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Effects of dietary whole cottonseed and crude protein level on rumen protozoal population and fermentation parameters

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Abstract

In this investigation in vitro and in vivo trials were performed to determine the efficacy of a cottonseed to limit protozoal population and fermentation parameters. The composition of diets given to the different treatments were as follow: (1) control (without whole cottonseed), 16% crude protein (CP), 3.2% ether extract (E.E.); (2) 20% whole cottonseed, 16% CP, 6.5% E.E.; (3) 20% whole cottonseed, 13% CP, 6.4% E.E. and (4) 20% crushed whole cottonseed, 13% CP, 6.4% E.E. DM disappearance (DMD) and fermentation characteristics of the treatments were determined by in vitro incubation studies. In the in vivo trial, ruminal fluid was taken by rumenocentesis (3 h after feeding) on days 1, 3, 5, 7, 9, 11, 14, 21 and 28 from four sheep fed about treatment diets. The pH and protozoal counts were determined in each sample, while ammonia nitrogen and volatile fatty acid (VFA) were determined in samples taken on days 7, 14, 21 and 28. The in vitro DMD after 24 h incubation decreased ($p < 0.01$) with the addition of cottonseed in diets 3 and 4 and DMD after 72 h incubation was highest ($p < 0.01$) for the control diet. The fractional rate of gas production (c) for the control and diet 2 was higher ($p < 0.05$) than for the diets 3 and 4. Feeding crushed whole cottonseed decreased molar proportion of propionate ($p < 0.05$) and increased molar proportion of butyrate ($p < 0.01$). Low crude protein level increased the molar proportion of propionate ($p < 0.05$) and decreased molar proportion of butyrate ($p < 0.05$) and cellolytic protozoa population ($p < 0.05$). Feeding cottonseed decreased ($p < 0.05$) the total protozoa population from approximately 500,000 to 250,000 ml^{-1} and Holotrich and cellulolytic protozoa disappeared from the rumen of sheep and only *Entodinium* sp., remained. This was associated with lower concentration of ammonia nitrogen in rumen fluid of sheep fed diets 4 ($p < 0.05$) and 2 ($p < 0.01$). It was concluded that cottonseed reduced rumen fauna and ammonia nitrogen, but had no effect on ruminal VFA while the crushed whole cottonseed decreased molar proportion of isovalerate only. In vivo molar proportion of propionate and butyrate and valerate were increase and decrease, respectively, by decreasing CP percentage in treatment diets.

Keywords: Sheep; Cottonseed; Crude protein; Volatile fatty acid; Protozoa; NH_3N

1. Introduction

The rumen has been the subject of many studies to understand the biological activities of different microorganisms and their relations. Williams and Coleman (1992) showed different metabolic functions of protozoa present in the rumen; some may or may not be beneficial to the ruminal host. The ciliated protozoa consume and digest rumen bacteria, and increase recycling of microbial nitrogen in the rumen (Jouany, 1996), resulting in decreased amino acid supply to the intestine (Veira et al., 1984). Such decrease in the amino

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acid supply to the intestinal is reported to be as high as 20–28% (Ivan et al., 1991). Consequently, the elimination of protozoa in the rumen is desirable when animal performance is limited by the availability of amino acids for absorption (Jouany and Ushida, 1999). In addition, ciliated protozoa contribute to ruminal production of methane and associated loss of feed energy (Hegarty, 1999). Methanogens associated with ciliated protozoa are responsible for 9–25% of methanogenesis in ruminal fluid (Newbold et al., 1995). Nitrogen and methane are both important environmental pollutants, and numerous studies have been done to manipulate the protozoal population in the rumen, including total defaunation. Some of the techniques, using chemical substances to remove protozoa from the rumen have been tested experimentally, but none of these techniques are practical, because of toxicity problems, either to the rumen bacteria or to the host animal (Williams and Coleman, 1992). Recently, there has been an increased interest in plant secondary metabolites as possible defaunting agents, but there are presently no specific antiprotozoal agents commercially available. Different defaunation techniques have been reviewed recently (Hegarty, 1999) and it has been concluded that oil, milk fat (Kreuzer and Kirchgessner, 1987; Machmuller and Kreuzer, 1999) and unsaturated C18 fatty acids (Newbold and Chamberlain, 1988) are toxic to protozoa. Ivan et al. (2001) reported that feeding sunflower oil (6% of dietary dry matter) decreased the total protozoal numbers in rumen fluid samples from approximately 1,000,000 to less than 200,000 ml^{-1} within 6 days. Cottonseed oil is rich in linoleic acid and can be used as a supplement to decrease protozoal population. However, using plant oil in ruminant diets is very expensive and not economically viable; an alternative approach is to utilize oilseeds such as whole cottonseed.

