Sheep & Goat Research Journal
Volume 18: 2003

Contents:

Effect of Ethanol Supplementation on In Vitro Digestion and VFA Production and Growth Performance of Newly Weaned Lambs

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Summary

The objectives of these studies were to determine the effects of ethanol (ETOH) supplementation on DM digestibility and VFA production in an in vitro system and on growth performance of newly weaned lambs. For Exp. 1, four ruminally cannulated beef heifers were used as ruminal fluid donors for an in vitro trial. Two of these heifers received a commercial rice hulls-based supplement containing ETOH (Corner Post, Free Choice Enterprises, Richland, Iowa) for 14 d before ruminal fluid collection (adapted), whereas the other two heifers received no supplement (unadapted). Substrates included 0.5 g oat hay (OH) or 0.44 g oat hay plus 0.06 g ETOH supplement (ES). Data were analyzed as a split-plot with adaptation to ETOH supplement as the main-plot and type of substrate as the sub-plot factor. At 6 h of incubation, in vitro dry matter disappearance (IVDMD) tended to be greater (P = 0.09) for adapted than unadapted ruminal fluid, and OH had greater ($P \geq 0.02$) IVDMD, total VFA concentrations, and molar proportions of acetate, butyrate, isovalerate, and valerate than ES. Adaptation to ETOH supplement improved (P = 0.03) 48-h IVDMD of OH and ES. At 12 h, ruminal fluid adapted to ETOH supplement increased (P = 0.03) total VFA from OH substrate. By 24 h of incubation, adaptation or dietary substrate effects on total VFA and molar proportions of individual VFA were not significant ($P \leq 0.60$). For Exp. 2, 24 Rambouillet crossbred lambs (25.0 ± 0.4 kg) were blocked by body weight and allotted to one of 12 pens resulting in two lambs per pen with four replications per treatment. Lambs were fed no supplement (CON), the commercial ETOH supplement (ES), and the ETOH supplement dried (DRY) to evaluate the commercial supplement without the ETOH. Experimental treatment differences were evaluated using orthogonal contrast comparing CON vs. supplement and ES vs. DRY. Overall (d-0 to d-42) DMI and ADG were not different for CON vs supplement (P = 0.71 and 0.63, respectively) or ES vs DRY (P = 0.83 and 0.41, respectively). We conclude that adaptation of ruminal microflora to ETOH supplementation will improve diet digestibility, but the improvement in diet digestibility is not sufficient to enhance growth performance of newly weaned lambs.

Key Words: Lambs, Ethanol, Supplementation, Digestion, Growth

Introduction

As a feed additive for ruminants, ethanol (ETOH) has been suggested to stimulate effective use of feed ingredients (Pradhan and Hemken, 1970). Ethanol introduced into the rumen with the diet is transformed by the microorganisms into VFA, with the main end-product being acetate (Durix et al., 1991). Chalupa et al. (1964) showed that supplemental ETOH increased cellulose digestion, suggesting that ETOH may provide energy for microbial activity. Proper utilization of ETOH by microorganisms must consider adaptation via an appearance of new bacterial strains, an increased number of existing types, or an increased enzyme production per bacterial cell (Pradhan and Hemken, 1970). Thus, improvements in diet digestibility in vitro may not be observed unless the ruminal microflora are adapted to supplemental ETOH. Moreover, increased ruminal digestion may improve production of livestock consuming ETOH. Kreul et al. (1993) reported that dietary ETOH stimulated ADG and gain efficiency of steers in a feedlot trial. We hypothesized that sheep production could be improved if provision of supplemental ETOH results in increased diet digestibility. Therefore, the objectives of these experiments were to determine the effects of ETOH supplementation on IVDMD and VFA production using adapted vs unadapted ruminal fluid and on growth performance of newly weaned lambs.

