

http://www.uem.br/acta ISSN printed: 1806-2636 ISSN on-line: 1807-8672

Doi: 10.4025/actascianimsci.v36i2.23089

# Glycerin and essential oils in the diet of Nellore bulls finished in feedlot: animal performance and apparent digestibility

Lorrayny Galoro da Silva, Juliana Akamine Torrecilhas, Mariana Garcia Ornaghi, Carlos Emanuel Eiras, Rodolpho Martin do Prado and Ivanor Nunes do Prado

Departamento de Zootecnia, Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. \*Author for correspondence. E-mail: inprado@uem.br

**ABSTRACT.** Current research studied the effect of partial replacing corn by glycerin and essential oils addition in the diet of Nellore bulls finished in feedlot. Thirty bulls with average weight 400 ± 34.1 kg were housed in collective pens (10 x 20 m²) for 63 days. The bulls were randomly assigned to 3 diets (10 bulls per treatment): CON – Control (without glycerin or Essential® oils); GLY – Glycerin (15% on dry matter - DM); and GEO – Glycerin (15% on DM) and Essential® oils (3 g animal day¹). Three different markers were used to estimate apparent digestibility in the diets: indigestible dry matter –iDM; indigestible neutral detergent fiber – iNDF; and purified lignin – LIPE®. Feed efficiency and animal performance were not affected by the corn partial replacing by glycerin. Partial corn replacing by glycerin and Essential® oils addition in the diets increased the DM and organic matter apparent digestibility for bulls. The glycerin addition in the diet decreased NDF, fibrous carbohydrate and non-fibrous carbohydrate digestibility. Apparent digestibility estimated by markers iDM, iNDF and LIPE® were similar to all nutrients in the diet.

Keywords: cashew nut oil, castor oil, glycerin, markers.

# Glicerina e óleos essenciais na dieta de bovinos Nelore terminados em confinamento: desempenho animal e digestibilidade aparente

**RESUMO.** Neste estudo foi avaliado o efeito da substituição parcial do milho pela glicerina e óleos essenciais (Essential®) na dieta de bovinos da raça Nelore terminados em confinamento Trinta touros com peso médio de 400 ± 34,1 kg foram alojados em baias coletivas (10 x 20 m²) por 63 dias. Os touros foram divididos aleatoriamente em três dietas (10 touros por tratamento): CON - controle (sem glicerina ou óleos essenciais); GLI - Glicerina (15% na matéria seca - MS) e GEO - Glicerina (15% na MS) e Essential® (óleo de caju líquido e mamona, 3 g animal dia-¹). Três marcadores diferentes foram utilizados para estimar a digestibilidade aparente das dietas: matéria seca indigestível - MSi, fibra em detergente neutro indigestível - FDNi e lignina purificada - LIPE®. A substituição do milho pela glicerina e adição de Essential® nas dietas aumentou a digestibilidade da MS e matéria orgânica para os animais. A inclusão de glicerina na dieta reduziu a digestibilidade aparente da FDN, carboidratos fibrosos e não fibrosos. Os marcadores das iDM, FDNi e LIPE® estimaram de forma semelhante a digestibilidade aparente de todos os nutrientes de todas as dietas experimentais.

Palavras-chave: óleo da castanha de caju, óleo de mamona, glicerina, marcadores.

#### Introduction

Intensive systems of cattle production improve animal performance and meat quality (PRADO et al., 2008; DUCATTI et al., 2009; PRADO et al., 2009; FUGITA et al., 2012); however it demands higher investments and technological alternatives (SILVA et al., 2010). In recent years, beef cattle producers have operated with a narrow profit margin. To improve the production of beef cattle finished in feedlot, it is necessary to employ high energy density diets (NRC, 2000).

In order to increase the energy density in diets it is necessary to use cereals and co-products from

agribusiness sector (MARQUES et al., 2000; PRADO et al., 2000; ABRAHÃO et al., 2006). Previous studies demonstrated that glycerin is an excellent energy source and can partially replace corn grain in ruminant diets (MACH et al., 2009; EIRAS et al., 2013; FRANÇOZO et al., 2013; EIRAS et al., 2014). However carbohydrates can be rapidly degraded, which can disturb ruminal fermentation (MARTINS et al., 1999; GIGER-REVERDIN et al., 2002). Therefore, various substances have been used to control ruminal fermentation, mainly additives from plant extracts and oils to replace ionophores (BENCHAAR et al., 2008; VALERO et al., 2011; ZAWADZKI, et al., 2011a and b).

Previews researches reported that plants extracts and essential oils contain secondary metabolites (terpenoids, phenolic compounds and others) that show antimicrobial activity (BENCHAAR et al., 2008). Castor and cashew oils contain a high percentage of compounds (NAUGHTON, 2000; TREVISAN et al., 2006) that confer antimicrobial activity as the ricinoleic acid and anacardic, cardanol and cardol acids respectively (KUBO et al., 2003; SHIN et al., 2004; TREVISAN et al., 2006). According to Dorman and Deans (2000), this antimicrobial activity can be attributed to structural hydroxyl groups that have a high affinity for the lipids of bacterial cell membranes.

Thus, this work was conducted to study the effects of partial replacement of corn grain by glycerin and Essential® oils addition on animal performance, feed intake, feed efficiency, apparent digestibility, and three markers to estimate apparent digestibility of Nellore bulls finished in a feedlot.

# Material and methods

#### Animals, housing and diets

This study was approved by Department of Animal Production of State University of Maringá (CIOMS/OMS, 1985) and performed at Farm at Colorado city, Paraná, Brazil south. Thirty Nellore bulls were randomly assigned to 1 of 3 diets (10 bulls per treatment): CON – Control (without glycerin or Essential® oils); GLY – Glycerin (15% on a dry matter – DM); and GEO – Glycerin (15% on a dry matter – DM) and Essential® oils (3 g animal day¹). At beginning of the experimental period, the bulls weighed (400 ± 34.1 kg and 22 ± 2 months old) and were housed in collective pens of 200 m² on ground floors, equipped with feeders of 60 cm deep and 10 m length and drinkers with a capacity of 250 liters of watering. The concentrate and corn silage

intake were daily recorded in the experimental period when the bulls reached a final BW of  $482 \pm 27.9$  kg.