The objectives of the present study were to test efficacy of adding whole cottonseed and different CP level to suppress protozoal numbers in the rumen and measure their effects on rumen fermentation parameters. The effects of the cottonseed and CP level on in vitro dry matter disappearance, kinetics of gas production and volatile fatty acid concentration were also studied.

2. Material and method

2.1. Experimental diets

Treatment diets were different in crude protein (CP) level and amount and form of whole cottonseed. The composition of diets given to the different treatments were as follow: (1) control (without whole cottonseed), 16% CP; (2) 20% whole cottonseed, 16% CP, 6.5% ether extract (E.E.); (3) 20% whole cottonseed, 13% CP, 6.4% E.E. and (4) 20% crushed whole cottonseed, 13% CP, 6.4% E.E. mineral and vitamin additions were the same in all treatment diets. Ingredient and nutrient composition of the experimental diets is provided in Table 1.

Table 1
Ingredient and chemical composition of the diets (DM basis)

Item	Diet[a]			
	1	2	3	4
Ingredient (%)				
Alfalfa hay	14.0	19.3	12.0	12.0
Wheat straw	5.6	6.0	6.0	6.0
Corn silage	34.9	29.4	33.0	33.0
Barley grain, ground	8.5	8.0	17.5	17.5
Corn grain, ground	12.0	2.9	7.5	7.5
Cottonseed, whole with lint	0.0	20.0	20.0	20.0
Cottonseed meal	12.3	0.0	0.0	0.0
Soybean meal	10.3	9.5	0.0	0.0
Wheat bran	1.0	2.9	2.0	2.0
Dicalcium phosphate	0.6	0.5	0.6	0.6
Limestone	0.2	0.2	0.2	0.2
Sodium bicarbonate	0.3	0.3	0.3	0.3
Vitamin A, D, and E premix[b]	0.4	0.4	0.4	0.4
Trace-mineralized Salt[c]	0.5	0.5	0.5	0.5
Chemical composition				
DM	48.7	47.0	49.5	49.5
ME (Mcal/kg DM)	2.47	2.51	2.5	2.5
Ether Extract	3.2	6.5	6.4	6.4
CP	16.2	15.7	13.1	13.3
NDF	37.5	43.2	45.3	45.6
Forage NDF	28.8	29.7	27.5	27.5
ADF	24.5	31.3	32.5	33.2
Lignin	4.5	8.6	8.8	8.9
NSC[d]	39.9	30.3	30.7	30.3
Ash	3.3	4.3	4.5	4.4

[a] Diets were (1) control, 16% CP; (2) 20% whole cottonseed, 16% CP; (3) 20% whole cottonseed, 13% CP and (4) 20% crushed whole cottonseed, 13% CP.

[b] Contains 5,000,000 IU of Vitamin A; 5,000,000 IU of Vitamin D and 500,000 IU of Vitamin E per kg.

[c] Composition: 75.15% NaCl, 20.5% Dynamad, 3.046% Mn, 1.025% Cu-sulphate, 0.253% Zn-sulphate, 0.015% EDDI-80 and 0.011% Na-selenide.

[d] NSC, non-structural carbohydrate; NSC = 100 − (CP + NDF + EE + ash).

2.2. In vitro degradation

In vitro DM disappearance of the dried treatment diets was determined in five replications (five syringes, each ones as a replicate) after incubation with ruminal fluid for 24 and 72 h using the Menke's gas test apparatus (Mir et al., 2000). Approximately 250–300 mg of dried sample