Material & Methods

Experiment I

Four Angus x Gelbvieh heifers (avg BW = 430 kg) with ruminal cannulae were used as ruminal fluid donors for determination of IVDMD and VFA. Two of these heifers received a commercial rice hulls-based supplement containing approximately 16% ETOH (Corner Post, Free Choice Enterprises, Richland, Iowa; adapted), whereas the other two heifers received no supplement (unadapted). All four heifers grazed a native (predominantly bromegrass) pasture before and during ruminal fluid collection. Animal care and sampling procedures were approved by the University of Wyoming Animal Care and Use Committee.

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Triplicate 0.5 g oat hay (OH) or 0.44 g OH plus 0.06 g ETOH supplement (ES) were weighed into 50 mL centrifuge tubes to determine IVDMD and VFA. The composition of OH and ES are shown in Table 1. Tubes were inoculated with 28 mL of McDougall's buffer solution and 7 mL of ruminal fluid (Tilley and Terry, 1963). Ruminal fluid was collected following 14 d of adaptation to ETOH supplementation. Ruminal fluid was strained through four layers of cheesecloth and composited within adaptation treatment before inoculating the tubes. Following inoculation with ruminal fluid the air space in each tube was cleared with CO_2 and capped. Tubes were placed in a 39° C water bath and incubated for 6, 12, 24 and 48 h. Tubes were removed from the water bath and centrifuged at 1000 x *g* for 15 min. From tubes incubated for 6, 12, and 24 h, 10 mL of supernatant was extracted, acidified with 1 mL of 7.2 N H_2SO_4 and frozen. Remaining supernatant was aspirated from all tubes and residues were digested with pepsin in 1 N HCL to measure IVDMD.

Thawed ruminal fluid samples (4° C for 24 h) were centrifuged at 10,000 x *g* for 10 min and 2.5 mL of supernatant added to tubes containing 0.5 mL 25% metaphosphoric acid and 2 g/L of 2-ethyl-butyric acid as internal standard (Goetsch and Galyean, 1983). Samples were then centrifuged for 10 min at 10,000 x *g* and the supernatant analyzed for concentrations of VFA by gas-liquid chromatography using a Hewlett Packard 5890 Series II gas chromatograph equipped with a 15m x .53 mm (i. D.) column (Nukol: Supelco, Bellefonte, PA) with temperature ramp of 110° C to 150° C at 8° C per min. Helium was used as carrier gas with column flow rate of 20 mL/min. Injector and detector temperatures were 250° C.

All data were analyzed using the GLM procedure of SAS (Release 7.0, ver. 4.1, 1998; SAS Institute Carry, NC) for a split-plot in a completely random design. The model included the adaptation effect as the main-plot tested against source of ruminal fluid within the main-plot effect (error a) and type of substrate and the adaptation x substrate interaction as the sub-plot tested against residual error (error b).

Experiment II

Two weeks after weaning, 24 Rambouillet cross-bred lambs (25.0 ± 0.4 kg) were blocked by BW and allotted to one of 12 pens resulting in two lambs per pen with four replications per treatment. The basal diet consisted of ad libitum access to oat hay plus 0.91 kg/d of dehydrated alfalfa pellets to meet NRC (1985) recommendations for CP for early weaned lambs. Lambs were fed one of three experimental supplements at 0600 daily for 42 d. Supplement treatments were control (CON), ETOH supplement (ES) using a commercial product containing approximately 16% ETOH (Corner Post, Free Choice Enterprises, Richland, Iowa), and the commercial product dried at 55° C for a minimum of 96 h (DRY), which removed 88% of the ETOH (Table 2). Both ES and DRY were fed at 45.4 g of DM/d according to the manufacture's recommendations. Body weights were obtained immediately before feeding on the first 2 d and the last 2 d of the feeding trial. Gain efficiency was expressed as kg of BW gained per 100 kg DMI. Animal care and sampling procedures were approved by the University of Wyoming Animal Care and Use Committee.

Individual dietary components were ground through a Wiley mill (1-mm screen), and analyzed for DM, OM, CP, and NDF (AOAC, 1990; Table 2). Animal performance data were separated into the first and second 21-d periods, and overall 42-d period. Data were analyzed (SAS, Release 7.0, ver. 4.1, 1998; SAS Institute Carry NC) as a randomized complete block design with pen as the replicate. Experimental treatment differences were evaluated using orthogonal contrast comparing CON vs. supplement and ES vs. DRY (Steel and Torrie, 1980).

