in this trial was 12.7 kg/day, this value is appropriate for the breed and animal growth stage.

Table 3. Performance

Indices	Experimental diets, g[1]				SEM	P value	
	0	1	2	4		linear	quadratic
Initial BW[2], kg	382	382	395	382	2.37	-	-
Final BW, kg	488	494	484	477	3.40	0.001	0.050
ADG[3] kg/day	1.83	1.92	1.84	1.82	0.03	0.028	0.031
Intake kg DM/day	13.0	12.6	13.0	12.3	0.08	0.745	0.876
FCR[4]	8.0	7.3	7.5	7.0	0.14	0.142	0.735
Feed efficiency	0.14	0.15	0.14	0.15	0.002	0.375	0.709

[1] inclusion of 0, 1, 2 or 4 g of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet; BW - body weight; [3]ADG - average daily gain; [4] FCR - feed conversion ratio

There was no linear or quadratic effect (P>0.05) for feed conversion or feed efficiency in relation to the inclusion of RA in the diets. The control diet had a feed conversion ratio that was 8.15%, 6.52%, and 11.28% higher than in the diets with the addition of 1, 2, and 4 g of RA/day, respectively.

For the variables of final body weight and average daily gain (ADG), a quadratic effect (P<0.05) was observed, and therefore the lowest ADG and final body weight were obtained in the group receiving 4 g of RA. The regression equation presenting the ADG is described by Y = 1.82 + 0.05X - 0.017X2; R2 = 0.47. Analysis of this function showed that the optimal level of inclusion of RA was 1.47 g/day and the ADG estimated for this level of inclusion was 1.87 kg/day.

Table 4. Red blood cells and white blood cells counts

Indices	Experimental diets, g[1]				SEM	P	
	0	1	2	4		linear	quadratic
Erythrocytes, 10^6/mm^3	8.2	8.2	8.2	8.2	0.14	0.340	0.154
Haematocrit, %	36.5	34.3	35.4	35.6	0.64	0.432	0.116
Haemoglobin, g/dl	10.7	11.0	10.9	11.0	0.16	0.123	0.450
MCV[2], fl	45.6	43.4	44.5	44.6	0.63	0.654	0.390
MCH[3], pg	13.7	13.1	13.4	13.5	0.19	0.679	0.560
MCHC[4], g/dl	29.5	30.4	29.9	29.6	0.34	0.334	0.865
Absolute value/mm^3							
leucocytes	12562	17647	15105	16356	-	0.112	0.042
neutrophil	3277	5637	4457	4784	-	0.569	0.089
lymphocytes	8394	11044	9719	10127	-	0.231	0.034
eosinophil	309	325	317	288	-	0.987	0.453
monocytes	380	549	465	384	-	0.450	0.648
basophil	47	51	49	42	-	0.543	0.568
Fibrinogen, mg/dl	64.0	55.1	60.0	62.4	0.02	0.833	0.452

[1] inclusion of 0, 1, 2 or 4 g of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet; [2] MCV - corpuscular volume; [3] MCH - mean corpuscular haemoglobn; [3] MCHC - mean corpuscular haemoglobn concentraton

Haemogram and leucogram

There was no effect (P>0.05) on the RBC (red blood cell count) due to the addition of RA in the diet, suggesting that the use of RA did not affect the red blood cells and the health of animals.

In general, the addition of RA to the diets did not affect most variables in the WBC (P>0.05). There was, however, a quadratic effect (P<0.05) for leukocytes and lymphocytes and the lowest value was found for the group receiving the control diet. The regression equation obtained for the absolute values of leukocytes is provided by Y = 13.359 + 2.656X - 694X2; R2 = 0.45, where the optimal level of inclusion of RA was 1.91 g/day and the estimated value of leukocytes was

15.903 cells. The absolute values for lymphocytes was observed at the equation Y = 8.802 + 1.436X - 290X2; R2 = 0.44, where the optimal level of inclusion of RA was 1.78 g/day and the estimated value of lymphocytes was 10.458 cells.

