

Different Ratios of Alfalfa Hay to Corn Silage on Lactation Performance of Holstein Dairy Cattle

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INTRODUCTION

Alfalfa is the third largest crop, next to corn and soybeans, grown in the United States. Alfalfa forage has been a major component in dairy cattle diets because of its many nutritional and physical attributes. Most importantly, alfalfa is less rumen filling than most other forages. This is primarily due to its lower neutral detergent fiber (NDF) content and higher rate of NDF digestibility than most other forages, which allows it to clear the rumen more quickly, leaving more space for greater intake of the entire diet. Nutritionally, alfalfa has a higher protein content than most other forages, with an amino acid profile more like milk, giving alfalfa protein a better amino acid profile than most other forages. It also has a higher rumen degraded protein content than most other forages, which helps support better microbial growth in the rumen. Less recognized, alfalfa has a higher positive cation exchange capacity than most other forages, which can help maintain a higher rumen and metabolic pH, which helps support the opportunity for higher milk fat production (Robinson, 2014). Finally, alfalfa works well in rotational cropping systems with corn to help improve soil health and soil drainage and reduce plant disease pressure. Through alfalfa's ability to fix atmospheric nitrogen, alfalfa can provide 40 to 120 lb nitrogen/acre to support a subsequent crop in rotational cropping programs.

Despite these many benefits, alfalfa content in dairy cow diets have been in decline. Since 1999 in California alone, alfalfa inclusion in high producing dairy cow diets has declined approximately 50%, from 28% to 14% of the diet dry matter (Robinson, 2019). Much of this reduction has been related to perceptions that alfalfa is expensive, more difficult to grow, and lower yielding relative to corn silage. However, these observations ignore all the benefits that alfalfa contributes to animal performance, soil health, and the sustainability benefits that result from rotational cropping. Furthermore, many of these aspects are overlooked by feed formulation programs that are parameterized on traditional nutritional constraints, where alfalfa use can be minimized or removed from the diet entirely by least cost optimizations.

Therefore, there is a need to better define the benefits of alfalfa in the dairy landscape so that they can be better represented in feed formulation and nutrient planning programs.

OBJECTIVE

The objective of this study was to identify potential positive associative effects between alfalfa and corn silage on improving the amount and efficiency of milk protein and milk fat in high producing cows. We hypothesized that as alfalfa incrementally replaced corn silage, there would be a significant boost of milk fat output and either maintain or modestly improve milk protein.

MATERIALS AND METHODS

Experimental procedures were approved by the William H. Miner Agricultural Research Institute Animal Care and Use Committee (protocol no. 2021AUR01). The animal study was conducted at the Charles J. Sniffen Dairy Research Center of the William H. Miner Agricultural Research Institute (Chazy, NY) from February 11 to May 13, 2021.

Experimental Design and Management of Cows

Cows were housed in a pen with sand-bedded free stalls, fed once per day at 12:30 using the Calan Broadbent Feeding System (American Calan Inc., Northwood, NH), and milked three times daily at 04:30, 12:30, and 20:30 in a double-twelve parallel milking parlor (Xpressway Parallel Stall System, BouMatic LLC, Madison, WI). Cows had free access to water via troughs available in pen. Animals were trained to access feed from a Calan Broadbent Feeding System (American Calan Inc.) before the beginning of the experiment.

The experiment was carried out as a randomized complete block design study. Due to cow availability, the experiment was separated into 2 enrollment phases. Forty-five lactating Holstein cows [15 primiparous and 30 multiparous; mean \pm standard deviation: 2.02 \pm 0.89 lactation, 111 \pm 27 days in milk (DIM), 679 \pm 55 kg of body weight (BW)], and 60 lactating Holstein cows (30 primiparous and 30 multiparous; mean \pm SD: 1.97 \pm 1.25 lactation, 104 \pm 22 DIM, 679 \pm 70 kg of BW) were enrolled at the beginning of enrollments 1 and 2, respectively. Each enrollment consisted of a 1-wk covariate period and 4-wk experimental period. At the end of each covariate period, cows were blocked by parity (i.e. primiparous vs. multiparous), milk yield, and DIM into blocks of 5 cows. Cows within each block were assigned randomly to one of the five experimental diets (Table 1) composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: (1) 10:90 (**10ALF**), (2) 30:70 (**30ALF**), (3) 50:50 (**50ALF**), (4) 70:30 (**70ALF**), (5) 90:10 (**90ALF**). The 50ALF diet was also used as the covariate diet in both enrollments. Water was added to 50ALF (8% as fed), 70ALF (10% as fed), and 90ALF (25% as fed) diets to reduce dry matter (DM) of the diets. The diets were formulated using AMTS.Cattle.Professional 4.16.1 (Agricultural Modeling and Training Systems, LLC, Groton, NY) with Cornell Net Carbohydrate and Protein System (CNCPS) biology to meet nutrient requirements of a lactating dairy cows with 726 kg BW, 3.00 body condition score (BCS), 110 DIM, 43.0 kg/d milk, 3.75% milk fat, 3.10% milk true protein, and 26.3 kg/d dry matter intake (DMI).

Concentrates included beet pulp, two concentrate mixtures [high-protein low-starch (**HPLS**) vs. low-protein high-starch (**LPHS**)], BergaFat F100 (Berg+Schmidt America, LLC, Libertyville, IL), and Calfat (Volac Wilmar USA, Perland, TX). The high-protein low-starch concentrate mixture was mainly composed of ground corn, AminoMax, canola meal, and soybean meal, and was formulated to contain 32.4% crude protein (CP), 13.7% NDF, and 20.2% starch. The low-protein high-starch concentrate mixture primarily consisted of ground corn, and had a formulated to contain 13.3% CP, 8.9% aNDFom, and 62.0% starch. These two concentrate mixtures were produced by Poulin Grain Inc. (Newport, VT), and were included in the diets at different ratios to balance for differences in the nutrient composition between the range in which alfalfa hay and corn silage were included in the diets.

