Effect of glycerine and essential oils (Anacardium occidentale and Ricinus communis) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system

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Abstract

The effect of corn substitution by glycerine and essential oils on animal performance, apparent digestibility and red and white blood cells of crossbred bulls finished in feedlot was evaluated. Thirty bulls with average weight of 311±28.8 kg and 22±2 month-old were allocated in three diets: CON (without glycerine or essential oils), GLY (with glycerine) and GEO (with glycerine and essential oils). The bulls were fed a diet of sorghum silage, cracked corn, soybean meal, urea, limestone and mineral salt. Three grams of cashew and castor oil/animal/day were included in GEO diet. Animals were kept in feedlot for 115 days and slaughtered at average weight of 467±40.6 kg. No differences (P>0.05) among diets regarding final body weight, average daily gain and feed conversion were reported. Ether extract intake was higher (P<0.05) in CON diet compared to the others. Dry matter, organic matter and crude protein digestibility was higher (P<0.05) in GLY diet compared to CON. Acid detergent fibre digestibility was higher (P<0.05) in GEO compared to GLY diet. Non-fibrous carbohydrate, fibrous carbohydrate and ether extract digestibility were similar (P>0.05) among diets. No effect of glycerine and essential oil addition on total blood cholesterol, triglycerides, haemogram, leukogram and plasmatic proteins was observed. Corn replacement by glycerine and essential oils addition did not affect (P>0.05) carcass weight, dressing and conformation, carcass length and cushion thickness.

Introduction

Since the emergence of the biodiesel industry in Brazil, its production has helped the conservation of the environment by reducing global warming gases and has also contributed to social development by job generation (Visser et al., 2011). Biodiesel is a biodegradable fuel derived from renewable sources that may be obtained by different processes such as cracking, esterification or transesterification (Gerpen, 2005). Recent increase in biodiesel production provided a rise in available quantities of glycerine generated from the transesterification of vegetable oils (Crandall, 2004). Glycerine could thus be included in ruminant diets as an energetic feed ingredient and substituted for other ingredients such as cereals (Eiras et al., 2013, 2014; Françozo et al., 2013). In fact, glycerine may be converted into glucose in the liver of cattle and may provide energy for cellular metabolism (Freely and Ferrell, 2000; Krechbel, 2008).

When glycerine is fed to ruminants, there is an increase in the population of the Megasphaera and Selenomonas genuses, the main glycerine fermenters in the rumen (Krueger et al., 2010). Therefore, antibiotics could act on the behalf or against these genuses, which would have a direct effect on the final product, i.e. the meat.

Antibiotics are commonly provided for animals to prevent disease and metabolic disorders and to improve feed efficiency (Bergen and Bates, 1984). However, in recent years, public concern on routine use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic resistant-bacteria that could represent risks to human health (Russell and Houlihan, 2003). Consequently, considerable efforts have been employed towards the development of alternatives to antibiotics (Benchaar et al., 2006; Zawadzki et al., 2011a, 2011b). Plant extracts offer an opportunity in this regard (Zhang et al., 2010), since several plants produce secondary metabolites with antimicrobial properties (Kim et al., 2013). These compounds have been shown to modulate rumen fermentation and to improve nutritional utilisation in ruminants (Geraci et al., 2012; Hristov et al., 2008).

The current study evaluated the effects of feeding glycerine as an energetic ingredient and essential oils on performance, digestibility, carcass characteristics and blood cells of crossbred bulls finished in feedlot.

Materials and methods

Place, animals, housing and diets

The experiment, approved by the Department of Animal Production of the State University of Maringá (CIOMS/OMS, 1985), was conducted at the Experimental Station of Iguatemi, Maringá, Brazil.

Thirty crossbred bulls (½ Aberdeen Angus vs ½ Nellore) were used in a completely randomised design. The bulls were randomly assigned to 1 of the 3 diets (ten bulls by treatment): CON, diet without glycerine or essential oils; GLY, replacement of 16.1% corn by glycerine in the diet; GEO, replacement of 16.1% corn by glycerine in the diet and addition of essential oils from cashew and castor (3 g/animal/day). Glycerine was used, in current study, as an energetic component of the diet and its inclusion was counterbalanced mainly by reducing corn grain contents in order to obtain three iso-energetic diets. Essential oils...
were used as additive to improve ruminal fermentation and meat quality.

