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The impact of feeding condensed distillers byproducts on rumen microbiology and metabolism[1]

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Abstract

In vitro fermentation experiments and a feeding trial were conducted to determine how distillers byproducts impact rumen microbiology and metabolism. The in vitro rate of lactic acid disappearance was not stimulated by direct addition of distillers byproducts to mixed rumen contents collected from a steer adapted to a high concentrate diet. However, if animals were fed condensed distillers byproducts for several weeks, rumen microbiology and metabolism were affected. Culturable counts of starch-degrading and lactic acid utilizing bacteria increased, and this coincided with a two-fold increase in the in vitro rate of lactic acid fermentation. These data suggest distillers byproducts could be used to selectively manipulate the rumen microbial population and improve the capacity of the rumen microorganisms to utilize lactic acid, the causative agent of acute ruminal acidosis and morbidity in cattle.

Keywords: Rumen fermentation; Microbiology; Lactate; Distillers byproducts

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1. Introduction

Feeding rations comprised almost entirely of cereal grain(s) are common in beef feedlot systems and, if improperly managed, can result in ruminal acidosis. Feedlot acidoses are primarily caused by rapid production of organic acids and endotoxins by rumen bacteria, and the ruminal concentration of lactic acid is considered to serve as an index of the severity of acidosis. While the biochemical bases of subacute acidosis are not well defined, the feeding of ionophores has helped to minimize its negative impact upon feedlot profitability (Russell and Strobel, 1989).

In acute conditions, rapid production of both D- and L-isomers of lactic acid 'sour' the rumen fermentation, create a systemic acidosis, and can result in animal death (Slyter, 1976). The acute condition can be avoided by gradual adaptation to high grain diets, which promotes balanced development of lactic acid-producing and -utilizing bacteria (Mackie et al., 1978; Mackie and Gilchrist, 1979). This is currently achieved by replacing forage with starch over a period as long as 28 days, but the length of the adaptation period and the need to handle forage seriously impact feedlot profitability. Alternative methods that result in a more rapid, yet balanced development of the rumen microbiota offers the potential for a more time- and cost-effective means of adaptation to finishing rations.

Grain starch is also used for the production of fuel ethanol, and, as this industry grows, so too will the volume of its byproducts. These byproducts include the unfermented fractions of the grain, as well as the yeast cells plus metabolic intermediates. It is common for the byproducts following ethanol production to be separated into solids and soluble fractions, and the latter is condensed by heating the mixture, generating a viscous end-product known as condensed distillers solubles. The US feedlot industry offers a productive means to dispose of these end-products and a number of feeding trials have been conducted to evaluate the nutritional value of these byproducts (Firkins et al., 1985; Larson et al., 1993). Most, if not all, the attention has focused upon the macronutrients (lipids, protein and cellulose) present in these byproducts, and their impact upon animal growth. However, recent research has indicated that distillers byproducts alter ruminal fermentation patterns, favoring increased molar proportions of propionate (Ham et al., 1994).

Preliminary analyses in our laboratory identified fumarate (0.1–1 mM), malate (1.2–6.2 mM), and succinate (3–16 mM) in several different sources of condensed distillers byproducts. Nisbet and Martin (1990, 1991) have previously reported that malate or fumarate can improve both lactate fermentation and growth of *Selenomonas ruminantium*, a rumen bacterium present in high numbers in grain-fed animals. In addition, we also detected high concentrations of lactic acid in the condensed byproducts, similar to levels reported by Huhtahnen and Nasie (1992). Dietary lactic acid can also elevate the capacity of the rumen microflora to utilize this acid (e.g. Huntington and Britton, 1979b), therefore condensed distillers byproducts might be a useful alternative to forage during adaptation to high grain diets. This may offer the ruminant animal further protection against acute and subacute acidosis, enhance feedlot performance, and improve profitability. The following studies were undertaken to determine whether and how distillers byproducts impact rumen microbes and metabolism.

