Short Communications

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Functional oils affect blood and bacteriological parameters in sheep¤

Los aceites funcionales afectan parámetros sanguíneos y bacteriológicos en ovinos

Óleos funcionais afetam parâmetros sanguíneos e bacteriológicos em ovinos

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Abstract

Background: Feeding ionophores to sheep is intended for improving feed efficiency. Functional oils (FO) are an alternative to the use of ionophores. **Objective:** To evaluate the effect of feeding FO to sheep on blood parameters and fecal bacteria. **Methods:** Five sheep were used in a Latin square design with five treatments: 190, 285, 380, 570, and 675 g/t FO in the diet. White cell count and levels of blood glucose, total protein, urea nitrogen, and fecal bacteria presence in feces were determined. **Results:** The FO levels did not affect serum parameters. *Salmonella* spp was found only in feces of sheep fed 190 mg/t FO. **Conclusion:** Functional oils can be added to sheep diets to reduce the presence of *Salmonella* spp in the feces.

Keywords: *cashew oil, castor oil, feed supplement, ionophores, sheep fattening.*

Resumen

Antecedentes: Los ionóforos se suministran a los ovinos para mejorar la eficiencia alimenticia. Una alternativa al uso de ionóforos son los aceites funcionales (FO). **Objetivo:** Evaluar los efectos del suministro de FO en el alimento sobre los parámetros sanguíneos y bacterias fecales de ovinos. **Métodos:** Cinco ovejos

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fueron usados en un diseño de cuadrado latino con cinco tratamientos: 190, 285, 380, 570 y 675 g/t FO en la dieta. Se realizó conteo de leucocitos y niveles de glucosa, proteína total y nitrógeno ureico, y se determinó la presencia de bacterias fecales. **Resultados:** La inclusión dietaria de FO no afectó los parametros séricos. Se encontró *Salmonella* spp únicamente en heces de los ovinos que habían consumido 190 mg/t FO. **Conclusión:** Se puede agregar FO a la dieta de ovinos para reducir la presencia de *Salmonella* spp en heces.

Palabras clave: *aceite de anacardo, aceite de ricino, engorde de corderos, ionóforos, suplemento alimenticio.*

Resumo

Antecedentes: Na produção animal, ionóforos são ingeridos por ovinos para melhorar a eficiência alimentar. Alternativa ao uso de ionóforos são os óleos funcionais (FO). **Objetivos:** Avaliar os efeitos de FO em ovinos por meio da alimentação sobre os parâmetros sanguíneos e bacteriológicos. **Métodos:** Cinco ovinos foram usados em Quadrado Latino com cinco tratamentos: 190, 285, 380, 570 e 675 g/t FO nas dietas. Contagem de leucócitos e níveis de glicose, proteína total, nitrogênio ureico e presença de bactérias nas fezes foram determinados. **Resultados:** Os níveis de FO não afetaram os níveis séricos dos fatores mencionados. *Salmonella* spp foi encontrada somente em fezes de ovinos que haviam ingerido dietas com 190 mg/t FO. **Conclusão:** Óleos funcionais podem ser adicionados em dietas para ovinos para reduzir a presença de *Salmonella* spp nas fezes.

Palavras chave: *ionóforos,* óleo *de caju, óleo de mamona, suplemento alimentar, terminação de cordeiros.*

Introduction

Functional oils are defined as oils that have an action beyond their nutritional value (Murakami *et al.*, 2014) and are not derived from essences or spices (Bess *et al.*, 2012), but from oil plants such as castor seeds (*Ricinus communis*) and cashew nutshell (*Anacardium occidentale;* Silva *et al.,* 2014).

Castor seeds contain ricin, a water-soluble toxin. Although these seeds are toxic, castor oil is not, because ricin is not fat-soluble (Gaillard and Pepin, 1999). Additionally, ricinoleic acid has antimicrobial properties (Maenz and Forsyth, 2005). Antimicrobial action in castor oil comes from anacardic acid and cardol. Anacardic acid has been shown to inhibit the growth of gram-positive bacteria (Lima *et al*., 2000). On the other hand, oil from cashew nutshell has also antimicrobial and antioxidant activities (Oliveira *et al*., 2011). This study was conducted to determine how feeding functional oils could affect blood parameters and presence of *Escherichia coli*, *Clostridium* spp, and *Salmonella* spp in sheep feces.

Materials and methods

Ethical considerations

The study was approved by the Animal Ethics Committee of Instituto Federal Goiano de Rio Verde, Brazil (number 21/11, February 09, 2012).

