Use of indirect calorimetry to evaluate utilization of energy in lactating Jersey dairy cattle consuming diets with increasing inclusion of hydrolyzed feather meal

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ABSTRACT

A study using indirect calorimetry and 12 lactating multiparous Jersey cows (53 ± 23 d in milk at the beginning of the experiment; mean $\pm$ standard deviation) was conducted to evaluate the utilization of energy in cattle consuming diets containing increasing hydrolyzed feather meal (HFM). A triplicated 4×4 Latin square design with 35-d periods (28-d adaption and 4-d collections) was used to compare 4 different dietary treatments. Treatments contained (DM basis) HFM at 0% (0HFM), 3.3% (3.3HFM), 6.7% (6.7MFM), and 10.0% (10HFM). Diets were formulated such that HFM replaced blood meal and nonenzymatically browned soybean meal. With increasing HFM, linear increases were observed for dietary NE_L content (1.61, 1.64, 1.69, and 1.70 ± 0.042 Mcal/kg of DM for 0HFM, 3.3HFM, 6.7MFM, and 10HFM, respectively), and the efficiency of converting ME to NE_L (0.708, 0.711, 0.717, and 0.719). Apparent total-tract digestibility of CP linearly decreased with increasing HFM (63.4, 61.1, 59.9, and $58.6 \pm 1.46\%$ for 0HFM, 3.3HFM, 6.7MFM, and 10HFM, respectively), whereas long-chain fatty acid digestibility increased with increasing HFM (77.2, 77.7, 78.5, and $80.6 \pm 1.30\%$). With increased inclusion of HFM, fecal N excretion increased (199, 230, 239, 237 ± 12.1 g/d for 0HFM, 3.3HFM, 6.7MFM, and 10HFM, respectively), whereas urinary N excretion decreased (166, 151, 155, and 119 ± 14.8 g/d). Increasing the concentration of HFM resulted in a quadratic effect on DMI (19.6, 20.2, 20.3, and 19.1 ± 0.79 kg/d for 0HFM, 3.3HFM, 6.7MFM, and 10HFM, respectively) and milk yield (31.7, 32.0, 31.9, and 29.7 ± 1.32 kg/d). Increasing HFM linearly decreased the milk protein concentration (3.34, 3.29, 3.23, and 3.23 ± 0.158 for 0HFM, 3.3HFM, 6.7MFM, and 10HFM, respectively) and yield (1.05, 1.05, 1.02, and 0.96 ± 0.040 kg). The inclusion of

HFM did not affect energy-correct milk yield (average of 39.3 ± 1.54). Results of this study suggest that HFM can increase dietary NE_L content compared with blood meal and nonenzymatically browned soybean meal and maintained energy-corrected milk yield; however, feeding HFM at greater than 6.7% of diet DM decreased DMI, and protein availability may have been reduced with increased HFM, leading to a linear decrease in milk protein concentration and yield.

Key words: hydrolyzed feather meal, digestibility, dairy cow, nutrition, energy

INTRODUCTION

The production of milk for human consumption has become more efficient over time. In an evaluation of inputs needed to produce 1 billion kg of milk, Capper et al. (2009) concluded that compared with 1944, dairy producers in 2007 used 21% fewer animals, 23% less feed, 35% less water, and 10% less land. In the United States and in confined housing systems, it is generally accepted that feed costs represent the single greatest cost to produce milk (Wolf, 2010). To reduce this expense, dairy producers often purchase byproduct feeds as a less costly source of nutrients to feed cattle (Bradford and Mullins, 2012). The use of feed byproducts also contributes to the sustainability of the dairy industry (Salami et al., 2019) and supports efficiencies of human food production. Byproducts represent a feed input that from a human consumption perspective would be considered a waste product (VandeHaar and St-Pierre, 2006). In support of this suggestion, Karlsson et al. (2018) reported that feeding diets containing a high proportion of byproducts, to replace human-edible cereal grains, resulted in a 3-fold increase in human-edible feed conversion efficiency. A major challenge with byproducts is that although they are generally cost effective they vary in chemical composition and digestibility.

