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EFFECTS OF ENSILED WHOLE STILLAGE AND FESCUE HAY ON RUMEN CHARACTERISTICS IN SHEEP*

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INTRODUCTION

The addition of corn whole stillage to mature fescue hay has recently been demonstrated to improve its overall digestibility particularly the cellulose containing acid detergent fiber (ADF) fraction (Heitmann and Britton, 1983). This has been demonstrated also for other distiller's fermentation by-products (Burroughs et al., 1950; Little et al., 1964 and 1967; Potter et al., 1966; and Chen et al., 1976). Furthermore, a large increase in dry matter digestibility was observed with small increases in the amount of wet distillers grains in the diet (Heitmann and Britton, 1983). This increase (33%) in dry matter digestibility between two such similar diets is unusual and cannot be explained entirely by the 5-10% increase in whole stillage content.

Since most of the dry matter digestion and almost all of the cellulolytic activity is of rumen microbial origin, studying the effects of corn whole stillage on rumen microbial population and their end products, volatile fatty acids (VFA), was of interest. Although greater than 99.99% of the total microbial numbers in the reticulorumen are bacteria, almost 50% of the total microbial volume is protozoa (Warner, 1962). Therefore, the objective of this study was to observe the effects of corn whole stillage on ruminal pH, protozoa concentration and population, and VFA concentration.

EXPERIMENTAL PROCEDURE

Animals. Eight mature, crossbred wethers weighing an average of 94 kg were randomly assigned to total confinement digestion stalls. Each stall was equipped with individual feed bunks and urine and fecal pans. Water and trace mineralized salt were available to the wethers on a free choice basis. The sheep were kept under continuous artificial lighting and were subjected to ambient temperatures (15-33°C). Prior to data collection, all animals were thoroughly adapted to the digestion stalls and laboratory personnel.

Diets. The sheep were fed either mature fescue hay, 70:30 corn whole stillage:fescue hay silage, or 65:35 corn whole stillage:fescue hay silage, gross weight, on a wet basis. The 70:30 and 65:35 silages were chosen since they showed marked differences in overall digestibilities, with the 70:30 being more digestible as shown in previous work (Heitmann and Britton, 1983). The silages were mixed by hand and ensiled in silos made from 200 ℓ steel barrels double lined with polyethylene bags. The can liners were individually sealed and the individual silos remained sealed until required for feeding. The silages were allowed to ensile for approximately four weeks prior to use. Samples for proximate analysis were collected by random grab samples on a daily basis. The proximate analyses of the whole stillage, fescue hay, and 70:30 and 65:35 stillage:hay silage are given in Table 1.

Experimental protocol. The eight wethers were randomly divided into two groups of four each. Group 1 received the 70:30 stillage:hay silage diet and Group 2 was assigned the 65:35 silage. Initially, both groups were fed chopped, mature fescue hay in 1-kg aliquots at 0800 and at 1600 h. Following a 15-day adaptation period, rumen fluid samples were taken 2 hours following the morning feeding for a 5-day period. Approximately 100 ml of rumen fluid were aspirated with a suction-strainer apparatus. A 10-ml aliquot was set aside and immediately analyzed for pH. A second 10-ml aliquot was diluted with an equal quantity of 40% formalin and the diluted sample was stored at room temperature until determinations of protozoa concentrations and identifications could be performed. The remaining 80 ml were sealed in brown glass screw top jars and immediately cooled by immersion in ice and then frozen until VFA analysis could be performed.

A silage feeding phase immediately followed the 5-day fescue period. Group 1 received the 70:30 stillage:hay silage and Group 2 received the 65:35 diet. Each wether received 4 kg/day of silage given in two equal portions at 0800 and at 1600 h. Rumen fluid samples were taken for a 5-day adaptation period, and immediately following this, for a final 5-day experimental period. Rumen fluid samples were treated exactly as in the fescue period.

Analyses. The pH of the rumen fluid was immediately determined following aspiration using a Fisher Accumet Model pH meter standardized with a pH 7 phosphate buffer prior to each testing.

The procedures used in the staining, diluting, counting and identification of rumen ciliate protozoa are similar to those of Boyne et al. (1957).