Data Collection, Sampling Procedures, and Analytical Methods

Feed Ingredients and Diets. High-quality rectangular alfalfa bales were purchased from a hay producer in Ohio and delivered to the William H. Miner Agricultural Research Institute before the beginning of the experiment. Alfalfa bales were chopped using a Haybuster bale processor (DuraTech Industries International, Inc., Jamestown, ND) with 7.62- and 5.08-cm screens.

Corn silage, alfalfa hay, straw, total mixed ration (TMR), and orts were sampled three times per week, and the beet pulp, two concentrate mixtures, and fat sources (i.e., BergaFat F100 and Calfat) were sampled once per week. Following collection, one portion of these feed samples was used to determine DM by drying for 18 to 24 h in a forced-air oven at 105°C. The proportions of feed ingredients in TMR were adjusted on an as-fed basis when DM values of ingredients were outside the normal range. Dry matter values of TMR and orts were used to calculate DMI. Another portion of each feed sample (i.e., forages, concentrates, and TMR; no orts) was stored at -20°C and then composited every 2 weeks. The biweekly composited samples were then shipped to Cumberland Valley Analytical Services (Waynesboro, PA), and were analyzed for CP (method 990.03; AOAC International, 2000), soluble CP (Krishnamoorthy et al., 1982), neutral detergent insoluble protein [Van Soest et al., 1991; Leco FP-528 Nitrogen Combustion Analyzer (LECO Co., St. Joseph, MI)], aNDFom (Van Soest et al., 1991), acid detergent fiber (ADF; method 973.18; AOAC International, 2000), acid detergent lignin (ADL; Goering and Van Soest, 1970), starch (Hall, 2009), sugars (Dubois et al., 1956), crude fat (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), and minerals (method 985.01; AOAC International, 2000). Composited samples of forages were analyzed for 24-h in vitro NDF digestibility (Goering and Van Soest, 1970). Additionally, 7-h in vitro starch digestibility of corn silage and two concentrate mixtures, and fermentation analysis of corn silage were measured. Composited samples of fat sources were analyzed for fat using the acid hydrolysis method (method 954.02; AOAC International, 2000).

Furthermore, another portion of forages (i.e., corn silage, long and chopped alfalfa hay, and straw), TMR and orts samples was used to determine particle size distribution on an as-fed basis using a Penn State Particle Separator (Lammers et al., 1996) modified to include a 4-mm screen. In addition, forages, TMR, and orts samples were composited biweekly for measurement of particle size distribution using the same method as mentioned above. The physical effectiveness factor (pef) of TMR and orts was determined as the proportion of particles collected on ≥ 4.0 -mm screen. Physically effective NDF (peNDF) was calculated as the NDF content in TMR multiplied by its pef (Mertens, 1997).

Dry Matter Intake. Dietary ingredients were mixed and offered as TMR once daily using a Super Data Ranger mobile mixer (American Calan Inc.) and orts were collected before fresh feed delivery. Feed offered and orts were recorded by individual cow to calculate DMI.

Milk Yield and Composition. Milk yields were recorded electronically at each milking during the covariate and treatment periods (ProVantage Information Management System; Bou-Matic, Madison, WI). Milk samples from six consecutive milkings for each cow were collected weekly. The milk samples were analyzed for fat, true protein, lactose (anhydrous), solids nonfat, urea nitrogen, and de novo, mixed, and preformed fatty acids by mid-infrared procedures (CombiScope FTIR 300 Hp; Delta Instruments, Drachten, The Netherlands; Wojciechowski and Barbano, 2016; Wojciechowski et al., 2016; Woolpert et al., 2016). Somatic cell counts were analyzed by flow cytometry (CombiScope FTIR 300 Hp, Delta Instruments, Drachten, The Netherlands). Daily milk samples were mathematically composited after analysis in proportion to milk yield at each sampling within a day. Somatic cell count was transformed and analyzed as somatic cell score (SCS) according to Shook et al. (1993) using the equation: $SCS = \log_2(SCC/100) + 3$ where SCC is in units of 1,000 cells/mL. Fat-corrected milk (4.0%) was calculated as $0.4 \times \text{kg of milk} + 15 \times \text{kg of fat}$ (NRC, 2001). Solids-corrected milk was calculated according to Tyrrell

and Reid (1965): $[(12.3 \times \text{kg of fat}) + (6.56 \times \text{kg of solids non-fat}) - (0.0752 \times \text{kg of milk})]$. Energy-corrected milk was calculated using a formula modified to account for use of true protein instead of total protein (Tyrrell and Reid (1965); Mark Stephenson, University of Wisconsin; <https://dairymarkets.org/PubPod/Reference/Library/Energy%20Corrected%20Milk>): $0.327 \times \text{kg of milk} + 12.95 \times \text{kg of fat} + 7.65 \times \text{kg of true protein}$.

Feed and Energy Efficiency. Feed efficiencies were calculated by dividing milk yield, FCM, and ECM yield by DMI. Energy conversion efficiency was estimated by the following equation: $ECE = \text{ECM} / \text{ME intake (Mcal/d)}$ (Mäntysaari et al., 2012). Metabolizable energy was estimated using AMTS with cow input variables and feed analysis from wk 4 of the treatment period for each enrollment.

Body Weight and Body Condition Score. Body weight was measured using an Allweigh computerized scale (Allweigh Scale System Inc., Red Deer, Canada), and BCS was determined by 3 trained individuals using a 5-point scale with 0.25 increments (Ferguson et al., 1994) once per week over the duration of the study.

Rumination. Rumination was monitored and recorded (SCR DataFlow II; SCR North America) for the duration of the study. Rumination data were summarized as a daily mean and then a weekly mean for the covariate period and week 4 of the treatment period.

Statistical Analyses

The final dataset used for analysis included 104 cows (45 primiparous and 59 multiparous). One cow was removed from the study during the covariate period because of mastitis that did not respond to treatment.

