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Improvements of in situ degradability of grass hay, wet brewer's grains, and soybean meal with addition of clay in the diet of Holstein cows

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ABSTRACT

Clay-based feed additives have been widely examined as tools for combating the negative impacts of both aflatoxin (**AF**) infected feedstuffs as well as subacute ruminal acidosis (**SARA**). The objective of this study was to determine the ruminal degradability of feedstuffs in response to 3 concentrations of dietary clay in lactating dairy cows. Treatments were: no clay (**CON**), **10**, or **20 g/kg** of dietary DM as clay (EcoMix®, UMG, Ukraine). Samples (8 g) of dried alfalfa hay (**AH**), grass hay (**GH**), wet brewer's grains (**WBG**), ground corn (**GC**), corn silage (**CS**), or soybean meal (**SBM**) were placed into polyester bags (3 replicates per feed) and incubated for 0, 2, 4, 8, 12, 48, 72, or 96 h in 3 rumen-cannulated cows fed one of three treatments. Recovered bags were analyzed for DM, aNDF, ADF, starch, CP for all feedstuffs, and total fatty acids (**TFA**), saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**), and polyunsaturated fatty acids (**PUFA**) for GH, WBG, and CS. Soluble (**S**), digestible (**D**), and undigested (**U**) fractions; and fractional rate of digestion (**K_d**) and effective degradability (**ED**), were estimated for each feedstuff, treatment, and cow combination. Statistical analysis was preformed using the MIXED procedure of SAS. Grass hay S of DM exhibited a quadratic treatment effect (CON = 1.4, 10 g/kg = 1.7, and 20 g/kg = 1.2 g/kg). There was an increase in WBG D of DM as concentration of clay in the diet increased (CON = 5.9, 10 g/kg = 6.6, and 20 g/kg = 7.6 g/kg). There was a quadratic treatment effect for DM S (CON = 2.6, 10 g/kg = 3.4, and 20 g/kg = 1.5 g/kg) and ED (CON = 4.8, 10 g/kg = 5.7, and 20 g/kg = 3.9 g/kg) of SBM. There was a decrease in SBM S of starch (CON = 6.3, 10 g/kg = 4.7, and 20 g/kg = 3.3 g/kg) and an increase in SBM D of starch (CON = 7.7, 10 g/kg = 7.7, and 20 g/kg = 8.2 g/kg) as concentration of clay in the diet increased. There was an increase in GH D of SFA (CON = 3.4, 10 g/kg = 7.8, and 20 g/kg = 9.1 g/kg DM) and a decrease in both GH U (CON = 6.0, 10 g/kg = 1.9, and 20 g/kg = 0.0 g/kg DM) and K_d (CON = 0.07, 10 g/kg = 0.03, and 20 g/kg = 0.01 h^{-1}) of SFA as concentration of clay in the diet increased.

Abbreviations: 10 g/kg, 10 g/kg treatment; 20 g/kg, 20 g/kg treatment; ADF, acid detergent fiber; AF, aflatoxin; AH, alfalfa hay; CON, control treatment; CS, corn silage; CP, crude protein; D, digestible fraction; DM, dry matter; DMI, dry matter intake; ED, effective degradability; GH, grass hay; GC, ground corn; HPDDG, high protein corn distiller's grains; U, undigested fraction; K_d, fractional digestion rate; K_p, assumed rate of passage; ME, metabolizable energy; MUFA, monounsaturated fatty acids; aNDF, neutral detergent fiber; NE_L, net energy of lactation; NFC, non-fiber carbohydrates; NRC, National Research Council; PUFA, polyunsaturated fatty acids; S, soluble fraction; SARA, subacute ruminal acidosis; SEM, standard error of mean; SFA, saturated fatty acids; SBM, soybean meal; TFA, total fatty acids; WBG, wet brewer's grain; WDDGS, wheat dried distiller's grains with solubles

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There was a decrease in WBG S of SFA as concentration of clay in the diet increased (CON = 0.8, 10 g/kg = 0.4, and 20 g/kg = 0.3 g/kg). Corn silage D of TFA exhibited a quadratic treatment effect (CON = 7.3, 10 g/kg = 3.3, and 20 g/kg = 4.5 g/kg). In conclusion, the addition of clay at 10 g/kg of total dietary DMI maximized S for GH and SBM DM as well as ED for SBM. Also, the addition of clay at 20 g/kg of total dietary DMI maximized degradability of SFA for GH.