2. Materials and methods

2.1. In vitro studies

Rumen contents were collected from a ruminally cannulated steer fed a dry-rolled corn diet every 2 h using a timed feeder. Rumen contents were mixed by hand and samples were taken from different sites until a 4 liter plastic bucket was filled. The collected material was filtered through two layers of cheesecloth to remove large feed particles, and the fluid was collected in a 1 liter glass container. The container was then sealed and transferred to an anaerobic chamber filled with a CO_2/H_2 (96:4 v/v; Coy Instruments, Ann Arbor, MI), and 400 ml was processed for 1 min using a high speed homogenizer (Brinkmann Instruments, Westerbury, NY). Triplicate 85 ml aliquots of the homogenized material were dispensed into 110 ml serum bottles (Bellco Glass, NJ), which contained 15 ml of either deionized water (control), condensed distillers solubles, or uncondensed distillers solubles, and then sealed with rubber stoppers. The sealed bottles were removed from the anaerobic chamber and placed in a 39°C water bath.

After temperature equilibration, 5 ml aliquots were removed from each bottle prior to addition of 1 ml of 1 M L(+)-lactic acid (Sigma Chemical Co., St. Louis, MO). Subsequently, 5 ml samples were taken at 10 min intervals for 1 h. Each individual sample was immediately placed into a centrifuge tube containing 0.1 ml of concentrated H_2SO_4 and the acidified mixture was centrifuged at $10\,000 \times g$, at 4°C for 15 min. The supernatant was then frozen, thawed and re-centrifuged to remove precipitated proteins. Supernatants were stored at −20°C until analysed for lactic acid.

2.2. Animals and diets

Six ruminally cannulated steers (460 kg average weight) were maintained in individual stalls and a controlled environment. Diets (Table 1) were fed to meet ad libitum intake once daily at 08:00 h with orts removed twice weekly. Following an adaptation period of 23 days, all six animals were fed the dry-rolled corn (DRC-control) diet, with Rumensin and Tylan added (Elanco, Indianapolis, IN). After 4 weeks, the rumen contents of all the animals were sampled by procedures described above. Subsequent to this sampling, three steers (referred to as Group A) continued on the same DRC-control diet, while the three remaining animals (Group B) were placed on a final diet to which condensed distillers solubles were added as 15% of the ration dry matter (replacing corn; Table 1). The byproducts (corn-based) were obtained from Chief Ethanol Fuels (Hastings, NE). The animals were maintained on these diets for an additional 5 weeks, and the rumen contents were again sampled for estimation of microbiological counts and in vitro rates of lactic acid disappearance.

2.3. Microbiological counts

The media used to enumerate total culturable, amylolytic and lactilytic bacteria are described in Table 2. The plate media were prepared at least 48 h prior to use and were stored at 39°C. On both sampling days, total rumen contents were collected from each

Table 1
Composition of diets fed to animals

Item	Treatment [a]	
	DRC control	15% CDS
Dry-rolled corn	78.87	63.87
Condensed solubles [b]	–	15.00
Alfalfa hay	10.00	10.00
Liquid 32 [c]	4.13	4.13
Cane molasses	2.00	2.00
Supplement	5.00	5.00
Finely ground corn	3.14	3.14
Limestone	1.20	1.20
Dicalcium phosphate	0.16	0.16
Potassium chloride	0.18	0.18
Salt	0.30	0.30
Rumensin premix [d]	0.02	0.02
Tylan premix [e]	0.01	0.01

[a] % dry matter (DM) basis: DRC, dry-rolled corn; CDS, condensed solubles.
[b] CDS contains (% DM basis): 23% crude protein (CP), 3.6% neutral fiber, 21.6% fat, 14% starch, 6.6% ash.
[c] Contains (% DM basis): 50.6% CP (as urea), 0.55% P, 0.95% Ca, 2.61% K, 0.35% Mg, 90000 IU vitamin A, 18000 IU vitamin D, 22 IU vitamin E (kg^{-1} DM).
[d] 132 g of monensin kg^{-1} premix.
[e] 88 g of tylosin kg^{-1} premix.

Table 2
Composition of media used to enumerate total culturable, amylolytic and lactilytic bacteria

Ingredient [b]	Reference	Selective medium [a]		
		Total culturable	Amylolytic	Lactilytic [d]
Clarified rumen fluid (ml 100 ml^{-1})		40	40	–
Mineral solution I [c] (ml 100 ml^{-1})		2.5	2.5	2.5
Mineral solution II [c] (ml 100 ml^{-1})		2.5	2.5	2.5
Glucose		0.05	–	–
Cellobiose		0.05	–	–
Xylan		0.05	–	–
Starch		0.05	0.3	–
Maltose		0.05	–	–
Yeast extract		–	–	0.2
Trypticase		0.05	0.05	2.0
DL-Lactic acid [e]	Mackie and Heath (1979)	0.2	–	2.0
Volatile fatty acids	Caldwell and Bryant (1966)	–	–	1.0
Trace elements	Schaefer et al. (1980)	–	–	1.0
Hemin	Caldwell and Bryant (1966)	–	–	2.0