At the beginning of the experimental period, the bulls weighed 311±28.8 kg and were 22±2 months old. Animals were kept in 10 m² individual pens with concrete floors, equipped with 60 cm deep and 2 m long feeders, and watering place. The bulls were fed on corn silage and concentrate in separate troughs, with ad libitum intake, twice a day, at 08:00 and 15:00 h. Diets were weighed daily so that the refusals represented 5% of the total.

The diet formulation and quantity supplied were designed to provide a weight gain of 1.4 kg/day, following National Research Council (2000) recommendations. The body weight (BW) of bulls and intake of concentrate and corn silage were recorded daily. After 115 days on experimental trial bulls had a final BW of 467±27.9 kg.

The diets had a ratio of 50% roughage (corn silage) and 50% concentrate. Concentrate was composed of cracked corn, soybean meal, glycerine, urea, limestone and mineral salt (Table 1). All the diets were formulated to be iso-nitrogenous and iso-energetic (Table 2).

Glycerine was produced in a soy-diesel facility and its chemical composition was determined at the Institute of Technology of Paraná (TECPAR; Curitiba, Brazil), as follows: water, 23.2 g/kg; ashes – 47.6 g/kg; glycerol, 812 g/kg; methanol, 3.32 mg/kg; sodium, 11.6 g/kg; potassium, 79.1 mg/kg; chloride, 35.8 mg/kg, magnesium, 16.3 mg/kg; phosphorus, 239.0 mg/kg.

Essential oils contained ricinoleic and anacardic acid, and cardanol and cardol. Ricinoleic acid was obtained from castor oil (extracted from the castor seed – *Anacardium occidentale*). Both oils were used for the solidification of the essential oils formulated by Oligo Basics Agroindustrial Ltd.® (Cascavel, Brazil).

### Performance and feed intake

Bulls were weighed at the beginning of the experiment and once every 28 days (after fasting from solid food for 16 h). Daily feed intake was estimated as the difference between the supplied feed and the refusals in the trough. During the collection period, samples of the supplied feed and refusals were collected daily and a representative composite sample was drafted per animal in each treatment.

### Apparent total-tract digestibility

Faecal collections were performed starting on the 40th day of the feedlot period, to estimate apparent digestibility coefficient of dry matter and other nutrients. Faecal samples (approximately 200 g wet weight) were collected from the rectum of each bull twice a day (minimum 3 h interval between samples) during five consecutive days. Samples were pooled by bulls using the 5 days samples and were dried at 55°C for 48 h. Then, the material was ground in a mill and passed through a 2 mm sieve to estimate apparent total-tract digestibility and passed through a 1 mm sieve in preparation for the chemical analyses.

Indigestible dry matter (iDM) was used as an internal marker to estimate the flux of faecal dry matter (Zoula et al., 2002). Samples milled through a 2 mm sieve were packed (20 mg of DM/cm²) in previously weighed 4×5 cm Ankom® bag (filter bags F57) and incubated for 240 h in the rumen of a Holstein bull (Casali et al., 2008) fed a mixed diet of equal parts of forage (corn silage) and concentrate (the same concentrate used in the treatments). After incubation, the bags were removed, washed with water until cleaned and dried in a ventilated oven at 55°C for 72 h. Bags were then removed and oven-dried again at 105°C. The iDM was estimated by the difference in sample weight before and after rumi-

| Ingredients          | CON | GLY | GEO |
|----------------------|-----|-----|-----|
| Sorghum silage       | 411 | 411 | 411 |
| Corn                 | 326 | 326 | 326 |
| Soybean meal         | 84.2| 84.2| 84.2|
| Glycerine            | -   | 161 | 161 |
| Limestone            | 6.93| 6.93| 6.93|
| Mineral salt         | 4.31| 4.31| 4.31|
| Urea                 | 6.89| 6.89| 6.89|
| Essential oils       | -   | -   | 0.42|

CON, diet without glycerine or essential oils (*i.e.* control); GLY, diet with glycerine; GEO, diet with glycerine and essential oils.

