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Effects of *Yucca schidigera* Based Feed Additive on In Vitro Dry Matter Digestibility, Efficiency of Microbial Production, and Greenhouse Gas Emissions of Four Dairy Diets

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Abstract: The present study evaluated the effects of a feed additive (synthesized from *Yucca schidigera*) on some fermentation variables. In the first of two experiments, seven concentrations of the feed additive were evaluated using the in vitro batch culture technique to determine the optimum dose to use in the second experiment. The substrates used were a total mixed ration (TMR) and alfalfa hay. The levels of inclusion were 0 (control), 0.5, 1, 2, 4, 6, and 8 g/head/d. After this initial evaluation, 2 g/head/d was selected for the second experiment. For the second study, four dietary substrates (two corn silages and two TMR; collected from different dairy farms in the Piedmont, North Carolina, area) were used. Incubation times were 3, 6, and 24 h and treatments were 0 (control) and 2 g/head/d of the feed additive. Inclusion of the feed additive did not affect ($p > 0.05$) in vitro dry matter disappearance. Additionally, the feed additive had no effect ($p > 0.05$) on short-chain fatty acid concentrations, microbial mass, and efficiency of microbial production. Methane production was reduced by 22.7% with feed additive inclusion. Similarly, lower ($p = 0.013$; 18%) carbon dioxide concentration was observed in the feed additive treatment. Ammonia and hydrogen sulfite concentrations were similar ($p > 0.05$) for both treatments. Inclusion of the feed additive at 2 g/head/d decreased methane and carbon dioxide concentrations in most of the diets. The energy saved by reducing the amount of methane produced was not partitioned into valuable products such as short-chain fatty acids and microbial mass.

Keywords: batch culture; feed additives; ammonia; methane; saponin

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1. Introduction

According to an Environmental Protection Agency report, cattle are estimated to produce 212 Mt of CO_2eq per year in the United States, representing about 3.4% of total greenhouse gas (GHG, CH_4, N_2O, and CO_2) emissions [1]. Although beef cattle production contributes about 17 kg CO_2eq/kg carcass beef to as high as 40 CO_2eq/kg carcass beef [2], dairy cattle remain the major contributor to total GHG emissions [1]. With increasing populations and changes toward animal-based foods, there will be increased demand for livestock products [3] with a concomitant increase in food-related GHG emissions. There are many strategies and solutions being put forward as potential approaches to mitigate GHG emissions. One such solution is that reducing animal products (meat, milk, etc.) consumption will reduce GHG emissions by about 0.7 to 7.3 gigatons CO_2eq yearly [4]. Another is that the introduction of a meat and dairy tax could cut carbon emissions by up to 1 billion metric tons by changing consumer demand and could be an environmentally substantial strategy to mitigate GHG emissions [5]. Mitigating GHG emissions in ruminants without reducing productivity will help reduce global GHG emissions.

Inclusion of feed additives in the diets of ruminants can minimize energy losses such as CH_4 and ammonia nitrogen, both of which reduce animal performance and contribute to

GHG emissions in the environment [6]. Plant secondary metabolites have well-recognized antimicrobial and antiprotozoal activities and may help to control ruminal bacteria and protozoa [7,8]. They have been known to have both inhibitory and stimulatory effects on rumen microbial activities [9], with dose-dependent effects. Studies have shown that plant secondary metabolites can be species-specific [10]. For example, saponins have been known to stimulate the growth of *Prevotella ruminicola* but inhibit the growth of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* [10]. A major source of saponins for both production and experimental purposes is a desert plant, *Yucca schidigera*. We hypothesize that improved feed efficiency and abatement of GHG emissions can be achieved by using a *Y. schidigera* based feed additive. The present study also evaluated the effect of saponins from *Y. schidigera* on similar substrates. Our specific objectives were to (1) identify the optimum dose of the additive, (2) determine dry matter degradation and fermentation gas kinetics, (3) estimate the efficiency of microbial production, (4) estimate total short-chain fatty acids, and (5) estimate the greenhouse gas emissions in four dairy cow diets.

2. Materials and Methods

All animal procedures were approved by the North Carolina A&T Institutional Animal Care and Use Committees prior to experimentation.