Results and Discussion

Experiment I

At 48 h of incubation, an adaptation to ETOH supplement x diet interaction was observed (P = 0.03), where adaptation to ETOH supplement improved 48 h IVDMD of both OH and ES (Figure 1). Likewise, IVDMD of OH was greater (P = 0.01) at 6 h than OH + ES (Table 3). Adapted ruminal fluid had greater ($P \leq$ 0.01) IVDMD at 6 and 12 h, but unadapted ruminal fluid had slightly greater (P = 0.06) IVDMD at 24 h of incubation. Consistent with our results, Chalupa et al. (1964) noted that ETOH stimulated the digestion of cellulose with in vitro cultures. In contrast, Durix et al. (1991) observed no improvement in OM, NDF, or ADF digestion with ETOH addition to a semi-continuous culture system. However, these authors and others (Richardson et al., 1958) also reported improved growth and efficiency of steers supplemented with ETOH, suggesting either stimulation of ruminal digestion and (or) direct utilization of ETOH or it's metabolites as an energy source. Further stimulation of digestion with adaptation was also supported by the work of Pradhan and Hemken (1970), who noted that adaptation to ETOH supplementation was important for its proper utilization by the ruminal microflora.

No interactions ($P \geq 0.50$) between adaptation to ETOH supplementation and dietary substrate were observed for total VFA concentrations at 6 or 24 h of incubation. Such an interaction however, was detected (P = 0.03) for total VFA production at 12 h, wherein adaptation to ETOH supplement increased total VFA concentrations for ES but not for OH (data not shown). At 6 h of incubation, total VFA concentrations were greater ($P \leq 0.01$) for OH than ES, reflecting the improvement in IVDMD observed for the OH (Table 4). An adaptation to ETOH supplementation x dietary substrate interaction was also detected ($P \leq 0.04$) for molar proportions of acetate, propionate, butyrate, isovalerate, and valerate (Table 5). Ruminal fluid collected from tubes inoculated with microflora adapted to ETOH had greater molar proportions of acetate, with acetate differing between dietary substrate for adapted ruminal fluid but not unadapted ruminal fluid. Molar proportions of propionate and butyrate were greater with unadapted ruminal fluid and ES was greater than OH. In vitro molar proportions of isovalerate and valerate were increased with ES for adapted ruminal inoculum, which was not the case for unadapted fluid. Molar proportions of isovalerate increased ($P \leq 0.03$) with ES at 12 h and by adaptation to ETOH at 24 h, but other effects on individual VFA were not noted ($P \geq 0.15$). By 24 h no differences ($P \geq 0.60$) in total VFA were detected among adaptation or dietary treatments (Table 4).

Other researchers (Pradhan and Hemken, 1970; Durix et al., 1991) have observed similar modifications in molar proportion of VFA. The observed decrease in molar proportions of propionate for adapted ruminal fluid in our study is likely attributed to the relative increase in molar proportions of acetate. When fed to mature cows ETOH increased total ruminal VFA and the molar proportions of acetate by 4 h after feeding, but this improvement diminished by 8 h (Chalupa et al., 1964) after feeding. Caldwell (1995) illustrated that it is possible for bacteria to synthesize butyrate during the metabolism of ETOH, which may explain greater molar proportions of butyrate observed at 6 h for adapted microbes in our study. Durix et al. (1991) noted that increased VFA immediately following feeding or inoculation reflects metabolism of the ETOH itself rather than stimulation of nutrient digestibility because 77 to 80% of radiolabeled supplemental ETOH was recovered as acetate with the remainder of the ETOH carbon recovered mainly as butyrate, caproate and valerate. However, Durix et al. (1991) also observed significant appearance of isovalerate when ETOH was added to a semicontinuous culture system. Valerate and isovalerate are growth factors for cellulolytic bacteria (Andries et al., 1987), and the provision of these minor acids has stimulated digestion of fiber in vitro (Argyle and Baldwin, 1989) and in vivo (Maeng and Baldwin, 1976). The improvements in isovalerate and valerate observed herein with adaptation to ETOH and (or) ETOH supplementation could have contributed to greater IVDMD at 6, 12, and 48 h for adapted ruminal microbes.