### Table 2. Chemical composition of ingredients and diets (g/kg on dry matter).

| Ingredients          | DM  | OM  | Ashes, | CP  | NDF | ADF | TC  | NFC | EE  | TDN |
|----------------------|-----|-----|--------|-----|-----|-----|-----|-----|-----|-----|
| Sorghum silage       | 242 | 937 | 63.4   | 49.6| 516 | 291 | 865 | 349 | 22.1| 600 |
| Corn                 | 886 | 986 | 13.6   | 84.0| 96.3| 40.8| 862 | 766 | 40.3| 900 |
| Soybean meal         | 893 | 928 | 72.4   | 473 | 139 | 256 | 863 | 766 | 13.3| 800 |
| Glycerine            | 875 | -   | 478    | 10.0| -   | -   | 139 | 10.0| -   |      |
| Limestone            | 980 | -   | 950    | -   | -   | -   | -   | -   | -   |      |
| Mineral salt         | 980 | -   | 952    | -   | -   | -   | -   | -   | -   |      |
| Urea                 | 980 | -   | 945    | 2.800| -   | -   | -   | -   | -   |      |
| Diets                |     |     |        |     |     |     |     |     |     |     |
| CON                  | 424 | 952 | 47.9   | 110 | 271 | 164 | 618 | 547 | 29.9| 757 |
| GLY                  | 424 | 944 | 58.4   | 110 | 255 | 157 | 673 | 418 | 23.1| 752 |
| GEO                  | 424 | 944 | 58.4   | 110 | 255 | 157 | 673 | 418 | 23.1| 752 |

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; TC, total carbohydrates; NFC, non-fibrous carbohydrates; EE, ether extract; TDN, total nutrients digestible; CON, diet without glycerine or essential oils (*i.e.* control); GLY, diet with glycerine; GEO, diet with glycerine and essential oils.
nal incubation. Faecal excretion was calculated by the following equations:

$$FE = \frac{DMI}{DMCF}$$

where: $FE =$ faecal excretion (kg/day); $DMI =$ DM intake (kg/day); DMCF = DM concentration in faeces (kg/day). The apparent digestibility coefficients (ADC) for DM and nutrients were calculated by the formula: $DC = [(\text{intake} – \text{excreted})/\text{intake}] \times 100$.

**Chemical analyses**

Dry matter (DM) content of the ingredients (silage and concentrate mix) was determined by oven-drying at $65^\circ C$ for 24 h. Analytical DM content of the oven-dried samples was determined by drying at $105^\circ C$ for 24 h following (AOAC, 1998) (method 930.15). Organic matter (OM) content was calculated as the difference between DM and ash contents where ash was determined by combustion at $550^\circ C$ for 5 h. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined by methods described by Mertens (2002). Nitrogen (N) content was calculated by Kjeldahl method (AOAC, 1998) (method 976.05). Total carbohydrates (TC) were estimated by the equation: $TC = 100 - (% \text{ CP} + % \text{ EE} + % \text{ ash})$ (Sniffen et al., 1992). Non-fibre carbohydrates (NFC) were determined as the difference between TC and NDF. Total digestible nutrient (TDN) content of the diets was obtained by methodology described by Kearl (1982).

**Blood measurements**

Blood was collected from the jugular vein at beginning and end of the experimental period (115 days). Before blood collection, the bulls were kept at fasting for 14 h. Then 5 mL of blood were collected into tubes containing ethylenediamine tetracetic acid anticoagulant (disodium salt of ethylene diaminotetracetic acid) and kept refrigerated until centrifugation at $3,500 \text{ rpm}$ during $15 \text{ min}$ for plasma harvesting.

Further haemogram (erythrocytes, haemoglobin, haemacrit, MCV, MCH and MCHC) and leukogram (eosinophils, segments, lymphocytes and monocytes) measurements were determined following methodology described by Jain and Jain (1993). Enzymatic and colorimetric methods (LABORCLIN®, Pinhais, Brazil) were employed to determine total cholesterol, following methodology described by Allain et al. (1974). Enzymatic and colorimetric tests (LABORCLIN®) were employed to determine total triglycerides following methodology described by Fossati and Prencipe (1982).

**Results and discussion**

**Animal performance and feed intake**

Partial replacement of corn by glycerine as energy source in the diet for crossbred bulls in feedlot did not affect ($P>0.05$) final live weight, average daily gain (ADG), feed intake (DM, CP, NDF and ADF) and feed efficiency, with the exception of EE intake (Table 3).