Hydrolyzed feather meal (**HFM**) is a feed byproduct produced from clean, undecomposed feathers that are

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cooked under pressure (AAFCO, 2016). The CP content of HFM is approximately 85%, and when fed to dairy cattle, a high proportion of this protein bypasses rumen fermentation and directly supplies protein to the small intestine (NRC, 2001). Thus, HFM can be used to replace more expensive sources of RUP such as blood meal or nonenzymatically browned soybean meal. The intestinal digestibility of HFM protein may, however, be less than other commonly fed protein sources (Waltz et al., 1989). Surprisingly, only a few studies have sought to evaluate the inclusion of feather meal on nutrient utilization in dairy cattle (Rakes et al., 1968; Harris et al., 1992; Grant and Haddad, 1998). From these studies, the effects of HFM on DMI, milk yield, and milk protein yield were dependent on inclusion, dietary CP content, blood content of HFM, and digestibility of HFM. More recent studies in which diets were formulated to induce an AA deficiency have reported that HFM may result in decreased milk protein yield (Stahel et al., 2014; Giallongo et al., 2017). The crude fat content of HFM ranges from 6 to 14% of DM (Cotanch et al., 2007). Because dietary fat can increase dietary energy content (Andrew et al., 1991; NRC, 2001), increased inclusion of HFM may also affect energy utilization by lactating cows. Therefore, the objectives of this research was to evaluate the overall feeding value of HFM when fed to lactating dairy cattle and to test the effects of HFM on total-tract CP digestibility and energy utilization. We hypothesized that increased inclusion of HFM would decrease CP digestibility leading to decreased milk protein yield; however, due to the contribution of fat the supply of energy would increase.

MATERIALS AND METHODS

Experimental Design, Animals, and Treatments

The University of Nebraska–Lincoln Animal Care and Use Committee Animal approved animal care and experimental procedures. The experiment used 12 multiparous lactating Jersey cows averaging ($\pm$ SD) 53 ± 28 DIM (21 to 107) and 445.3 ± 45.2 kg of BW at the beginning of the experiment. Cows were housed in individual tiestalls equipped with rubber mats in a temperature-controlled (20°C) barn at the Dairy Metabolism Facility in the Animal Science Complex at the University of Nebraska–Lincoln and milked at 0700, 1400, and 2100 h, representing AM, midday, and PM milking. All cows were less than 63 d pregnant at the end of the last experimental period; thus, fetal energy was assumed to be zero (NRC, 2001).

The experimental design was a 3 times replicated 4×4 Latin square with 35-d periods. Animals were blocked into each square by milk production. Treatments alter-

nated over 4 experimental periods and measurements were collected on each animal consuming each treatment within the same period (Kononoff and Hanford, 2006). The study was conducted with a total of 4 experimental periods each being 35 d in duration. Each period included 28 d for ad libitum diet adaptation, targeting about 5% refusals during that time, followed by 4 d of collection with 4 d of 95% ad libitum feeding to reduce the amount of refusals.

Cows were fed 1 of 4 treatment diets that contained HFM on a DM basis at (1) 0% (**0HFM**), (2) 3.3% (**3.3HFM**); (3) 6.7% (**6.7HFM**); and (4) 10% (**10HFM**; Table 1). The HFM was a blend of 2 sources (Pilgrim's, Greeley, CO; Simmons Foods, Siloam Springs, AR). Target inclusion of HFM was achieved by replacing blood meal and nonenzymatically browned soybean meal. The TMR was mixed in a Calan Data Ranger (American Calan Inc., Northwood, NH) and fed once daily at 0900 h.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and frozen at -20°C and later analyzed for DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), lignin (Goering and Van Soest, 1970), sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (2003.05; AOAC International, 2000), ash (943.05; AOAC International, 2000), and minerals (985.01; AOAC International, 2000) at Cumberland Valley Analytical Services Inc. (Hagerstown, MD). Gross energy (**GE**; Parr 6400 Calorimeter, Moline, IL) was measured at the University of Nebraska–Lincoln laboratory. Samples of HFM, blood meal, and nonenzymatically browned soybean meal were analyzed for RUP and digestible RUP according to Ross et al. (2013). Total mixed rations were sampled (500 g) on each day of each collection period and were frozen at -20°C . The TMR sample was used to determine particle size using the Penn State Particle Separator according to Heinrichs and Kononoff (2002). On each day of the collection period, refusals were sampled and frozen at -20°C . The samples were composited by period and individual cow. A subsample was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for nutrient analysis of DM, N, NDF with sodium sulfite, starch, and ash as described previously. A subsample was also used to measure GE (Parr 6400 Calorimeter) at the University of Nebraska–Lincoln laboratory.