Animals

The experiment was conducted with five male Santa Inés sheep. The sheep were 14 months old and weighed 35 Kg. Sheep were kept in confinement and fed diets formulated to fulfill NRC (2007) requirements during 14 d. Functional oils (FO) consisted of a mixture of cashew oil, castor oil, and silica. The oils included cardol (40 g/Kg), ricinoleic acid (90 g/Kg), and cardanol (200 g/Kg). Sheep were allocated in individual boxes for adaptation during two weeks before the experimental period. The boxes were cleaned daily and washed weekly. A 5×5 Latin square design was used, with five treatments: 190, 285, 380, 570, and 675 g/t FO in the diet. On the 15th d, blood samples were collected and analyzed for white cell count (WCC), total protein (TP), glucose, and blood urea nitrogen (BUN). The reference values for TP, glucose and BUN are 6.0-7.9 g/dL, 50-80 mg/dL, and 17.12-42.8 mg/dL, respectively (Meyer and Harvey, 2004). Fecal samples were collected directly from the rectum to isolate bacteria. Isolation of *Clostridium* spp, *Salmonella* spp, and *E. coli* followed the methodology described in Brasil (2003).

Statistical analysis

An ANOVA and F-test were used to analyze the results. When F was significant, Tukey test was performed to compare the means at 5% probability.

Results

Functional oil levels did not influence WCC, glucose levels, or TP (p>0.05). Inclusion of 570 and 675 g/t FO resulted in lower BUN levels (p<0.05; Table 1).

Functional oils did not influence the presence of *Clostridium* sp nor *E. coli* in feces (p>0.05; Table 2); *Clostridium* sp and *E. coli* were present in all the samples. However, *Salmonella* spp was only found in the sheep fed 190 g/t FO.

Discussion

White cell count was not influenced, indicating that treatments did not affect the immune system of the sheep. White cell counts were within the normal reference levels for sheep (Meyer and Harvey, 2004).

Glucose was not affected by FO levels. Glucose results, between 81.97 and 97.45 mg/dL, were above the reference values reported by Meyer and Harvey (2004). Functional oils act in the rumen by changing ion transport in the cellular membrane of bacteria (Calsamiglia *et al*., 2007). They select for gram-negative bacteria and increase propionic acid (di Lorenzo, 2011) that is transformed into glucose by gluconeogenesis in the liver.

Table 1. White blood cell count (WCC) and levels of glucose, total protein (TP), and blood urea nitrogen (BUN) in sheep fed diets containing functional oils.

Parameters	Functional oil levels (g/t)					SEM
	190	285	380	570	675	
Neutrophils (%)	26.01	26.57	27.78	27.23	21.38	1.35
Lymphocytes (%)	43.95	42.17	39.12	41.93	50.74	2.77
Eosinophils (%)	3.85	4.09	4.58	3.07	5.88	1.08
Monocytes (%)	0.18	0.58	0.73	0.54	0.61	0.25
Glucose (mg/dL)	81.97	97.27	83.27	97.45	96.77	6.07
Total protein (mg/dL)	7.44	6.98	7.87	7.44	5.57	0.58
Urea nitrogen (mg/dL)	20.54^{a}	17.61^{ab}	18.58^{ab}	11.76^{b}	13.21^{b}	1.99

Different superscript letters ($^{a,\ b}$) between columns indicate significant difference (p<0.05). SEM: Standard error of the mean.

Table 2. Bacterial presence in feces of sheep fed diets containing functional oils.

Parameters*	Functional oil levels (g/t)					SEM
	190	285	380	570	675	
Escherichia coli	3/5	4/5	2/5	2/5	5/5	0.20
Salmonella spp	1/5	0/5	0/5	0/5	0/5	0.09
Clostridium spp	1/5	1/5	1/5	1/5	1/5	0.17

*Number of samples with bacterial growth/total number of samples.

Regarding total protein in blood, values were similar to those described by Kaneko (1997) and Contreras (2000). Thus, it can be inferred that inclusion of 190 g/t FO did not change the hepatic function and plasmatic protein production of the animals. Hypoproteinemia intensity is usually an indicator of the severity of gastrointestinal helminthiasis.

Blood urea nitrogen results were within reference values (17.12 to 42.8 mg/dL) published by Meyer and Harvey (2004). Plasmatic concentration of urea is positively correlated with intake of nitrogenous compounds (Valadares *et al.,* 1999). Protein catabolism and its excretion rate depend on glomerular filtration rate and its reabsorption in renal tubules (Kaneko *et al.,* 1997). Blood urea nitrogen is related to the efficiency of use of dietary protein, so high blood BUN concentrations could indicate inefficiencies in the dietary usage of protein, and also that high amounts of energy are being lost (Pessoa *et al.,* 2009).

No difference was observed in fecal egg and oocyte numbers among treatments; all fecal samples contained *E. coli* and *Clostridium* spp colonies, but only samples from animals treated with 190 g/t FO tested positive for *Salmonella* spp. This indicates that only higher doses of FO can inhibit certain bacteria, as noted by Kubo *et al.* (1993). Ruminants are born with sterile gastrointestinal tracts, but it becomes colonized with several types of bacteria within a few hours after birth, including species of *Lactobacillus*, *E. coli* and anaerobic bacteria, such as *Clostridium* and others (Quinn *et al.,* 2012). Frias and Kozusny (2013) assessed the microbiota of healthy sheep and noted the presence of *E. coli* and *Clostridium* sp. In conclusion, functional oils can be added to sheep diets at 570 g/t to reduce blood urea nitrogen and presence of *Salmonella* spp in the feces.

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