Blood metabolites

There was no difference (P > 0.05) for total protein and aminotransferase (AST) activity (Table 5). For urea and blood urea nitrogen (BUN), a quadratic effect (P<0.05) was observed and the lowest value was found for the group receiving 2 g of RA/day. The regression equations that represent the concentrations of urea and BUN are given by Y = 29.87 – 5.55X + 0.83X2; R2 = 0.64 and Y = 13.94 – 2.58X + 0.38X2; R2 = 0.64, respectively. The optimum addition level of RA to the diets for urea and BUN was 3.40 g of RA/day, where the critical concentration of urea was 20.48 mg/dl and of BUN, 9.60 mg/dl.

An increasing linear effect (P < 0.05) was observed for the concentrations of albumin Y = 2.65 + 0.17X; R2 = 0.92 and the hepatic enzyme, alkaline phosphatase Y = 343.64 + 12.64X; R2 = 0.62. A decreasing linear effect was observed, however, for the concentration of gamma glutamyl transferase: Y = 31.20 – 4.84X; R2 = 0.54.

Table 5. Blood metabolites

Indices	Experimental diets, g[1]				SEM	P	
	0	1	2	4		linear	quadratic
mg/dl							
urea	28.4	29.1	19.1	21.4	1.34	0.340	0.002
blood urea nitrogen	13.2	13.6	8.9	10.0	1.34	0.340	0.002
g/dl							
total protein	6.0	6.1	7.0	6.9	0.07	0.282	0.122
albumin	2.7	2.8	2.9	3.4	0.05	0.029	0.908
UI/l							
AST[2]	66.1	70.6	61.6	69.1	2.64	0.654	0.178
ALP[3]	338	377	350	399	26.68	0.017	0.238
GGT[4]	25.5	37.2	16.5	11.6	5.72	0.004	0.327

[1] inclusion of 0. 1. 2 or 4 g of ricinoleic acid from of castor oil (*Ricinus communis* L.) per animal/day in total diet; [2] AST - asparate amnotransferase; [3] ALP - alkaline phosphate; [4] GGT - gamma glutanyl transferase

DISCUSSION

Performance. With regard to dry matter intake, some data from the literature show that essential oils have attractive and palatable properties that influence intake by animals (Wallace, 2004) (Table 3). Other authors, however, observed no effect on the dry matter intake of beef cattle supplemented with essential oils

(Meyer et al., 2009). Hence, reports on the effect of essential oils on dry matter intake are contradictory. Benchaar et al. (2008), supplementing beef cattle with 2 and 4 g/day of essential oil observed an increase in dry matter intake when compared with non-supplemented animals. Likewise, Alçiçek et al. (2004) claimed that the addition of essential oils maximized dietary intake by animals. Cardozo et al. (2008), when testing the effects of the essential oil of pepper (*Capsicum annuum*) also observed an increase in the intake of water and dry matter.

With regard to the feed and conversion ratios in a similar experiment, Meyer et al. (2009) observed no effect on feed efficiency in cattle supplemented with essential oils compared with the control diet. Moreover, Benchaar and McGinn (2008) when evaluating beef cattle fed 0.2 or 4 g/day of essential oils found a quadratic effect on feed efficiency, with it being better in the animals fed with 2 g of essential oil.

The results for ADG and BW in this trial disagree with a large number of studies with essential oils, since Beauchemin et al. (2006) and Meyer et al. (2009) when supplementing beef cattle with essential oils, observed no difference in ADG compared with the control diets. Both authors emphasize that the lack of effect on performance is due to the similarity in dry matter intake of animals in different treatments. After all, according to NRC (2001), dry matter intake accounts for 60-90% of the variations in animal performance.

The effect of RA on dry matter intake and ADG could be related to the selection of microorganisms in the ruminal flora that produce propionate, which might reduce the dry matter intake, while maintaining or even increasing animal performance, as noted in the tests. Other studies should be conducted, however, in order to establish the actual mechanism of RA action in the ruminal environment. The use of RA in ruminant nutrition has only recently been attempted, but according to its chemical characteristics and physical mechanism of action, it seems similar to most of the essential oils extracted from plants. The actual mechanism of action has not been completely established, however, requiring new trials in order to obtain concrete results.