1. Introduction

Clay-based feed additives have been examined as tools for combating the negative impacts of both aflatoxin (**AF**) infected feedstuffs as well as subacute ruminal acidosis (**SARA**) on the rumen environment (Sulzberger et al., 2016, 2017). When consumed by dairy cows, the AF derivative, aflatoxin B_1, is converted to aflatoxin M_1 and is commonly considered a toxic carcinogen (IARC, 2002; Kutz et al., 2009). Supplementation of clay-based additives have proven useful in minimizing AF absorption in the gastrointestinal tract and, subsequently, reducing the concentration of aflatoxin M_1 in milk (Sulzberger et al., 2017; Pate et al., 2018). Increasing the concentrate-to-forage ratio in dairy rations can subsequently drop rumen pH below a healthy threshold and leave the cow susceptible to SARA (Shaver et al., 2000). The use of buffering feed additives, like bentonite clay, alleviate the negative impacts of decreased rumen pH due to their high capacity for H^+ exchange within the rumen, leading to alkalization (Yong et al., 1990; Cruywagen et al., 2015). Although the use of clay additives can be beneficial, limited research is available which analyze potential side-effects of including a clay additive in the diet.

Bentonite clay has the capacity to absorb heavy metals, bacteria, and toxic agents such as AF (Trckova et al., 2004). Specifically, phyllosilicates (including bentonite) consists of 4 oxygen atoms and a silicon ion that form a tetrahedral configuration (Di Gregorio et al., 2014). This structure allows for internal absorption of mono- and divalent ions, which includes AF but also many essential mineral nutrients (Di Gregorio et al., 2014). Due to its great binding affinity, clays pose a potential risk for interacting with critical nutrients (Phillips, 1999). Chung et al. (1990) reported a reduction in zinc utilization with the addition of 0.5 or 1 g/100 g dietary clay fed to chicks. Similarly, Gowda et al. (2007) observed a marked decrease in the apparent gut absorption of copper, zinc, iron, and manganese for sheep fed 5 g/kg of the diet DM as clay. Although previous work has detailed interactions between clay supplementation and mineral availability, there is a lack of research investigating how supplementation of clay may interact with other vital

Table 1
Ingredient composition of diet with addition of 0 g/kg clay (**CON**), and clay added to the diet in **10 g/kg** and **20 g/kg** of the dietary DMI of Holstein cows.

Ingredient	g/100 g of DM
Alfalfa silage	6.12
Alfalfa hay	6.94
Corn silage	35.09
Cottonseed	3.26
Wet brewer's grains	8.16
Ground shelled corn	25.09
Soy hulls	4.74
Soybean meal, 48% CP	2.45
Expeller soybean meal[b]	1.22
Blood meal[c]	1.43
Urea	0.33
Rumen-inert fat[a]	1.43
Limestone	1.14
Salt	0.30
Dicalcium phosphate	0.30
Magnesium oxide	0.12
Sodium bicarbonate	0.78
Potassium carbonate	0.30
Calcium sulfate	0.12
Mineral and vitamin mix[d]	0.53

[a] Energy Booster 100 (Milk Specialties Global, Eden Prairie, MN).
[b] SoyPLUS (West Central Cooperative, Ralston, IA).
[c] Perdue AgSolutions LLC, Binghamton, NY.
[d] Mineral and vitamin mix was formulated with 5% Mg, 10 g/kg S, 7.5% K, 2.0 g/kg Fe, 3.0 g/kg Zn, 3.0 g/kg Mn, 5000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D_3, and 7700 IU/kg of vitamin E.

nutrients available in feedstuffs. Thus, it is important to understand the potential effects that clays have on the degradability of feedstuffs commonly fed to lactating ruminants to determine nutrient availability.