Statistical computations were performed using the Statistical Analysis System (version 9.4; SAS Institute Inc., Cary, NC). Data from the analysis of feed ingredients and diets were analyzed using the MEANS procedure of SAS and were reported as descriptive statistics (mean \pm standard deviation).

Data from the cows were analyzed as a randomized block design with a covariate period. Cow was defined as the experimental unit since the treatment was applied to each cow through the use of an individual feeding bin. Data that were collected over time (e.g. intake, milk yield, milk composition, body weight, and body condition score) were reduced to either a covariate period mean or a treatment period mean. The last week of the treatment period was used for final analysis for the treatment period. Data were subjected to analysis of covariance using the MIXED procedure of SAS (Littell et al., 1996). The model included a covariate and the fixed effect of treatment and enrollment. If enrollment was not significant ($P > 0.25$) then it was removed from the model. Enrollment remained in the model for milk (kg), BCS, MUN, chain length, de novo and mixed FA (g/100g FA), milk/DMI, and SNF (kg). Block was a random effect in the model. Linear, quadratic, and cubic orthogonal contrast were tested using the CONTRAST statement of SAS. Significance was concluded when $P \leq 0.05$ and tendencies discussed when $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Dietary and Ingredient Nutrient Composition

Table 1 details the ingredient composition of the diets fed during the covariate and treatment periods. Figure 1 contains photographs of the five diets to visually demonstrate their physical form. The diets varied in the amount of corn silage replaced with alfalfa hay. Beet pulp and two concentrate mixes were varied in their inclusion across diets in an effort to maintain similar NDF and starch among diets (Table 2). The amount of CP in the diets increased with greater inclusion of alfalfa hay but overall metabolizable protein was formulated to be similar as predicted by the CNCPS model. The chemical composition, *in vitro* digestibility, and fermentation analysis for individual ingredients used in the treatment period are presented in Table 3. All values were within expected ranges for the feed ingredients included in the diets.

The particle size of the individual ingredients, TMR, and orts are provided in Table 4. With the inclusion of the chopped alfalfa hay, the *pef* was only 0.29 for the 90ALF diet, while the *pef* for the 10ALF, 30ALF, 50ALF, and 70ALF were 0.63, 0.57, 0.49, 0.40, respectively during the first week of the treatment period in enrollment one (data not shown). Likely as a result, two cows (i.e., 1 for 70ALF and 1 for 90ALF) were diagnosed with a displaced abomasum during the treatment period (7 and 11 d after start of treatment period). To address the low *pef* of the diets with higher inclusion of chopped alfalfa hay, 1.5% DM chopped straw was added into all the diets in the first enrollment to replace equal amounts of both alfalfa hay and corn silage on an as-fed basis to increase *peNDF* and also minimize changes across diets. In enrollment 2, 50% alfalfa hay mixed in the diets was chopped as in enrollment one, and the other 50% of alfalfa hay was added without chopping (i.e., long alfalfa hay) although the mixing procedure did reduce the length of the unchopped hay. No straw was included in the diets during enrollment two. The inclusion of the unchopped alfalfa hay increased the *pef* of the 90ALF diet to 0.40 in enrollment two versus 0.30 in enrollment one. The other diets were similar between enrollments.

Overall, these five TMR were much smaller in particle size than silage-based diets typically fed to lactating cattle in the US; but importantly, they were very similar in particle distribution to diets commonly fed in the Parma region of Italy where dry forage diets predominate in the production of Parmigiano Reggiano cheese (Heinrichs et al., 2021).

In our study, we used alfalfa hay rather than silage because there was no available source of high-quality alfalfa silage that met our specifications for NDF (i.e., approximately 30 to 35% of DM). Consequently, we sourced sufficient alfalfa hay from one location in Ohio for the entire study that then had to be chopped prior to feeding. As an experimental model hay was judged to be our best option because it ensured more uniformity and consistency during the study than silage would have. Even though many dairy farmers feed silage rather than hay, the dietary model that we used with dry hay should be largely applicable to silage systems in terms of the cow's lactation responses. Specifically, previous research comparing alfalfa hay and silage found that they were often similar in the DMI and FCM responses elicited (Broderick, 1985; Broderick, 1995). In general, practical on-farm considerations would be potential leaf losses when drying and baling alfalfa and the challenge of chopping and feeding dry alfalfa hay versus similar quality silage, including whether or not to add water to the ration (as we experienced in our study).

Table 5 summarizes predicted metabolizable energy (ME), metabolizable protein (MP), and lysine profiles for the five diets based on cow characteristics and feed analyses determined during the study (i.e., wk 4 for treatment for cow responses and wk 3 and 4 for feed analyses) using

AMTS with the CNCPS biology model. Overall, MP supply increased from 2802 to 2960 g/d as the ratio of alfalfa hay to corn silage increased (or from 107 to 112 g/kg of DMI). Our goal was to deliver a similar amount of MP as alfalfa proportion increased, but in fact the 50, 70, and 90ALF diets provided between 2.8 and 4.6% more MP per kg of DMI. As the proportion of alfalfa hay in the diet increased, supply of lysine also increased by about 5.7% for the 50, 70, and 90 ALF diets. Likewise, as the alfalfa-to-corn silage ratio increased, supply of methionine increased by about 7%. These changes reflect the relative composition of lysine in corn versus alfalfa protein (Park et al., 2020).

Lactational Performance Responses During the Treatment Period

There was no significant effect ($P \geq 0.25$) of enrollment, despite the differences in particle size of the alfalfa hay or inclusion of wheat straw, on DMI, ECM or SCM yield, or efficiency of ECM or SCM production (ECM/DMI or SCM/DMI; data not shown).

The lactation responses to the different ratios of alfalfa and corn silage are presented in Table 6 including the linear, quadratic, and cubic effects of these diets. Compared to previous studies that are referenced in this discussion, the cows in our study were higher in DMI and milk production, averaging 26.6 kg/d in DMI and 44.3 kg/d in milk yield. The sole exception was the study by Wattiaux and Karg (2004) for which their cows had DMI and milk yield similar to our study. It is important to emphasize that our study is unique given the wide range of dietary alfalfa-to-corn silage ratios fed and the high level of production which should make the results directly applicable to progressively managed dairy herds.