Bulls fed on diets with partial replacing of corn by glycerine had lower ($P<0.05$) EE intake due to low EE level in glycerine ($P<0.01$), as shown in Table 2. The low EE intake in diets without glycerine (0.2 kg/animal/day) had no effect on the intake of the other nutrients. A study from Mach et al. (2009) did not report any effect of glycerine inclusion in the diet (4, 8, 12 and 16% DM) on performance and feed intake in cattle finished in feedlot and fed on a high-concentrate level. Likewise, Gomes et al. (2011) reported no negative effect on animal performance and feed intake with the addition of glycerine or essential oils (i.e., control). GIL, diet with glycerine; GEO, diet with glycerine and essential oils; BW, body weight; ADG, average daily gain; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract. *Ten bulls were considered per diet; †DM intake (kg)/average daily gain (kg). **Means followed by different letters were significantly different.

**Table 3. Animal performance, feed intake and dry matter conversion of crossbred bulls finished in feedlot.**

| Items                     | CON     | GIL     | GEO     | SEM     | P      |
|---------------------------|---------|---------|---------|---------|--------|
| Initial BW, kg            | 311     | 312     | 311     | 27.9    | 0.99   |
| Final BW, kg              | 470     | 473     | 458     | 40.4    | 0.69   |
| ADG, kg/day               | 1.39    | 1.40    | 1.28    | 0.21    | 0.36   |
| DM intake, kg/day         | 8.34    | 8.12    | 8.37    | 0.99    | 0.84   |
| DM intake, %/BW           | 2.22    | 2.17    | 2.27    | 0.19    | 0.48   |
| CP intake, kg/day         | 0.90    | 0.87    | 0.91    | 0.11    | 0.70   |
| NDF intake, kg/day        | 2.78    | 2.62    | 2.64    | 0.35    | 0.54   |
| NDF intake, %/BW          | 0.74    | 0.70    | 0.72    | 0.07    | 0.52   |
| EE intake, kg/day         | 1.35    | 1.22    | 1.22    | 0.18    | 0.21   |
| EE intake, %/BW           | 0.75    | 0.65    | 0.69    | 0.04    | 0.01   |
| DM conversion, kg         | 6.10    | 5.88    | 6.58    | 0.69    | 0.06   |

CON, diet without glycerine or essential oils (i.e. control); GIL, diet with glycerine; GEO, diet with glycerine and essential oils; BW, body weight; ADG, average daily gain; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract. *Ten bulls were considered per diet; †DM intake (kg)/average daily gain (kg). **Means followed by different letters were significantly different.

**Statistical analysis**

The experimental design was completely randomised with three treatments and ten replications. Results were statistically interpreted by SAS (2004):

$$Y_{ij} = \mu + T_i + e_{ij}$$

where, $Y_{ij} =$ dependent variables; $\mu =$ overall mean; $T_i =$ treatment effect; $e_{ij} =$ residual error. When the means were different, the Tukey’s test was used to compare them.
of up 30% of DM glycerine in diets for lambs finished in feedlot. On the other hand, Parsons et al. (2009) found that the addition of glycerine (8% on DM) in the diet reduced the final body weight (BW) and decreased feed efficiency of cattle in feedlot and fed a high-concentrate level. However, Françozo et al. (2013) observed that glycerine addition (15% DM) in the diet for bulls finished in feedlot increased ADG and improved feed efficiency. Thus, glycerine may be supplemented up to 15% DM in diets for crossbred bulls finished in feedlot and fed on 50% concentrate and 50% corn silage.

The addition of essential oils to the diets of bulls had no effect (P>0.05) on final live weight, ADG, feed intake (kg DM/day or in relation to BW) and feed conversion (Table 3). In beef cattle, Benchaar et al. (2006) evaluated the performance of animals fed on corn silage and supplemented with 2 or 4 g/day of a compound based of thymol, eugenol, vanillin and limonene. Data reported that ADG and feed intake were not affected by supplements. However, the inclusion of 2 g/day of the compound with essential oils improved feed efficiency. In a recent work, Zawadzki (2013) did not verify any effect when essential oils (Anacardium occidentale and Ricinus communis) were added on the final live weight of Purunã bulls finished in feedlot. Dry matter feed intake was equivalent to 2.2% BW of animals and thus within an intake expected between 2.0 and 2.5% of BW for bulls finished in feedlot and fed on a diet with 50% roughage and 50% concentrate (Ito et al., 2010; Maggioni et al., 2010). Mean DM conversion was approximately 6.2 kg of DM for 1 kg of BW gain and may be attributed to the genetic quality of animals (½ Angus vs ½ Nellore), their sexual condition (bulls) and the diet’s high energy density (75% TDN). Usually, crossbred bulls fed on a high energy density diet have feed conversion of 7 kg of DM intake for 1 kg of BW gain (Fugita et al., 2012; Maggioni et al., 2009).