The oxidation of ETOH gives rise to increased NADH + H^+ and NADPH + H^+ and ATP being produced when acetate is formed (Caldwell, 1995). Thus, provision of supplemental ETOH will provide the ruminal bacteria with energy shortly after consumption by the host animal. Supplemental ETOH also enhanced production of valerate and isovalerate. An increase in these minor VFA may increase growth and propagation of the cellulolytic bacteria. As a result, diet digestibility may be improved with provision of supplemental ETOH.

Experiment II

As illustrated in Table 6, DMI was not different ($P \geq 0.71$) among treatments. The DMI results from this trial would indicate that supplement with or without ETOH did not stimulate feed intake. This is in agreement with Head (1959) who reported no significant increase in feed intake by steers with ad libitum access to straw and a liquid supplement containing 6% ETOH. However, Drori and Loosli (1959) showed a 2% increase in feed intake for steers supplemented with 2% ETOH when fed grass hay. Richardson et al. (1958) also reported increased feed intake of roughages by steers receiving a liquid supplement containg 3% ETOH.

There were no differences in ADG for CON vs. supplement ($P = 0.63$) or ES vs. DRY ($P = 0.41$). Moreover, there were no significant ($P \geq 0.34$) changes in gain efficiency during the feeding trial. It was anticipated that lambs fed ETOH supplement would have had greater ADG and gain efficiency because results of our in vitro experiment suggested that digestion of oat hay was enhanced when ruminal fluid was adapted to the ETOH supplement. Lack of difference in lamb performance among dietary treatments in the current study suggest that the response detected in the in vitro experiment was not sufficient to elicit a response in animal performance. Results of the animal performance trials were consistent with those of Bates et al., (1958) who reported that supplemental ETOH did not increase weight gain of dairy heifers fed a high-roughage diet. In contrast, Kreul et al. (1993) reported an improvement in ADG by steers supplemented with ETOH during the finishing phase of a feedlot trial. Perhaps ETOH helped maintain fiber digestion, lessening the digestive disturbances often noted with concentrate diets (Galyean and Owens 1991), allowing for the improved animal performance in the study of Kreul et al. (1993).

Conclusions

Ethanol supplementation can potentially increase diet digestibility following adaptation of the ruminal microflora to the ethanol supplement. However, the increase in diet digestibility may not be great enough to elicit a response in growth performance of newly weaned lambs. Therefore, supplementing with the commercial ethanol supplement used in these studies would not be expected to improve ruminant livestock production.

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Table 1. Chemical composition of oat hay and ethanol supplement (Exp. 1).

Item	Oat hay	Ethanol supplement[a]
DM, %	95.9	75.3
OM, % of DM	90.7	86.4
CP, % of DM	8.10	7.49
NDF, % of DM	67.2	61.2
ADF, % of DM	38.9	46.8

[a]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland, Iowa).

Table 2. Chemical composition of dehydrated alfalfa (DEHY), oat hay (OH), ethanol supplement[a] (ES), and dry ethanol supplement (DRY) used in Exp. 2.

Item	DEHY	OH	ES	DRY[b]
DM, %	92.6	92.7	74.6	88.7
OM, % of DM	90.1	90.8	86.5	86.5
CP, % of OM	18.8	7.9	8.1	8.1
NDF, % of OM	52.8	63.4	44.6	44.6

[a]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland, Iowa; adapted).

[b]Commercial supplement was dried in a forced-air oven at 55° C for a minimum of 96 h to remove ethanol.