Holotricha were differentiated from the sub-class spirotricha, order entodiniomorphida, depending on whether the cilia completely covered the protozoa or were arranged in a spiral band about the oral region. The holotricha were then determined to be either Isotricha sp. or Dasytricha ruminantium depending upon the size, shape and location of the mouth. The entodiniomorphida were divided into either Diplodinium sp. or Entodinium sp. depending on the number of ciliated zones, the Diplodinium having a dorsal ciliated zone in addition to the oral zone (Hungate, 1966). The presence of Epidinium and Ophsyoscolex were quite low and eratic and were consequently disregarded in this study.

VFA analysis was performed in the following manner. The frozen rumen fluid samples were thawed and a 25-ml aliquot was centrifuged at 4°C and 3,020 x G for five minutes. A 5-ml aliquot of the supernatant was removed and acidified with 1 ml of 25% metaphosphoric acid. The acidified sample was centrifuged at 4°C and 12,100 x G for 10 minutes to remove any protein precipitate. A 5-μl aliquot of the supernatant was analyzed on a Bendix gas chromatograph, Model 2600, with a 1.8 m x 2.0 mm glass column packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh chromosorb WaW[3].

Statistical analyses. All data were analyzed by a two-way analysis of variance to detect significant differences due to level of whole stillage in the silages and a Duncan's New Multiple Range Test was used to determine the significance of differences among fescue hay, silage adaptation and silage feeding periods according to the Statistical Analysis System (1979).

RESULTS

Ruminal pH. The mean ruminal pH values are presented by group and treatment in Table 2. The ruminal pH of animals receiving the 65:35 stillage:hay diets was 0.10-0.15 higher than that of the 70:30 group over all phases of the experiment. However, this was not due to type of silage fed but rather to the random allocation of animals when the groups were formed. Since no differences were found between groups, the data from both groups were combined to increase statistical sensitivity. Although pH tended to decrease with the stillage:hay silage feeding, these decreases were not significant.

Ciliate protozoa. Rumen ciliate protozoa concentrations are given by group, treatment and species in Table 3. Differential protozoa counts showed that the feeding of the whole stillage:fescue hay silages caused a significant reduction in protozoa numbers as compared with the feeding of fescue hay alone. However, no differences were found between experimental groups. Therefore, the data from the two groups were combined. For the combined groups, the population of Isotricha sp. decreased (P<.01) from 0.6 x 10^4/ml on fescue to 0.03 x 10^4/ml when the sheep were fed whole stillage:fescue hay silages. Dasytricha ruminantium had an average population of 2.81 x 10^4/ml on the fescue diet but was effectively (P<.05) eliminated from the microbial population during silage feeding. Diplodinium sp. progressively decreased (P<.05) from an average of 0.37 x 10^4 ml during the fescue phase to an average of 0.03 x 10^4/ml during the silage phase. Entodinium sp. showed no change with diet. As a result, the total population of protozoa decreased (P<.01) from 8.74 to 5.67 x 10^4/ml during the transition from fescue to silage feeding.

Volatile fatty acids. Individual and total volatile fatty acids are presented by group and treatment in Table 4. Differences between experimental groups were not significant. As with protozoa, the data from the two groups were combined. The total volatile fatty acid concentration increased sharply (P<.05) from 31.1 mM/l on fescue hay to 63.4 mM/l on whole stillage:fescue hay silages. The feeding of fescue hay resulted in rumen volatile fatty acid profile of 23.8 mM/l acetate, 5.02 mM/l propionate, 0.35 mM/l isobutyrate, 1.55 mM/l butyrate, and 0.39 mM/l isovalerate. Valerate was not present in any of the rumen fluid samples from fescue-fed sheep. Feeding whole stillage:fescue hay silages increased the individual concentrations of all volatile fatty acids to give a final profile of 42.1 mM/l acetate, 11.6 mM/l propionate, 0.89 mM/l isobutyrate, 6.84 mM/l butyrate, 1.02 mM/l isovalerate, and 0.94 mM/l valerate. These values are extremely similar to those reported by Muntiferring, et al. (1983) on mature cross-bred wethers fed a 66:34 whole stillage:fescue hay silage.