2.1. Batch Culture Study

An initial in vitro batch culture study was conducted for optimum dose determination of a feed additive, Saport (Saport®, Land O'Lakes, Inc., Arden Hills, MN, USA). The study was arranged as a 2 (treatments) × 2 (substrates) × 6 (doses) factorial design. Treatments were *Hibiscus sabdariffa* and Saport. Substrates were alfalfa hay and a total mixed ration (TMR). The TMR consists of grain products, processed grain byproducts, plant protein products, roughage products, molasses products, and multivitamin and mineral supplements. Inclusion levels of the feed additive were at 0, 0.5, 1, 2, 4, 6, and 8 g/head/d. The dose that produced the least greenhouse gases with higher DM disappearance was selected for the second study. At the end of the study, 2 g/head/d was selected for the second study. Both studies were conducted similarly. The second study was arranged as a 2 (treatments) × 4 (substrates) factorial design. Treatments were 0 (control) and 2 g/head/d of the feed additive and substrates were 2 corn silages and 2 TMR collected from dairy farms in the Piedmont, North Carolina, area.

About 0.5 ± 0.05 g of the substrates was weighed using a Mettler XPR 204S balance scale (Mettler-Toledo, Columbus, OH, USA) into triplicate Ankom bags (F57, ANKOM Technology, Macedon, NY, USA) and sealed. The bags were previously soaked in acetone for 3–5 min and air-dried in a Fume hood for approximately 2 h before further drying overnight in an oven. The sealed bags were folded vertically and put inside 100 serum bottles. Rumen fluids were sampled 3 h after feeding from 2 canulated Holstein dairy cows (5 years; 900 ± 70 kg BW) fed corn silage and alfalfa hay and 18% protein supplement. The cows were dry when rumen fluid was sampled from them. The rumen fluid was filtered through 4 layers of cheesecloth, and the pH was measured immediately with a pH meter (B20PI, SympHony Benchtop Meters, VWR, West Chester, PA, USA).

Approximately 45 mL of McDougall's artificial saliva and 15 mL of strained ruminal fluid were dispensed into each serum bottle. The bottles were immediately sealed and incubated for 24 h at 39 °C. Gas production was measured using a manometer at 3, 6, and 24 h. An aliquot of the total gas was sampled and used to estimate methane, CO_2, ammonia, and hydrogen sulfide concentrations. Dry matter disappearance was determined after 6 and 24 h. Additionally, total short-chain fatty acids (SCFA) concentration, microbial mass, and efficiency of microbial production were determined after 24 h. Details on how in vitro true and apparent dry matter digestibility, efficiency of microbial production (PF; partitioning factor), microbial mass, and short-chain fatty acids are analyzed and calculated have been described previously by Anele et al. [11]. The batch fermentation was repeated on a different day to make 2 replications.

2.2. Chemical Analyses

Substrates were analyzed as per the procedures of AOAC [12] for DM (976.63), N (2001.11), and ether extract (EE; 2003.06). Neutral detergent fiber was determined according to the procedures of [13] using heat-stable α-amylase (ANKOM Technology, Macedon, NY, USA) with sodium sulfite. The aNDF value was expressed inclusive of residual ash.

2.3. Statistical Analysis

Data generated were subjected to the MIXED procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA) in a 2 × 4 factorial arrangement. Treatment effects and interactions were examined by using the probability of difference (PDIFF) option. Means were separated using Fisher's LSD, and significant differences were declared at $p \leq 0.05$.

3. Results

The chemical composition of the substrates used in the present study is presented in Table 1. The choice of similar substrates (corn silage 1 and 2 and TMR 1 and 2) did not result in similar chemical composition for the two silages and TMR. The CP content varied from 7.63% DM in one of the corn silages to 27.1% DM in one of the TMR. Neutral detergent fiber content was lower, and EE content was higher for the TMR versus corn silage. Inclusion of the feed additive had no effect ($p > 0.05$) on asymptotic gas, rate of gas production, and lag time (Table 2). Greater ($p < 0.05$) asymptotic gas and rate of gas production were noted for the TMR substrate compared to corn silage. No difference was noted for lag time. Differences were also noted within the substrates (corn silage_1 vs. corn silage_2 and TMR_1 vs. TMR_2).

Table 1. Chemical composition (% DM) of substrate.

	Dry Matter (g/kg)	Crude Protein	Ether Extract	NDF [1]
Corn silage_1	975	7.63	3.42	46.2
Corn silage_2	875	8.29	4.86	60.5
Total mixed ration_1	972	22.9	10.2	25.0
Total mixed ration_2	919	27.1	12.1	29.2

[1] NDF, neutral detergent fiber.

Table 2. Effects of treatments on fermentation characteristics of four dairy diets.