[a] Sodium carbonate, resazurin and agar were added to all media at 0.4 g, 0.0001 g and 1.5 g 100 ml^{-1}, respectively, and deionized water was used to make up to volume.
[b] Expressed as g 100 ml^{-1} unless otherwise indicated.
[c] Final concentration and composition identical to that of Caldwell and Bryant (1966).
[d] Modified from Mackie and Heath (1979).
[e] Neutralized with sodium hydroxide.

animal 4 h post-feeding and pH was measured within 5 min of collection. Rumen contents were mixed and a 10–15 g portion was added quantitatively to a 250 ml erlenmeyer flask while being flushed with a continuous stream of CO_2. Each flask was then transferred to the anaerobic chamber, and the contents were diluted ten-fold with sterile anaerobic diluent (Table 2). The diluted contents were then homogenized for 1 min, and serial, ten-fold dilutions in sterile diluent were made in 18 mm × 150 mm anaerobic tubes (Bellco, NY), fitted with butyl rubber stoppers.

For each animal, 0.1 ml aliquots of 10^{-6} through 10^{-10} dilutions were spread in triplicate on the selective plate media using sterilized glass spreaders. Plates were then incubated at 39°C in a CO_2 atmosphere for 7 days until colony forming units (CFU) were estimated. Counts are based upon the lowest dilution that provided at least 20 CFU per plate.

Direct counts of protozoa were determined from a second sample (1–1.5 g) of mixed rumen contents, which was preserved in methylene blue/formal saline solution (Ogimoto and Imai, 1981). Protozoal counts were determined using a Sedgewick Rafter counting chamber (Clay Adams, NY) using previous described methods (Purser and Moir, 1959). For each sample, 30 fields of view (10 fields × 3 separate fillings) were counted.

2.4. Measurement of lactic acid and other fermentation products

L-Lactic acid in collected samples was determined by measuring the increase in NADH following the addition of β-NAD^+ and L(+)-lactate dehydrogenase (LDH) to collected samples, following previously described procedures (Huntington and Britton, 1979b). Each sample was corrected for non-specific NAD^+ reduction by subtracting the activity in similarly prepared tubes lacking LDH. Total lactic acid was quantified by high performance liquid chromatographic analysis. A Waters high performance liquid chromatograph equipped with a HPX-87H Bio-Rad column (Richmond, CA) and UV detector (210 nM) was used. The mobile phase consisted of degassed, filtered 0.015 N H_2SO_4, and the column was operated at a flow rate of 0.7 ml min^{-1} at 50°C.

Volatile fatty acids were quantified by gas chromatography using previously described methods for sample preparation and detection (Erwin et al., 1961; Harmon et al., 1985).

2.5. Statistical analyses

Lactate disappearance rates were calculated by regression of time points taken within the linear range of the assay. Differences between sampling periods within groups of total culturable, differential counts of bacteria, total protozoa, LDH activity and in vitro rates of lactate disappearance were analyzed according to the following model: $y_{ij} = \mu + \alpha_i + e_{ij}$, where α_i is the effect of the ith treatment ($i = 1,2$), and e_{ij} is the random residual. All statistical analyses were performed using software of the Statistical Analysis Systems Institute Inc. (1985). Differences are considered to be statistically significant where the probability of a Type I error was calculated to be less than 10%.

3. Results

3.1. In vitro experiments

The disappearance rate of added lactic acid from mixed rumen contents in vitro was measured when either water, condensed solubles or uncondensed solubles were added to these incubations at a final concentration of 15% (v/v). Mean disappearance rates were determined to be 0.10, 0.08, and 0.07 mmol min^{-1} for the control, condensed solubles and thin stillage additions, respectively (Fig. 1), and these differences were not statistically significant. Addition of the condensed solubles increased the L-lactic acid concentration in these incubations to approximately 27 mM, but this appeared to have minimal effects upon the fermentative capacity of the microbial population. The volatile fatty acid analysis of the samples collected in these and other initial in vitro experiments showed that, as lactate was fermented, the concentrations of butyrate and propionate tended to increase (data not shown). These observations are consistent with the pathways of lactate fermentation utilized by lactilytic bacteria such as *S. ruminantium* (Nisbet and Martin, 1990, 1991) and *Megasphaera elsdenii* (Counotte et al., 1981).