The use of in situ digestion methods has long been considered an effective way of evaluating the nutritive adequacy of feedstuffs for dairy cows (Nocek, 1988). Techniques have been heavily researched over the years to develop a standard procedure for comparison of in situ digestion that helps explain what happens to the diverse rumen ecology during digestion (Vanzant et al., 1998). Because of the possible effects of clay on rumen environment discussed above, understanding the effects of clay in the diet of the animal on in situ feedstuff digestibility is an important step in understanding the mechanisms within the rumen environment that would affect nutrient availability. Therefore, the objective of this study was to determine the ruminal degradability of 6 different feedstuffs including alfalfa hay, grass hay, wet brewer's grains, ground corn, corn silage, and soybean meal in response to 3 concentrations of dietary clay in lactating dairy cows. We hypothesized that, based on the potential for clay to alter the rumen environment, the addition of clay to the diet of lactating cows would alter the degradability of feedstuffs commonly fed to dairy cows.

2. Materials and methods

2.1. Animals and treatments

All experimental procedures were approved by the University of Illinois (Urbana-Champaign, IL, USA) Institutional Animal Care and Use Committee. Three rumen-cannulated Holstein cows (lactation number: 5.3 ± 2.3; days in milk: 217 ± 47 days; DMI: 24.0 ± 0.7 kg/day; milk yield: 17.6 ± 9 kg/day) were housed in tie-stall barns with individual feed bunks and fed the same TMR ad libitum to meet NRC requirements (Tables 1 and 2; NRC, 2001). Cows were assigned to 1 of 3 treatments in a 3 × 3 Latin square design with 3, 21-d periods. The adaption phase occurred from d 1 to 17 of each period, while the digestibility phase of the experiment occurred from d 18 to 21 (96 h) of each period. Cows were fed at 1400 h and were milked three times daily at 700, 1400, 2200 h. The 6 feeds used for degradability analysis were: alfalfa hay (**AH**), grass hay (**GH**), corn silage (**CS**), ground corn (**GC**), wet brewer's grain (**WBG**), and soybean meal (**SBM**). Chemical composition of each feed ingredient can be found in Table 3. Treatments were: TMR without clay (EcoMix®, UMG, Ukraine; **CON**), TMR with either 10 g/kg (**10 g/kg**) or 20 g/kg (**20 g/kg**) dietary DMI addition of clay to the TMR as a topdress per the manufacturers' recommendations. As described in Sulzberger et al. (2017), the clay was analyzed by x-ray diffraction, which indicated the presence of vermiculite, nontronite, and montmorillonite (Frederick Seitz Materials Research Laboratory, University of Illinois, Urbana-Champaign). Each treatment (including CON) was mixed with 500 g of

Table 2
Mean chemical composition (g/100 g of DM unless otherwise noted) and standard deviation of basal diet fed to Holstein cows with addition of 0 g/kg clay (**CON**), and clay added to the diet in **10 g/kg** and **20 g/kg** of the dietary DMI.

	Mean	SD[a]
DM	49.2	0.8
CP	16.2	0.5
ADF	23.0	1.6
aNDF	34.0	2.6
Lignin	3.85	0.78
NFC	35.5	2.5
Starch	24.8	2.3
Crude fat	5.86	0.33
Ash	8.39	0.61
ME[b]	2.60	0.05
NE_L, MJ/kg of DM[b]	6.32	0.07
Ca	1.26	0.21
P	0.36	0.04
Mg	0.27	0.04
K	1.28	0.19
Na	0.39	0.06
S	0.23	0.03
Fe, mg/kg	494	81
Zn, mg/kg	87.8	17.6
Cu, mg/kg	17.5	2.8
Mn, mg/kg	91.2	12.8
Mo, mg/kg	0.82	0.16

Abbreviations: ADF, acid detergent fiber; Ca, calcium; CON, control treatment; CP, crude protein; Cu, copper; DM, dry matter; DMI, dry matter intake; Fe, iron; K, potassium; ME, metabolizable energy; Mg, magnesium; Mn, manganese; Mo, molybdenum; Na, sodium; aNDF, neutral detergent fiber; NE_L, net energy of lactation; NFC, non-fiber carbohydrates; P, phosphorous; S, sulfur; SD, maximum standard deviation of mean; Zn, zinc.