		M [1]	K [2]	L [3]
Corn silage_1	Control	136	6.45	1.63
	Saport	119	8.44	2.01
Corn silage_2	Control	116	11.3	2.56
	Saport	115	9.22	2.17
Total mixed ration_1	Control	150	14.2	2.10
	Saport	137	12.9	1.74
Total mixed ration_2	Control	140	13.8	2.06
	Saport	147	14.0	2.02
Average (Substrate)	Corn silage	122 [b]	8.83 [b]	2.09
	TMR	143 [a]	13.7 [a]	1.98
Average (Treatment)	Control	135	11.4	2.09
	Saport	130	11.1	1.98
SEM [4]		5.2	0.939	0.403
LSD [5] (Interaction)		35	7.72	0.933
p-value (Main effect of substrate)		0.004	<0.001	0.572
p-value (Main effect of treatment)		0.290	0.679	0.728
p-value (Interaction)		0.384	0.189	0.751

Significant differences in each column are indicated by different superscripts ($p < 0.05$). [1] M, asymptotic gas; [2] k, rate of gas production; [3] L, lag time; [4] SEM, standard error of means; [5] LSD, Fisher's least significant difference.

Except for TMR_2, inclusion of the feed additives decreased (22.7%; $p < 0.001$) methane production in all the other diets (Table 3). Similarly, lower ($p = 0.013$; 18%) carbon dioxide concentration was observed in the feed additive treatment. Ammonia and hydrogen sulfite concentrations were not affected ($p > 0.05$). For the main effect of substrate, higher ($p < 0.001$) methane, carbon dioxide, ammonia, and hydrogen sulfite concentrations were consistently noted for TMR versus corn silage.

Table 3. Effects of treatments on in vitro methane (CH_4, mg/g DM), CO_2 (mg/g DM), ammonia (NH_3, mmol/g DM), and hydrogen sulfide (H_2S, mmol/g substrate DM) of four dairy diets.

		CH_4	CO_2	NH_3	H_2S
Corn silage_1	Control	2.68	33.9	1.06	0.95
	Saport	1.78	23.5	0.73	0.57
Corn silage_2	Control	2.42	31.9	1.15	0.97
	Saport	2.00	26.1	1.16	0.73
Total mixed ration_1	Control	4.99	43.8	1.89	1.71
	Saport	3.11	33.3	1.56	1.46
Total mixed ration_2	Control	3.64	42.5	1.87	1.56
	Saport	3.72	42.4	1.75	1.54
Average (Substrate)	Corn silage	2.22 ^{b}	28.9 ^{b}	1.03 ^{b}	0.80 ^{b}
	TMR	3.87 ^{a}	40.5 ^{a}	1.77 ^{a}	1.57 ^{a}
Average (Treatment)	Control	3.43 ^{a}	38.0 ^{a}	1.49	1.29
	Saport	2.65 ^{b}	31.3 ^{b}	1.30	1.07
[1] SEM		0.469	6.49	0.233	0.245
[2] LSD (Interaction)		3.22	18.9	1.16	1.15
p-value (Main effect of substrate)		<0.001	<0.001	<0.001	<0.001
p-value (Main effect of treatment)		0.019	0.013	0.247	0.203
p-value (Interaction)		0.190	0.466	0.858	0.905

Significant differences in each column and for each main effect are indicated by different superscripts ($p < 0.05$). [1] SEM, standard error of means; [2] LSD, Fisher's least significant difference.

Inclusion of the feed additive had no effect ($p > 0.05$) on dry matter disappearance (Table 4). Additionally, the feed additive had no effect ($p > 0.05$) on the concentrations of short-chain fatty acids, microbial mass, and partitioning factor. Similar to the trend noted for methane, carbon dioxide, ammonia, and hydrogen sulfite concentrations, TMR also had higher ($p < 0.001$) apparent and truly degraded DM, efficiency of microbial production, microbial mass, and short-chain fatty acid concentrations compared to corn silage.

Table 4. Effects of treatments on in vitro apparent dry matter digestibility (IVADMD) and in vitro true dry matter digestibility coefficients (IVTDMD), efficiency of microbial production (PF; partitioning factor, mg/mL), microbial mass (Mmass, g/kg DM), and short-chain fatty acids (SCFA, mmol/g DM) of four dairy diets.

		IVADMD	IVTDMD	PF	Mmass	SCFA
Corn silage_1	Control	0.443	0.635	1.93	197	3.96
	Saport	0.473	0.624	1.91	164	3.93
Corn silage_2	Control	0.436	0.589	1.50	143	4.36
	Saport	0.462	0.603	1.49	156	4.43
Total mixed ration_1	Control	0.595	0.896	2.41	309	4.52
	Saport	0.580	0.887	2.33	308	4.50
Total mixed ration_2	Control	0.546	0.846	2.45	288	3.99
	Saport	0.561	0.846	2.28	273	4.20
Average (Substrate)	Corn silage	0.453 ^{b}	0.613 ^{b}	1.71 ^{b}	165 ^{b}	4.17 ^{b}
	TMR	0.570 ^{a}	0.868 ^{a}	2.37 ^{a}	294 ^{a}	4.30 ^{a}
Average (Treatment)	Control	0.505	0.741	2.07	234	4.21
	Saport	0.519	0.740	2.00	225	4.27

