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Rumen Fermentation and Nutrient Digestibility in Beef Steers Fed Rations Containing Either Cotton Seed or Sunflower Seed

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Abstract: The hypothesis tested was that a high inclusion level of oil seeds in the ration of ruminants does not negatively affect ruminal function. Rumen-fistulated beef steers were fed concentrates containing either cotton seed or sunflower seed, each at two levels so that the crude fat concentrations were 3 and 6%. The ration also contained urea-treated rice straw. When the concentrates with low fat content were supplied, concentrate intake was significantly higher when sunflower seed instead of cotton seed was included. An increase in the inclusion level of cotton seed significantly raised concentrate intake whereas extra sunflower seed significantly lowered concentrate intake. The experimental concentrates did not affect the intake of rice straw. An increase in oil seed intake slightly but systematically reduced the group means of apparent digestibility of neutral and acid detergent fiber by up to 1.2% units. The type of oil seed did not influence ruminal pH but an increased intake caused a significant increase at 3 h post feeding. There were no dietary effects on ruminal concentrations of ammonia and volatile fatty acids and on the number of viable bacteria. It is concluded that the incorporation of extra fat in ruminant rations in the form of oil seed may only have a minor effect on rumen fermentation and macronutrient digestibility but dry matter intake may be influenced by the type of oil seed.

Key words: Sunflower seed, cotton seed, rumen fermentation, apparent digestibility, beef steers, oil seed

INTRODUCTION

The use of high fat rations for ruminants remains a topic of interest. The intake of extra fat by dairy cows in early lactation may meet the gap between energy consumed with the diet and the energy needed for milk production and body maintenance (Coppock and Willks, 1991). In addition, the feeding of supplemental fats rich in polyunsaturated fatty acids has been shown to increase the level of polyunsaturated fatty acids in meat (Felton and Kerley, 2004). Even though, ruminal bacteria hydrogenate ingested polyunsaturated fatty acids still there is a small fraction that will reach the tissues (Banks *et al*., 1983). Ruminant food stuff enriched in polyunsaturated fatty acids at the expense of saturated fatty acids may contribute to lowering serum cholesterol concentrations and thereby to reducing the risk of coronary heart disease in humans (Willett, 2007; Harris, 2010). High fat levels in the ration of ruminants inhibit ruminal fermentation and thus diminish the utilization of dietary fiber (Coppock and Willks, 1991; Vafa *et al*., 2009). Fatty acids that ar released in the rumen disturb the function of microbial cell membranes (Calsamiglia *et al*., 2007). However, the degree of the toxic effect of fatty acids on ruminal bacteria depends on the amount and type of fat. Especially, oils with a high degree of unsaturation disturb ruminal fermentation (Coppock and Willks, 1991; Hristov *et al*., 2009; Vafa *et al*., 2009). There is evidence that oil seeds are less toxic than purified oils which might relate to a protective

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effect of the pericarp that reduces exposure of the oil inside the seeds to ruminal bacteria (Felton and Kerley, 2004). If the intake of oil seeds rather than supplemental oils indeed has a limited effect on rumen fermentation, it may be reasoned that high inclusion levels of oil seeds may not negatively affect ruminal function. This reasoning was tested in the present experiment with rumen-fistulated beef steers.

The effects of high intakes of cotton seed and sunflower seed were compared with regard to feed intake, macronutrient digestibility and rumen function. The oil seeds were incorporated into diet concentrates at two levels so that the concentrations of fat were 3 and 6%.

MATERIALS AND METHODS

Animals and dietary treatments: Four rumen-fistulated crossbred Thai native x Brahman steers, aged about 2.5 years were used. The steers had been treated for intestinal worms and were injected with a mixture of vitamins A, D and E. The steers were fed four rations according to a 4×4 Latin-square design with 21 days per period. The steers were housed individually. The experimental concentrates contained either cotton seed or sunflower seed at two levels and were formulated so that the calculated fat concentrations were 3 or 6% while the content of crude protein was constant.

Table 1 shows the ingredient composition of the concentrates. Extra oil seed was added to the concentrates in association with changes in the amounts of rice pollard, soybean meal and cassava chips. The animals were fed a combination of the concentrates and urea treated rice straw. The analyzed composition of the concentrates and roughage is shown in Table 2. Both the concentrate and rice straw were supplied in an amount that was equivalent to 1.0% of body weight.