The concentrations of the volatile fatty acids on a mol/100 mol basis are given in Table 5. Again, no differences were noted between the 70:30 and 65:35 whole stillage:fescue hay silages and the data from the two groups were combined. Of the total volatile fatty acid concentration on fescue hay, 75.1 mol/100 mol was acetate, 16.5 mol/100 mol propionate, 1.5 mol/100 mol isobutyrate, 5.3 mol/100 mol butyrate, and 1.7 mol/100 mol isovalerate. The concentration of acetate on a mol/100 mol basis decreased and that of butyrate increased significantly with the whole stillage to give a final ratio of 66.4 mol/100 mol acetate, 18.4 mol/100 mol propionate, 1.5 mol/100 mol isobutyrate, 10.6 mol/100 mol butyrate, 1.7 mol/100 mol isovalerate and 1.4 mol/100 mol valerate. Again, these values are very similar to those reported by Muntiferring et al. (1983).

DISCUSSION

Ruminal pH. The pH of whole stillage alone was 3.4 and that of the whole stillage:fescue silages 3.8-4.0. However, the natural buffering capacity of the ruminant was quite adequate to maintain the pH of the rumen between 6.4-6.6 even during the silage feeding periods. Therefore, any effects of whole stillage on rumen protozoa populations, volatile fatty acid concentrations, or digestibilities must be independent of pH. The addition of a buffer such as sodium bicarbonate to the diet for the sole purpose of maintaining a physiological rumen pH would seem unnecessary.

Ciliate protozoa. The striking observation with respect to whole stillage:fescue hay silage effects upon protozoa is the complete disappearance of the holotricha and a 90% reduction in the numbers of the diplodinium. Purser and Moir (1966) and Dehority and Purser (1970) reported a negative association of water consumption and holotrich population. The whole stillage:fescue hay silages contain a large quantity of water (65-68%) and, therefore, may have increased the quantity and rate of passage of the liquid particulate fraction resulting in a washing out effect on the holotrich and diplodinium. However, holotrich disappearance may have been due to a lack of soluble carbohydrates with the whole stillage (Hungate, 1966).

Volatile fatty acids. Concentrations of individual and total volatile fatty acids increased significantly with the whole stillage:fescue silage diets. Final concentrations, both mM/l and mol/100 mol, were very similar to values reported on mature crossbred wethers fed a 66:34 whole stillage:fescue hay silage (Muntiferring et al., 1983).

Ruminal volatile fatty acid concentrations are directly related with their production rates. Weston and Hogan (1967) derived an equation estimating total volatile fatty acid production from concentrations. The equation is $Y = 0.068X - 1.75$, where Y is the total volatile fatty acid production in moles/day and X is the volatile fatty acid concentration. Applying this equation to the data from the current study, it can be estimated that volatile fatty acid production increased from 0.37 to 2.56 moles/day as the sheep changed from fescue to whole stillage:fescue diets. Using these estimated production rates, the concentrations in mol/100 mol as a percentage from Table 5 and the gross energy values for acetate (209.4 Kcal/mole), propionate (367.2 Kcal/mole), butyrate (524.3 Kcal/mole), and valerate (681.6 Kcal/mole) from Blaxter (1962) it can be estimated that the energy available from volatile fatty acids increased 7.5 fold from 98 to 743 Kcal/day with the feeding of whole stillage:fescue silage over fescue alone. Furthermore, there was a 5-fold increase in available energy from volatile fatty acids when intake, 481 g dry matter of fescue hay/day and 713 g dry matter of whole stillage:fescue silage/day, was accounted for from 0.2 Mcal/kg dry matter to 1.0 Mcal/kg dry matter. This accounted for 40-50% of the total digestible energy reported earlier (Heitmann and Britton, 1983) for a 70:30 and a 65:35 whole stillage:fescue silage (2.6 and 1.9 Mcal/g dry matter).

Summary. The differences in digestibilities between the 70:30 and 65:35 whole stillage:fescue hay silages observed in an earlier report (Heitmann and Britton, 1983) can not be explained by differences in rumen pH, protozoa, or volatile fatty acids. Protozoa populations decreased with the addition of whole stillage and this could not have been due to pH effects but may have been due to a rumen wash out effect or to a lack of soluble carbohydrates. Importantly, the addition of whole stillage to mature fescue hay and the subsequent ensiling of the product increases rumen volatile fatty acid concentration 2-fold and production and available energy from volatile fatty acids 5-fold after adjustment for feed intake.

