2009

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Proceedings, Western Section, American Society of Animal Science

Vol. 60, 2009

EFFECTS OF LEVEL OF SUPPLEMENTAL FLAX OIL ON RUMINAL DISAPPEARANCE OF SOYBEAN MEAL

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ABSTRACT[1]: Reductions in forage intake can influence the extent of ruminal degradability of protein and supplemental fats can reduce forage intake. Therefore, the effects of supplemental flax oil on in situ disappearance of soybean meal was evaluated utilizing six ruminally cannulated beef cows (Initial BW = 639 ± 30.2 kg) grazing bromegrass pasture. Cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Supplements were dosed intraruminally at 0730 daily for 28 d. In order to confirm that the level of flax oil fed reduced forage intake, masticate was collected from each cow and TiO_2 was dosed twice daily starting on d 13. Then on d 22, in situ bags (50-µm pore size) containing 5 g of soybean meal were inserted into the rumen and collected at 0, 3, 6, 9, 12, 15, 18, 24, and 48 h after insertion. Effective ruminal degradation was estimated using a combination of this experiment's non-linear regression data and previously determined fluid passage rates. Despite a numerical decline in forage intake ranging from 10,144 to 8,219 g/d as dietary inclusion of flax oil increased, no differences ($P \geq 0.29$) were observed across treatments. Total tract digestion (g/d) and digestibility (% of intake) of DM, N, and NDF did not differ ($P \geq 0.14$) with the exception of total tract NDF digestibility, which tended ($P = 0.06$) to decrease linearly with flax oil inclusion. In situ N disappearance was not different ($P \geq 0.18$) at 0, 3, 6, 9, 12, 15, 18, and 48 h incubation times. However, N disappearance tended ($P = 0.13$) to increase linearly at 24 h. Nitrogen fractions A and B did not differ ($P \geq 0.54$) with level of flax oil. Likewise, no differences ($P \geq 0.26$) in ERD ruminal degradability of N were observed. Overall, supplemental fat fed at levels reported herein were not sufficient to alter the ruminal disappearance of soybean meal when cows grazed brome grass pasture.

Key Words: Fat, In situ, Ruminal degradable protein

Introduction

One of the most notable effects of feeding fat to beef cattle is a depression in dietary intake (Pavan and Duckett, 2008; Scholljegerdes and Kronberg, 2008). A definitive cause of intake depression is unknown, however, research has shown that dietary fats can reduce dry matter intake by lowering ruminal digestion of fiber (Devendra and Lewis, 1974) or by altering hormonal regulation of intake (Choi and Palmquist, 1996). Nevertheless, a decrease in intake with fat feeding may have an effect on ruminal protein digestibility because previous work (Scholljegerdes et al., 2005) has demonstrated that ruminal degradability of protein is altered when intake is restricted.

A reduction in intake may alter the extent of ruminal digestion (Riewe and Lippke, 1969) and a feedstuff's ruminally undegradable protein value (Shadt et al., 1999). Metabolizable protein can often be limited in forages that livestock traditionally graze (Anderson et al., 1988). In addition, cattle fed fats can also be limited in metabolizable protein (Palmquist et al., 1993; Brokaw et al., 2001) due to a reduction in microbial protein production. Therefore metabolizable protein deficiencies may be further exacerbated when fats are fed to grazing cattle. However, it is not known whether or not the depression in forage intake associated with fat feeding is sufficient to cause a change in the proportion of protein degraded in the rumen. The hypothesis for this experiment is that by feeding high levels of fat, forage intake will be depressed and degradable protein content of soybean meal will be altered. Therefore, the objectives of this study were to examine the effects of level of flaxseed oil on the ruminal disappearance of soybean meal.