**Apparent total-tract digestibility coefficient**

Dry matter, OM and CP apparent digestibility coefficients for bulls fed GLY (72.1, 72.7 and 67.8%) and GEO (73.2, 73.6 and 70.9%) diets were higher (P<0.05) than those of CON (66.7, 67.4 and 64.3%, respectively). However, DM, OM and CP apparent digestibility coefficients were similar between GLY and GEO diets. Thus, the replacing of corn by glycerine in the diets improved apparent digestibility (Table 4).

On the other hand, ADF apparent digestibility was higher (P<0.05) for CON diet (54.5%) than in GLY (49.6%), with GEO diet being 53.2% (Table 4).

However, NDF, TC, NFC, FC and EE apparent digestibility was similar (P>0.05) for bulls fed on the three diets. Replacing corn by glycerine did not influence (P>0.05) apparent digestibility of carbohydrates. Trabue et al. (2007) observed that 80% of glycerine disappeared from the rumen 24 h after feeding. Also, Bergner et al. (1995) reported that the total glycerine content was fermented in the rumen 4 h after feeding. Results in vivo and in situ suggested that the inclusion of glycerine might change the proportion of bacteria, benefiting populations of *Megasphaera* and *Selenomonas*, the main glycerine fermenters, at levels up to 20% glycerine without reducing NDF digestibility (Krueger et al., 2010). Since glycerine had a high fermentation rate in the rumen with increased levels of volatile fatty acids, data would partially explain the high digestibility of DM, OM and CP in this experiment. However, a study with different glycerine levels in ruminant diets showed no negative or positive effect on apparent digestibility up to a maximum of 10% glycerine inclusion (Ramos and Kerley, 2012). Levels above 15% significantly reduced the apparent digestibility coefficients of all nutrients of the diets (Farias et al., 2012; Ramos and Kerley, 2012). Thus, the maximum level of corn substitution by glycerine, without any modification in the apparent digestibility of bulls, would be between 10 and 15% of diet DM. However, this maximum substitution level also depended on other factors such as glycerine quality (Farias et al., 2012).

The addition of essential oils in diets for crossbred bulls finished in feedlot did not affect (P>0.05) the apparent digestibility of DM, OM, CP, NDF, ADF, TC, NFC, FC and EE (Table 4). The supplementation of ruminant diets with essential oils increases the concentration of volatile fatty acids in the rumen which could indicate an increase in feed efficiency according to a limited number of studies (Benchaar et al., 2008). The addition of 1.5 mg/L of a mixture of essential oils increased total production of volatile fatty acids in continuous culture, although no increase in OM digestibility was observed (Castillejos et al., 2007). However, in other in vivo studies, the essential oils added to the diets had no effect on final concentration and on the ratio of fatty acids in the rumen of cattle and sheep (Beauchemin and McGinn, 2006). It is likely that the production and ratio of fatty acids in the rumen could depend also on the diet type (Benchaar et al., 2008). Benchaar et al. (2006) showed that the addition of essential oils to alfalfa silage diets to dairy cattle increased the total concentration of fatty acids, but decreased diets based on corn silage. However, Busquet et al. (2006) studied various components of plant extracts and plants’ secondary metabolites and found that high doses of these products could even reduce feed digestion. Several studies showed that the addition of essential oils to the diet changed the ratio of volatile fatty acids in the rumen and improved feed efficiency, similarly to monensin (Busquet et al., 2006).