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TABLES

TABLE 1

Proximate analysis of whole stillage, fescue hay, and whole stillage:fescue hay silages, %

	Whole Stillage	Fescue Hay	Silages[a], wet basis	
			70:30	65:35
Dry matter	6.9	97.4	32.1	35.2
Crude protein[b]	27.5	7.4	12.5	11.9
Crude fiber[b]	12.0	41.3	31.5	35.0
Ether extract[b]	8.1	1.0	6.8	4.2
Ash[b]	2.7	6.6	6.8	5.1
NFE[b]	54.3	41.1	34.9	34.6
ADF[b]	20.2	48.8	47.8	48.8

[a]Determined after a four-week ensiling period.

[b]Expressed as a percentage of dry matter.

TABLE 2

Ruminal pH, means and standard errors

	Number of Animals	Fescue Hay	Silage Adaptation	Silage
Group 1[a]	4	6.48 ± 0.10	6.48 ± 0.02	6.41 ± 0.10
Group 2[b]	4	6.63 ± 0.04	6.63 ± 0.07	6.51 ± 0.07
Groups 1 & 2	8	6.57 ± 0.05	6.56 ± 0.05	6.47 ± 0.06

[a]Wethers fed the 70:30 whole stillage:fescue hay silage.

[b]Wethers fed the 65:35 whole stillage:fescue hay silage.

TABLE 3

Rumen ciliate protozoa populations (10^4/ml), means and standard errors

	Fescue Hay	Silage Adaptation	Silage Trial
Group 1 (4)[a]			
Isotricha sp.	0.51 ± 0.11	0.00 ± 0.00*	0.00 ± 0.00*
Dasytricha ruminantium	2.75 ± 0.45	0.09 ± 0.08*	0.00 ± 0.00*
Diplodinium sp.	0.32 ± 0.15	0.06 ± 0.06*	0.02 ± 0.02**
Entodinium sp.	4.01 ± 1.58	5.18 ± 2.28	4.97 ± 1.48
Total	7.59 ± 1.93	5.33 ± 2.34*	4.99 ± 1.47*
Group 2 (4)[b]			
Isotricha sp.	0.67 ± 0.14	0.03 ± 0.02*	0.04 ± 0.05*
Dasytricha ruminantium	2.85 ± 1.43	0.02 ± 0.01**	0.00 ± 0.00**
Diplodinium sp.	0.42 ± 0.20	0.18 ± 0.05	0.03 ± 0.02**
Entodinium sp.	5.94 ± 3.19	5.09 ± 1.41	6.10 ± 2.54
Total	9.60 ± 4.47	5.60 ± 1.30	6.18 ± 2.53
Groups 1+2 (8)			
Isotricha sp.	0.60 ± 0.09	0.02 ± 0.01*	0.03 ± 0.02*
Dasytricha ruminantium	2.81 ± 0.79	0.05 ± 0.03*	0.00 ± 0.00*
Diplodinium sp.	0.37 ± 0.12	0.13 ± 0.04*	0.03 ± 0.01*
Entodinium sp.	5.11 ± 1.85	5.13 ± 1.15	5.64 ± 1.47
Total	8.74 ± 2.53	5.48 ± 1.13**	5.67 ± 1.48**

[a]Animals fed the 70:30 whole stillage:fescue hay silage.

[b]Animals fed the 65:35 whole stillage:fescue hay silage.

*Significantly different from fescue hay, P<.05.

**Significantly different from fescue hay, P<.01.

TABLE 4

Volatile fatty acid concentration (mM/1) by group and treatment, means and standard errors