Dry matter intake was not affected by diet. In fact, as the ratio of alfalfa to corn silage ranged between 10:90 and 90:10 (DM basis) DMI only varied by 0.5 kg/d and averaged about 3.9% of BW. Previous studies have reported variable responses in DMI as ratio of alfalfa to corn silage varied, with many showing no effect on DMI (Dhiman and Satter, 1997; Wattiaux and Karg, 2004; Erdman et al., 2011; Arndt et al., 2015); some showing increased DMI as alfalfa increased (Brito and Broderick, 2006; Mullins et al., 2009; Weiss et al., 2009); and one showing a positive effect of corn silage on DMI (Uddin et al., 2020). In evaluating these previous studies, it is clear that ration formulation strategy plays an important role in the relative intake and milk yield response of cows to varying proportions of alfalfa and corn silage (i.e., forage percentage in the ration, carbohydrate content, and significant use of forage or non-forage sources of fiber). Clearly, with our formulation approach and using alfalfa hay, DMI was unaffected across a wide range of alfalfa hay to corn silage ratios.

Likewise, yield of milk and either energy- or solids-corrected milk was unaffected by the ratio of alfalfa and corn silage. Efficiency of ECM and SCM production was also unaffected by the ratio of the forages. There was a trend ($P = 0.10$) for a cubic effect on milk/DMI with cows fed the 70ALF diet having slightly lower efficiency, but generally cows on all five diets had high dairy production efficiencies. As with DMI, previous reports on the effect of alfalfa-to-corn silage ratio on milk yield and its efficiency of production have been variable. Many studies have observed no effect of the ratio on ECM or FCM yield (Kleinschmit et al., 2007; Mullins et al., 2009; Erdman et al., 2011) and a few have shown a positive response of greater corn silage (Groff and Wu, 2005; Uddin et al., 2020).

Several studies have concluded that a blend of alfalfa and corn silage that avoids the extremes seems to be desirable to maximize SCM or ECM yield. Arndt et al. (2015) found a quadratic effect of ratio of alfalfa silage to corn silage between 20:80 and 80:20 (DM basis) on fat-

and protein-corrected milk yield with the predicted maximum being at 50:50 alfalfa to corn silage. Weiss et al. (2009) found that ECM yield was maximized for diets containing 75:25 alfalfa silage to corn silage (DM basis). Dhiman and Satter (1997) concluded that corn silage and alfalfa silage in a ratio between 1/3 to 2/3 corn silage was optimal for milk yield and most efficient use of dietary N.

Despite the overall lack of diet effect on ECM or SCM production, there are several responses in milk composition in our study that are worth mentioning in relation to alfalfa and corn silage ratio. Over a wide range of alfalfa hay to corn silage ratios, content and production of milk fat was high and unaffected, averaging about 4.0% and 1.8 kg/d. Whether the low dietary particle size across all treatments contributed to this lack of response in milk fat yield should be considered. It is possible that with greater particle size hay or silage that milk fat may have responded to varying proportions of alfalfa and corn silage. Nonetheless, the high milk fat content in all diets indicates healthy rumen conditions as rumen pH and milk fat have been reported to be positively related (Allen, 1997).

There was a significant ($P = 0.04$) cubic effect of diet on milk true protein output. There was also a significant effect of increasing proportion of corn silage on lactose percentage ($P = 0.01$) and lactose output ($P = 0.06$) with both measures increasing as corn silage ratio increased. There was a significant linear ($P = 0.001$), quadratic ($P = 0.002$), and cubic effect on MUN ($P = 0.002$). Milk urea nitrogen was reduced between the 10 and 30 ALF diets, and then it increased incrementally for the 50, 70, and 90 ALF diets. Although the difference in MUN among the five diets was relatively small, it may be that the greater soluble protein of alfalfa hay complemented the rumen fermentable starch provided by the corn silage and diet, resulting in a stimulation of microbial protein production in the rumen. This would make sense given that milk true protein was greatest for the 30ALF diet and MUN was the lowest. For the higher alfalfa diets (50, 70, and 90ALF), MUN increased likely reflecting an oversupply of rumen degradable protein, although milk protein output generally remained similar to the 10ALF diet.

Observed changes in milk fatty acid fractions were all very small and likely not biologically meaningful. Nonetheless the slight quadratic effect on de novo fatty acids and unsaturation index as alfalfa proportion increased may suggest an optimal ratio of alfalfa hay and corn silage between 30:70 and 50:50. Greater proportion of de novo fatty acids in milk fat and lower unsaturation index both indicate better conditions for rumen fiber fermentation and synthesis of milk fat (Woolpert et al., 2016).

Changes in BW and body condition were minimal among the five diets and almost certainly not of biological importance.

There was a significant linear ($P < 0.001$) effect on rumination. The amount of time that cows spent rumination per day decreased from 499 to 396 min/d from the 10ALF diet to the 90ALF diet, respectively. Overall, these rumination times are greater than previously reported for finely chopped alfalfa hay diets (443 min/d; Cavallini et al., 2018) with the exception of the 90ALF diet. For lactating dairy cows fed a wide range of diets the average range of rumination has been reported as being between 420 to 480 min/d (Haan, 2020). The diets that we fed were within this expected range with the exception of the 90ALF diet that was slightly less. Overall, even though the peNDF content of these diets was less than ordinarily fed in the US, the rumination activity as well as the milk fat content were well within desirable ranges.

CONCLUSIONS

The ratio of alfalfa hay to corn silage was varied between 10:90 and 90:10 in diets containing 62% forage (DM basis). Cows in this study were high producing and averaged 26.6 kg/d DMI and 44.3 kg/d milk yield with 4.1% fat and 3.0% true protein. Regardless of the proportion of alfalfa and corn silage in the diet, the yield and efficiency of production of ECM and SCM were similar. These results indicate that you could feed a ration formulated using our approach that ranged between 10:90 and 90:10 alfalfa to corn silage and expect similar intake and milk production. In addition, modest, but statistically significant, changes in milk true protein output and milk urea nitrogen indicate that between 30:70 and 50:50 alfalfa to corn silage may be optimal. Small changes in milk fatty acid metrics support this same conclusion. Daily rumination time was within the expected range for lactating dairy cattle with the exception of the diet containing the 90:10 alfalfa-to-corn silage ratio.