[a] Maximum standard deviation between all samples.

[b] NRC (2001).

Table 3
Mean chemical composition (g/100 g of DM) and standard deviation of ingredients used to determine degradability in Holstein cows with addition of 0 g/kg clay (**CON**), and clay added to the diet in **10 g/kg** and **20 g/kg** of the dietary DMI.

	Mean	SD[a]
Alfalfa hay		
DM	82.10	0.05
CP	20.67	0.80
Starch	1.37	0.55
ADF	56.83	2.74
aNDF	67.17	4.45
Grass hay		
DM	86.97	0.014
CP	9.02	0.56
Starch	0.84	0.31
ADF	52.02	0.95
aNDF	80.54	1.72
Brewers grain		
DM	38.15	0.06
CP	35.37	1.97
Starch	3.17	0.06
ADF	37.53	0.50
aNDF	58.03	0.95
Corn		
DM	85.83	0.005
CP	7.95	0.26
Starch	68.25	1.90
ADF	3.65	0.50
aNDF	9.15	1.05
Corn silage		
DM	30.98	0.03
CP	7.05	0.24
Starch	19.93	1.91
ADF	34.68	1.20
aNDF	50.0	2.82
Soybean meal		
DM	89.56	0.01
CP	58.48	7.60
Starch	1.88	0.30
ADF	15.46	6.34
aNDF	51.70	9.07

Abbreviations: ADF, acid detergent fiber; CON, control treatment; CP, crude protein; DM, dry matter; aNDF, neutral detergent fiber; SD, standard deviation of mean.

[a] Maximum standard deviation between all samples.

ground corn and top-dressed at feeding. Topdress consumption was assessed visually after each application. Cows readily consumed all topdress applications.

2.2. Sampling and bag preparation

Feed samples were weighed, dried for 24 h at 60 °C, and weighed again for DM analysis. Alfalfa hay and GH were ground to fit through a 1-mm screen in a Thomas-Wiley laboratory mill (Arthur H. Thomas, Philadelphia, PA). Polyester forage bags (Dacron, Ankom Technology, Macedon, NY, USA) measuring 10 × 20 cm with a 50-μm pore size were used in this study. Bags were filled with 8 g of DM to achieve a 20 mg/cm^2 of DM feed to surface area ratio. Bags were filled with dried and ground AH or GH; and dried, unground CS, GC, WBG, or SBM. Corn silage was left unground to more closely mimic CS as it is presented to dairy cows. Each bag was heat sealed twice to ensure no feed particles escaped and to minimize DM infiltration (i.e. rumen contents entering into the bag). Bags (n = 3 replicates per feedstuff) were placed into large mesh garment bags to prevent the loss of bags in the gastrointestinal tract. Each mesh garment bag filled with polyester bags were soaked in warm water (45 °C) for 30 min and placed into the ventral portion of the rumen on d 18 of each period. Six washers (275 g) were placed into each of the mesh garment bags to ensure that bags were submerged in the lower rumen. Bags were removed, noting their identifications at 0, 4, 8, 12, 24, 48, 72, 96 h post insertion and were immediately placed in ice water to stop fermentation. Care was taken to minimize air exposure that could interfere with proper fermentation, and remaining bags were placed back into the ventral rumen. Bags were hand rinsed in cold running water until the water was clear, then bags were immediately frozen (−20 °C) for at least 24 h. After freezing, the bags were thawed and rinsed on a rinse cycle of a washing machine (Roper RTW4641BQ1,Whirlpool Corp., Benton Harbor, MI, USA) 2 times to reduce microbial content. Bags were dried for 24 h at 60 °C to calculate nutrient of disappearance.