	Fescue Hay	Silage Adaptation	Silage Trial
Group 1[a]			
Acetate	29.1 ± 6.18	33.4 ± 3.85	43.8 ± 4.51*
Propionate	5.81 ± 0.89	7.70 ± 0.70	11.3 ± 0.65*
Isobutyrate	0.35 ± 0.03	0.59 ± 0.03*	0.93 ± 0.13**
Butyrate	1.82 ± 0.23	5.19 ± 0.54*	7.64 ± 1.07*
Isovalerate	0.39 ± 0.07	0.85 ± 0.08*	1.16 ± 0.17*
Valerate	0.00 ± 0.00	0.78 ± 0.13*	1.00 ± 0.13*
Total	37.46 ± 7.22	48.47 ± 5.29*	65.84 ± 6.35*
Ac:Pr	5.01 ± 0.04	4.34 ± 0.06*	3.88 ± 0.13**
Group 2[b]			
Acetate	18.6 ± 1.40	27.5 ± 2.26*	40.3 ± 2.31**
Propionate	4.22 ± 0.33	5.93 ± 0.51	11.9 ± 0.83*
Isobutyrate	0.35 ± 0.03	0.47 ± 0.02*	0.87 ± 0.09**
Butyrate	1.27 ± 0.08	2.81 ± 0.28*	6.03 ± 0.29**
Isovalerate	0.39 ± 0.05	0.56 ± 0.09*	0.87 ± 0.06*
Valerate	0.00 ± 0.00	0.11 ± 0.02	0.88 ± 0.09*
Total	24.81 ± 1.82	37.54 ± 2.69*	60.95 ± 3.40*
Ac:Pr	4.41 ± 0.24	4.64 ± 0.23	3.39 ± 0.36*
Groups 1+2			
Acetate	23.8 ± 2.63	30.4 ± 1.47*	42.1 ± 1.63**
Propionate	5.02 ± 0.29	6.82 ± 0.29	11.6 ± 0.37*
Isobutyrate	0.35 ± 0.02	0.53 ± 0.01*	0.89 ± 0.05**
Butyrate	1.55 ± 0.07	4.00 ± 0.19*	6.84 ± 0.30**
Isovalerate	0.39 ± 0.03	0.76 ± 0.03*	1.02 ± 0.05**
Valerate	0.00 ± 0.00	0.45 ± 0.03*	0.94 ± 0.07**
Total	31.19 ± 2.06	42.96 ± 1.98*	63.45 ± 2.34**
Ac:Pr	4.74 ± 0.11	4.45 ± 0.20	3.63 ± 0.27*

[a]Animals fed the 70:30 whole stillage:fescue hay silage.

[b]Animals fed the 65:35 whole stillage:fescue hay silage.

*Significantly different from fescue hay, P<.05.

**Significantly different from fescue hay, P<.01.

TABLE 5

Volatile fatty acids, molar percent, means and standard errors

	Fescue Hay	Silage Adaptation	Silage Trial
Group 1[a]			
Acetate	75.8 ± 2.38	68.9 ± 0.51*	66.7 ± 2.17*
Propionate	16.2 ± 1.33	16.1 ± 0.69	17.4 ± 1.18
Isobutyrate	1.25 ± 0.43	1.31 ± 0.18	1.49 ± 0.35
Butyrate	5.17 ± 0.72	10.4 ± 0.08*	11.1 ± 1.67*
Isovalerate	1.51 ± 0.72	1.82 ± 0.15	1.81 ± 0.30
Valerate	0.00 ± 0.00	1.39 ± 0.39*	1.43 ± 0.36*
Group 2[b]			
Acetate	74.4 ± 0.66	73.3 ± 0.82	66.0 ± 1.35*
Propionate	16.8 ± 0.85	16.4 ± 1.71	19.4 ± 0.57*
Isobutyrate	1.61 ± 0.31	1.47 ± 0.32	1.44 ± 0.18
Butyrate	5.42 ± 0.58	7.11 ± 0.56*	10.2 ± 0.87**
Isovalerate	1.85 ± 0.52	1.55 ± 0.42	1.55 ± 0.19
Valerate	0.00 ± 0.00	0.19 ± 0.07*	1.43 ± 0.22**
Group 1+2			
Acetate	75.1 ± 1.01	71.4 ± 0.99*	66.4 ± 1.10**
Propionate	16.5 ± 0.69	16.3 ± 0.95	18.4 ± 0.67*
Isobutyrate	1.46 ± 0.24	1.40 ± 0.19	1.46 ± 0.16
Butyrate	5.30 ± 0.38	8.52 ± 0.73*	10.6 ± 0.81**
Isovalerate	1.68 ± 0.39	1.67 ± 0.24	1.66 ± 0.16
Valerate	0.00 ± 0.00	0.79 ± 0.29*	1.43 ± 0.18**

[a]Animals fed the 70:30 whole stillage:fescue hay silage.

[b]Animals fed the 65:35 whole stillage:fescue hay silage.

*Significantly different from fescue hay, P<.05.

**Significantly different from fescue hay, P<.01.