The bulls were fed twice a day (8:00 am and 3:00 pm). The diets were weighed daily so that the refusals represented 5% of the total. Both diet formulation and quantity supplied were designed to provide a weight gain of 1.3 kg day<sup>-1</sup>, according to NRC (2000). The concentrate used was based on corn, soybean meal, glycerin, urea, limestone and mineral salt. Diets provided showed the ratio of 50% roughage (corn silage) and 50% concentrate (Table 1). All the diets were formulated to be sonitrogenous and isoenergertics (Table 2).

Table 1. Composition (% on DM) of the diets.

| Ingredients  | CON1 | GLY <sup>2</sup> | GEO <sup>3</sup> |
|--------------|------|------------------|------------------|
| Corn silage  | 49.9 | 49.9             | 49.9             |
| Corn cracked | 40.1 | 24.6             | 24.6             |
| Soybean meal | 8.92 | 8.92             | 8.92             |
| Glycerin     | -    | 15.0             | 15.0             |
| Limestone    | 0.50 | 0.50             | 0.50             |
| Mineral salt | 0.50 | 0.50             | 0.50             |
| Urea         | 0.10 | 0.54             | 0.54             |

<sup>1</sup>Diet without glycerin or Essential® oils. <sup>2</sup>Diet with glycerin. <sup>3</sup>Diet with glycerin and Essential® oils

The glycerin used in the diets was produced in a soy-diesel facility (BIOPAR, Rolândia, Paraná State and South Brazil) and the chemical composition was determined at the Institute of Technology of Paraná (TECPAR). The glycerin contained water, 23.2 g kg<sup>-1</sup>; ashes, 47.6 g kg<sup>-1</sup>; glycerol, 812 g kg<sup>-1</sup>; sodium, 11.6 g kg<sup>-1</sup>; methanol, 3.32 mg kg<sup>-1</sup>; potassium, 79.1 mg kg<sup>-1</sup>; chloride, 35.8 mg kg<sup>-1</sup>; magnesium, 16.3 mg kg<sup>-1</sup>; phosphorus, 239 mg kg<sup>-1</sup>; and crude energy 15.3 MJ kg<sup>-1</sup>.

#### Performance, feed intake and feed efficiency

The bulls were weighed at the beginning of the experimental period and thereafter at 21 days intervals, after fasting from solid food for a period of 16 hours, to evaluate animal performance. The daily feed intake was estimated by difference between supplied feed and refusals in the trough. The refused feed represented 5% of the total.

Table 2. Ingredients and composition (% DM) of the diets.

| Ingredients                | $DM^1$ | $OM^2$ | Ash  | $CP^3$ | $EE^4$ | TDN⁵ | $NDF^6$ | $ADF^7$ | $TC^8$ | NFC <sup>9</sup> | $FC^{10}$ |
|----------------------------|--------|--------|------|--------|--------|------|---------|---------|--------|------------------|-----------|
| Corn silage                | 31.5   | 95.5   | 4.41 | 7.78   | 2.88   | 56.0 | 52.4    | 29.4    | 84.9   | 32.5             | 52.5      |
| Corn cracked               | 88.6   | 98.6   | 1.36 | 8.40   | 4.03   | 90.0 | 9.63    | 4.08    | 86.2   | 76.8             | 9.63      |
| Soybean meal               | 89.3   | 92.8   | 7.24 | 47.3   | 1.13   | 81.5 | 13.9    | 9.86    | 44.3   | 30.4             | 13.9      |
| Glycerin                   | 88.0   | 94.5   | 5.50 | 1.00   | 10.0   | 90.0 |         |         |        |                  |           |
| Limestone                  | 99.2   | 2.67   | 97.3 |        |        |      |         |         |        |                  |           |
| Mineral Salt <sup>11</sup> | 99.0   | 2.00   | 98.0 |        |        |      |         |         |        |                  |           |
| Urea                       | 97.5   |        | 100  | 282    |        |      |         |         |        |                  |           |
| Diets                      |        |        |      |        |        |      |         |         |        |                  |           |
| CON <sup>12</sup>          | 46.4   | 94.6   | 4.43 | 11.6   | 3.12   | 70.4 | 31.2    | 17.1    | 80.1   | 49.0             | 31.1      |
| GLY <sup>13</sup>          | 46.4   | 93.6   | 5.47 | 10.7   | 3.98   | 70.0 | 29.7    | 16.5    | 67.1   | 43.5             | 23.5      |
| GEO <sup>14</sup>          | 46.4   | 93.6   | 5.47 | 10.7   | 3.98   | 70.0 | 29.7    | 16.5    | 67.1   | 43.5             | 23.5      |

<sup>1</sup>Dry matter. <sup>2</sup>Organic matter. <sup>3</sup>Crude protein. <sup>4</sup>Ether extract. <sup>5</sup>Total digestible nutriments. <sup>6</sup>Neutral detergent fiber. <sup>7</sup>Acid detergent fiber. <sup>8</sup>Total carbohydrates. <sup>9</sup>Non-fibrous carbohydrates. <sup>10</sup>Fibrous carbohydrates. <sup>11</sup>Guarantee levels (per kg): calcium - 175 g; phosphorus - 100 g; sodium - 114 g; selenium - 15 g; magnesium - 15 g;

During the collection period, samples of the supplied feed and the refused feed were collected and a representative composite sample was drafted per animal in each diet for analysis. Dry matter conversion (DMC) was calculated using the following equations: DMC = (Daily average gain / Dry matter intake) x 100.