Overall, given the agronomic benefits of alfalfa, our results suggest that cows will perform well on diets containing as much as 90% of the forage as alfalfa with minimal corn silage compared with high corn silage rations. An optimal ratio of the two forages where milk true protein is maximized, MUN is minimized, and milk fatty acid metrics are optimized is about 30:70 to 50:50 alfalfa hay and corn silage. Based on our study and previously published research, this translates into diets containing between 20 to 25% alfalfa and up to 35% alfalfa in the ration dry matter.

In the future, based on these dairy performance results and our knowledge of the agronomic advantages of alfalfa, sustainable dairy-forage programs can utilize higher alfalfa-to-corn silage ratios than is commonly practiced today within the dairy industry.

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TABLES AND FIGURES

Table 1. Ingredient composition (% of dry matter) of the diets with different ratios of corn silage to alfalfa hay fed to lactating dairy cattle.

Item	Diets ¹				
	10ALF	30ALF	50ALF ^{2,3}	70ALF ³	90ALF ³
Conventional corn silage	56.40	43.46	31.05	18.61	5.69
Alfalfa hay ⁴	5.69	18.63	31.05	43.42	56.38
Straw ⁵					
Beet pulp	7.26	5.65	4.84	1.61	0.00
Berga fat ⁶	1.61	1.21	1.38	1.55	1.37
Calfat ⁷	1.00	0.86	0.86	1.03	1.00
Concentrate mixture					
Ground corn	6.79	14.95	18.50	24.02	29.87
AminoMax Pro Poulin ^{8,9}	5.32	3.96	3.29	2.71	1.82
Canola meal	5.31	3.30	2.34	1.34	-
Soybean meal	3.90	2.43	1.72	0.99	-
Wheat middlings	0.92	0.57	0.41	0.23	-
PGI Amino Enhancer ⁹	0.62	0.95	1.08	1.32	1.56
Sugar	1.56	1.12	0.91	0.71	0.42
Salt	0.47	0.45	0.44	0.45	0.45
Sodium sesquicarbonate	0.79	0.78	0.76	0.79	0.79
Calcium carbonate	1.42	0.88	0.63	0.36	-
Urea	0.39	0.25	0.17	0.23	-
Magnesium oxide	0.30	0.32	0.33	0.36	0.38
Trace min/vit mix ^{9,10}	0.12	0.12	0.12	0.12	0.12
Smartamine M ¹¹	0.06	0.06	0.07	0.07	0.08
XPC Yeast culture ¹²	0.04	0.05	0.05	0.05	0.05
Rumensin ¹³	0.01	0.01	0.01	0.01	0.01

¹The diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

²The 50ALF diet was used as the covariate diet.

³Water was added to 50ALF (8% as fed), 70ALF (10% as fed), and 90ALF (25% as fed) to reduce DM of the diets.

⁴Alfalfa hay in enrollment two was included as 50% chopped and 50% unchopped (i.e., long).

⁵In enrollment one, chopped straw was added into all the diets at 1.5% dry matter to replace equal amounts of both alfalfa hay and corn silage on an as fed basis.

⁶Berg + Schmidt America, LLC; Libertyville, IL.

⁷Volac Wilmar USA, Perland, TX

⁸Afgritech, LLC, Watertown, NY.

⁹Poulin Grain Inc., Newport, VT.

¹⁰Micronutrients, Indianapolis, IN.

¹¹Adisseo USA, Inc.; Alpharetta, GA.

¹²Diamond V, Cedar Rapids, IA.

¹³Elanco Animal Health, Greenfield, IN.

Table 2. Calculated chemical composition of the diets¹ (% of dry matter, unless otherwise noted; mean \pm standard deviation). Values presented here are from week 3 and 4 of the treatment period for each enrollment.