Total fecal and urine output was collected from each individual cow during the collection period for 4 con-

secutive days. Personnel were assigned and present during all times of sample collections and when possible, manually collected feces with a 3-quart scoop (Miller Manufacturing, Eagan, MN) upon defecation, but when missed, feces landed on a 137 × 76 cm rubber mat (Snake River Supply, Idaho Falls, ID) that was placed on the floor behind the cow and collected immediately. The feces were deposited multiple times a day from the rubber mats into a large 121-L (32 gallon) garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce nitrogen losses before subsampling. The feces were subsampled (500

g) every day and dried at 60°C in a forced-air oven for 48 h and then composited by cow and period before being ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground fecal sample were sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for nutrient analysis of DM, N, NDF with sodium sulfide, starch, and ash using the same methods as feed ingredients. Total urine was collected by inserting a 30-French Foley catheter into each cow's bladder with a stylus. The balloon was inflated to 55 mL with physiological saline and Tygon tubing drained into a plastic carboy (15

Table 1. Ingredient inclusion and chemical composition of treatments containing hydrolyzed feather meal (% of DM unless otherwise indicated)¹

Item	0HFM	3.3HFM	6.7HFM	10HFM
Ingredient				
Corn silage	40.2	40.2	40.2	40.2
Alfalfa hay	8.00	8.00	8.00	8.00
Brome hay	4.44	4.44	4.44	4.44
Ground corn	11.2	11.2	11.2	11.2
Beet pulp	9.33	9.33	9.33	9.33
Feather meal	—	3.33	6.66	10.0
Nonenzymatically browned soybean meal ²	5.22	3.48	1.74	—
Bloodmeal	4.44	2.96	1.48	—
Whey, deproteinized	3.78	3.78	3.78	3.78
Soybean hulls	3.78	3.78	3.78	3.78
Molasses	3.11	3.11	3.11	3.11
Soybean meal	1.11	1.11	1.11	1.11
Tallow	0.89	0.89	0.89	0.89
Ca-salts of long-chain fatty acids ³	0.89	0.89	0.89	0.89
Urea	0.56	0.44	0.33	0.22
Rumen-protected Lys ⁴	0.22	0.22	0.22	0.22
Rumen-protected Met ⁵	0.13	0.13	0.13	0.13
Mineral-vitamin mix ⁶	2.75	2.75	2.75	2.75
Chemical composition ⁷				
DM	61.4 (0.40)	61.6 (0.27)	61.6 (0.30)	61.3 (0.82)
CP	17.1 (0.67)	17.3 (0.59)	17.6 (0.61)	17.9 (0.71)
Crude fat	3.44 (0.38)	3.70 (0.41)	3.97 (0.46)	4.23 (0.54)
Fatty acids	3.46 (0.14)	3.65 (0.14)	3.85 (0.14)	4.05 (0.16)
16C fatty acids	0.82 (0.04)	0.88 (0.04)	0.94 (0.04)	1.00 (0.04)
18C fatty acids	2.37 (0.12)	2.48 (0.12)	2.60 (0.12)	2.71 (0.13)
ADF	19.9 (0.75)	20.7 (1.06)	21.4 (1.44)	22.1 (1.84)
NDF	31.8 (1.51)	32.1 (1.51)	32.3 (1.54)	32.6 (1.62)
Lignin	3.15 (0.28)	3.90 (0.38)	4.65 (0.49)	5.40 (0.61)
Starch	25.8 (1.00)	25.8 (0.78)	25.8 (0.76)	25.9 (0.95)
Ash	7.53 (0.18)	7.45 (0.07)	7.37 (0.09)	7.29 (0.20)
RDP ⁸	8.1	8.3	8.6	8.8
RUP ⁸	8.9	9.1	9.3	9.5
Lys, ⁸ % of MP	6.39	6.07	5.75	5.41
His, ⁸ % of MP	2.67	2.41	2.12	1.82
NE _L , ⁸ Mcal/kg of DM	1.55	1.55	1.56	1.58