2.3. Chemical analysis

Three replicates of each time point from each treatment were combined to make a composite sample per cow and were sent to a commercial laboratory (Rock River Lab, Watertown, WI, USA) for analysis via wet chemistry methods (Schalla et al., 2012). The samples were analyzed for neutral detergent fiber (**aNDF**), acid detergent fiber (**ADF**), crude protein (**CP**), starch, and DM content. The aNDF was analyzed using sulfite and alpha amylase along with a pre-mixed neutral detergent solution (Goering and Van Soest, 1970), and expressed inclusive of residual ash. The ADF was analyzed using the Ankom200 fiber analyzer (Ankom Technology, Fairport, NY, USA) and expressed inclusive of residual ash. The CP content of the samples was measured using the combustion method (976.06) to determine N content and then multiplying the N content by 6.25 (AOAC, 1995). Starch was measured using alpha amylase, amyloglucosidase, and sodium acetate buffer by the procedure described by Hall and Mertens (2008). Grass hay, WBG, and CS samples were analyzed for fatty acid composition using a modification of the method developed by Sukhija and Palmquist (1988) as described by Lock et al. (2013). Briefly, dried, ground samples were weighed to yield ∼10 to 50 mg of FA, and cis-10 C17:1 (1:1 mg/ml acetone) was added as the internal standard. Samples were dissolved in toluene at twice the volume of the internal standard in acetone solution (2:1 mL/mL). A 5% methanolic sulfuric acid solution was added at two-thirds the sample volume and samples were incubated overnight at 50 °C. Once at room temperature, samples were neutralized with twice their volume using a 5% aqueous sodium chloride solution and fatty acid methyl esters extracted using n-hexane. The solvent layer was washed with a 6% aqueous potassium bicarbonate solution and dried over anhydrous sodium sulfate. The fatty acid methyl esters were filtered through silica gel and charcoal, solvents removed under nitrogen flux at 37 °C, the fatty acid methyl esters weighed, and a 1% solution with n-hexane prepared on a weight basis, which was used for GLC analysis (Lock et al., 2013).

2.4. Statistical analysis

Statistical analysis was performed using the MIXED procedure of SAS (v9.4 Institute Inc., Cary, NC). A non-linear model was created and analyzed using the NLIN procedure of SAS based on the subdividing of feed in which the sum of the soluble feed (**A**), digestible feed (**B**), and undigested feed (**C**) was equal to 1. Nutrient disappearance data from the bags was used to fit a nonlinear function to model digestion. Lag was excluded from the parameters in order to meet convergence criteria. If all convergence criteria were met, a dataset was created for results including soluble (**S**), digestible (**D**), and undigested (**U**) fractions; and fractional digestion rate (**K_d**) using the following model:

Table 4

Least squares means and associated standard errors of soluble fraction, digestible fraction, undigested fraction, fractional rate of digestion (**K_d**), and effective degradability (**ED**) for grass hay, wet brewer's grains, and soybean meal dry matter, ADF, and starch with the addition of 0 g/kg clay (**CON**), and clay added to the diet in **10 g/kg** and **20 g/kg** of the dietary DMI as determined by 10 × 20 cm polyester bags inserted into the rumen of lactating Holstein cows for 0, 4, 8, 12, 24, 48, 72, 96 h.

	Treatment[a]				*P*-value[c]	
	CON	10 g/kg	20 g/kg	SEM[b]	Linear	Quadratic
Grass hay						
DM, g/kg						
Soluble	1.4	1.7	1.2	0.5	0.31	0.03
K_d, h^{-1}	0.026	0.015	0.022	0.003	0.30	0.02
Wet brewer's grains						
DM, g/kg						
Digestible	5.9	6.6	7.6	0.6	0.04	0.84
K_d, h^{-1}	0.18	0.07	0.03	0.08	0.08	0.62
ED[d]	4.4	4.1	3.1	1.1	0.02	0.54
Soybean meal						
DM, g/kg						
Soluble	2.6	3.4	1.5	0.5	0.18	0.04
Undigested	0.9	0.2	1.1	0.4	0.55	0.02
ED[4]	4.8	5.7	3.9	0.3	0.07	0.002
ADF, g/kg DM						
Digestible	6.3	4.7	3.3	0.9	0.03	0.94

Abbreviations: ADF, acid detergent fiber; CON, control treatment; CP, crude protein; DM, dry matter; ED, effective degradability; K_d, fractional digestion rate; K_p, assumed rate of passage; aNDF, neutral detergent fiber; SEM, standard error of mean.