3.2. Microbiological studies

The pH of rumen contents ranged between 5.3 and 5.8 4 h post-feeding, and there appeared to be no treatment effect. Although detailed measurements were not made on a

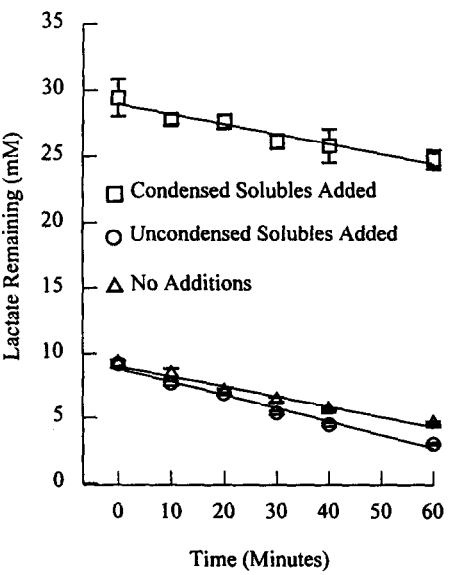

Fig. 1. The in vitro rates of lactic acid disappearance (expressed as mmol min^{-1}) from rumen fluid with either distilled water, condensed solubles or thin stillage added to 15% (v/v).

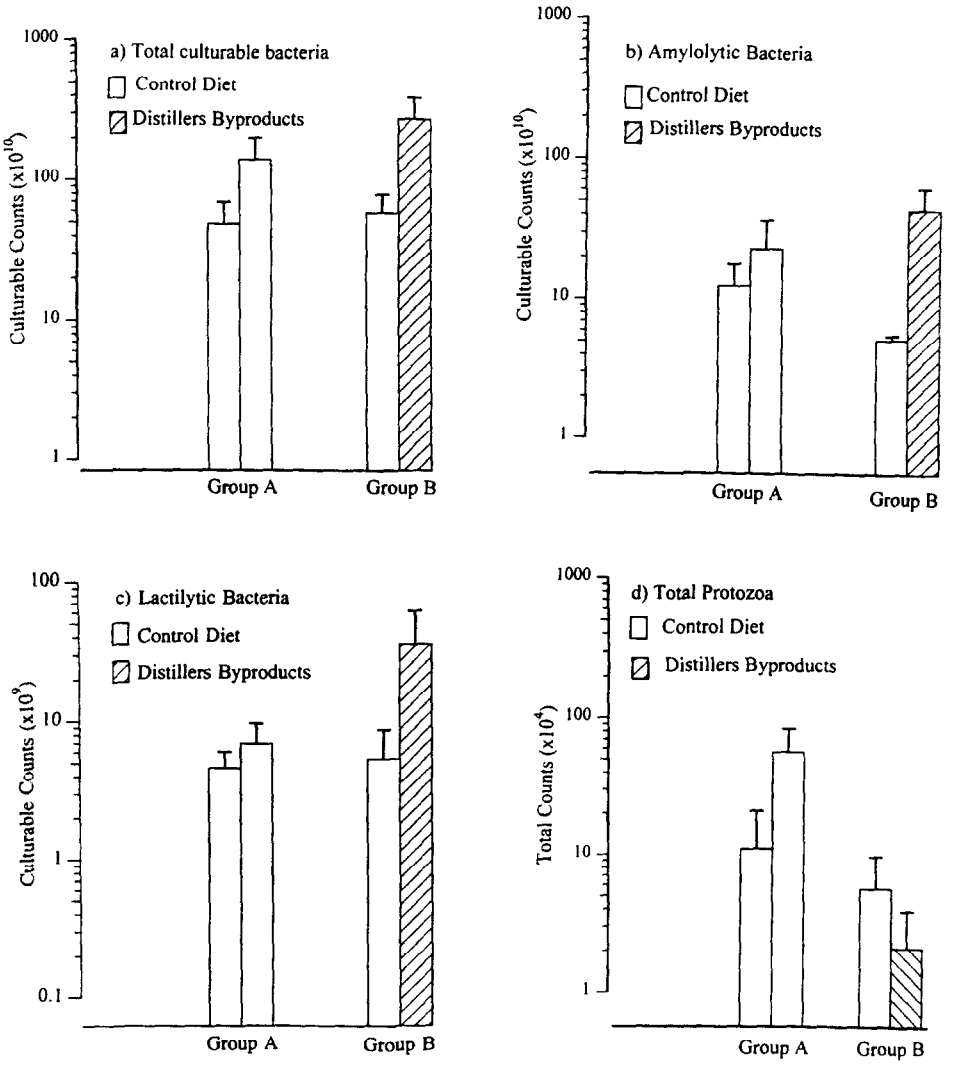

Fig. 2. Culturable counts of (a) total, (b) amylolytic (starch-degrading), and (c) lactilytic (lactic acid-utilizing) rumen bacteria, plus (d) ciliate protozoa, in rumen digesta collected from animals fed either a DRC-control diet, or a diet containing condensed solubles at 15% of dietary DM. Animals in Group A were fed the DRC-control diet throughout the entire experimental period. Animals in Group B were fed the same DRC-control diet for 4 weeks (open bar) then fed the condensed solubles containing diet for a period of 5 weeks (cross-hatched bars). The bars represent the means (+SEM) from the two sampling periods.

daily basis, the animals maintained consistent levels of feed intake throughout the experiment, suggesting that there were no obvious signs of acidosis.

Counts of total culturable bacteria were found to be very high in both groups of animals (range $48–273 \times 10^{10}$ g^{-1}; Fig. 2(a)), and estimates were highest in the second sampling period. The increases in amylolytic (starch-degrading) and lactilytic (lactate-

Table 3
The mean (± SEM) rates of lactate disappearance (mmol min^{-1}) from the in vitro incubations of rumen fluid to which 10 mM L(+)-lactate was added [c]

	Group A	Group B
Period 1	0.18 ± 0.065 [a]	0.17 ± 0.026 [a]
Period 2	0.14 ± 0.016 [a]	0.38 ± 0.045 [b]

[a,b] Values with unlike superscripts differ ($P < 0.07$).
[c] Animals in Group A were fed dry-rolled corn during both sampling periods. Animals in Group B were fed dry-rolled corn (Period 1) then switched to a diet containing 15% (DM basis) condensed distillers byproducts. Values are the means of three animals and triplicate observations.