¹Treatments: 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal.

²Soypass (LignoTech, Overland Park, KS).

³Megalac (Church & Dwight Co. Inc., Princeton, NJ).

⁴Ajipro (Ajinomoto, Chicago, IL).

⁵Smartamine M (Adisseo, Alpharetta, GA).

⁶Contained per kilogram: 403 g of CaCO₃, 204 g of NaHCO₃, 160 g of Ca₂PO₄, 113 g of MgO, 87 g of salt, 18 g of vitamin premix (14,850 IU/g vitamin A, 3,850 IU/g vitamin D, and 90 IU/g of vitamin E), and 15 g of trace mineral premix (180,000 mg/kg Zn, 150,000 mg/kg Mn, 25,000 mg/kg Cu, 2,600 mg/kg I, 2,300 mg/kg Co, 1,000 mg/kg Fe, and 820 mg/kg Se).

⁷Values determined by Cumberland Valley Analytical Services, Waynesboro, PA; values denoted as mean (SD).

⁸Estimated with NRC (2001) using mean DMI, and milk production and composition from Table 6.

quart; Midwest Can Company, Franklin Park, IL) behind the cow. Using the funnel spout of the plastic carboy, urine was deposited into a 55-L plastic container 4 times a day and was acidified with 100 mL of HCl before subsampling (500 mL). Daily subsamples were frozen at -20°C until analysis. Before analysis urine was thawed and boiled to remove the water. To boil the urine, samples were placed in a 600-mL beaker in a boiling water bath (Ankom Technology, Macedon, NY). After water was boiled away, the remaining paste was lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY). Once lyophilized, sample size was reduced using mortar and pestle and then analyzed at the University of Nebraska–Lincoln for DM (100°C oven for 24 h), N (Leco FP-528, Leco Corp.), and GE (Parr 6400 Calorimeter).

Milk production was measured daily and milk samples were collected during the AM, midday, and PM milking times for 4 consecutive days at the end of each period. Two tubes were collected during each milking (100 mL); one 50-mL conical tube was frozen at -20°C and one tube was sent off to DHIA preserved using 2-bromo-2-nitropropane-1,3 diol. Samples were sent to Heart of America DHIA (Kansas City, MO) and were analyzed for fat, protein, lactose, and MUN using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). The frozen milk sample was lyophilized and composited by cow and period for nutrient analysis. Milk samples were analyzed at the University of Nebraska–Lincoln for DM, N, and GE using the same methods as were used for urine. To determine DM output, feed ingredient, refusals, feces, and urine samples were dried at 60°C in a forced-air oven for 48 h and milk samples were lyophilized. Feed ingredients, refusals, and feces were ground as previously described with the feces. Water intake was measured using DLJGHT garden hose water meter (DLJ Meter, Hackensack, NJ) while each cow was inside the headbox.