Materials and Methods

Six ruminally cannulated Angus beef cows (Initial BW = 639 ± 30.2 kg) were used in a completely randomized design to evaluate the effects of supplemental flax oil on in situ disappearance of soybean meal. Cows were allowed to graze a predominately bromegrass pasture starting on June 11, 2008 until July 8, 2008. All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of dietary flax oil inclusion (0, 3, or 6%) was based on predicted total dietary intake for cows as suggested by the NRC (2000). The level of fat supplementation fed in this trial are similar to those known to depress forage intake (Scholljegerdes and Kronberg, 2008). All supplements were dosed intraruminally at 0730 for 28 d.

Although in situ analysis was the primary focus of this experiment, it was important to confirm that our diets indeed influenced intake. Therefore, cattle were allowed 12 d to adapt to diet. Then on d 13, masticate from each cow was collected as described by Brokaw et al. (2001), after which TiO_2 dosing commenced (5 g boluses inserted intraruminally, twice daily). Titanium dioxide was dosed for a total of 10 d with 5 d of adaptation and 5 d of fecal collection occurring at 0730 and 1930. On d 20, just prior to dosing of 200 mL of Co-EDTA into the rumen (Uden et al., 1980), whole ruminal contents were collected and 10

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mL of rumen fluid was strained through 8 layers of cheesecloth and acidified with 7.2 N H_2SO_4. Rumen fluid collections occurred at 0, 6, 12, 18, 24, and 36 hr relative to Co-EDTA dosing. Then on d 22, duplicate in situ bags (50-µm pore size, ANKOM Technology, Fairport, NY) containing 5 g of soybean meal were inserted into the rumen and collected at 0, 3, 6, 9, 12, 15, 18, 24, and 48 h after insertion. In situ bags were rinsed and processed as described by Scholljegerdes et al., 2005. On d 28 rumen fluid was collected from each cow for IVDMD determination of masticate and soybean meal.

Masticate, in situ residue, and fecal samples were analyzed for DM and ash (AOAC, 1990), N (Carlo Erba Model NA 1500 Series 2 N/C/S analyzer (CE Elantech, Lakewood, NJ), and neutral detergent fiber (ANKOM 200 fiber analyzer, ANKOM Technology, Fairport, NY). Fecal samples were analyzed for TiO_2 according to the procedures of Myers et al. (2004) using a spectrophotometer (DU -640, Beckman Instruments, Inc., Fullerton, CA). .

Ruminal fluid samples were centrifuged at 20,000 × g for 20 min and a 2.5-mL aliquot was added to 0.5 mL 25% (wt/vol) metaphosphoric acid containing 2 g/L of 2-ethyl-butyric acid (Goetsch and Galyean, 1983). These samples were analyzed for concentrations of VFA as described by Scholljegerdes and Kronberg (2008). Concentration of NH_3 in ruminal fluid was determined using the phenol-hypochlorite procedure of Broderick and Kang (1980). Ruminal fluid Co concentrations were determined by atomic absorption spectroscopy using an air/acetylene flame (Model 3110, Perkin Elmer, Inc., Norwalk, CT).

Intake was estimated from fecal OM output and in vitro OM indigestibility and partitioned between forage and supplement intakes. Protein fractions A and B, as well as protein degradation rate (k_d = %/h) were calculated with the model of Ørskov and McDonald (1970) using the NLIN procedure of SAS (SAS Inst., Inc., Cary, NC). Effective ruminal degradation (ERD) was calculated using the equations of Broderick (1994). Actual fluid passage rate (k_p = %/h) estimates were used to calculate supplement ERD because the supplement would most likely be associated with the fluid phase (Nocek, 1985).

All non-repeated measure data were analyzed with the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). All time course data were analyzed using the MIXED model of SAS as a completely randomized design experiment. Included in the model were the effects of treatment, time, and treatment × time. Autoregression order one was determined to be the most desirable covariance structure according to the Akaike's information criterion. Orthogonal contrasts were used to compare linear and quadratic responses to level of flax oil intake (Steel and Torrie, 1980). There was no treatment × time interactions ($P \geq 0.16$) for any of the variables measured.

