Original Research Article

Timing of High-Energy Supplementation to Forage Feed Continuous Culture in the Early-Season Pasture

ABSTRACT

A continuous culture study was conducted to duplicate the daily meal pattern of pasture-grazed cattle and evaluate the time of two supplements differing in their composition (one complex: SUPP A and one simplistic: SUPP B) on their effects on nutrient digestion and metabolism of ruminal microorganisms. SUPP A contained ingredients containing substantial amounts of both structural and non-structural carbohydrates (NSC), fat, and protein, all having fractions with differing rates of availability. SUPP B's predominate organic matter ingredients were only corn grain and soybean meal and contained approximately 3% less crude protein than SUPP A. All treatments were fed forage at five distinct times, mimicking forage intake on pasture, with supplements being fed at either a morning (AM) or evening (PM) feeding. The treatments were a) SUPP A fed AM, b) SUPP B fed AM, c) SUPP A fed PM, and d) Supp B fed PM. The results showed that dry matter, organic matter, and NSC digestibility's were unaffected by supplement type or time of feeding. Supplement A showed greater fiber digestion than SUPP B, regardless of time of supplementation. The PM feeding decreased the digestion of both neutral detergent fiber and acid detergent fiber in SUPP B with no effect on fiber digestion in SUPP A. Culture pH remained more stable over the 24-hour day with SUPP B feeding than it did with SUPP A feeding while average pH was not affected by supplement or time of feeding. Total volatile fatty acid flow was unaffected by treatment, as were flows of acetic and propionic acids. Butyric acid flow decreased significantly on both supplements with the PM feeding. As expected, there were fewer total branch chain volatile fatty acids generated with SUPP B. Microbial growth measured in g microbial N flow/day increased for both supplements at the PM feeding. Flow of by-pass feed nitrogen was unaffected by supplement but was lower with PM feeding. Microbial efficiency was increased as a result of the PM feedings. The timing of a large amount of supplementation relative to the timing of daily forage meals can alter the effects seen in continuous culture work and may translate to effects *in-vivo* systems as well.

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Keywords: Continuous culture, pasture, supplementation, fermentation

1. INTRODUCTION

Based on nutrient content, early-season pastures in the Appalachian region can be of substantial quality. Predictive modeling systems such as those found in several versions of the beefand dairy nutrient requirement reports[1] and numerous other implicative

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reports[2,3] suggest that ruminants grazing moderate to high-quality cool season pastures typically consume excess digestible protein. When consumed, excess rumen degradable protein will be degraded by rumen micro-organisms to ammonia, with substantial amounts ultimately absorbed by the animal and lost as urea. The provision of readily fermentable carbohydrates to the rumen micro-organisms at times when ammonia is released may aid in efficiency of which dietary nutrients from high-quality pastures are used. Furthermore, appropriate supplementation of desirable energy sources at this time may be beneficial to varied production practices, including but not limited to pasture finishing[4,5] and pasture-based dairying[6] where the pasture contribution is viewed as a dietary contribution and not the sole dietary source of nutrients.

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Too numerous to list all, but previous work [7,8,9] has demonstrated that timing of supplementation will affect productivity of grazing animals with results ranging from negative to positive. The variability in results is relatedto many factors such as energy source (carbohydrates (**CHO**) vs. lipid vs. protein) as well as actual source of each of these. This timing of supplement delivery often occurs at times of convenience for both the livestock producer and the researcher looking for detailed effects. This methodology is often approached with a simplistic idea that pasture animals eat forage, a single entity that contains all the nutrients that an animal will receive unless supplemented. Continuing this "simplistic" approach, the supplement delivers "extra" nutrients. In fact, this is far from a "simplistic" event when consideration is given that both feeds (forage & supplement) contain many different nutrients (crude protein (**CP**), CHO, lipids, etc.) that have differing fractions of each that become available/exposed to microbial attack at different times once consumed. Couple this with the understanding that grazing animals do not eat a single or uniformed size meals throughout a 24-hour day. Beef cattle grazing cool-season pastures in the Appalachian region of the U.S. have three major meal bouts throughout a typical day[10]: the largest meal occurring in the morning followed by an intermediate-sized meal from late morning to early midday and a subsequently larger meal in the late afternoon to early evening with a much smaller bout occurring early afternoon. This scenario creates a complex interaction of previously consumed nutrients being available to interact with more recently consumed nutrients and most likely affects the synchrony of nutrient availability to the micro-organisms in the rumen. The objective of this study was to duplicate the daily meal pattern of pasture- grazed cattle in a continuous culture system and evaluate the time of two supplements differing in their composition on their effects on nutrient digestion and metabolism of ruminal microorganisms.