Table 3. In vitro DM digestibility (%) inoculated with ruminal fluid adapted[a] or unadapted to ethanol supplement[b] with oat hay (OH) or oat hay plus ethanol supplement (ES) as substrates.

Item	Adapted	Unadapted	SE[c]	*P*[d]	OH	ES	SE[c]	*P*[d]
6-h incubation	44.1	29.0	2.2	<0.01	37.5	35.6	0.07	0.01
12-h incubation	42.8	35.7	2.3	0.01	40.2	38.4	0.77	0.63
24-h incubation	40.0	42.0	1.0	0.06	41.7	40.0	0.58	0.07

[a]Ruminal fluid with 14 d adaptation to ethanol supplement vs no supplement (Unadapted).

[b]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland, Iowa; adapted).

[c]n = 4.

[d]P-value of the observed effect.

Table 4. Concentrations of VFA following in vitro incubations inoculated with ruminal fluid adapted[a] or unadapted to ethanol supplement[b] with oat hay (OH) or oat hay plus ethanol supplement (ES) as substrates.

Item	Adapted	Unadapted	SE[c]	*P*[d]	OH	ES	SE[c]	*P*[d]
6-h incubation								
Total VFA, mM	44.2	29.7	10.2	0.42	43.5	30.4	3.1	0.005
12-h incubation								
VFA, mol/100 mol								
Acetate	66.3	68.9	1.4	0.32	65.4	69.8	2.1	0.15
Propionate	23.0	20.3	2.2	0.48	23.8	19.5	2.4	0.43
Butyrate	8.0	8.3	0.9	0.81	8.1	8.2	0.3	0.68
Isobutyrate	0.44	0.60	0.18	0.60	0.56	0.48	0.08	0.53
Isovalerate	0.75	0.80	0.02	0.21	0.71	0.84	0.03	0.01
Valerate	1.5	1.1	0.3	0.52	1.5	1.1	0.2	0.21
24-h incubation								
Total VFA, mM	41.5	47.5	3.0	0.29	44.0	45.1	1.7	0.65
VFA, mol/100 mol								
Acetate	67.2	67.2	2.1	0.99	67.3	67.1	0.8	0.85
Propionate	21.7	22.2	2.4	0.89	22.1	21.7	0.5	0.54
Butyrate	8.0	8.2	0.3	0.82	7.8	8.4	0.3	0.21
Isobutyrate	0.80	0.46	0.62	0.73	0.70	0.56	0.12	0.43
Isovalerate	1.11	0.86	0.03	0.03	0.94	1.03	0.04	0.20
Valerate	1.2	1.1	0.04	0.56	1.1	1.2	0.05	0.34

[a]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland, Iowa; adapted).

[b]Ruminal fluid with 14 d adaptation to ethanol supplement vs no supplement (Unadapted).

[c]n = 4.

[d]P-value of the observed effect.

Table 5. Molar proportions of VFA following 6-h incubations of oat hay (OH) or oat hay plus ethanol supplement[a] (ES) inoculated with ruminal fluid adapted vs unadapted to ethanol supplement[b].

	Adapted		Unadapted			
VFA	OH	ES	OH	ES	SE[c]	*P*[d]
	mol/100 mol					
Acetate	72.9	70.2	68.1	68.2	0.53	0.01
Propionate	18.3	19.8	21.4	20.9	0.44	0.04
Butyrate	6.90	7.94	8.38	8.48	0.13	<0.01
Isobutyrate	0.14	0.16	0.28	0.40	0.10	0.60
Isovalerate	0.69	0.80	0.82	0.84	0.01	<0.01
Valerate	1.01	1.15	1.06	1.07	0.02	<0.01

[a]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland, Iowa; adapted).

[b]Ruminal fluid with 14 d adaptation to ethanol supplement vs no supplement (Unadapted).

[c]n = 4.

[d]P-value observed for the adaptation C dietary substrate effect.

Table 6. Growth performance, DMI and gain efficiency of lambs consuming control (CON), ethanol (ETOH) and dried (DRY) supplemented diets.