Results and Discussion

Forge DM intake did not differ ($P = 0.29$) across treatments (Table 1). This is surprising, due to the fact that when ground flaxseed was fed to provide a diet of 3% total fatty acids for grazing beef cattle (Scholljegerdes and Kronberg, 2007), forage DM intake was significantly reduced. In the current trial, diets consumed were 1.1, 4.5, and 9.0% total fatty acids for 0, 3, and 6% treatments, respectively. Furthermore, Pavan et al. (2007) observed a linear decrease in forage DM intake when corn oil was supplemented at 0, 0.75 and 1.5 g/kg of BW, which equates to a diet that was 2.5, 4.9, and 8.0% total fatty acid. Likewise, total N and NDF intake did not differ ($P \geq 0.14$) across treatment. No differences were observed ($P \geq 0.14$) for DM, N, or NDF digested (g/d). However, there was a tendency for NDF digestibility (% of intake) to decline linearly ($P = 0.06$) with increasing levels of flax oil. A reduction in fiber digestibility has been reported previously when cattle were fed soybean oil (Whitney et al., 2000) and corn oil (Pavan et al., 2007). Total tract DM and N digestibility did not differ ($P \geq 0.20$) across treatments.

Ruminal pH and NH_3 did not differ ($P \geq 0.38$) across dietary treatments (Table 2). Fluid passage rate (%/h) differed quadratically ($P = 0.03$) with cattle receiving 3% flax oil having the lowest values for passage rate. We had expected a linear decrease in forage intake accompanied with a linear decrease in fluid passage rate. Therefore, it is not clear as to why there was a quadratic response in fluid passage rate. Total ruminal VFA concentration did not differ ($P = 0.35$), however, the molar proportion of acetate and propionate differed quadratically ($P = 0.03$). Whereas, proportions of butyrate did not change ($P = 0.25$) with flax oil supplementation. The increase in propionate as oil level increased in the diet is similar to that reported by Kucuk et al. (2004), when lambs were fed increasing levels of soybean oil.

Supplemental flax oil did not affect ($P \geq 0.18$) in situ N degradability (% digested) of soybean meal at 0, 3, 6, 9, 12, 15, 18 or 48 h. Whereas 24 h N degradability tended to be greater (Linear, $P = 0.13$) as flax oil inclusion increased in the diet. Soybean meal N fractions, A and B, did not differ ($P \geq 0.20$) with flax oil inclusion. Degradation rate was not different ($P = 0.20$) despite values ranging from 6.0 to 12.0%/h due in part to large standard errors. Though it is difficult to find published values for in situ degradability of soybean meal in grazing cattle; ruminal N degradability of soybean meal for animals not fed flax oil was similar to that reported by others who fed silage-based diets (Spears et al., 1985; Stern et al., 1994). When ERD was calculated using a k_p that declined linearly as fat levels in the diet increased similarly to those herein (5.7, 6.86, and 6.47 %/h; Scholljegerdes and Kronberg, 2008); no difference ($P \geq 0.26$) was observed (data not shown). However, when actual k_p was utilized (Table 2), ERD tended to differ (Quadratic, $P = 0.08$) with animals consuming 3% flax oil having the greatest ERD compared to 0 or 6% with 6% being greater than 0%. The increased ERD observed in cattle supplemented with 3% flax oil was likely due to a slower fluid passage rate compared to other treatments. Likewise ruminally undegradable protein tended to increase (Quadratic, $P = 0.08$) for cattle fed 3% flax oil compared to 0 or 6%. Palmquist et al. (1993) suggested that cattle fed high-fat diets may have an increased requirement for

ruminally undegradable protein presumably due to a decrease in microbial protein synthesis. Data from our experiment would suggest that deficiencies in ruminally undegradable protein may be further exacerbated due to the tendency for the proportion of protein fermented in the rumen to be greater when fats are fed to grazing cattle.

