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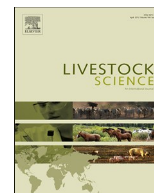
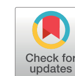


Effects of dietary soybean oil and coated folic acid on ruminal digestion kinetics, fermentation, microbial enzyme activity and bacterial abundance in Jinnan beef steers



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ABSTRACT

This study was conducted to evaluate the effects of dietary soybean oil (SO) and coated folic acid (CFA) supplementation on ruminal digestion kinetics, fermentation, microbial enzyme activity and bacterial abundance in Jinnan beef steers. Eight ruminally cannulated Jinnan beef steers, averaging 15 months of age and 415 ± 5.6 kg of body weight (BW; Mean ± S.E.), were used in a replicated 4 × 4 Latin square design by a 2 × 2 factorial arrangement. Two levels of CFA (0 mg [FA-] or 115 mg per kg dietary DM [FA +]) were supplemented to the diets without or with SO supplementation (0 g [SO −] or 30 g per kg dietary dry matter [DM] [SO +]). Steers were fed a total mixed ration with ensiled corn forage to concentrate ratio of 50:50 on a DM basis. The SO × CFA interaction was observed for ruminal pH, total VFA concentration, molar proportions of acetate and propionate, the ratio of acetate to propionate and ammonia N content. Ruminal pH increased with SO supplementation, but decreased with CFA supplementation. Ruminal total VFA concentration, acetate molar proportion and acetate to propionate ratio decreased with SO supplementation, but increased with CFA supplementation. Ruminal ammonia-N content decreased with SO or CFA supplementation. No SO × CFA interaction was observed for nutrient degradability and digestibility. Degradability of neutral detergent fibre (NDF) and digestibility of DM, organic matter, NDF and acid detergent fibre decreased with SO supplementation, but increased with CFA supplementation. Degradability and digestibility of crude protein was not affected by SO supplementation, but increased with CFA supplementation. Pectinase activity was unaltered, whereas, other microbial enzyme activity, bacterial abundance and total PD excretion were affected by SO × CFA interaction. Activities of carboxymethyl-cellulase, cellobiase and xylanase and abundance of *R. albus, R. flavefaciens, F. succinogenes* and *B. fibrisolvens* decreased with SO supplementation, but increased with CFA supplementation. Activities of pectinase, α-amylase and protease as well as total bacterial abundance were not affected by SO supplementation, but increased with CFA supplementation. Total protozoal abundance decreased with SO supplementation, but was not affected by CFA supplementation. Abundance of *P. ruminicola* and *R. amylophilus* increased with SO or CFA supplementation. Total PD excretion increased with CFA supplementation, but decreased with SO supplementation. The results suggested that ruminal fermentation, NDF degradability, protozoal abundance, cellulolytic bacterial abundance and enzyme activity decreased with dietary SO supplementation, but increased with CFA supplementation. The supplementation of CFA in the SO diet could relieve the negative effects of SO on ruminal fermentation.

1. Introduction

The supplementation of soybean oil (SO) increased dietary energy density, energy utilization, milk conjugated linoleic acid content and decreased ruminal protozoal abundance and methane production in ruminants (Veria et al., 2001; Yang et al., 2009; Mao et al., 2010). However, the lipid coating in the surface of feed particle and microbes would form with dietary SO supplementation, causing an inhibition to nutrient digestion and microbial growth (Mao et al., 2010; Fiorentini et al., 2013). Some studies found that ruminal total volatile fatty acids

Abbreviation: ADF, acid detergent fibre; BW, body weight; CP, crude protein; CFA, coated folic acid; DM, dry matter; ED, effective degradability; aNDF, amylase-treated neutral detergent fibre; OM, organic matter; PD, purine derivatives; TMR, total mixed ration; VFA, volatile fatty acids

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(VFA) concentration, acetate production, fibre degradability and cellulolytic bacterial abundance decreased with SO supplementation in cows or lambs (Ferreira et al., 2016; Yang et al., 2009). Nevertheless, other studies indicated that folic acid (FA) supplementation promoted the growth of ruminal microbes (Slyter and Weaver, 1977; C. Wang et al., 2016, 2017). Therefore, dietary FA supplementation would promote the biohydrogenation of unsaturated fatty acids in the rumen by stimulating microbial growth and reduce the negative effects of SO on ruminal fermentation and nutrient digestibility.

Folic acid could be synthesized by ruminal microorganisms (NRC, 2001). However, studies indicated that the synthesized FA could not meet the requirement of growing steers, and approximately 97% of dietary supplementary FA degraded by ruminal microorganisms (Zinn et al., 1987; NRC, 2001; C. Wang et al., 2017). Therefore, FA should be partly rumen-protected to meet the requirements of both ruminal microorganisms and the host. Studies indicated that FA supplementation promoted the growth of *R. albus* and *R. flavefaciens* (Bryant and Robinson, 1961; Slyter and Weaver, 1977), increased acid detergent fibre (ADF) degradability of high forage diets (Ragaller et al., 2010) and ammonia-N utilization in vitro (Wejdemar, 1996). Our previous studies found a linear increase in ruminal total VFA concentration, nutrient degradability, microbial protein synthesis, cellulolytic bacterial abundance and enzyme activity with increasing rumen-protected folic acid (RPFA) supplementation in steers (C. Wang et al., 2016, 2017). Nevertheless, the effects of caoted folic acid (CFA) on ruminal fermentation and microbial growth in steers fed diets with SO supplementation were not evaluated.