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2. MATERIAL AND METHODS

All animal usage occurred under approved IACUC protocol 05-1102. On the pastureland previously utilized[10], mid-May pasture (un-grazed since the previous October) was harvested in single passes via a battery powered hedge trimmer at a height of approximately 10 cm. This pastureland was divided into 8, one-hectare plots. To have enough immediate freezer space, forage (approximately 7 kg per plot) was harvested from all eight plots over a 3-day period between 0900 and 1500 with avoidance of forage being wet from precipitation. Immediately after each harvest, material was dumped on a single plastic tarp, transported to an on-site facility with a walk-in freezer (-18℃) with tarp spread on floor and forage loosely mixed and spread across the tarp to freeze overnight. Prior to the next day's forage collection, frozen forage was collected as un-macerated material and loosely stored in the same freezer in lidded 121 L plastic trashcans. The morning after the 3rd days collection, all frozen forage was dumped on the same tarp in the above freezer mixed with a pitchfork, collected into 1 of 3 large heavy duty black trash bags and immediately transported (< 20 minutes) to campus and stored frozen (-18℃) in a walk-in freezer. The week prior to the

initiation of the continuous culture fermentation (< 10 days after last pasture collection), one random location hand-grabbed pasture sample from all three bags was collected for dry matter (**DM**) determination. Based on the average DM value of these samples, a predetermined amount of frozen pasture forage was collected (10% excess of need) mixed with dry ice and ground through a 4 mm screen utilizing a Wiley Mill. After grinding, frozen material was returned to the walk-in freezer where it remained for roughly 48 hours to degas. Ground-frozen-pasture was weighed into labeled individual plastic trays in amounts equivalent to 9.1g, 5.6g, 5.8g, 3.0g, and 6.5g of DM for culture feeding. Throughout the ground-frozen-forage weighing process, random samples were collected and composited for nutrient analysis. Supplements used in this investigation were approximately 5 kg composites derived from single ~ 907 kg batches utilized in separate independent animal feeding events. Supplements were ground through the same screen as pasture samples, thoroughly mixed and pre-weighed (30 g, as-fed) into small sealable plastic bags. Supplement A (**SUPP A**) was a multi-ingredient supplement containing various energy and protein sources, while supplement B (**SUPP B**) was much more simplistic, with its organic matter (**OM**) coming predominately from ground corn and soybean meal. Supplement A was formulated to contain 16% CP with an actual analysis of 16.4% CP, with 21.6% of CP being soluble. Supplement B was formulated to contain 13% CP with an actual analysis of 13.0% CP, with 21.4% of CP being soluble. Supplement A, in comparison to SUPP B, was used due to its diverse carbohydrate and protein fractions rates of availability, with the majority of its energy coming from relatively equal content of NSC (starch and sugar: 32.9%) and fiber (neutral detergent fiber (**NDF**): 32.8%), and from fat (ether extract (**EE**): 7.4%) supplied predominately as soybean oil (4.3% DM). Most of the energy found in SUPP B was from NSC (57.7%) as it only contained 9.9% NDF and 3.5% EE. Formulated composition and analyses of the grain supplements and pasture are shown in Table 1.