In conclusion, feeding fats may impact ruminal fermentation patterns and flow kinetics such that ruminal protein degradability is altered. However, the majority of responses reported herein were mere tendencies, therefore more work is warranted in order to better quantify the effect fat has on metabolizable protein supply in grazing cattle.

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Table 1. Effects of soybean meal and increasing levels of flax oil on forage intake and total tract nutrient digestibility in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Forage DM Intake	10144	9483	8219	1050	0.29	0.83
Total DM Intake	10431	10176	9337	1050	0.52	0.84
N Intake	127	119	103	8.4	0.14	0.74
NDF Intake	7515	6990	5839	678	0.18	0.73
Total tract						
DM digested, g/d	7174	6877	6384	800	0.54	0.93
DM digestibility, % of intake	68.8	67.6	68.1	0.96	0.64	0.53
N digested, g/d	97.9	90.7	84.3	8.7	0.35	0.97
N digestibility, % of intake	77.0	76.0	81.6	2.0	0.20	0.26
NDF digested, g/d	5401	4887	3971	507	0.14	0.77
NDF digestibility, % of intake	71.9	70.0	67.7	0.98	0.06	0.91

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 2. Effects of soybean meal and increasing levels of flax oil on ruminal pH, NH_3, fluid passage rate, and volatile fatty acids in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Ruminal pH	5.9	5.7	5.8	0.2	0.87	0.61
Ruminal NH_3, m*M*	4.3	5.0	5.5	0.8	0.38	0.92
Fluid passage rate, %/h	7.5	2.9	10.1	1.2	0.24	0.03
Ruminal total VFA, m*M*	66.4	73.2	67.1	4.8	0.92	0.35
Ruminal VFA, mol/100 ml						
Acetate	59.6	59.4	53.1	0.7	0.01	0.03
Propionate	21.9	22.1	27.4	0.0	0.01	0.03
Butyrate	14.3	14.7	14.2	0.3	0.72	0.25
Isobutryate	1.20	0.94	1.30	0.01	0.35	0.03
Isovalerate	1.9	1.7	2.5	0.2	0.09	0.08
Valerate	1.1	1.1	1.6	0.0	0.04	0.15
Acetate:propionate	2.7	2.7	1.9	0.1	0.01	0.03

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 3. Effects of increasing levels of flax oil on in situ N degradability (% digested) of soybean meal in grazing beef cows

	Treatments[1]				Contrast	
h	0	3	6	SEM[1]	Linear	Quadratic
0	38.5	35.2	36.5	3.9	0.74	0.66
3	44.7	45.9	51.2	6.7	0.38	0.69
6	52.0	45.8	54.3	8.1	0.26	0.55
9	53.0	53.4	68.0	10.7	0.21	0.88
12	63.2	72.4	78.3	6.3	0.18	0.84
15	70.7	76.6	86.4	6.6	0.19	0.82
18	78.6	85.6	90.4	4.8	0.18	0.86
24	80.6	90.4	95.1	4.8	0.13	0.69
48	87.5	92.9	98.4	4.5	0.19	1.0

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 4. Effects of increasing levels of flax oil in situ N fractions and degradability rate of soybean meal in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Fraction A	35.8	32.8	36.1	3.8	0.95	0.55
Fraction B	57.4	63.6	63.1	5.7	0.54	0.66
k_d, %/hr	6.0	8.3	12.0	2.6	0.20	0.84
ERD[3]	61.1	79.7	69.5	4.4	0.28	0.08
RUP[4]	38.9	20.3	30.5	4.4	0.28	0.08

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.
[3]ERD = Effective ruminal degradation = % Fraction A + {% Fraction B · [kd/(kd+kp)]} where kp = 7.5, 2.9, and 10.1%/h for the 0, 3, and 6% treatments, respectively.
[4]RUP = ruminally undegradable protein = 100 – ERD.