Heat production was determined through the headbox-type indirect calorimeters as described previously (Freetly et al., 2006; Foth et al., 2015). For each cow, a collection period of 23-h was used to measure O_2 consumption and CO_2 and CH_4 production. Gas data were adjusted to a 24-h period. Four headboxes were used and data were collected across 3 d during the 4-d collection period. Cows were adapted to headboxes for a minimum for 3 d before the start of the experiment. Feed was placed in the bottom of the headbox and cows were allowed ad libitum access to water from a water bowl placed inside the headbox. Free water intake was measured using a water meter (model DLJSJ75, Daniel L. Jerman Co., Hackensack, NJ) while each cow was

inside the headbox. Within the headbox, temperature and dew point were measured every minute during the 23-h collection interval using a probe (model TRH-100, Pace Scientific Inc., Mooresville, NC) and recorded using a data logger (model XR440, Pace Scientific Inc.). Line pressure was measured using a u-tube manometer (item 1221-8, Park Supply of America Inc., Minneapolis, MN) and barometric pressure of the room was measured using a barometer (Chaney Instruments Co., Lake Geneva, WI). Total volume of air flow through the headbox was measured using a gas meter (model AL425, American Meter, Horsham, PA) and corrected to standard temperature and pressure (0°C , 101.3 kPa) with adjustment for moisture content of exhaust air (Nienaber and Maddy, 1985). From the headbox, continuous samples of incoming and outgoing air were collected into separate aluminum Mylar bags (44 L, LAM-JAPCON-NSE, Pollution Measurement Corp., Oak Park, IL) using glass tube rotameters (model 1350E Sho-Rate “50,” Brooks Instruments, Hatfield, PA). Gas bags were analyzed for O_2 , CO_2 , and CH_4 using an Emerson X-stream 3-channel analyzer (Solon, OH) according to the method of Nienaber and Maddy (1985). Before the start of the experiment, system efficiency (head box and gas analyzer) was determined by burning 100% ethyl alcohol and measuring gas recoveries. Recoveries of O_2 consumption and CO_2 production were (average $\pm$ SD) $101.0 \pm 0.04\%$ and $100.8 \pm 0.04\%$, respectively.

Energy Calculations

Calculations for digestible energy (DE) and ME were as follows:

$$\text{DE (Mcal/d)} = \text{GE (Mcal/d)} - \text{fecal energy (Mcal/d)}, \quad [1]$$

$$\begin{aligned} \text{ME (Mcal/d)} &= \text{DE (Mcal/d)} - \text{urine energy (Mcal/d)} \\ &\quad - \text{methane energy (Mcal/d)}. \end{aligned} \quad [2]$$

Heat production (HP) was estimated as follows (Brouwer, 1965):

$$\begin{aligned} \text{HP (kcal/d)} &= 3.866 \times \text{O}_2 \text{ (L/d)} \\ &+ 1.200 \times \text{CO}_2 \text{ (L/d)} - 0.518 \times \text{CH}_4 \text{ (L/d)} \\ &\quad - 1.431 \times \text{urinary N excretion (g/d)}. \end{aligned} \quad [3]$$

The respiratory quotient was calculated using the ratio of carbon dioxide produced to oxygen consumed (L/L). Methane energy was estimated by multiplying CH_4 pro-

duction by its enthalpy (9.45 kcal/L). Unaccounted for energy was assumed to represent tissue energy, which was expressed on an NE_L basis as follows:

$$\begin{aligned} \text{residual energy (Mcal/d)} &= \text{ME (Mcal/d)} \\ &- \text{HP (Mcal/d)} - \text{milk energy (Mcal/d)}. \end{aligned} \quad [4]$$

$$\begin{aligned} \text{Tissue energy (TE; Mcal of } NE_L/\text{d)} &= \\ &\text{positive residual energy} \\ &\times k_T \text{ or negative residual energy} \times k_G/k_L, \end{aligned} \quad [5]$$

where k_T is the efficiency of utilizing body reserves for milk energy production, k_G is the efficiency of utilizing ME intake for tissue gain, and k_L is the efficiency of utilizing ME intake for milk production. Values of 0.89, 0.75, and 0.66 were used for k_T , k_G , and k_L , respectively (Moraes et al., 2015). Calculations for NE_L was as follows:

$$\begin{aligned} NE_L \text{ (Mcal/d)} &= \text{milk energy (Mcal of } NE_L/\text{d)} \\ &+ \text{TE (Mcal of } NE_L/\text{d)} + 0.080 \times \text{BW (kg)}^{0.75}. \end{aligned} \quad [6]$$