Continuous Culture System

All continuous culture work in this study was performed at theformer Rumen Fermentation Profiling Lab (West Virginia University, Morgantown, WV). In short, a 12-unit continuous culture system similar to [11] was used. Each fermenter had a working volume of 1164 ml, and all treatments were fermented in triplicate under the following conditions utilizing artificial saliva[12]: liquid dilution rate: 8.0 %/h, solids dilution rate: 4.0%/h, solids retention time: 25 h, temperature: 39^0 C, Feed Intake/24 h: 56.4 g of DM (Forage, 30g DM/d + 26.3 g DM/d SUPP A or 26.5 g DM/d SUPP B, respectively). Culture vessels were fed forage: Five times/d, 9.1 g DM at 0700 hr, 5.6 g at 1000 hr, 5.8 g at 1300 hr, 3.0 g at 1600 hr, and 6.5 g at 1800 hr. Supplements were fed to assigned culture vessels automatically: one time/d over a 45-minute interval at either 0700 hr (**AM**) or 1900 hr (**PM**). The pH was recorded immediately prior to each forage feeding. Inoculum for fermenters was obtained from two ruminally cannulated non-lactating Holstein cows adapted (10 d) and fed free-choice mixed grass-legume baleage and supplemented with 2.5 kg/hd/d of SUPP B. Rumen fluid was pooled before inoculating fermenters.

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Each treatment was evaluated with 9-day fermentations with effluent samples composited for analysis during the last three days. During the last 3 d the effluents were collected in an ice bath, and a 1-L sample was composited and saved for analysis. After the effluent was collected on d 9, the contents of the fermenters were allowed to settle, and the upper fluid layer was used for collection of microbes. Two 250-ml samples were taken from each fermenter and centrifuged at 4°C for 20 min at 200 × g. The supernatants were centrifuged for 15 min at 30,000 × g, the pellets were combined, resuspended in saline, and again centrifuged at 4°C for 15 min at 30,000 × g. The supernatants were discarded, and the

pellets were resuspended in 20 ml of a 50:50 mixture of distilled water and methanol and centrifuged for 15 min at 30,000 × g. The supernatants were poured off, and the pellets were resuspended in distilled water and lyophilized.

Chemical Analyses

Feed DM was determined by oven drying (100°C for 24 h). Effluent DM was determined by centrifuging a 34 to 40-g sample of effluent at 30,000 × g for 45 min. The supernatant was discarded, and the particulate matter was dried at 100°C for 24 h and reweighed. For digestibility determinations, digestibilities of DM (**DMD**) and OM (**OMD**) were corrected for microbial DM and OM. Determination of the NDF and acid detergent fiber (**ADF**) content in the feed was by the reported methods of [13] with modifications [14]. The adaptations for NDF and ADF analysis of continuous culture effluents were described by [15]. Total N in feed, effluents, bacterial and ammonia was determined [16] using an automated Tecator digestion system (Tecator, Inc., Herndon, VA). Ether extraction of the feed was performed [16]. Analysis of volatile fatty acids (**VFA**) was performed in accordance with the gas chromatographic separation procedure [17]. The GC was a Varian model 3300 with an FID detector (Varian, Inc., Palo Alto, CA). The column was a 2-m × 2-mm glass column packed with 10% SP-1200/1% H_3HPO_4 on 80/100 chromosorb WAW (Supelco,. Inc. Bellefonte, PA). Effluent and bacterial concentrations of purines were determined [18]. The sugars and starches of the feeds and effluents were determined [19], except that ferricyanide was used to detect reducing sugars.

Statistics

Data were analyzed as a completely random design using the PROC GLM procedure [20]. Fermenter vessel was the experimental unit. The treatment structure consisted of a 2 x 2 factorial, orthogonal contrasts were used to partition specific treatment effects. Contrast statements included the main effects comparing supplement type (SUPP A vs. SUPP B) and supplementation time (AM vs. PM). In addition, the interaction of main effects was evaluated (supplement type x supplementation time). Treatment means were calculated using the LSMEANS. Mean comparisons with *P*-values less than or equal to 0.05 were declared significant, and values less than or equal to 0.10 were considered tendencies.

3. RESULTS AND DISCUSSION

The pasture grass used as the base feed in this experiment was high quality. Its analysis revealed almost a 19% CP content, with approximately 27% of itsprotein being soluble. Roughly 43% of its CP was bound to the NDF fraction, with only 7.4% of its total protein being bound to ADF. This pasture grass contained 17.5% enzymatically determined NSC and 28.6% calculated non-fiber carbohydrates (**NFC**). Pasture grass was not fermented alone but accounted for 53% of the DM supplying nutrients to the continuous culture system.