#### Apparent digestibility in the entire digestive tract

Fecal collection was performed for a period of five days starting on the 40<sup>th</sup> day of the feedlot period. Fecal samples (approximately 200 g wet weight) were collected for each bull from rectum (minimum 1 hour intervals between samples) and were pooled by bull. After drying at 55°C for 72 hours, the samples were ground in a feed mill and passed through a 1-mm sieve in preparation for chemical analyses.

To estimate the excretion of the fecal dry matter, indigestible dry matter (iDM) and indigestible neutral detergent fiber (iNDF) were used as internal marker (ZEOULA et al., 2002) and purified lignin (LIPE®) external marker (FERREIRA et al., 2009). For iDM determination, the samples were milled through a 2 mm sieve, packed (20 mg of DM cm<sup>-2</sup>) in 4 x 5 cm Ankon<sup>®</sup> filter bags (F<sup>57</sup>) that had been previously weighed and incubated for 240 hours in the rumen of a crossbred cow (CASALI et al., 2008) fed a mixed diet of equal parts forage (corn silage) and concentrate (the same concentrate used in the treatments). After incubation, the bags were removed, washed with water until clean and dried in a ventilated oven at 55°C for 72 hours. The bags were then removed and oven-dried at 105°C. The iDM was estimated using the difference in sample weight before and after ruminal incubation. Fecal excretion was calculated using the following equations: FE = iDMI / iDMCF, where: FE = fecalexcretion (kg day-1), iDMI = indigestible dry matter intake (kg day-1) and iDMCF = indigestible dry matter concentration in faeces (kg day<sup>-1</sup>).

For iNDF determination, the samples (food and faeces) were milled through a 2-mm sieve, packed (20 mg of DM cm<sup>-2</sup>) in 4 x 5 cm Ankon® previously weighed filter bags (NOCEK, 1988) and incubated for 240 hours in the rumen of a crossbred cow (DETMANN et al., 2001) fed a mixed diet of equal parts forage (corn silage) and concentrate (the same concentrate used in the treatments). After incubation, the bags were removed, washed with water until clean and dried in a ventilated oven at 55°C for 72 hours. The bags were then removed and oven-dried at 105°C. After, 25 bags were placed in Ankon® machine. The samples were digested by

NDF solution and heated (100°C) under stirring for 60 min. After, the bags were removed, washed with heat distilled water for five min. when the bags were removed and placed in acetone for 3 a 5 min. After, the bags were dried in a ventilated oven at 65°C for 24 hours.

The iNDF was estimated using the difference in sample weight before and after ruminal incubation. Fecal excretion was calculated using the following equations: FE = iNDFI / iNDFCF, where FE = fecal excretion (kg day-1), iNDFI = indigestible neutral detergent fibre intake (kg day-1) and iNDFCF = indigestible neutral detergent fibre concentration in faeces (kg day-1). For two internal markers, the apparent digestibility coefficients for DM and other nutrients were estimated according to the formula: AD = [(Intake - Excreted) / intake] x100. For fecal dry matter output determination as markers utilization LIPE®, the following equations were used: FSMP = (G of marker intake / Faecal marker concentration) x 100. The digestibility coefficients of dry matter (DCDM) and other nutrients (DCN) were determined by equations: DCM = [(DM intake - Fecal excreted DM) / DM intake] x 100; and DCN = [(Nutrient intake -Fecal excreted nutrients) / Nutrient intake] x 100.

The DM content of the samples was determined by drying at 105°C for 24 hours according by method 930.15 (AOAC, 1990). The OM content was calculated as the difference between the DM and ash contents, with ash determined by combustion at 550°C for 5 hours by method 924.05 (AOAC, 1990). The aNDF content was measured according recommendations of Mertens (2002) using alphaamylase in the process. The nitrogen (N) content was determined by the Kjeldahl by method 976.05 (AOAC, 1990), and the total carbohydrates (TC) were calculated using the following equation according recommendations of Sniffen et al. (1992): TC = 100 -(CP% + EE% + Ash%). Non-fibrous carbohydrates (NFC) were determined as the difference between the TC and NDF. Total digestible nutrient (TDN) content of diets was obtained by the methodology described by Kearl (1982). The LIPE in feces was analyzed an infra-red spectrophotometer (Varian 099-2243) following methodology described by Ferreira et al. (2009).

# Statistical analysis

Data analysis in this experiment the package was employed (SAS, 2002). The results were interpreted by variance analyses and the differences were tested by Tukey test, being the variables measured following the model:

$$Y_{ij} = \mu + d_i + e_{ij},$$

where:

 $Y_{ij}$  = observation on animal j fed with diet i;

 $\mu$  = mean treatments;

 $d_i$  = effect of diet i;

1, 2, and 3; and  $e_{ij}$  = residual error.

#### Results and discussion

The partial corn replacing (38.5%) by glycerin, as energy source in the diets did not affect (p > 0.05) final live weight, average daily gain, dry matter intake (kg day-1 or as live weight ratio) and dry matter efficiency (Table 3). Mean values observed were: 482.2 kg; 1.28 kg day<sup>-1</sup>; 9.95 kg day<sup>-1</sup>; 2.25% on live weight and 8.20, respectively. Similar results were found on animal performance and feed efficiency by Mach et al. (2009) with glycerin addition of 4, 8 and 12% in the diets for cattle finished in feedlot. On the other hand, Farias et al. (2012) observed a linear decrease in performance, feed intake and feed efficiency in heifers finished on pasture due to the increased levels of glycerin (0, 3.3, 6.6 and 9.9% on DM) in the diet.