utilizing) bacteria tended to be greater in the animals fed condensed distillers byproducts (Group B; Fig. 2(b) and 2(c)). The mean culturable counts of amylolytic and lactilytic bacteria were increased in Group B from 5.2 to 43.3×10^{10} g^{-1}, and from 5.3 to 35.4×10^{9} g^{-1}, respectively. This can be compared to mean counts in Group A which increased from 12.3 to 22.7×10^{10} g^{-1}, and from 4.6 to 6.9×10^{9} g^{-1}, for amylolytic and lactilytic bacteria, respectively. The correlation between the culturable numbers of amylolytic bacteria and lactilytic bacteria was also high ($r^2 = 0.79$; $P < 0.05$). Protozoa concentrations in two of the three animals in Group B were below 5×10^{3} g^{-1} upon feeding condensed distillers byproducts (Fig. 2(d)). This compares to the protozoa concentrations in animals of Group A ranging between 3.0×10^{4} and 8.5×10^{5} g^{-1}.

In vitro rates of lactate disapppearance rates for both groups of animals in both sampling periods are shown in Table 3. The capacity of the rumen microbes to ferment lactate was increased approximately two-fold ($P < 0.07$) in animals fed condensed distillers byproducts, and a statistically significant correlation was also found to exist between culturable numbers of lactilytic bacteria and the in vitro rate of lactic acid disappearance ($r^2 = 0.65$; $P < 0.05$). There was little difference in LDH specific activity throughout the experiment.

4. Discussion

Adaptation of ruminant animals to diets containing large amounts of starch has been shown to be largely a function of changes in the rumen microflora (Slyter, 1976; Mackie et al., 1978; Mackie and Gilchrist, 1979; Leedle et al., 1982). Huntington and Britton (1979a,b) showed that the in vitro fermentation rate of lactic acid could be increased by intraruminal infusion of lactic acid for several days prior to sampling rumen contents. These workers also showed that, when animals were rapidly changed from a high roughage to a high concentrate diet, animals infused with lactic acid prior to grain feeding were able to maintain a higher ruminal pH, and were less prone to acute acidosis. Therefore, ways of rapidly increasing the population of lactilytic bacteria in the early stages of adjustment to a high grain diet remains an attractive hypothesis, to control acidosis.