[a] Treatments were: Basal TMR without clay (**CON**), basal TMR with 10 g/kg dietary addition of clay as a top-dress (**10 g/kg**), or basal TMR with 20 g/kg dietary addition of clay as a top-dress (**20 g/kg**).

[b] Greatest standard error of mean.

[c] Contrasts statements were: **linear** = linear treatment effect; and **quadratic** = quadratic treatment effect.

[d] Effective degradability (**ED**) = soluble fraction + digestible fraction × $[K_d/(K_d + K_p)]$. Rate of passage from the rumen (**K_p**) assumed to be 0.06.

$$Y = B(e^{-K_d(t)}) + C$$

where B = digestible fraction, C = insoluble fraction, and K_d = fractional degradation rate of fraction B at time t (McDonald, 1981). Effective degradability (**ED**) was calculated using the following model (McDonald, 1981):

$$ED = A + B\left(\frac{K_d}{K_d + K_p}\right)$$

where A = soluble fraction (1 – B – C), B = digestible fraction, and K_d = fractional degradation rate. Although the rate of passage from the rumen (**K_p**) is affected by many factors [feed intake, diet composition, feed particle size, and moisture in feeds (Krizsan et al., 2010)], a value of 0.06 was assumed to estimate ED for all feedstuffs in the present experiment. Analysis included a linear mixed model that was created to measure treatment effects. Linear and quadratic contrasts were used to examine treatment effects of clay on digestion. Residual distribution was evaluated for normality using the UNIVARIATE procedure in SAS.

3. Results

3.1. Wet chemistry digestibility kinetics

Significant treatment differences for DM, ADF, and starch digestibility kinetics of GH, WBG, and SBM are available in Table 4. We observed that GH DM soluble fraction had a positive quadratic treatment effect (P = 0.03), while GH DM fractional rate of digestion (K_d) exhibited a negative quadratic treatment effect (P = 0.02). We observed that WBG DM digestible fraction increased linearly as concentration of clay in the diet increased (P = 0.04), while WBG DM ED decreased linearly and DM K_d tended to decrease linearly as concentration of clay in the diet increased (P = 0.08 and 0.02, respectively). We observed that SBM DM soluble fraction exhibited a positive quadratic treatment effect (P = 0.04), while SBM DM undigested fraction exhibited a negative quadratic treatment effect (P = 0.02). Also, SBM DM ED had a positive quadratic treatment effect (P = 0.002). Additionally, we observed that SBM digestible fraction of ADF decreased linearly as concentration of clay in the diet increased (P = 0.03).

There were no treatment difference for the linear or quadratic treatment effects for all other degradability measurements for AH, GH, WBG, GC, CS, and SBM DM, aNDF, ADF, starch, or CP.

3.2. Fatty acid digestibility kinetics

Significant treatment differences for SFA, MUFA, and TFA digestibility kinetics of GH, WBG, and CS are available in Table 5. We

Table 5
Least squares means and associated standard errors of soluble fraction, digestible fraction, undigested fraction, and fractional rate of digestion (**K_d**) for grass hay, wet brewer's grains, and corn silage saturated fatty acids (**SFA**), monounsaturated fatty acids (**MUFA**), and total fatty acids (**TFA**) with the addition of 0 g/kg clay (**CON**), and clay added to the diet in **10 g/kg** and **20 g/kg** of the dietary DMI as determined by 10 × 20 cm polyester bags inserted into the rumen of lactating Holstein cows for 0, 4, 8, 12, 24, 48, 72, 96 h.