The glycerin and Essential® oils addition in the diets did not affect (p > 0.05) performance animal, feed intake and feed efficiency (Table 3). Few studies have been published on essential oils addition as additives in the diets for beef cattle finished in feedlot on animal performance, feed intake and feed efficiency. Benchaar et al. (2006) evaluated the animal performance fed with corn silage and supplemented with 2 or 4 g day-1 of a compound based on thymol, eugenol, vanillin and limolene and observed no effect of essential oils addition on animal performance and feed intake. In other studies, Meyer et al. (2010) working with cattle fed with high grain diets based on corn tested a product (Crina Ruminants®) composed of multiple active ingredients of numerous essential oils, such as thymol, eugenol, vanillin, guaiacol, limolene and a control (without additive addition) and did not observe difference on feed intake.

No effects were found in partial corn replacing by glycerin and Essential® oils addition in the diets (p > 0.05) on the fecal output, crude protein and ether extract digestibility among the diets (Table 4). On the other hand, the DM, and OM apparent digestibility were higher (respectively, p < 0.01 and p < 0.02) for bulls fed with glycerin and Essential® oils than control diet (Table 4).

**Table 3.** Effect of glycerin and functional oil on animal performance, feed intake and feed efficiency of Nellore bulls finished in feedlot

| I  |       | Diets            |                  | V/C (0/ \ | 1 .     |
|--|-------|------------------|------------------|-----------|---------|
| Item   | CON1  | GLY <sup>2</sup> | GEO <sup>3</sup> | VC(%)     | p value |
| Initial live weight, kg                                      | 399.6 | 399.5            | 403.8            | 8.77      | 0.45    |
| Final live weight, kg  | 477.2 | 477.6            | 491.8            | 8.74      | 0.39    |
| Average daily gain, kg                                       | 1.23  | 1.23             | 1.39             | 5.81      | 0.78    |
| Dry matter intake, kg  | 9.82  | 9.78             | 10.2             | 8.60      | 0.91    |
| Dry matter intake, % LW <sup>-1</sup>                        | 2.24  | 2.23             | 2.28             | 0.78      | 0.45    |
| Organic matter intake, kg                                    | 9.29  | 9.15             | 9.59             | 8.60      | 0.76    |
| Crude protein intake, kg                                     | 1.14  | 1.14             | 1.20             | 8.61      | 0.34    |
| Ether extract intake, kg                                     | 0.40  | 0.32             | 0.33             | 8.75      | 0.12    |
| Neutral fiber detergent intake, kg                           | 3.06  | 2.91             | 3.04             | 8.58      | 0.35    |
| Acid fiber detergent, kg                                     | 1.68  | 1.61             | 1.69             | 8.59      | 0.86    |
| Total carbohydrate intake, kg                                | 7.87  | 7.71             | 8.08             | 8.55      | 0.25    |
| Non-fibrous carbohydrate intake, kg                          | 4.81  | 4.80             | 5.03             | 8.56      | 0.31    |
| Fibrous carbohydrate intake, kg                              | 3.06  | 2.90             | 3.04             | 8.51      | 0.37    |
| Total digestible nutrients intake, kg                        | 6.92  | 6.85             | 7.18             | 8.60      | 0.82    |
| Dry matter conversion <sup>5</sup> , kg DM ADG <sup>-1</sup> | 8.69  | 8.32             | 7.60             | 6.54      | 0.65    |

<sup>1</sup>Diet without glycerin or Essential<sup>®</sup> oils; <sup>2</sup>Diet with glycerin; <sup>3</sup>Diet with glycerin and Essential<sup>®</sup> oils; <sup>4</sup>Variation coefficient.

**Table 4.** Effect of glycerin and functional oils on fecal output and apparent digestibility of Nellore bulls finished in feedlot.

| Item                      |       | Diets            | VC (%) <sup>4</sup> | p value |         |
|---------------------------|-------|------------------|---------------------|---------|---------|
| Item                      | CON1  | GLY <sup>2</sup> | GEO <sup>3</sup>    | VC (70) | p value |
| Fecal output, kg day-1    | 3.74  | 3.73             | 3.72                | 11.2    | 0.08    |
| Dry matter                | 61.7b | 64.6a            | 63.2a               | 4.32    | 0.01    |
| Organic matter            | 64.9b | 65.8a            | 66.1a               | 7.49    | 0.02    |
| Crude protein             | 63.0  | 64.0             | 64.9                | 10.0    | 0.28    |
| Ether extract             | 72.5  | 74.0             | 73.9                | 9.48    | 0.08    |
| Neutral detergent fiber   | 59.0a | 58.0b            | 58.4ab              | 3.52    | 0.04    |
| Fibrous carbohydrates     | 56.3a | 55.0b            | 55.3ab              | 8.93    | 0.02    |
| Non-fibrous carbohydrates | 79.0a | 77.9b            | 78.7ab              | 4.64    | 0.03    |

<sup>1</sup>Diet without glycerin or Essential<sup>®</sup> oils; <sup>2</sup>Diet with glycerin; <sup>3</sup>Diet with glycerin and Essential<sup>®</sup> oils; <sup>4</sup>Variation coefficient.

Previous studies (DONKIN, 2008) reported 63, 60, 61 and 63% for DM digestibility in the diets with 0, 5, 10 and 15% glycerin levels inclusion, respectively. The apparent digestibility results of DM and OM indicate that glycerin supplementation in the diet potentially improves rumen fermentation and feed digestibility in the total digestive tract of beef cattle. Wang et al. (2009) found that the total tract digestibility of DM, OM, NDF and CP were increased linearly with increasing glycerin in the diet.