Table 4. *Cont.*

	IVADMD	IVTDMD	PF	Mmass	SCFA
[1] SEM	0.0139	0.0130	0.085	12.3	0.150
[2] LSD (Interaction)	0.160	0.307	0.96	166	0.59
p-value (Main effect of substrate)	<0.001	<0.001	<0.001	<0.001	0.002
p-value (Main effect of treatment)	0.165	0.847	0.247	0.308	0.572
p-value (Interaction)	0.361	0.764	0.762	0.285	0.833

Significant differences in each column are indicated by different superscripts ($p < 0.05$). [1] SEM, standard error of means; [2] LSD, Fisher's least significant difference.

4. Discussion

Results from the present study showed that the feed additive did not have the same effect on similar substrates (corn silages (2) or TMR (2)). This could be explained by the differences in their chemical composition. The additive has a high content of saponins, which are known to have a depressing effect on gas production, protein degradation, total VFA, and microbial population [14–16]. Contrary to expectation, the feed additive did not decrease asymptotic gas in any of the substrates in the present study. One could only speculate why the additive did not reduce but rather increase asymptotic gas in TMR_2. However, considering what is known about in vitro gas production, one could argue that a higher content of crude protein and neutral detergent fiber in TMR_2 versus TMR_1 could be responsible for the observed higher asymptotic gas. This is consistent with the general knowledge that only fermentable substrates contribute to gas production. The assumption that diets with higher fermentable substrates produce the most gas is consistent with the diets in the present study. Rate of gas production was also numerically lower for the feed additive treatment compared with the control. This is consistent with the saponin effect on the microbial population, which could have resulted in a reduction in their activities. Shorter lag time was also noted with the feed additive treatment.

Consistent with other studies, the saponin-based treatment reduced both methane and carbon dioxide concentrations in most of the diets [14,17]. Contrary to the assumption that substrates with a higher fiber content tend to produce more methane, both corn silage_1 and TMR_1 (with a lower NDF content) produced more methane than corn silage_2 and TMR_2 (with a higher NDF content). Another factor to consider is that both diets (corn silage_1 and TMR_1) had a lower ether extract content compared to the other two diets. Lipids are known to have a depressing effect on gas production [9]. The 22.7% reduction in methane concentration is similar to values (22–26%) reported by different authors [18,19]. Saponins are known to decrease methane concentration by decreasing the population and activities of methanogens [14,18]. Saponins are also known to have negative effects on protozoa resulting in a reduction in methane production [14]. Although saponins from the feed additive lowered methane production, the reduction could also be associated with reduced gas production noted with the feed additive inclusion. Previous studies reported saponins can alter the hydrogen utilization pathway resulting, in a lower concentration of H^+ available to methanogens with a concomitant reduction in methane production [20,21].

The 13% reduction in ammonia concentration with the feed additive inclusion noted in the present study is consistent with several other previous studies [14,17,22]. The magnitude of ammonia reduction due to saponin inclusion depends on the chemical structure, source, level of inclusion, and diet type [10,22]. Ammonia concentration was not reduced at a similar rate within the same diets (corn silage or TMR). Ammonia was reduced the most in corn silage_1 (31%), followed by TMR_1 (17%) and TMR_2 (6%). There was a slight increase of 0.01 mol/g DM in corn silage_2 (−0.01%). Ammonia production is a function of microbial breakdown of dietary protein, and contrary to this statement, the diets in the present study did not follow this trend. It could also be that diets with higher protein contents had a lower rate of protein degradation. Antiprotozoal activity

of saponins could also be likely responsible for the reduction in ammonia concentration of the substrates. Ruminal protozoa are responsible for approximately 10–40% of total ruminal ammonia concentration [23]. The concentration of hydrosulfide followed a similar trend as the ammonia concentration, with lower values noted with the inclusion of the feed additive.

In the present study, inclusion of the feed additive had no significant effect on major fermentation products such as microbial mass and total short-chain fatty acids. Ref. [24] reported an increase in apparent degraded DM with increasing levels of saponins (5–20 μL/g DM). Consistent with our observation, [22] reported that saponin inclusion did not affect apparent degraded DM.

5. Conclusions

Overall, inclusion of the feed additive at 2 g/head/d resulted in a significant reduction in the concentrations of methane and carbon dioxide. The energy saved by reducing the amount of methane produced was not partitioned into valuable products such as short-chain fatty acids and microbial mass. Additionally, our study highlighted that there could be a significant variation in the effect of saponins from *Y. schidigera* even on the same type of diet.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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