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Digestion coefficients for treatments are shown in Table 2. Previous reports suggest that free oil addition as seen in SUPP A may decrease digestibility of fiber. However, this was not evident in this study. Digestibility's of DM, OM, and NSC were unaffected ($P \geq 0.11$) by supplement type or time of feeding. Acid detergent fiber and NDF digestions were affected by supplement ($P = 0.0001$) and feeding time ($P \leq 0.02$) and showed a significant interaction ($P \leq 0.004$). Supplement A showed greater fiber digestion than SUPP B at both times of

supplementation. The PM feeding decreased the digestion of both NDF and ADF in SUPP B with no effect on fiber digestion in SUPP A. Crude protein digestion was not different between the two supplements, however, CP digestion of both supplements increased with the PM feeding.

Average pH was not affected by supplement or time of feeding (Table 3). Figure 1 shows average pH plotted at each of the five forage feeding times. The fermentation pH was significantly lower ($P \leq 0.05$) at 0700 hr for supplement B, however at 1600 and 1800 hr supplement B was higher($P \leq 0.05$) than A. Interestingly, it appears that pH remained more stable over the 24 hr day with SUPP B feeding than it did with SUPP A feeding. This may indicate that the dietary composition of SUPP A allowed greater synchrony of nutrients available for bacterial incorporation and or synthesis even within an artificial buffer-controlled environment resulting in the base forage feeding having a greater effect on daily pH changes. Total VFA flow was unaffected by treatment, as were flows of acetic, propionic, and isovaleric acids. Butyric acid flow decreased significantly on both supplements with the PM feeding. As one might expect with a higher CP diet, isobutyric and valeric acids were greater ($P \leq 0.04$) with SUPP A when compared to SUPP B.

Effluent N was partitioned into ammonia and non-ammonia N with non-ammonia N being partitioned further into microbial and bypass feed nitrogen (Table 4). As one might expect with a supplement containing greater soluble CP content, ammonia N was higher ($P = 0.001$) with SUPP A than SUPP B. Time of supplement feeding did not affect ammonia concentration, but there was a numerical trend ($P = 0.18$) for the PM feedings to have lower ammonia than the AM feedings. Non-ammonia N flow was greatest ($P = 0.0001$) with SUPP A, with the PM feeding tending ($P = 0.06$) to increase flow of non-ammonia N with both supplements. Microbial growth measured in g microbial N flow/day increased ($P = 0.01$) for both supplements at the PM feeding. Supplement A showed a numeric trend ($P = 0.11$) to increase flow of microbial N. Flow of by-pass feed N was unaffected by supplement but was lower ($P = 0.05$) with the PM feeding.

The presence of a rumen allows for a diverse usage of feed ingredients, many of which cannot be used effectively by monogastric animals. Ultimately the niche created by the rumen is that non-protein nitrogen or poor-quality protein sources can be turned into much higher quality protein sources. This upscaling is often measured or reported as microbial efficiency and can be reported via different means. Three measures of microbial efficiency are presented in Table 5. All three are in general agreement as to the effects of the treatments in this investigation. Microbial efficiency tended to increase ($P \leq 0.09$) as a result of the PM feedings. The efficiency at which degraded feed N was converted to microbial N was greatest in the PM ($P = 0.05$) with SUPP B having a more efficient conversion ($P = 0.002$) ofdegraded feed N to microbial N than SUPP A.

A partial compositional analysis of the microbes (Table 6) revealed that total N content was both numerically greater ($P = 0.16$) onSUPP A and with PM feeding ($P = 0.15$). Bacterial ash was unaffected by treatment. Purine-N as a percent of total microbial N was greater ($P = 0.003$) withmicrobes harvested from SUPP B than from SUPP A and showed a numerical trend ($P = 0.19$) to decrease with PM feedings.

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TABLE 1. Feed Composition and Analysis, %Dry Matter Basis.