Given the effects of FA and SO on ruminal fermentation described above, we hypothesized that a combination of FA and SO would reduce the negative effects of SO on ruminal fermentation, resulting in an improved nutrient utilization. Therefore, the objective of the study was to evaluate the effects of dietary SO and CFA supplementation on ruminal fermentation, nutrient digestibility, abundance of protozoa and bacteria, microbial enzyme activity and microbial protein synthesis in steers.

2. Materials and methods

2.1. Animals and experimental design

The experimental scheme was authorized by the Animal Care and Research Committee of Shanxi Agriculture University. Eight ruminally cannulated Jinnan beef steers, averaging 15 months of age and 415 ± 5.6 kg of body weight (BW; Mean ± S.E.), were allocated into a replicated 4 × 4 Latin square design by a 2 × 2 factorial arrangement. One factor was without or with SO supplementation (0 g [SO−] or 30 g per kg dietary dry matter [DM] [SO+]). Another factor was the level of CFA (0 mg [FA-] or 115 mg per kg dietary DM [FA+]). The supplementation of SO and CFA containing 100 g FA per kg were hand-mixed into the top one-third of the morning ration to ensure complete consumption. The supplement of CFA was produced according to the method of C. Wang et al. (2016) and manufactured by Shanxi Jushuoyuan Biological Technology Co., LTD., Taiyuan, China. Degradability of CFA in the rumen and in the small intestine were 30% and 50%, respectively, as determined in situ using rumen and duodenum cannulated cattle (C. Wang et al., 2016). Steers were fed a total mixed ration (TMR) with ensiled corn forage to concentrate ratio of 50:50 on a DM basis (Table 1). Corn was harvested at full ripening stage immediately after removal of the ear corn by rotary mower and forage harvester equipped with a pickup head attachment. Corn forage was ground through a tub grinder with a 6.35-cm sieve and was ensiled. Each experimental period included 25 days with 15 days of adaption and 10 days of sampling collection. Animals were raised in single pens (3.5 × 3 m) during adaption period and in metabolism cages for the sampling period. Animals were fed twice daily at 0700 and 1900 h and water was provided *ad libitum*. On day 13, animals were confined to 95% of their voluntary intake measured during the first 12 days to guarantee no residues at the collection periods. At the beginning and end of each period, the body weight of animals was measured.

Table 1
Ingredients and chemical composition of diets with (SO+) or without soybean oil (SO−) supplementation (in g/kg dry matter).

Item	SO−	SO+
Ingredients		
Ensiled corn forage	500	500
Corn grain, ground	346	316
Wheat bran	30	30
Soybean oil	0	30
Soybean meal	40	40
Cottonseed cake	60	60
Calcium carbonate	8.0	8.0
Salt	5.0	5.0
Calcium hydrogen phosphate	5.0	5.0
Sodium bicarbonate	5.0	5.0
Mineral and vitamin premix[a]	1.0	1.0
Chemical composition		
Organic matter	943	952
Crude protein	116	118
Ether extract	2.62	5.51
Neutral detergent fibre	476	474
Acid detergent fibre	295	293
Calcium	3.62	3.81
Phosphorus	2.45	2.53

[a] Contained per kg premix: 100 mg Co, 8,500 mg Cu, 50,000 mg Fe, 30,000 mg Mn, 30,000 mg Zn, 300 mg I, 300 mg Se, 7500,000 IU vitamin A, 1200, 000 IU vitamin D, and 40, 000 IU vitamin E.

2.3. Data collection and sampling procedures

Samples of feeds and refusals were collected once daily for DM determination. Feeds offered and refusals were dried in an oven at 55 °C for 48 h, and ground to pass through a 1 mm screen with a cutter mill (110, Qingdao Ruixintai instrument Co., Ltd., Qingdao, China) for further chemical analysis. Ruminal fermentation parameters were determined from day 24 to 25 of each period. Ruminal fluid was sampled (approximately 200 mL) from several sites (reticulum, dorsal and ventral sac) at 07:00, 10:00, 13:00 and 16:00 h. Values of pH were immediately determined using a pH meter (PHS-3C, Shanghai Leijun experimental instrument Co., Ltd., Shanghai, China). The ruminal fluid samples were filtered and divided into four portions: the first portion (5 mL) was combined with 1 mL of 250 g/L (w/v) metaphosphoric acid for preservation and used to measure VFA. The second portion (5 mL) was combined with 1 mL of 20 g/L (w/v) sulphuric acid for preservation and used to measure NH_3-N. These two portions were kept frozen at −20 °C prior to analysis. The third portion (50 mL) was kept in a centrifuge tube and frozen at −80 °C for DNA extraction. The fourth portion (50 mL) was used to determine microbial enzyme activity.

During each experimental period, the entire volume of urine excreted by each steer was gathered immediately by using urine collection aprons into plastic containers. Urine samples accounting for 1% of the urine output were collected immediately and placed into a glass reagent bottle with sufficient 10% sulphuric acid to maintain pH lower than 3.0. At the end of each period, urine samples were pooled by animal, diluted five times with distilled water, and then separated into two subsamples. The subsamples were kept frozen at −20 °C until further analysis.

2.4. Ruminal degradation

Degradability of ensiled corn forage and concentrate were determined by using the nylon bag technique from day 16 to 18 during each sub-period according to the method of Liu et al. (2008). The air-dried ensiled corn forage and concentrate were milled to 2.5 mm (110, Qingdao Ruixintai instrument Co., Ltd., Qingdao, China). About 3 g of

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