During the preliminary stages of this work, three different sources of byproducts were found to contain in excess of 100 mM lactic acid. Moreover, other workers have determined similar concentrations in condensed byproducts resulting from yeast fermentaton of barley grain (Huhtahnen and Nasie, 1992). While the high concentrations of lactic acid in distillers byproducts appears to be fairly common, the origin of this fermentation end-product remains obscure. The most likely explanation in the authors' opinion is that lactic acid bacteria contaminate the byproducts and ferment any residual sugars to lactic acid.

Water-soluble extracts from commercial fungal and yeast probiotic cultures, as well as malate and fumarate, stimulate lactate utilization and cell yield of *S. ruminantium* (Nisbet and Martin, 1990, 1991). Our preliminary analyses showed that both types of distillers byproducts contained detectable (millimolar) concentrations of these dicarboxylic acids. Therefore, the in vitro studies were conducted to determine whether distillers byproducts had an immediate, stimulatory effect upon ruminal fermentation of lactate. The disappearance rates were consistent with previously reported values for animals fed grain based diets (Kunkle et al., 1976) and there were no significant differences between treatments. Based upon these findings, it seems likely that the concentrations of dicarboxylic acids required for an immediate stimulation of ruminal lactate fermentation can not be met by the current levels of byproduct feeding, or the number of *S. ruminantium* and other lactilytic bacteria that utilize the C_4 randomizing pathway for lactate fermentation were low in the rumen contents of the donor animal. That butyrate concentration also increased as lactate was fermented supports the contention that *M. elsdenii* was an important member of the rumen lactilytic population in these initial in vitro experiments.

Culturable counts of bacteria, as well as ciliate protozoa, were determined from digesta samples collected from the six animals at a time post-feeding when ruminal fermentation was likely to be very active. This is reflected in ruminal pH being close to the pK_a values for acetic, propionic and butyric acids and the high numbers of culturable bacteria. Moreover, the contents of the ventral ruminal sacs of most animals seemed to be dry, indicative of less saliva and buffering capacity introduced to the rumen reticulum when animals are fed grain-rich diets. Samples of rumen contents were used as collected from the animals, and were treated with a high speed homogenizer, which is very effective at removing bacteria from plant tissue. Plates were also allowed to incubate for a period of 1 week prior to making counts of CFU. All these factors probably had an impact upon the high numbers of total culturable bacteria per gram of contents. Between 9% and 25% of the total culturable population were amylolytic, while the lactilytic population increased from 0.5% to 1.3% of total culturable bacteria when condensed byproducts were fed. This range in values is similar to those obtained from sheep fed varying amounts of grain–molasses (Mackie and Gilchrist, 1979), and upon which the sampling and cultivation methods used in this study are based. Moreover, the correlation between in vitro rates of lactic acid disappearance and the culturable counts of lactilytic bacteria was also high and statistically significant. Thus, feeding condensed distillers byproducts appears to enrich for a larger population of lactilytic bacteria in the rumen, facilitating faster rates of lactate utilization.

All animals possessed ciliated protozoa, and populations were largely comprised

Entodinia, although large holotrichs were occasionally observed. While there appeared to be no effect from feeding condensed solubles upon the types of protozoa present (data not shown), for two out of three animals ciliate protozoal numbers decreased ten- to 100-fold. Dietary lactic acid and low ruminal pH can reduce rumen protozoal populations below detectable concentrations, which permits an increase in both the numbers of amylolytic bacteria and the rate of ruminal starch digestion (Mackie et al., 1978; Mendoza et al., 1993). Our findings are consistent with these previous microbiological observations.

5. Conclusions

The studies presented here show that distillers byproducts contain appreciable quantities of nutrients in addition to protein, structural polysaccharides and lipids, which can affect rumen microbiology and fermentation. The results of the in vitro and animal studies support the contention that distillers byproducts enhance the capacity of the microbial population to utilize lactic acid, by increasing the relative numbers of lactilytic bacteria. Therefore, one possible use for these byproducts may be as an alternative to forage during adaptation periods to high grain diets.

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