Ingredient	Supplement A	Supplement B	Pasture Grass

Ground Corn	40.94	87.58	
Soybean Hulls	37.39	-	
Wheat Midds	8.90	-	
Soybean Oil	4.27	-	
Corn Gluten Meal	2.28	-	
Soybean Meal 48	1.57	7.57	
Limestone	1.50	1.95	
Sodium Bicarbonate	1.28	1.28	
Magnesium Oxide	0.71	0.75	
Dicalcium phosphate	0.64	0.35	
Sodium Chloride	0.50	0.50	
Rumensin 80	0.02	0.02	
Analysis, %DM			
Dry Matter	88.78	86.99	24.56
CrudeProtein	16.36	13.01	18.86
Soluble Protein (%CP)	21.62	21.35	27.13
NDF-CP (%CP)			42.82
ADF-CP (%CP)			7.35
NDF	32.82	9.87	43.85
ADF	20.83	2.94	26.65
Lignin (%NDF)			8.88
NSC[1]	32.93	57.70	17.49
Starch	28.72	54.27	5.49
Sugar	4.21	3.43	12.00
Ether Extract	7.43	3.54	3.23

Comment [k2]: DM

Comment [k3]: CP

Ash	8.45	12.89	8.91
NFC[2]	34.94	60.69	28.64

[1]Enzymatic determination of starch and sugar.

[2]Calculated Non-Fiber Carbohydrate = 100 – (%NDF + %CP + %EE + %Ash)

TABLE 2. Effects of Treatments on Nutrient Digestion Coefficients.

	Diets						
	Supplement A		Supplement B		*P* =		
Item[1]	AM	PM	AM	PM	Sup.	Time	Sup. x Time
Digestion, %							
DM	68.1	67.9	67.6	64.7	.58	.63	.67
OM	44.2	46.5	41.7	41.1	.11	.70	.53
ADF	57.9	60.0	48.0	33.5	.0001	.02	.004
NDF	49.8	49.0	38.7	20.5	.0001	.006	.009
NSC[2]	72.7	75.0	68.2	78.5	.91	.16	.35
CHO[3], g/d	20.9	21.2	20.0	19.6	.21	.93	.68
CP	55.5	59.6	48.9	59.5	.39	.04	.30

Comment [k4]: Where are the SEM or SD? They should be add.

[1] AM = Supplement fed at 0700 hr, PM = Supplement fed at 1900 hr, Sup. = supplement

[2] Nonstructural carbohydrate (sugars + starch)

[3] Total carbohydrate digested (NDF + NSC), g/day

TABLE 3. Volatile fatty acid (VFA) production and average daily fermenter pH.

	Diets						
	Supplement A		Supplement B		*P* =		
Item[1]	AM	PM	AM	PM	Sup.	Time	Sup. x Time
Average pH	5.91	5.75	5.87	5.92	.30	.41	.11
VFA, mmoles/d							
Total	219.2	220.6	223.4	208.7	.61	.38	.30
Acetic	115.3	109.8	121.3	106.6	.83	.14	.48
Propionic	58.0	65.2	61.6	67.6	.61	.28	.92
Butyric	31.0	26.7	29.8	25.8	.57	.05	.93
Isobutyric	1.22	1.11	0.95	1.01	.04	.79	.29
Valeric	9.40	17.05	7.31	6.78	.008	.08	.05
Isovaleric	0.85	0.68	0.66	0.79	.72	.86	.18
A-P ratio	2.04	1.69	1.99	1.65	.84	.17	.99

[1] AM = Supplement fed at 0700 hr, PM = Supplement fed at 1900 hr, Sup. = supplement

Comment [k5]: ?

TABLE 4. Nitrogen partitioning.

	Diets						
	Supplement A		Supplement B		*P* =		
Item[1]	AM	PM	AM	PM	Sup.	Time	Sup. x Time
Ammonia N, mg/dl	4.77	3.64	1.94	1.57	.001	.18	.47
Non-ammonia N, g/d	1.68	1.71	1.60	1.62	.0001	.06	.33
Microbial N, g/d	0.83	0.93	0.71	0.89	.11	.01	.40
NANMN[2], g/d	0.85	0.78	0.89	0.73	.90	.05	.35

[1]AM = Supplement fed at 0700 hr, PM = Supplement fed at 1900 hr, Sup. = supplement

[2]Non-Ammonia, Non-Microbial N (bypass feed N)

TABLE 5. Grams of microbial N produced per kg digested DM, OM and carbohydrate, and efficiency of N uptake.