Tissue energy for body protein and fat deposit was calculated as follows (Freetly et al., 2006; van Kneegsel et al., 2007):

$$\begin{aligned} \text{TE as body protein (TE}_P\text{; Mcal/d)} &= \\ \text{retained N (g/d)} \times 5.88 \text{ (g of protein/g of N)} \\ &\times 0.0057 \text{ (Mcal/g of protein)}, \end{aligned} \quad [7]$$

$$\begin{aligned} \text{TE as body fat (Mcal/d)} &= \text{TE (Mcal/d)} \\ &- \text{TE}_P \text{ (Mcal/d)}. \end{aligned} \quad [8]$$

We determined NE_M and k_L as described by Kebreab et al. (2003). For this, ME was corrected for the ME that was used for tissue retention and milk energy was corrected for milk energy from tissue mobilization. Corrected ME and milk energy were calculated as follows:

$$\begin{aligned} \text{corrected ME} &= \text{ME (kg/d)} \\ &- |\text{positive residual energy (Mcal/d)}|/k_G, \end{aligned} \quad [9]$$

$$\begin{aligned} \text{corrected milk energy} &= \text{milk energy (Mcal/d)} \\ &- |\text{negative residual energy (Mcal/d)}| \times k_T, \end{aligned} \quad [10]$$

where k_G and k_T are as described above. Corrected ME and corrected milk energy were expressed as a function of metabolic body weight (MBW ; $\text{kg}^{0.75}$).

Statistical Analysis

Data were analyzed in R (v3.5.1) using the lmer package (Kuznetsova et al., 2017). The model included the fixed effects of treatment and the random effect of period, square, and cow nested in square. To determine NE_M and k_L , corrected milk energy was regressed against the fixed effect of treatment and the interaction of treatment with corrected ME. Random effects of period, square, and cow nested in square were included. The fixed effect of treatment was not significant ($P = 0.55$) and was thus removed. Linear, quadratic, and cubic effects of treatments were tested using the emmeans package (Lenth, 2018). All data are presented as least squares means $\pm$ largest standard error. Significance was declared at $P \leq 0.10$.

RESULTS AND DISCUSSION

This experiment was designed to test the effects of replacing blood meal and nonenzymatically browned soybean meal with HFM on energy and N utilization, DMI, and milk production and composition. Compared with feeding a diet with soybean meal, total-tract CP digestibility of diets containing HFM are often lower (Waltz et al., 1989; Harris et al., 1992), and decreased milk protein concentration has also been observed (Harris et al., 1992). Furthermore, the crude fat content of HFM can be as high as 14% of DM (Cotanch et al., 2007); therefore, increased inclusion of HFM may increase dietary energy density. We expected that increased HFM inclusion would decrease digestible protein supply leading to decreased milk protein yield, while dietary energy density would increase.

Increasing inclusion of HFM increased CP from 17.1 to 17.9 and fatty acids from 3.46 to 4.05% of diet DM (Table 1). The blend of HFM from 2 sources used in the current study was (mean $\pm$ SD) $92.5 \pm 2.12\%$ CP and $7.5 \pm 0.26\%$ crude fat (Table 2). When analyzed according to Ross et al. (2013), estimated total-tract digestible CP for the HFM blend, blood meal, and nonenzymatically browned soybean meal was (mean $\pm$ SD) 50.3 ± 1.98 , 94.3, and 98.0% of CP, respectively. We have recently reported that the ruminal and intestinal digestibility of HFM can be highly variable and dependent on processing methods (Buse et al., 2019). Tabular values for RUP of HFM, blood meal, and nonenzymatically browned soybean meal for a cow eating a 50% roughage diet at 4.0% of BW are 65.4, 77.5, and 79.4% of CP, respectively, and the RUP digestibility values are 65, 80, and 93%, respectively (NRC, 2001). Therefore, using NRC (2001), estimated RDP content increased with increasing HFM inclusion (8.1 to 8.8% of DM for 0HFM and 10HFM, respectively).