**Plasma metabolites**

Although total cholesterol and triglycerides levels in blood plasma were similar for all the three diets (P>0.05) at baseline collection (Table 5), total cholesterol and triglycerides levels in the bloodstream were relatively high (118 and 50 mg/dL, respectively). Total cholesterol levels in the plasma of 24-month-old male animals are generally between 36 and 120 mg/dL (Pogliani and Birgel Junior, 2007). Likewise, triglycerides levels in the plasma...
sma of cattle without stress might range between 16 and 34 mg/dL (Pogliani and Birgel Junior, 2007). High levels of total cholesterol and triglycerides in the blood in the initial collection may be attributed to the lipolysis of adipose tissue (Erickson et al., 1992) due to catecholamine release (Chilliard et al., 2000) and to the animals’ low body conditions (DiMarco et al., 1981). Animal organism under stress conditions releases catecholamine’s which inhibit glucose synthesis and thus stimulate glucose production from adipose tissue (Chilliard et al., 2000). As has been reported for the initial levels of total cholesterol and triglycerides, blood plasma levels at the end of the experiment (115 days) did not differ (P>0.05) among animals fed on glycerine and with added essential oils (Table 6). Thus, the substitution of corn by glycerine as an energy source and the inclusion of essential oils did not alter the blood metabolites of bulls finished in feedlot. Regardless of diets plasma levels of total cholesterol and triglycerides were lower (P<0.05) on the last day of the experiment (90.5 and 12.4 mg/dL) when compared to those on the first day (118 and 50.0 mg/dL, respectively; Table 6). Lower levels of total cholesterol and triglycerides in blood plasma may be related to the animals’ physiological state (Godoy et al., 2004; Pogliani and Birgel Junior, 2007).

**Blood haemogram and leukogram**

There was no effect (P>0.05) on red blood cells count (erythrocytes, haemoglobin, haematocrit, MCV, MCH and MCHC) caused by the addition of glycerine and essential oils in the diets (Table 6). Haematological evaluation in cattle assessed disease in animals or evaluated groups of animals in a herd to detect hidden diseases and to guide clinical decisions (Jones and Allison, 2007). Rate variables for red blood cells observed in this study agreed with the reference rates described by Biondo et al. (1998) and Jones and Allison (2007).

There was no effect (P>0.05) on white blood cell count (leucocytes, eosinophil’s, segmented, lymphocytes and monocytes) caused by different diets (Table 6). The measurement of white blood cells in the current study monitored the health of bulls and detected cell behaviour upon supplementation with glycerine and essential oils, due to its recent introduction into ruminant nutrition (Gandra et al., 2012). Results for all groups agreed with reference rates for cattle at this growth phase (Biondo et al., 1998; Jones and Allison, 2007).

### Table 5. Cholesterol and triglycerides levels in blood plasma of crossbred bulls finished in feedlot.

| Parameters                      | Diets° | RV# | SEM | P     |
|--------------------------------|--------|-----|-----|-------|
| Cholesterol, mg/dL             |        |     |     |       |
| Initial                        | 122    | 111 | 36-120 | 28.7 | 0.65  |
| Final                          | 80.4   | 90.2 |       | 28.8  | 0.29  |
| Triglycerides, mg/dL           |        |     |     |       |
| Initial                        | 56.0   | 46.2 | 16-35  | 13.0  | 0.20  |
| Final                          | 12.8   | 12.5 |       | 7.28  | 0.97  |

**RV, reference values; CON, diet without glycerine or essential oils (i.e. control); GLY, diet with glycerine; GEO, diet with glycerine and essential oils.**