The increased digestibility of nutrients in diets containing glycerin may be explained by the metabolism of glycerin into volatile fatty acids by gram-negative bacteria in the rumen and the absorption of fatty acids by the gastrointestinal mucosa. Biohydrogenation and absorption occur rapidly in the rumen, reducing the amount of material to be transported and metabolized in the gut of the animals. However, glycerin addition in the diets decrease NDF, FC and NFC apparent digestibilities (respectively, p < 0.04, p < 0.02and p < 0.03), where the glycerin associated with Essential® oils. NDF, FC and NFC apparent digestibilities were similar among treatments (Table 4). On the other hand, we expected improves NDF, FC and NFC apparent digestibility, due absent of fiber in the glycerin. According to Krehbiel (2008), the disappearance rates of glycerin in rumen may increase with the adaptation of animals to intense fermentation of volatile fatty acids by rumen bacteria. Adding glycerin to ruminants diets alters the pattern of rumen fermentation, linearly reducing the proportion of acetate/propionate as the dose of glycerin is increased, favoring production of propionate in the rumen by gram-negative bacteria (WANG et al., 2009).

Corroborating these findings, Kijora et al. (1998) stated that after seven days of adaptation by microbial organisms the metabolisation of glycerin occurs more rapidly and 85% of glycerin disappears in less than two hours, reaching a total disappearance within six hours (BERGNER et al., 1995). Wang et al. (2009) observed an increase in the digestibility of DM, EE, OM, NDF and ADF (quadratic response) when compared to the control treatment.

Likewise, Benchaar et al. (2006) observed no effect on DM, OM and CP digestibility's in bulls fed grass legume silage-based diets supplemented with essential oils. On the other hand, previous research reported antimicrobial activity by compounds extracted from cashew nutshell (HIMEJIMA; KUBO, 1991; MUROI et al., 1993; DORMAN; DEANS, 2000) and castor oil (NOVAK et al., 1961; SHIN et al., 2004) which could improve rumen fermentation. Likewise, others researches show great potential of the essential oil to manipulate rumen fermentation (BENCHAAR et al., 2007; BENCHAAR; REATHEAD, 2011). However, in this study neither difference was found when associated glycerin and Essential® oils.

The three markers used to estimate faecal output and the apparent digestibility of dry matter and other nutrients presented similar values (p > 0.05, Table 5). The means values for apparent digestibilities of DM were 65.7, 63.3 and 61.4% for iDM, iNDF and LIPE® markers, respectively. Dettmann et al. (2001) suggested that the iDM utilization as internal marker potentially removes all soluble and insoluble compounds digestible. These authors evaluated the internal iDM, iNDF and iADF and found that iNDF and iDM were the best alternative to the indirect determination the diet digestibility.

According to such results, the iDM use for digestibility determination can have the advantage once this marker did not require analysis of detergent system; which can reduce the cost of reagents and laboratory procedures. However,

according to Huhtanen et al. (1994), although iDM can produce exact results, the contaminants presence may affect the results. These contaminants can remain adhered to the mesh tissue after incubation, even after total washing. On the other hand, the iADF marker demonstrated variable behavior. The authors suggested that this result could be due to the ADF technical analysis obtained by these sequential methods; which would allow the accumulation of errors.

**Table 5.** Comparison of different markers used to estimate fecal output and apparent digestibility of Nellore bulls finished in feedlot.

| Item                       | Markers          |                   |                   | -VC (%) <sup>4</sup> |         |  |
|----------------------------|------------------|-------------------|-------------------|----------------------|---------|--|
| rtem -                     | iDM <sup>1</sup> | iNDF <sup>2</sup> | LIPE <sup>3</sup> | - VC (%)             | p value |  |
| Fecal output, kg day-1     | 3.51             | 3.77              | 3.94              | 17.5                 | 0.36    |  |
| Dry matter                 | 65.7             | 63.2              | 61.3              | 9.49                 | 0.29    |  |
| Organic matter             | 67.7             | 65.4              | 63.7              | 9.20                 | 0.34    |  |
| Crude protein              | 65.5             | 62.9              | 61.6              | 14.7                 | 0.31    |  |
| Ether extract              | 74.9             | 73.1              | 69.7              | 10.1                 | 0.29    |  |
| Neutral detergent fiber    | 59.6             | 58.0              | 56.9              | 5.64                 | 0.21    |  |
| Fibrous carbohydrate       | 58.6             | 55.6              | 52.8              | 9.84                 | 0.80    |  |
| Non-fibrous carbohydrate   | 79.5             | 78.0              | 76.5              | 4.39                 | 0.18    |  |
| Total carbohydrate         | 62.5             | 59.8              | 57.7              | 10.9                 | 0.28    |  |
| Total digestible nutrients | 65.4             | 63.0              | 63.2              | 9.76                 | 0.48    |  |

<sup>1</sup>Indigestible dry matter; <sup>2</sup>Indigestible neutral detergent fiber; <sup>3</sup>Purified lignin®; <sup>4</sup>Variation coefficient.

The digestibility is influenced by associative effects, intake level, passage rate and the interactions among these factors (BERCHIELLI et al., 2000). The authors also reported that the nutrients digestibility when incubated for an insufficient time, especially iDM, there is a decrease in food digestibility due the exposure time reduction of the sample for ruminal microbes, leading to unrealistic indigestible reproduction of samples. Since there is no consensus in the literature on incubation time to allow better represent the indigestible portion of the samples are observed variable periods as: 96 (RUIZ et al., 2001), 144 (ZEOULA et al., 2002), 240 (CLIPES et al., 2006) and 288 hours (HUHTANEN et al., 1994). Berchielli et al. (2000) evaluated internal markers on digestibility trials and concluded that iNDF showed similar results to digestibility estimated when compared to digestibility estimated by total collection method. Likewise, Zeoula et al. (2002) found that fecal recovery of iNDF not differ among markers. Thus, the results for apparent digestibility of DM and OM were similar to that estimated from the fecal total collection.