Sample collection and analysis: Feces samples were collected quantitatively during the last 5 days of each period. Roughage, concentrates and feces samples were ground and chemically analyzed as described earlier (Jansen *et al*., 2000). Rumen fluid was collected through the ruminal fistula at 0, 3 and 6 h post morning feeding. The pH of rumen fluid samples was measured immediately. The samples were then filtered through four layers of cheese cloth. Samples were divided into two portions. One portion of ruminal fluid was mixed with a 50% H_2SO_4 solution in a 10:1 ratio. The mixture was centrifuged at 16,000×g for 15 min and the supernatant was stored at -20°C prior to ammonia-N measurement (Bremner and Keeney, 1965) and the analysis of volatile fatty acids using HPLC (model RF-10 AXmugiL, Shimadzu, Japan) according to Zinn and Owen (1986). The second portion of ruminal fluid was fixed with a solution of 10% formalin in saline and stored at 4°C. Total counts of bacteria and protozoa were determined according to Galyean (1989) with the use of a hemocytometer. A blood sample was taken from the jugular vein at the same time as ruminal fluid sampling and used for the analysis of blood urea-N (Crocker, 1967).

Table 1: Ingredient composition of the experimental concentrates

	Cotton seed (fat)		Sunflower seed (fat)	
Ingredients	3%	6%	3%	06%
Rice pollard	17.0	7.00	18.0	15.0
Whole cotton seed	15.0	30.0	-	-
Sunflower seed	-	-	8.00	15.0
Soybean meal	15.0	9.00	8.00	15.0
Cassava chips	44.5	45.5	47.5	46.5
Constant components[1]	8.50	8.50	8.50	80.5
Total	100	100	100	100

[1]The constant components consisted of g: urea, 3.0; molasses, 3.0; di-calcium phosphate, 1.0; limestone, 1.0; premix, 0.3; sulfur, 0.2

Table 2: Analyzed composition of the experimental concentrates and the urea-treated rice straw

	Cotton seed (fat)		Sunflower seed (fat)		
Macronutrient	3%	6%	3%	6%	Rice straw
Organic matter	91.1	91.3	90.8	91.2	84.0
Crude protein	18.0	17.8	17.7	19.0	4.3
Crude fat	3.6	6.8	3.2	6.1	0.6
Neutral detergent fiber	32.9	34.7	36.7	28.1	77.4
Acid detergent fiber	20.3	16.1	19.3	16.3	56.2
Acid detergent lignin	9.3	5.9	7.1	9.5	6.4
Crude ash	8.9	8.7	9.2	8.8	16.0

Statistical analysis: The data are shown as treatment means and SEM for four animals. Statistical analysis of the data was done using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL 1998). The data were subjected to two-way ANOVA to identify statistically significant effects of oil seed type, oil seed level and the interaction between type and level. The level of statistical significance was pre-set at $p<0.05$.

RESULTS AND DISCUSSION

There was a significant interaction between the type and amount of oil seed with regard to concentrate intake (Table 3). When the concentrates with low fat content were supplied, concentrate intake was significantly higher when sunflower seed instead of cotton seed was included. An increase in the inclusion level of cotton seed significantly raised concentrate intake whereas extra sunflower seed significantly lowered concentrate intake. The experimental concentrates did not affect the intake of rice straw. The effects of amount and type of oil seed on concentrate intake were mirrored by the total dry matter intake. The apparent digestibilities of dry matter, organic matter, neutral detergent fiber and acid detergent fiber were not significantly influenced by the experimental rations (Table 4).

However, an increase in oil seed intake systematically reduced the group means of apparent digestibility of neutral and acid detergent fiber. For the concentrates with cotton seed the decrease on average was 0.7% units and for those with sunflower seed the average decrease was 1.0% units.