	Treatment[a]				P-value[c]	
	CON	10 g/kg	20 g/kg	SEM[b]	Linear	Quadratic
Grass hay						
SFA, g/kg DM						
Digestible	3.4	7.8	9.1	1.2	0.02	0.34
Undigested	6.0	1.9	0.0	1.2	0.02	0.46
K_d, h^{-1}	0.07	0.03	0.01	0.02	0.04	0.75
MUFA, g/kg DM						
Soluble	1.2	1.0	1.3	0.1	0.56	0.02
K_d, h^{-1}	0.20	0.14	0.18	0.02	0.52	0.05
Wet brewer's grains						
SFA, g/kg DM						
Soluble	0.8	0.4	0.3	0.2	0.01	0.28
MUFA						
K_d, h^{-1}	0.04	0.02	0.05	0.001	0.001	< 0.0001
Corn silage						
TFA, g/kg DM						
Digestible	7.3	3.3	4.5	1.5	0.03	0.02
K_d, h^{-1}	0.01	0.19	0.04	0.04	0.38	0.02

Abbreviations: CON, control treatment; DM, dry matter; K_d, fractional rate of digestion; MUFA, monounsaturated fatty acids; SEM, standard error of mean; SFA, saturated fatty acids; TFA, total fatty acids.

[a] Treatments were: Basal TMR without clay (**CON**), basal TMR with 10 g/kg dietary addition of clay as a top-dress (**10 g/kg**), or basal TMR with 20 g/kg dietary addition of clay as a top-dress (**20 g/kg**).

[b] Greatest standard error of mean.

[c] Contrasts statements were: **linear** = linear treatment effect; and **quadratic** = quadratic treatment effect.

observed that GH digestible SFA increased linearly ($P = 0.02$) and that GH undigested SFA and K_d of SFA decreased linearly ($P = 0.02$ and 0.04, respectively) as concentration of clay in the diet increased. Also, GH soluble MUFA and K_d of MUFA exhibited quadratic treatment effects ($P = 0.02$ and 0.05, respectively). We observed that WBG soluble SFA decreased linearly as concentration of clay in the diet increased ($P = 0.01$) and that WBG MUFA K_d exhibited a linear and quadratic treatment effects ($P < 0.001$). WE observed that CS digestible fraction of TFA exhibited a linear and quadratic treatment effect ($P = 0.02$ and 0.03, respectively) and that CS TFA K_d exhibited a positive quadratic treatment effect ($P = 0.02$).

There were no treatment difference for the linear or quadratic treatment effects for all other degradability measurements for AH, GH, WBG, GC, CS, and SBM TFA, SFA, MUFA, and PUFA.

4. Discussion

The purpose of this study was to determine the ruminal degradability of 6 different feedstuffs including: alfalfa hay, grass hay, wet brewer's grains, ground corn, corn silage, and soybean meal in response to 3 concentrations (0 g/kg, 10 g/kg or 20 g/kg) of dietary clay fed to rumen-cannulated lactating dairy cows. We hypothesized that, based on the potential for clay to alter the rumen environment, the addition of clay to the diet of lactating cows would alter the degradability of feedstuffs commonly fed to dairy cows.

The extent of ruminal degradation of a feedstuff influences the utilization of nutrients by the ruminant animal. In the current study, GH exhibited the greatest DM soluble fraction when clay was included in the diet at 10 g/kg. However, 10 g/kg inclusion rate had the lowest K_d for GH DM. A slower rate of degradation in the rumen for GH DM for the 10 g/kg treatment may have accounted for greater calculated soluble fraction of GH DM based on a constant K_p. Similarly, WBG DM digestible fraction increased as clay was added to the diet. However, WBG DM ED decreased as clay was added to the diet, likely due to reduced K_d as inclusion rate of clay in the diet increased. Furthermore, DM degradability values for SBM were comparable to the values found by Maxin et al. (2013). Soybean meal DM soluble fraction and ED were greatest when clay was included in the diet at 10 g/kg. Not surprisingly, SBM DM undigested fraction was lowest for the 10 g/kg treatment compared to CON and 20 g/kg. In all, these results indicated that the 10 g/kg treatment maximized GH DM solubility and SBM DM solubility and ED. Additionally, increasing concentrations of clay in the diet increased WBG DM digestibility. When clays, including montmorillonites (bentonite clay), are placed in water or humid situations like the rumen, macroscopic swelling occurs, forming colloidal suspensions (Brown and Brindley, 1980; Newman, 1987). Moreover, previous research has indicated that clay supplementation has an effect on ruminal pH and thus microbial population (Yong et al., 1990; Sulzberger et al., 2016). Rindsig et al. (1969) observed an increased acetate and decreased propionate concentration in the rumen of clay-fed cows. In the present study, authors hypothesize that the swelling properties of clay along with a change in rumen pH modified rumen microbial population, and thus the degradability of feedstuffs. For instance, altered rumen environment and microbial population may have been responsible for the decrease in K_d of GH and WBG, which would consequently increase DM solubility of those feedstuffs, as seen in the current study.