### Table 6. Haematogram, leukogram and plasma protein levels of crossbred bulls finished in feedlot.

| Parameters                      | Diets° | RV# | SEM | P     |
|--------------------------------|--------|-----|-----|-------|
| Erythrocyte, mil/mm³            |        |     |     |       |
| Initial                        | 8.18   | 8.39 | 8.50 | 8.91  | 8.37  | 9.35  | 5-10  | 1.13  | 0.06  |
| Final                          | 8.39   | 8.50 | 8.91 | 8.37  | 9.35  |       |       |       |       |
| Haemoglobin, g/dL              |        |     |     |       |
| Initial                        | 11.1   | 12.8 | 12.5 | 12.9  | 11.4  | 13.5  | 8-15  | 1.48  | 0.23  |
| Final                          | 12.8   | 12.5 | 12.9 | 11.4  | 13.5  |       |       |       |       |
| Haematocrit, %                 |        |     |     |       |
| Initial                        | 33.8   | 38.5 | 37.0 | 36.7  | 33.6  | 38.5  | 24-46 | 3.61  | 0.90  |
| Final                          | 38.5   | 37.0 | 36.7 | 33.6  | 38.5  |       |       |       |       |
| MCV, fl                        |        |     |     |       |
| Initial                        | 40.5   | 45.2 | 40.3 | 42.8  | 39.1  | 41.9  | 40-50 | 4.17  | 0.06  |
| Final                          | 45.2   | 40.3 | 42.8 | 39.1  | 41.9  |       |       |       |       |
| MCH, pg                        |        |     |     |       |
| Initial                        | 13.5   | 15.3 | 13.9 | 14.8  | 13.6  | 14.6  | 11-17 | 1.12  | 0.49  |
| Final                          | 15.3   | 13.9 | 14.8 | 13.6  | 14.6  |       |       |       |       |
| MCHC, %                        |        |     |     |       |
| Initial                        | 32.9   | 33.6 | 34.1 | 34.7  | 33.5  | 35.0  | 30-36 | 1.73  | 0.06  |
| Final                          | 33.6   | 34.1 | 34.7 | 33.5  | 35.0  |       |       |       |       |
| Platelets, mil/mm³             |        |     |     |       |
| Initial                        | 395    | 243 | 506  | 375   | 472   | 420   | 100-800 | 220  | 0.30  |
| Final                          | 243    | 506 | 375  | 472   | 420   |       |       |       |       |
| Leucocytes, mil/mm³            |        |     |     |       |
| Initial                        | 15.3   | 15.9 | 15.8 | 17.5  | 17.3  | 16.8  | 4000-12,000 | 26.8 | 0.23  |
| Final                          | 15.9   | 15.8 | 17.5 | 17.3  | 16.8  |       |       |       |       |
| Eosinophil’s, mil/mm³          |        |     |     |       |
| Initial                        | 448    | 448 | 678  | 484   | 543   | 738   | 80-2400 | 444  | 0.36  |
| Final                          | 448    | 678 | 484  | 543   | 738   |       |       |       |       |
| Segmented, mil/mm³             |        |     |     |       |
| Initial                        | 3.74   | 7.00 | 10.6 | 7.12  | 11.1  | 9.51  | 1800-9000 | 2.35 | 0.63  |
| Final                          | 7.00   | 10.6 | 7.12 | 11.1  | 9.51  |       |       |       |       |
| Lymphocytes, mil/mm³           |        |     |     |       |
| Initial                        | 10.3   | 7.78 | 10.6 | 7.12  | 11.1  | 9.51  | 1800-9000 | 2.35 | 0.63  |
| Final                          | 7.78   | 10.6 | 7.12 | 11.1  | 9.51  |       |       |       |       |
| Monocytes, mm³                 |        |     |     |       |
| Initial                        | 943    | 916 | 866  | 581   | 762   | 1232  | 80-840 | 414  | 0.20  |
| Final                          | 916    | 866 | 581  | 762   | 1232  |       |       |       |       |
| Plasma proteins, mg/dL         |        |     |     |       |
| Initial                        | 6.45   | 7.11 | 6.35 | 7.25  | 6.35  | 6.90  | 5.7-8.0 | 0.51  | 0.65  |
| Final                          | 7.11   | 6.35 | 7.25 | 6.35  | 6.90  |       |       |       |       |

RV, reference values; CON, diet without glycerine or essential oils (i.e. control); GLY, diet with glycerine; GEO, diet with glycerine and essential oils. **Ten bulls were considered per diet,References values from Pogliani et al. (1998) and Jones and Allison (2007).**
with high percentage of protein and high density-energy and all necessary minerals, it was normal to detect an increase in red blood cells due to increased metabolism. Therefore, no inflammatory process was reported in the bulls during the experimental period.

On the other hand, the levels of white blood cells (leucocytes, eosinophil’s, segmented, lymphocytes and monocytes) in the three diets were similar (P>0.05) on the first and final day of the experiment (Table 6). Similarly, plasma protein levels were similar (P>0.05) on the first and final day of the experiment (Table 6). The bulls were reared in a pasture system from birth until their entrance into the feedlot. During the period in which the animals were kept on a grazing system, parasitic infestation might have occurred several times and might have triggered an immune response in the animals, with an increase in defence cells (leucocytes, eosinophil’s, segmented, lymphocytes and monocytes) in the blood of the animals. On the other hand, the levels of white blood cells were no different (P>0.05) between bulls on the three diets since the beginning of the experimental period. On the other hand, the levels of white blood cells were no different (P>0.05) between bulls on the three diets since the beginning of the experimental period.
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