					P	
	CON	ETOH[a]	DRY[b]	SE[c]	CON x supplement[d]	DRY x ES[e]
Initial BW, kg	25.2	25.6	25.4	0.25	0.42	0.55
Final BW, kg	31.5	32.8	31.5	0.72	0.47	0.51
DMI, kg/d	1.14	1.14	1.15	0.02	0.71	0.83
ADG, kg/d	0.15	0.15	0.16	0.01	0.63	0.41
Gain efficiency[f]	13.1	12.9	14.2	0.88	0.70	0.34

[a]Commercial rice hulls-based supplement containing approximately 16% ethanol (Corner Post, Free Choice Enterprises, Richland) was fed at the manufacturer's recommendations to provide 7.2 g/d ethanol.

[b]The commercial product was dried at 55∞ C for a minimum of 96 h, which removed 88% of the ETOH.

[c]n = 12.

[d]Control diet vs supplemented diets.

[e]Ethanol supplement vs dried supplement.

[f]Gain efficiency was expressed as kg of gain/100 kg of DMI

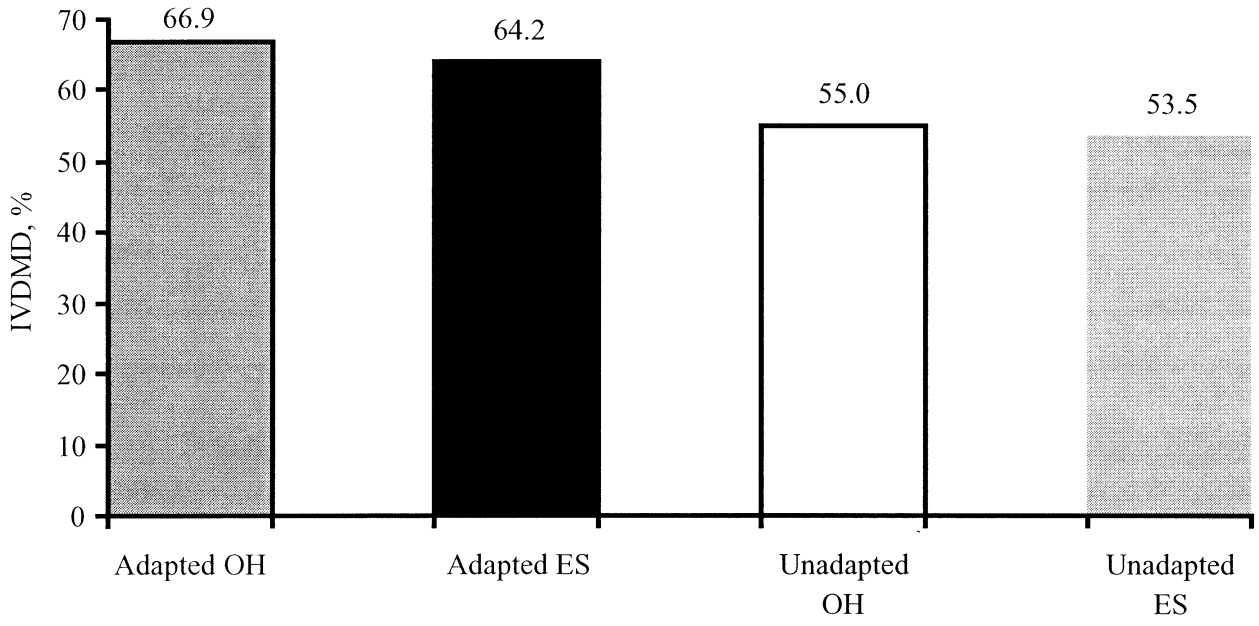

Figure 1. An illustration of the adaptation C dietary substrate interaction noted (*P* = 0.03) for IVDMD following 48 h of incu bation. Oat hay (OH) or oat hay plus ethanol supplement (Corner Post, Free Choice Enterprises, Richland, Iowa; ES) was inoculated with ruminal fluid adapted vs unadapted to the ethanol supplement. Pooled SE = 0.10.