The external marker LIPE® satisfactorily estimated the nutrients digestibilities; which did not differ from digestibility determined with the use of two other

internal markers (iNDF and iDM). Ferreira et al. (2009) found that there was no difference in DM digestibility of Tifton hay when they compared the results obtained from fecal total collection and LIPE® marker. The LIPE® has been evaluated and considered as external marker with relevant characteristics, presenting accurate results for digestibility studies (FERREIRA et al., 2009). The main advantage to use LIPE<sup>®</sup> as a marker is that it provides greater stability during passage through the animal's gastrointestinal tract. Moreover, its concentration and path through presents low variation. Furthermore, LIPE® marker is fully recover able in faeces. The LIPE® use has been considered promising in several species such as rabbits, sheep, horses, pigs, poultry and cattle (RODRIGUES et al., 2010).

#### Conclusion

The glycerin derived from biodiesel production has nutritional characteristics suitable for inclusion in ruminants diet, indicating that corn may be replaced by glycerin (38.5% DM) which corresponds to the inclusion of 15% glycerin in total dry matter of diet without affecting animal performance, feed intake, feed efficiency but increasing nutrient digestibility on DM. Moreover, the addition of Essential® oils to the diet of feedlot cattle showed no effect on animal performance, feed intake, feed efficiency and nutrient digestibility. Thus, the addition of Essential® oils to the diet of feedlot cattle with a ratio of concentrate and roughage 50% and 50% (low concentration) would not be required. The internal iDM and iNDF to estimate fecal output and digestibility of nutrients similarly to external marker LIPE® in cattle finished in feedlot.

# Acknowledgements

This project was supported by the Araucaria Foundation, funding from the State of Paraná and the Brazilian Council for Research and Technological Development (CNPq). The authors gratefully acknowledge Processing Inc. (BIOPAR, Rolândia, Paraná State, south Brazil) for providing the crude glycerin used in this research and Oligo Basics for providing Essential® oils. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the Department of Animal Science, State University of Maringá, Paraná State, Brazil.

### References

ABRAHÃO, J. J. S.; PRADO, I. N.; MARQUES, J. A.; PEROTTO, D.; LUGÃO, S. M. B. Avaliação da

substituição do milho pelo resíduo seco da extração da fécula de mandioca sobre o desempenho de novilhas mestiças em confinamento. **Revista Brasileira de Zootecnia**, v. 35, n. 2, p. 512-518, 2006.

AOAC-Association of Official Analytical Chemists. **Official Methods ot Analysis**. 14th ed. Arlington: AOAC, 1990.

BENCHAAR, C.; CALSAMIGLIA, S.; CHAVES, A. V.; FRASER, G. R.; COLOMBATTO, D.; MCALLISTER, T. A.; BEAUCHEMIN, K. A. A review of plant-derived essential oils in ruminant nutrition and production. **Animal Feed Science and Technology**, v. 145, n. 1-4, p. 209-228, 2008.

BENCHAAR, C.; CHAVES, A. V.; FRASER, G. R.; WANG, Y.; BEAUCHEMIN, K. A.; MCALLISTER, T. A. Effects of essential oils and their components on *in vitro* rumen microbial fermentation. **Canadian Journal of Animal Science**, v. 87, n. 3, p. 413-419, 2007.

BENCHAAR, C.; DUYNISVELD, J. L.; CHARMLEY, E. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. **Canadian Journal of Animal Science**, v. 86, n. 1, p. 91-96, 2006.

BENCHAAR, C.; GREATHEAD, H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. **Animal Feed Science and Technology**, v. 166, p. 338-355, 2011.

BERCHIELLI, T. T.; ANDRADE, P.; FURLAN, C. L. Avaliação de indicadores internos em ensaios de digestibilidade. **Revista Brasileira de Zootecnia**, v. 29, n. 3, p. 830-833, 2000.

BERGNER, H.; KIJORA, C.; CERESNAKOVA, Z.; SZAKACS, J. *In vitro* studies on glycerol transformation by rumen microorganisms. **Archiv für Tierernaehrung**, v. 48, n. 3, p. 245-256, 1995.

CASALI, A. O.; DETMANN, E.; VALADARES FILHO, S. C.; PEREIRA, J. C.; HENRIQUES, L. T.; FREITAS, S. G.; PAULINO, M. F. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos *in situ*. **Revista Brasileira de Zootecnia**, v. 37, n. 2, p. 335-342, 2008.

CIOMS/OMS-Council for International Organizations of Medical Services. **International guiding principles for biomedical research involving animals**. Geneva: WHO, 1985.

CLIPES, R. C.; DETMANN, E.; SILVA, J. F. C.; VIEIRA, R. A. M.; NUNES, L. B. M.; LISTA, F. N.; PONCIANO, N. J. Evaluation of acid detergent insoluble protein as an estimator of rumen non-degradable protein in tropical grass forages. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 58, n. 4, p. 694-697, 2006.

DETMANN, E.; PAULINO, M. F.; ZERVOUDAKIS, J. T.; VALADARES FILHO, S. C.; EUCLYDES, R. F.; LANA, R. P.; QUEIROZ, D. S. Cromo e indicadores internos na determinação do consumo de novilhos mestiços, suplementados, a pasto. **Revista Brasileira de Zootecnia**, v. 30, n. 5, p. 1600-1609, 2001.

DONKIN, S. S. Glycerol from biodiesel production: the new corn for dairy cattle. **Revista Brasileira de Zootecnia**, v. 37, n. SPE, p. 280-286, 2008.

DORMAN, H. J. D.; DEANS, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **Journal of Applied Microbiology**, v. 88, n. 2, p. 308-316, 2000.

DUCATTI, T.; PRADO, I. N.; ROTTA, P. P.; PRADO, R. M.; PEROTTO, D.; MAGGIONI, D.; VISENTAINER, J. V. Chemical composition and fatty acid profile in crossbred (*Bos taurus vs. Bos indicus*) young bulls finished in a feedlot. **Asian-Australasian Journal of Animal Sciences**, v. 22, n. 3, p. 433-439, 2009.