Item	Diet				
	10ALF	30ALF	50ALF ²	70ALF	90ALF
n	2	2	2	2	2
Dry matter, % ³	45.0 \pm 1.3	50.0 \pm 1.4	52.5 \pm 1.2	59.4 \pm 1.3	60.4 \pm 0.6
Crude protein (CP)	15.7 \pm 0.2	15.6 \pm 0.2	16.4 \pm 0.2	17.1 \pm 0.2	17.6 \pm 0.1
Soluble protein, % CP	44.3 \pm 5.5	41.0 \pm 3.3	38.4 \pm 1.4	35.7 \pm 0.6	32.8 \pm 2.6
Neutral detergent CP	1.6 \pm 0.0	1.5 \pm 0.0	1.5 \pm 0.0	1.4 \pm 0.1	1.3 \pm 0.1
Acid detergent fiber	19.4 \pm 0.0	19.1 \pm 0.0	19.3 \pm 0.0	18.9 \pm 0.1	18.8 \pm 0.1
Neutral detergent fiber (aNDF) ⁴	30.6 \pm 0.1	29.3 \pm 0.2	28.3 \pm 0.1	26.7 \pm 0.0	25.5 \pm 0.1
Acid detergent lignin	3.5 \pm 0.1	3.6 \pm 0.0	3.8 \pm 0.1	4.0 \pm 0.1	4.2 \pm 0.2
Nonfiber carbohydrates	42.1 \pm 0.7	44.0 \pm 0.8	43.7 \pm 0.8	43.8 \pm 0.7	44.4 \pm 0.7
Nonstructural carbohydrates	32.0 \pm 0.5	33.2 \pm 0.5	31.9 \pm 0.3	31.8 \pm 0.2	31.7 \pm 0.1
Starch	26.5 \pm 1.1	27.9 \pm 1.2	26.3 \pm 1.0	26.2 \pm 1.0	26.0 \pm 0.9
Sugar (ESC ⁵)	5.6 \pm 0.6	5.3 \pm 0.7	5.6 \pm 0.7	5.6 \pm 0.7	5.6 \pm 0.8
Ether extract	5.1 \pm 0.4	4.6 \pm 0.4	4.6 \pm 0.4	4.9 \pm 0.4	4.6 \pm 0.4
Ash	8.2 \pm 0.0	8.2 \pm 0.0	8.6 \pm 0.0	8.9 \pm 0.1	9.2 \pm 0.1
Calcium	1.16 \pm 0.00	1.06 \pm 0.00	1.1 \pm 0.00	1.14 \pm 0.00	1.13 \pm 0.00
Phosphorus	0.35 \pm 0.01	0.33 \pm 0.01	0.32 \pm 0.02	0.32 \pm 0.02	0.31 \pm 0.02
Magnesium	0.40 \pm 0.01	0.40 \pm 0.00	0.4 \pm 0.01	0.41 \pm 0.02	0.41 \pm 0.03
Potassium	1.20 \pm 0.00	1.36 \pm 0.02	1.56 \pm 0.04	1.75 \pm 0.06	1.94 \pm 0.08
Sulfur	0.20 \pm 0.00	0.20 \pm 0.00	0.21 \pm 0.00	0.21 \pm 0.00	0.22 \pm 0.00
Sodium	0.50 \pm 0.04	0.48 \pm 0.03	0.47 \pm 0.02	0.49 \pm 0.01	0.49 \pm 0.01
Chloride ion	0.49 \pm 0.01	0.55 \pm 0.02	0.62 \pm 0.04	0.7 \pm 0.06	0.77 \pm 0.07
Iron, mg/kg	245 \pm 12	253 \pm 18	270 \pm 51	271 \pm 87	282 \pm 121
Copper, mg/kg	18 \pm 0	17 \pm 1	18 \pm 1	19 \pm 2	19 \pm 2
Manganese, mg/kg	54 \pm 4	56 \pm 10	60 \pm 16	65 \pm 22	69 \pm 29
Zinc, mg/kg	87 \pm 11	87 \pm 10	86 \pm 10	87 \pm 10	88 \pm 10

¹The diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

²The 50ALF diet was also used as the covariate diet.

³Dry matter values were measured during week 3 and 4 of the treatment period. Sample n for each diet: 10ALF, 30ALF, 50ALF, and 70ALF n=13, 90ALF n=12.

⁴NDF with residual ash using α -amylase and without sodium sulfite

⁵Ethanol soluble carbohydrates.

Table 3. Chemical composition, in vitro digestibility, and fermentation analysis (mean \pm SD) of major ingredients¹ in the diets (% of DM, unless otherwise noted; treatment period; mean \pm standard deviation). Values presented here are from week 3 and 4 of the treatment period for each enrollment.

Item	Conventional corn					
	silage	Alfalfa hay	Straw	Beet pulp	HPLS ²	LPHS ²
Samples, n	2	3 ³	1	2	2	2
Dry matter, %	31.6 \pm 1.2	89.3 \pm 0.6	89.4	90.5 \pm 0.6	88.5 \pm 0.6	87.2 \pm 0.1
Crude protein (CP)	9.0 \pm 0.0	21.7 \pm 0.8	4.1	8.6 \pm 0.3	31.7 \pm 0.1	14.2 \pm 0.4
Soluble protein, % CP	4.9 \pm 0.7	8.3 \pm 1.0	1.6	2.1 \pm 0.6	10.9 \pm 1.3	3.0 \pm 0.1
Neutral detergent CP	1.0 \pm 0.2	1.6 \pm 0.3	1.5	4.6 \pm 0.4	2.3 \pm 0.4	0.9 \pm 0.2
Acid detergent fiber	22.4 \pm 0.6	28.4 \pm 2.9	53.4	27.7 \pm 0.4	10.4 \pm 0.1	3.7 \pm 0.5
Neutral detergent fiber (aNDFom) ⁴	37.4 \pm 0.9	34.1 \pm 3.1	80.9	38.9 \pm 2.5	15.7 \pm 0.1	10.6 \pm 1.1
Acid detergent lignin	3.0 \pm 0.0	6.3 \pm 0.6	8.9	3.8 \pm 0.2	4.0 \pm 0.1	1.4 \pm 0.4
Nonfiber carbohydrates	47.8 \pm 1.3	32.2 \pm 2.5	9.1	45.4 \pm 3.3	36.7 \pm 0.1	66.7 \pm 1.2
Nonstructural carbohydrates	36.4 \pm 1.4	11.5 \pm 0.7	2.4	15.3 \pm 0.3	35.4 \pm 0.0	65.8 \pm 2.1
Starch	35.8 \pm 1.9	3.4 \pm 0.8	1.4	0.3 \pm 0.1	22.2 \pm 0.7	62.6 \pm 3.6
Sugar (ESC ⁵)	0.7 \pm 0.5	8.0 \pm 0.8	1.0	15.1 \pm 0.2	13.2 \pm 0.7	3.3 \pm 1.5
Ether extract	2.6 \pm 0.6	2.0 \pm 0.4	0.78	1.2 \pm 0.4	3.4 \pm 0.1	3.2 \pm 0.1
Ash	4.3 \pm 0.0	11.4 \pm 0.3	6.59	10.6 \pm 0.5	14.8 \pm 0.4	6.3 \pm 0.2
Calcium	0.26 \pm 0.02	1.68 \pm 0.09	0.22	1.1 \pm 0.01	2.73 \pm 0.08	0.24 \pm 0.01
Phosphorus	0.24 \pm 0.01	0.33 \pm 0.02	0.05	0.09 \pm 0.01	0.68 \pm 0.01	0.33 \pm 0.01
Magnesium	0.14 \pm 0.00	0.23 \pm 0.02	0.08	0.26 \pm 0.00	1.04 \pm 0.05	0.79 \pm 0.04
Potassium	1.18 \pm 0.01	3.02 \pm 0.16	1.30	0.46 \pm 0.01	1.19 \pm 0.06	0.54 \pm 0.02
Sulfur	0.1 \pm 0.0	0.25 \pm 0.02	0.08	0.29 \pm 0.01	0.40 \pm 0.01	0.22 \pm 0.02
Sodium	0.01 \pm 0.0	0.05 \pm 0.01	0.02	0.05 \pm 0.01	1.74 \pm 0.17	1.30 \pm 0.04
Chloride ion	0.24 \pm 0.0	0.84 \pm 0.13	0.14	0.03 \pm 0.01	1.11 \pm 0.02	0.85 \pm 0.00
Iron, mg/kg	125 \pm 60	293 \pm 198	71	884 \pm 169	333 \pm 86	226 \pm 22
Copper, mg/kg	6 \pm 1	16 \pm 2	5	8 \pm 0	46 \pm 2	28 \pm 4
Manganese, mg/kg	19 \pm 3	57 \pm 41	21	58 \pm 6	126 \pm 14	83 \pm 3
Zinc, mg/kg	60 \pm 2	58 \pm 5	47	57 \pm 1	165 \pm 35	144 \pm 21
NE _L , Mcal/kg	1.64 \pm 0.02	1.35 \pm 0.06	0.99	1.38 \pm 0.05	1.55 \pm 0.02	1.87 \pm 0.03
7-h starch digestibility	61.3 \pm 4.7	-	-	-	61.0 \pm 1.8	53.4 \pm 0.7
30-h aNDFom digestibility	52.0 \pm 1.7	39.7 \pm 5.7	41.4	-	-	-