Cotton seed versus sunflower seed had induced higher group means of the digestibility of neutral and acid detergent fiber, this difference being noticeable for each inclusion level. The amount of oil seed had a marked, significant effect on the apparent digestibility of crude fat (Table 4). When the fat level of the concentrates was raised from 3-6% the increase in apparent fat digestibility was 8.7 and 6.8% units for cotton seed and sunflower seed, respectively. The type of oil seed did not influence ruminal pH at any time interval (Table 5). The inclusion of extra oil seed in the ration caused an increase in group mean ruminal pH values before and after feeding, except for the measurement at 6 h post feeding for the concentrate with cotttonseed. High versus low intake of oil seed caused a statistically significant increase in ruminal pH at 3 h post feeding. There were no systematic dietary effects on ruminal ammonia concentrations. When compared with pre-feeding values, the ammonia concentrations were increased at 3 h after feeding and then dropped. Dietary treatment had no significant effects on ruminal volatile fatty acid concentrations (Table 6) and viable bacteria (Table 7). It is noteworthy that the group mean

Table 3: Feed intake by the steers fed the experimental rations

Intake	Cotton seed (fat) 3%	Cotton seed (fat) 6%	Sunflower seed (fat) 3%	Sunflower seed (fat) 6%	SEM	ANOVA[1]
Concentrate (kg day^{-1})	1.83	1.97	1.97	1.85	0.03	T, A, TxA
Concentrate (g kg^{-1}BW$^{0.75}$)	34.4	35.9	35.8	34.5	0.24	T, A, TxA
Rice straw (kg day^{-1})	1.86	1.93	1.93	1.85	0.05	NS
Rice straw (g kg^{-1}BW$^{0.75}$)	35.1	35.3	35.2	34.4	0.33	NS
Total dry matter (kg day^{-1})	3.69	3.90	3.90	3.70	0.08	T, A, TxA
Total dry matter, (g kg^{-1}BW$^{0.75}$)	69.5	71.2	71.1	68.8	0.50	T, A, TxA

[1]Significance was calculated by analysis of variance: TxA = effect of interaction between type (T) and amount (A) of oil seed; NS = No Significant effect

Table 4: Apparent digestibility of macronutrients by the steers fed the experimental rations

Digestibility intake (%)	Cotton seed (fat) 3%	Cotton seed (fat) 6%	Sunflower seed (fat) 3%	Sunflower seed (fat) 6%	SEM	ANOVA[1]
Dry matter	79.6	79.9	80.0	78.9	0.62	NS
Organic matter	82.5	82.3	82.7	81.8	0.55	NS
Neutral detergent fiber	79.4	79.0	79.0	77.8	0.57	NS
Acid detergent fiber	76.6	75.9	76.1	75.3	0.78	NS
Crude fat	80.8	89.5	79.6	86.4	3.29	A

[1]Significance was calculated by analysis of variance: A = effect of amount of oil seed; NS = No Significant effect

Table 5: Ruminal pH values and ammonia-N concentrations in the steers fed the experimental rations

Items	Cotton seed (fat) 3%	Cotton seed (fat) 6%	Sunflower seed (fat) 3%	Sunflower seed (fat) 6%	SEM	ANOVA[1]
[1]Ruminal pH, hours after feeding						
0	7.05	7.16	7.00	7.14	0.09	NS
3	7.19	7.37	7.21	7.36	0.05	A
6	7.08	7.07	7.07	7.33	0.12	NS
Ammonia-N (mg dL^{-1}), hours after feeding						
0	12.7	12.2	12.3	15.8	2.79	NS
3	18.0	19.1	18.6	17.8	1.06	NS
6	14.5	13.3	14.3	11.4	1.58	NS

[1]Significance was calculated by analysis of variance: A = effect of amount of oil seed; NS = No Significant effect

Table 6: Ruminal concentrations of volatile fatty acids in the steers fed the experimental rations

Acids	Cotton seed (fat) 3%	Cotton seed (fat) 6%	Sunflower seed (fat) 3%	Sunflower seed (fat) 6%	SEM	ANOVA[1]
Acetate (mM)	59.1	58.8	62.9	58.3	2.11	NS
Propionate (mM)	30.1	31.7	27.3	31.5	1.66	NS
Butyrate (mM)	10.8	9.6	9.8	10.2	0.71	NS
Acetate:propionate ratio	2.13	2.05	2.34	1.96	0.18	NS

[1]Significance was calculated by analysis of variance: NS = No Significant effect

Table 7: Ruminal bacteria concentrations in the steers fed the experimental rations

Items	Cotton seed (fat) 3%	Cotton seed (fat) 6%	Sunflower seed (fat) 3%	Sunflower seed (fat) 6%	SEM	ANOVA[1]
Amylolytic $\times 10^{8}$CFU mL^{-1}	2.54	2.19	2.82	1.45	0.62	NS
Proteolytic $\times 10^{8}$CFU mL^{-1}	1.46	1.52	1.68	1.23	0.24	NS
Cellulolytic $\times 10^{10}$CFU mL^{-1}	6.89	8.03	8.76	7.98	0.97	NS