EIRAS, C. E.; MARQUES, J. D. A.; PRADO, R. M.; VALERO, M. V.; BONAFÉ, E. G.; ZAWADZKI, F.; PEROTTO, D.; PRADO, I. N. Glycerine levels in the diets of crossbred bulls finished in feedlot: Carcass characteristics and meat quality. **Meat Science**, v. 96, n. 2, Part A, p. 930-936, 2014.

EIRAS, C. E.; MARQUES, J. D. A.; TORRECILHAS, J. A.; ZAWADZKI, F.; MOLETTA, J. L.; PRADO, I. N. Glycerin levels in the diets for crossbred bulls finished in feed-lot: ingestive behavior, feeding and rumination efficiency. **Acta Scientiarum. Animal Sciences,** v. 35, n. 4, p. 411-416, 2013.

FARIAS, M. S.; SILVA, R. R.; ZAWADZKI, F.; EIRAS, C. E.; LIMA, B. S.; PRADO, I. N. Glycerin levels for crossbred heifers supplemented in pasture: Intake behavior. **Acta Scienciarum. Animal Sciences**, v. 34, n. 1, p. 63-69, 2012.

FERREIRA, M. A.; VALADARES FILHO, S. C.; INÁCIO, M.; MARCONDES, M. L. P.; PAULINO, M. F.; VALADARES, R. F. D. Avaliação de indicadores em estudos com ruminantes: digestibilidade. **Revista Brasileira de Zootecnia**, v. 38, n. 8, p. 1568-1573, 2009. FRANÇOZO, M. C.; PRADO, I. N.; CECATO, U.; VALERO, M. V.; ZAWADZKI, F.; RIBEIRO, O. L.; PRADO, R. M.; VISENTAINER, J. V. Growth performance, carcass characteristics and meat quality of finishing bulls fed crude glycerine-supplemented diets. **Brazilian Archives of Biology and Technology**, v. 56, n. 2, p. 327-336, 2013.

FUGITA, C. A.; PRADO, I. N.; JOBIM, C. C.; ZAWADZKI, F.; VALERO, M. V.; PIRES, M. C. O.; PRADO, R. M.; FRANÇOZO, M. C. Corn silage with and without enzyme-bacteria inoculants on performance, carcass characteristics and meat quality in feedlot finished crossbred bulls. **Revista Brasileira de Zootecnia**, v. 41, n. 1, p. 154-163, 2012.

GIGER-REVERDIN, S.; DUVAUX-PONTER, C.; SAUVANT, D.; MARTIN, O.; PRADO, I. N.; MÜLLER, R. Intrinsic buffering capacity of feedstuffs. **Animal Feed Science and Technology**, v. 96, n. 1, p. 83-102, 2002.

HIMEJIMA, M.; KUBO, I. Antibacterial agents from the cashew Anacardium occidentale (Anacardiaceae) nut shell oil. **Journal of Agricultural and Food Chemistry**, v. 39, n. 2, p. 418-421, 1991.

HUHTANEN, P.; KAUSTELL, K.; JAAKKOLA, S. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. **Animal Feed Science and Technology**, v. 48, n. 3, p. 211-227, 1994.

KEARL, L. C. Nutrient requirements of ruminants in developing countries. Utah: Utah State University, 1982.

KIJORA, C.; BERGNER, H.; GÖTZ, K. P.; BARTELT, J.; SZAKACS, J.; SOMMER, A. Investigation on the metabolism of glycerol in the rumen of bulls. **Arch Tieremahr**, v. 51, p. 341-348, 1998.

KREHBIEL, C. R. Ruminal and physiological metabolism of glycerin. **Journal of Animal Science**, v. 86, suppl., p. 392, 2008.

KUBO, I.; NIHEI, K.; TSUJIMOTO, K. Antibacterial action of anacardic acids against methicillin resistant *Staphylococcus aureus* (MRSA). **Journal of Agricultural and Food Chemistry**, v. 51, n. 26, p. 7624-7628, 2003.

MACH, N.; BACH, A.; DEVANT, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. **Journal of Animal Science**, v. 87, n. 2, p. 632-638, 2009. MARQUES, J. A.; PRADO, I. N.; ZEOULA, L. M.; ALCALDE, C. R.; NASCIMENTO, W. G. Avaliação da mandioca e seus resíduos industriais em substituição ao milho no desempenho de novilhas confinadas. **Revista Brasileira de Zootecnia**, v. 29, n. 5, p. 1528-1536, 2000.

MARTINS, A. S.; ZEOULA, L. M.; PRADO, I. N.; MARTINS, E. N.; LOYOLA, V. R. Ruminal in situ degradability of dry matter and crude protein of corn and sorghum silages and some concentrate feeds. **Revista Brasileira de Zootecnia**, v. 28, n. 5, p. 1109-1117, 1999.

MERTENS, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of AOAC International**, v. 85, n. 6, p. 1217-1240, 2002.

MEYER, U.; SCHWABE, A.; DÄNICKE, S.; FLACHOWSKY, G. Effects of by-products from biofuel production on the performance of growing fattening bulls. **Animal Feed Science and Technology**, v. 161, n. 3, p. 132-139, 2010.

MUROI, H.; KUBO, A.; KUBO, I. Antimicrobial activity of cashew apple flavor compounds. **Journal of Agricultural and Food Chemistry**, v. 41, n. 7, p. 1106-1109, 1993.

NAUGHTON, F. C. Castor oil. In: NAUGHTON, F. C. (Ed.). **Kirk-Othmer encyclopedia of chemical technology**. New York: John Wiley and Sons, 2000. p. 866.

NOCEK, J. E. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. **Journal of Dairy Science**, v. 71, n. 8, p. 2051-2069, 1988.