Lactic acid	3.0±0.1	-	-	-	-	-
Acetic acid	3.2±0.4	-	-	-	-	-
Propionic acid	0.1±0.0	-	-	-	-	-
Butyric acid	0±0	-	-	-	-	-
Total volatile fatty acids	6.1±0.3	-	-	-	-	-
Ammonia, % CP	0.9±0.0	-	-	-	-	-
pH	4.0±0.05	-	-	-	-	-

¹Bergafat and Calfat contained 99.9 and 91.2% crude fat, respectively.

²High-protein low-starch (HPLS) concentrate mix and low-protein high-starch (LPHS) concentrate mix.

³Chopped (n = 2) and unchopped (long; n = 1) alfalfa hay.

⁴NDF with residual ash using α -amylase and without sodium sulfite.

⁵Ethanol soluble carbohydrates.

Table 4. Particle size distribution (% as-fed, mean \pm standard deviation) of composited forage ingredients, total mixed rations, and Orts. Values presented here are from week 3 and 4 of the treatment period for each enrollment.

Distribution, % as-fed	Ingredient			
	Corn silage	Long alfalfa hay	Chopped alfalfa hay	Straw
Samples, n	2	1	2	1
>19.0 mm	8.6 \pm 0.9	38.1	8.6 \pm 1.4	26.2
8.0 to 19.0 mm	66.2 \pm 2.3	15.4	15.0 \pm 0.5	29.0
4.0 to 8.0 mm	13.6 \pm 0.5	11.2	13.4 \pm 0.4	19.0
<4.0 mm	11.6 \pm 0.9	35.3	63.0 \pm 0.5	25.8
pef	0.88 \pm 0.01	0.64	0.37 \pm 0.00	0.74
peNDF, %	33.0 \pm 1.1	24.1	12 \pm 0.9	60.1

Distribution, % as-fed	Diet ¹				
	10ALF	30ALF	50ALF ²	70ALF	90ALF
Samples, n	2	2	2	2	2
>19.0 mm	4.4 \pm 0.3	5.8 \pm 0.5	5.9 \pm 2.3	7.1 \pm 5.3	8.9 \pm 8.3
8.0 to 19.0 mm	45.8 \pm 0.2	37.6 \pm 0.2	31.7 \pm 1.2	23.4 \pm 0.8	15.6 \pm 1.5
4.0 to 8.0 mm	11.6 \pm 0.3	11.4 \pm 0.4	11.5 \pm 0.8	11.5 \pm 0.2	11.6 \pm 0.0
<4.0 mm	38.1 \pm 0.2	45.2 \pm 0.8	50.9 \pm 1.8	58.0 \pm 4.8	64.0 \pm 6.8
pef	0.62 \pm 0	0.55 \pm 0.01	0.49 \pm 0.02	0.42 \pm 0.05	0.36 \pm 0.07
peNDF, %	18.9 \pm 0.1	16.0 \pm 0.3	13.9 \pm 0.6	11.2 \pm 1.3	9.2 \pm 1.8

Distribution, % as-fed	Ort				
	10ALF	30ALF	50ALF	70ALF	90ALF
Samples, n	2	2	2	2	2
>19.0 mm	2.9 \pm 0.2	6.0 \pm 4.7	8.3 \pm 7.2	6.9 \pm 7.9	8.0 \pm 9.4
8.0 to 19.0 mm	52.1 \pm 2.1	39.5 \pm 4.4	33.2 \pm 2.1	19.9 \pm 0.5	13.3 \pm 0.7
4.0 to 8.0 mm	12.3 \pm 0.0	11.7 \pm 0.0	11.9 \pm 0.3	12.0 \pm 0.5	12.4 \pm 0.5
<4.0 mm	32.7 \pm 2.3	42.8 \pm 9.1	46.7 \pm 4.8	61.2 \pm 7.8	66.3 \pm 9.5
pef	0.67 \pm 0.02	0.57 \pm 0.09	0.53 \pm 0.05	0.39 \pm 0.08	0.34 \pm 0.10

¹The experimental diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

²The 50ALF diet was also used as the covariate diet.

Table 5. Model predictions from CNCPS (AMTS) of diets with different ratios of alfalfa hay to corn silage fed to Holstein dairy.