	Diets						
	Supplement A		Supplement B		*P* =		
Item[1]	AM	PM	AM	PM	Sup.	Time	Sup. x Time
G Mic. N/Kg:							
Digested DM	21.8	24.2	18.9	24.4	.37	.02	.29
Digested OM	36.6	38.7	34.3	40.4	.88	.05	.29
Digested CHO[2]	39.7	43.9	36.1	43.0	.45	.09	.64
CP Efficiency [3]	88.6	91.9	94.2	96.2	.002	.05	.60

[1]AM = Supplement fed at 0700 hr, PM = Supplement fed at 1900 hr, Sup. = supplement

[2]NDF + NSC digested

[3]% degraded feed N as Microbial N

Table 6. Partial composition of microorganisms.

	Diets						
	Supplement A		Supplement B		*P* =		
Item[1]	AM	PM	AM	PM	Sup.	Time	Sup. x Time
Nitrogen, %	8.98	9.21	8.25	8.99	.16	.15	.43
Ash, %	12.91	11.51	12.95	12.84	.71	.68	.73
RNA-N, % mN[2]	9.53	8.83	10.74	10.45	.003	.19	.58

[1]AM = Supplement fed at 0700 hr, PM = Supplement fed at 1900 hr, Sup. = supplement

[2]Purine N expressed as a % of microbial N.

Figure 1. Fermentation pH at time of forage feeding : Supplementeffects(P<0.05) at 0700, 1600 & 1800 hours.

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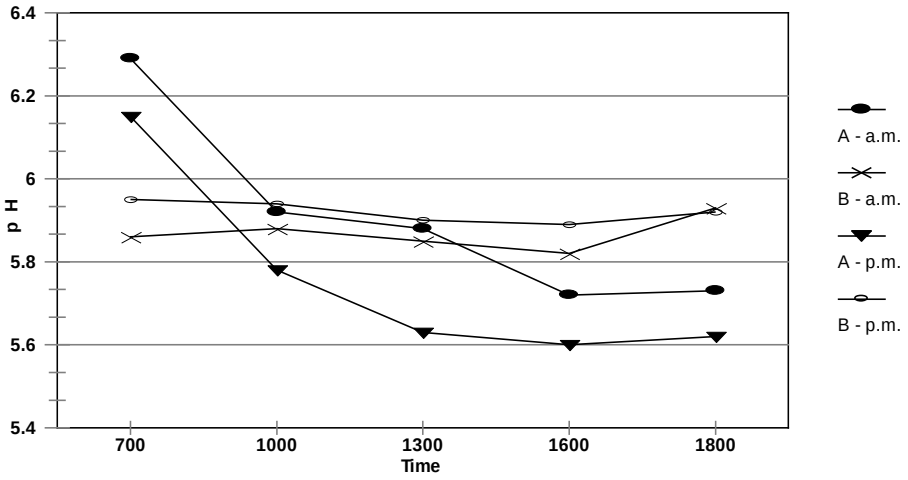

4. CONCLUSION

The timing of a large amount of supplementation relative to the timing of daily forage meals can alter the effects seen in continuous culture work and may translate to effects *in-vivo* systems as well. In this investigation PM feeding of supplements was more effective in eliciting changes than AM feeding, regardless of supplement type. This result was most likely due to the abundance of fermentative substrates in the PM stretching out the daily fermentation pattern. Supplement A, when given in the PM supported high levels of fiber and NSC digestion and slightly higher microbial growth than did AM supplementation with either supplement A or B. The PM supplementation with B was equal to A in most digestion and microbial growth responses and had the highest microbial efficiency, despite having 3.4% less CP. Albeit there where relatively high levels of degradable protein in the base forage, degradable protein probably prevented maximum responses to Supplement B.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

ADF = acid detergent fiber
CHO = Carbohydrates
CP = crude protein
DM = dry matter
DMD = DM digestibility
EE = ether extract
NFC = non-fiber carbohydrate
NSC = non-structural carbohydrates
NDF = neutral detergent fiber
OM = organic matter
OMD = OM digestibility
VFA = volatile fatty acid