NOVAK, A.; CLARK, G.; DUPUY, H. Antimicrobial activity of some ricinoleic acid oleic acid derivatives. **Journal of the American Oil Chemists' Society**, v. 38, n. 6, p. 321-324, 1961.

NRC-National Research Council. **Nutrient requirements of beef cattle**. 7th ed. Washington, D.C.: National Academy Press, 2000.

PRADO, I. N.; ITO, R. H.; PRADO, J. M.; PRADO, I. M.; ROTTA, P. P.; MATSUSHITA, M.; VISENTAINER, J. V.; SILVA, R. R. The influence of dietary soyabean and linseed on the chemical composition and fatty acid profile of the Longissimus muscle of feedlot-finished bulls. **Journal of Animal and Feed Sciences**, v. 17, p. 307-317, 2008.

PRADO, I. N.; PINHEIRO, A. D.; ALCALDE, C. R.; ZEOULA, L. M.; NASCIMENTO, W. G.; SOUZA, N. E. Níveis de substituição do milho pela polpa de citrus peletizada sobre o desempenho e características de carcaça de bovinos mestiços confinados. **Revista Brasileira de Zootecnia**, v. 29, n. 6, p. 2135-2141, 2000.

PRADO, R. M.; PRADO, I. N.; MARQUES, J. A.; ROTTA, P. P.; VISENTAINER, J. V.; SILVA, R. R.; SOUZA, N. E. Meat quality of the *Longissimus* muscle of bulls and steers (1/2 Nellore vs 1/2 Simmental) finished in feedlot. **Journal of Animal and Feed Sciences**, v. 18, n. 2, p. 221-230, 2009.

RODRIGUES, P. H. M.; GOMES, R. C.; SIQUEIRA, R. F.; MEYER, P. M.; RODRIGUES, R. R. Accuracy, precision and robustness of in vivo dry matter digestibility estimates by different markers in ovine. **Revista Brasileira de Zootecnia**, v. 39, n. 5, p. 1118-1126, 2010.

RUIZ, R.; VAN SOEST, P. J.; VAN AMBURGH, M. E.; FOX, D. G.; ROBERTSON, J. B. Use of chromium mordanted neutral detergent residue as a predictor of fecal output to estimate intake in grazing high producing Holstein cows. **Animal Feed Science and Technology**, v. 89, n. 3, p. 155-164, 2001.

SAS-Statistical Analyses System. **User's guide**: software 9. 1. Cary: SAS Institute Inc., 2002.

SHIN, S. Y.; KIM, H. R.; KANG, S. C. Antibacterial activity of various hydroxy fatty acids bioconverted by *Pseudomonas aeruginosa* PR3. **Agricultural Chemistry and Biotechnologye**, v. 47, p. 205-208, 2004.

SILVA, R. R.; PRADO, I. N.; CARVALHO, G. G. P.; JÚNIOR, S.; PAIXÃO, M. L.; FILHO, G. A. Níveis de suplementação na terminação de novilhos Nelore em pastagens: aspectos econômicos. **Revista Brasileira de Zootecnia**, v. 39, n. 9, p. 2091-2097, 2010.

SNIFFEN, C. J.; O'CONNOR, J. D.; VAN SOEST, P. J.; FOX, D. G.; RUSSELL, J. B. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. **Journal of Animal Science**, v. 70, n. 11, p. 3562-3577, 1992.

TREVISAN, M. T. S.; PFUNDSTEIN, B.; HAUBNER, R.; WÜRTELE, G.; SPIEGELHALDER, B.; BARTSCH, H.; OWEN, R. Characterization of alkyl phenols in cashew (*Anacardium occidentale*) products and assay of their antioxidant capacity. **Food and Chemical Toxicology**, v. 44, n. 2, p. 188-197, 2006.

VALERO, M. V.; ZAWADZKI, F.; FRANÇOZO, M. C.; FARIAS, M. S.; ROTTA, P. P.; PRADO, I. N.; VISENTAINER, J. V.; ZEOULA, L. M. Sodium monensin or propolis extract in the diet of crossbred (½ Red Angus vs. ½ Nellore) bulls finished in feedlot: chemical composition and fatty acid profile of the *Longissimus* muscle. **Semina: Ciências Agrárias**, v. 32, n. 4, p. 1617-1626, 2011.

WANG, C.; LIU, Q.; HUO, W. J.; YANG, W. Z.; DONG, K. H.; HUANG, Y. X.; GUO, G. Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. **Livestock Science**, v. 121, n. 1, p. 15-20, 2009.

ZAWADZKI, F.; PRADO, I. N.; MARQUES, J. A.; ZEOULA, L. M.; PRADO, R. M.; FUGITA, C. A.; VALERO, M. V.; MAGGIONI, D. Sodium monensin or propolis extract in the diet of Nellore bulls finished in feedlot: chemical composition and fatty acid profile of *Longissimus* muscle. **Semina: Ciências Agrárias**, v. 32, n. 4, p. 1627-1636, 2011a.

ZAWADZKI, F.; PRADO, I. N.; MARQUES, J. A.; ZEOULA, L. M.; ROTTA, P. P.; SESTARI, B. B.; VALERO, M. V.; RIVAROLI, D. C. Sodium monensin or propolis extract in the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics. **Journal of Animal and Feed Sciences**, v. 20, n. 1, p. 16-25, 2011b.

ZEOULA, L. M.; PRADO, I. N.; DIAN, P. H. M.; GERON, L. J. V.; CALDAS NETO, S. F.; MAEDA, E. M.; PRA PERON, P. D.; MARQUES, J. A.; FALCÃO, A. J. S. Fecal recuperation of internal markers in assay with ruminants. **Revista Brasileira de Zootecnia**, v. 31, n. 4, p. 1865-1874, 2002.

Received on February 21, 2013. Accepted on March 13, 2014.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.