Haemogram and leukogram. Haematological evaluations in cattle are used to assess disease in an animal or to evaluate groups of animals within a herd and also to detect hidden diseases and to guide clinical decisions (Weiss and Perman, 1992). The values of variables observed in this study for red blood cells, haematocrit, and haemoglobin are in accordance with the reference values obtained by Benatti et al. (2011) in a study on the blood profile of cattle. Furthermore, according to the values reported by Biondo et al. (1998) , the MCV and MCHC valued in our study are within the normal range for the breed and age of Nellore steers.

The increase in the absolute count of leukocytes and lymphocytes as the dose of RA increased may be related to some cumulative residual toxic effect of the plant

(*Ricinus communis* L.), but throughout the experimental period no clinical signs characteristic of infectious, neurological, or metabolic disease were observed in any animal. A quadratic response was observed, however, for all of the variables mentioned above, indicating that the maximum count was found for the level of 1 g of RA in animal diets, a fact that may not reflect a deleterious change in the immune system of the studied animals. A linear increase better characterizes any deleterious changes in the immune system, but this did not occur. Perhaps this increase in the white blood cell count was only a physiological adaptation to supplementation with RA.

According to Costa et al. (2000), leukocyte and lymphocyte counts are widely correlated, so the variation in total leukocytes reflects in particular the change in the total number of lymphocytes. The results for leukocyte counts are in agreement with Paes (2003), who studied the WBC of clinically healthy Nellore cattle subjected to confinement. The leukocyte and lymphocyte counts observed in this study for all treatments were within the ranges considered normal for Nellore steers in this age group (Costa et al., 2000).

The measurements of RBC and WBC in this trial were aimed at monitoring the health of animals and observing the behaviour of these cells upon supplementation with RA, due to its only recent introduction into ruminant nutrition. The results found in all groups are in accordance with the reference values for Nellore steers in this phase of growth. The quadratic effects seen for the leukocyte and lymphocyte counts can be linked to the better ADG observed for the same level of addition of RA. When the data for WBC and ADG are analysed together, the results suggest a better immune status of animals in the RA group.

Blood metabolites. According to Swenson et al. (1996), the BUN values found in this experiment are considered normal for cattle, and ranged from 10 to 30 mg/dl. Putrino et al. (2006) affirmed that BUN is used as an indicator of protein balance, and the limits indicating adequate protein intake are 9-12 mg/dl. Valadares et al. (1997) concluded that BUN concentrations from 13.52 to 15.15 mg/dl corresponded to maximum microbial efficiency and probably represent the limit above which there is loss of protein for Nellore steers fed diets containing 45% concentrate and, on average, 62.5% TDN.

Bortoli (2007), working with heifers fed a commercial product (Rumex®) consisting of several essential oils, saponins and yeast, observed plasma urea nitrogen values close to those found in this experiment (15.83 mg/dl). Likewise, Coneglian (2009) using the essential oil of castor and cashew in the diet of beef cattle, did not observe any effect on BUN levels, and the average value for all treatments was 14.72 mg/dl higher than those obtained in this study.

The values found for albumins showed an increase only for the addition of 4 g of RA/day. This increase did not constitute a clinical or nutritional concern,

since no effect was observed on total protein concentrations. The concentrations of gamma glutamyl transferase and alkaline phosphatase observed in the trial are within the reference values for Nellore. Despite the effects found by the inclusion of RA in the diet, no clinical changes were observed in the animals (Swenson et al., 1996).

In general, the biochemical profiles observed as a function of the addition of RA do not compromise the health and, especially, the performance of animals in feedlots. The changes found in BUN should be better established in further metabolism trials to elucidate the mechanism of RA action on the ruminal protein metabolism in beef cattle.

CONCLUSIONS

The inclusion of 1 to 2 g of ricinoleic acid (RA) in the diets of feedlot Nellore steers gave better performance without damaging the health of the animals. The results support the use of RA as a performance-modifying additive for feedlot cattle on high concentrate diets, replacing ionophores, with the reservation that more trials should be performed to confirm the mechanisms of RA action on ruminal metabolism.

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