Item	Diet ¹				
	10ALF	30ALF	50ALF	70ALF	90ALF
n	2	2	2	2	2
Metabolizable Energy (ME) Allowable Milk, kg/d	43.9±1.9 ²	44.6±1.6	44.7±2.4	45.2±0.6	43.0±0.1
Metabolizable Protein (MP) Allowable Milk, kg/d	42.9±1.7	43.1±1.2	44.8±1	46.5±0.0	45.8±0.7
ECM ³ /ME intake, kg/Mcal	0.70±0.00	0.70±0.01	0.70±0.01	0.68±0.00	0.71±0.01
ME, % requirement	99.0±0.7	99.9±1.5	98.9±0.3	102.9±0.2	99.1±1.0
MP, % requirement	99.2±0.7	97.5±0.8	101.1±0.0	104.7±0.9	103.6±1.9
MP, g/d	2802±52	2850±74	2923±61	2992±57	2960±32
MP, g/kg dry matter intake	107±0	107±1	110±0	111±2	112±2
Lysine, g	194±2	198±4	198±2	207±4	205±2
Lysine, % requirement	97.0±0.6	96.6±0.5	98.4±2.0	103.5±0.5	102.6±1.7
Methionine, g	71±1	73±2	73±0	77±1	76±0
Methionine, % requirement	100.8±0.3	101.0±1.0	102.4±1.9	108.7±0.3	107.9±1.5

¹The experimental diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

² Mean ± standard deviation.

³Energy-corrected milk.

Table 6. Dry matter intake, milk yield and composition, BW, and BCS in Holstein dairy cattle fed diets with different ratios of alfalfa hay to corn silage during week 4 of the treatment period.

Item	Diet ¹					SEM	<i>P</i> -value		
	10ALF	30ALF	50ALF ²	70ALF	90ALF		Linear	Quadratic	Cubic
DMI, kg/d	26.3	26.6	26.7	26.8	26.4	0.3	0.81	0.27	0.74
DMI, % of BW	3.82	3.85	3.86	3.91	3.91	0.05	0.12	0.92	0.78
Milk yield, kg/d	44.4	44.9	44.9	43.6	43.9	0.6	0.18	0.42	0.24
Milk/DMI, kg/kg	1.69	1.69	1.68	1.63	1.67	0.02	0.13	0.59	0.10
ECM ³ yield, kg/d	47.9	48.7	48.2	47.0	48.3	0.8	0.67	0.89	0.14
ECM/DMI, kg/kg	1.82	1.83	1.81	1.76	1.83	0.03	0.59	0.44	0.12
FCM ⁴ yield, kg/d	44.7	45.2	44.8	43.7	45.0	0.8	0.73	0.76	0.20
FCM/DMI, kg/kg	1.83	1.84	1.82	1.77	1.85	0.04	0.71	0.44	0.20
SCM ⁵ yield, kg/d	44.3	45.0	44.5	43.3	44.4	0.7	0.49	0.96	0.14
SCM/DMI, kg/kg	1.68	1.69	1.67	1.62	1.69	0.03	0.46	0.51	0.12
Milk									
Fat, %	4.08	4.06	4.02	4.01	4.22	0.13	0.58	0.30	0.57
Fat, kg/d	1.80	1.82	1.79	1.75	1.83	0.05	0.94	0.56	0.27
True protein, %	3.01	3.07	3.01	3.02	3.05	0.03	0.78	0.99	0.16
True protein, kg/d	1.33	1.37	1.35	1.31	1.33	0.02	0.39	0.43	0.04
Lactose, %	4.68	4.68	4.67	4.64	4.60	0.02	0.01	0.30	0.88
Lactose, kg/d	2.08	2.10	2.10	2.03	2.02	0.03	0.06	0.29	0.38
SNF, %	8.80	8.85	8.79	8.76	8.76	0.03	0.04	0.34	0.10
SNF, kg/d	3.90	3.97	3.94	3.82	3.84	0.05	0.10	0.29	0.15
MUN, mg/dL	9.8	8.5	10.4	11.0	12.0	0.3	<0.001	0.002	0.002
De novo FA ⁶ , g/100 g milk	0.95	1.01	1.01	0.96	1.01	0.04	0.51	0.58	0.21
De novo FA, g/100 g FA	24.76	25.86	25.82	25.22	25.58	0.24	0.19	0.03	0.007
Mixed origin FA, g/100 g milk	1.62	1.60	1.61	1.59	1.62	0.05	0.95	0.62	0.92
Mixed origin FA, g/100 g FA	42.07	41.51	41.60	41.44	40.81	0.31	0.007	0.72	0.23
Preformed FA, g/100 g milk	1.27	1.25	1.25	1.30	1.33	0.05	0.29	0.43	0.72
Preformed FA, g. 100 g FA	33.12	32.67	32.68	33.38	33.57	0.43	0.23	0.23	0.47
Unsaturation, double bonds/FA	0.2548	0.2506	0.2491	0.2521	0.2629	0.0045	0.22	0.05	0.72
Chain length, carbons/FA	14.67	14.57	14.56	14.62	14.62	0.03	0.87	0.004	0.10
BW, kg	689	694	692	691	671	5	0.008	0.007	0.44

BW change, kg	-9	-13	-12	-10	9	5	0.008	0.007	0.44
BCS	2.97	2.97	2.98	2.96	2.95	0.02	0.29	0.60	0.99
BCS change	0.00	0.00	0.00	0.02	0.03	0.02	0.29	0.62	0.97
Rumination, min/d	499	477	462	449	396	13	<0.001	0.25	0.26

¹The experimental diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

²The 50ALF diet was also used as the covariate diet.

³Energy-corrected milk.

⁴4% Fat-corrected milk.

⁵Solids-corrected milk.

Figure 1. Pictures of diets with different ratios of alfalfa hay to corn silage. The experimental diets composed of 62% forage and 38% concentrate, with the forage portion consisting of different ratios of alfalfa hay and corn silage: 10:90 (10ALF), 30:70 (30ALF), 50:50 (50ALF), 70:30 (70ALF), 90:10 (90ALF).