In the current study, as concentration of clay in the diet increased GH digestible SFA increased while GH undigested SFA decreased. Grass hay SFA K_d decreased linearly for CON, 10 g/kg, and 20 g/kg, respectively. As previously stated, increasing clay concentration may have altered rumen microbial population, thus altering feedstuff degradation rate and resulting in increased nutrient digestible fractions of feedstuffs, as seen in the current study. Grass hay soluble MUFA was greater for the CON and 20 g/kg treatments than for the 10 g/kg treatment. Similarly, GH and WBG MUFA K_d was greater for the CON and 20 g/kg treatments than the 10 g/kg treatment. In ruminant animals, unsaturated fatty acids are toxic to rumen bacteria and are thus biohydrogenated to saturated fatty acids (Viviani, 1970). Therefore, it is postulated that the addition of clay at 0 or 20 g/kg of the diet altered the rumen microbial pathways, such that the fractional rate of degradation was increased. However, the soluble MUFA was still greatest for these treatments compared to the 10 g/kg treatment. This also offers an explanation for the greatest amount of digestible SFA for the 20 g/kg treatment observed. These results indicated that the 20 g/kg treatment maximized SFA and MUFA solubility of GH. Similar to GH MUFA, CS digestible TFA was greater for the CON and 20 g/kg treatments than the 10 g/kg treatment. Corn silage TFA K_d was greatest for the 10 g/kg treatment compared to the CON and 20 g/kg treatments. As previously mentioned, this may be a result of altered rumen environment due to clay supplementation in the diet. These results indicated that the 20 g/kg treatment maximized CS digestible TFA but minimized TFA K_d.

In the present study, AH and GH were ground to pass through a 1 mm screen before insertion into the rumen. Grinding feeds is known to alter rate of digestion and rate of passage of feedstuffs from the rumen during digestibility trials (Galyean et al., 1979). Specifically, grinding feeds to a smaller particle size increases the rate at which feeds are passed through the rumen and thus can result in decreased digestibility (NRC, 2001). While there were no significant effects of clay on the degradability of AH, differences for GH DM S and K_d and GH SFA D, S, and K_d and MUFA S and K_d were observed. Generally, the addition of clay positively affected GH degradability compared to CON. These results could be a consequence of the altered particle size due to grinding rather than the feedstuff itself. Therefore, further research is needed to analyze the degradability of these forages without grinding, which may result in longer retention times in the rumen and thus fewer degradability differences for GH.

5. Conclusion

The addition of clay to the diet of lactating cows altered the in situ degradability of GH, WBG, SBM, and CS. Grass hay SFA digestible fraction, GH MUFA soluble fraction and K_d, and WBG MUFA K_d were maximized with the addition of clay in the diet. Wet brewer's grain DM ED decreased while SBM DM ED increased with the addition of clay. Finally, CS TFA D and K_d were maximized with the inclusion of clay in the diet. Results indicated that the addition of clay at 10 g/kg of total dietary DMI maximized solubility for grass hay and SBM DM as well as SBM ED. However, the addition of clay at 20 g/kg of total dietary DMI maximized fatty acid degradability.

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Declaration of Competing Interest

We acknowledge the affiliation of one of the co-authors with United Minerals Group. However, there are no conflicts of interest, financial or otherwise, including direct or indirect financial or personal relationships, interests, and affiliations, whether or not directly related to the subject of the paper.

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