Significance was calculated by analysis of variance: NS = No Significant effect numbers of viable bacteria were greater for the concentrate with 3% fat and sunflower seed but when the concentrate contained 6% fat the numbers were higher when cotton seed was included. When the concentrate contained cotton seed and the fat content was raised from 3-6% by including more cotton seed, the steers consumed 7.7% more concentrate. In contrast, an increase in the level of sunflower seed lowered concentrate intake by 6.1%. These effects resulted in a statistically significant interaction between the type and amount of oil seed with regard to concentrate intake. There was no significant concentrate effect on the intake of rice straw. Bernard *et al*. (2005) found that sunflower oil at a level of 3.5% of the dry matter intake did not affect feed intake in goats. Supplemental coconut oil at an intake level of 530 g^{-1} day did not affect dry matter intake in lactating cows (Hristov *et al*., 2009).

The inclusion of tallow in a total-mixed ration at a level of 2% of dry matter reduced feed intake by dairy cows (Onetti *et al*., 2002). When a total-mixed ration was fortified with 2% fish oil, dry matter intake by dairy cows dropped (Vafa *et al*., 2009). The feeding to dairy cows of rations containing of whole cotton seed with elevated concentrations of free fatty acids in the oil caused an increase in dry matter intake (Cooke *et al*., 2007). Taken together the literature data and present results, it would appear that the type and amount of fat in the ration have differential effects on feed intake.

The feeding of cotton seed or sunflower seed in the form of concentrates had no differential effect on the apparent digestibility of crude fat. An increase in the oil seed intake and thus an increase in fat intake, caused an increase in apparent fat digestibility. A high fat intake will increase the absolute amount of undigested crude fat in feces and thereby lowers the fraction of endogenous fat. This leads to an increase in the apparent, total gastrointestinal tract digestibility of crude fat. It is likely that the increased intake of oil seed did not enhance the efficiency of fat digestion. In fact, there is evidence that true fat digestibility falls when fat is added to the ration of steers (Moore *et al*., 1986).

The amount and type of dietary oil seed did not affect the apparent digestibilities of dry matter and organic matter. The type of oil seed did not affect the apparent digestibilities of neutral and acid detergent fiber but the addition of extra oil seed to the concentrate caused a decrease in the group mean values. Zhang *et al*. (2007) added 6.6% sunflower seed to the diet of ewes and found that that the apparent digestibilities of neutral and acid detergent fiber were not influenced. Sullivan *et al*. (2004) reported that the feeding of cotton seed at a level of 6% of total dietary dry matter did not lower the apparent digestibilities of neutral and acid detergent fiber in dairy cows. The discrepancy between the outcomes of this study and that of Zhang *et al*. (2007) and Sullivan *et al*. (2004) may be caused by a dose difference. When the steers were fed the concentrates containing the high level of sunflower seed and cotton seed, the oil seed levels in the total rations were about 7.5 and 15%.

These inclusion levels are higher than those in the studies of Zhang *et al*. (2007) and Sullivan *et al*. (2004). The observed decrease in fiber digestibility after higher intake of oil seed is much smaller than that reported by others after higher intake of supplemental oils (Coppock and Willks, 1991; Hristov *et al*., 2009; Vafa *et al*., 2009). Nevertheless, it was expected that high versus low intake of oil seed would have induced less cellulolytic bacteria, less fermentation, less acetate production, higher ruminal pH values and higher ruminal ammonia concentrations. The ruminal pH was indeed increased after the inclusion of extra oil seed in the concentrate but the amount of oil seed in the ration did not influence the number of viable cellulolytic bacteria in the rumen and the ruminal concentrations of acetate and ammonia. These data indicate that a high intake of oil seed only has a mild impact on rumen function.

CONCLUSION

In this study, the incorporation of extra fat in ruminant rations in the form of oil seed may only have a minor effect on rumen fermentation and macronutrient digestibility. This study did not include a direct comparison between purified oil and the same oil in the form of oil seed. Not with standing that the present data do indicate that the use of oil seeds offers a possibility to raise both the energy density of the ration and the intake of polyunsaturated fatty acids. This study shows that dry matter intake was stimulated by extra cotton seed in the concentrate but lowered by extra sunflower seed. Possible differences in feed intake as induced by the type of oil seed should be taken into account.

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