Table 2. Feed chemical analysis for alfalfa hay, brome hay, corn silage, and concentrate mixes (% of DM)^{1,2}

Item ³	Alfalfa hay		Brome hay		Corn silage		0HFM concentrate		10HFM concentrate		HFM ⁴	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, as is	88.9	0.80	88.9	2.93	42.8	1.40	89.9	0.39	90.6	0.34	91.9	0.49
CP	16.5	1.36	9.20	0.84	8.05	0.35	25.6	1.17	27.3	1.29	92.5	2.12
ADF	41.4	3.57	44.7	2.35	20.1	0.59	13.9	0.66	18.5	3.02	— ⁵	—
NDF	51.3	4.22	69.2	2.70	34.3	1.50	23.0	1.37	24.6	1.54	—	—
Lignin	8.95	1.31	6.25	0.83	2.95	0.45	2.05	0.29	6.81	0.87	—	—
ADICP	1.44	0.18	1.43	0.50	0.53	0.02	0.84	0.26	4.60	0.64	12.3	5.88
NDICP	2.62	0.42	4.49	0.67	0.68	0.06	3.26	0.98	5.65	1.63	16.6	10.6
Starch	1.33	0.38	1.38	1.70	42.7	1.73	17.9	1.31	18.1	2.56	—	—
Sugar	4.23	1.81	5.18	2.83	0.78	0.43	14.3	0.90	12.9	0.98	—	—
Crude fat	1.48	0.56	1.88	0.26	3.43	0.17	3.93	0.72	5.60	1.11	7.5	0.26
Fatty acids	0.92	0.08	0.62	0.07	2.78	0.15	4.73	0.19	5.97	0.25	—	—
Ash	9.53	0.49	9.71	0.32	3.71	0.35	10.2	0.45	9.73	0.48	1.33	0.54
Ca	1.05	0.12	0.42	0.06	0.18	0.01	1.86	0.20	1.97	0.26	0.42	0.21
P	0.36	0.01	0.29	0.01	0.22	0.02	0.46	0.03	0.45	0.03	0.26	0.10
Mg	0.23	0.02	0.13	0.02	0.14	0.00	0.52	0.03	0.55	0.03	0.03	0.00
K	3.62	0.04	2.21	0.15	0.95	0.09	1.30	0.04	1.03	0.03	0.12	0.01
S	0.20	0.02	0.20	0.02	0.15	0.02	0.32	0.06	0.69	0.07	2.32	0.08
Na	0.03	0.01	0.03	0.01	0.02	0.00	0.68	0.03	0.76	0.04	0.09	0.01
Cl	0.11	0.01	0.34	0.05	0.18	0.02	0.63	0.03	0.68	0.02	0.10	0.04

¹Mean and SD (n = 4) were calculated based on samples of each feedstuff collected during each period and estimated by a commercial feed testing laboratory (Cumberland Valley Analytical Services, Waynesboro, PA).

²0HFM concentrate = concentration with 0% hydrolyzed feather meal; 10HFM = concentration with 10% hydrolyzed feather meal (n = 4).

³ADICP = acid detergent insoluble crude protein; NDICP = neutral detergent insoluble crude protein.

⁴HFM = hydrolyzed feather meal blend from 2 sources (Pilgrim's, Greeley, CO; Simmons Foods, Siloam Springs, AR).

⁵Not determined.

This resulted in estimated RDP supplies that increased with increasing HFM inclusion, but were below NRC (2001) recommendations (RDP balance of −321, −276, −228, and −195 g/d for 0HFM, 3.3HFM, 6.7HFM, and 10HFM, respectively). For HFM, Lys and His content are lower than blood meal and nonenzymatically browned soybean meal, leading to decreased estimated Lys (6.39 to 5.41% of MP) and His (2.67 to 1.82% of MP) supply as HFM inclusion increased (NRC, 2001). Particle size distribution of TMR was similar across treatments (Supplemental Table S1; <https://doi.org/10.3168/jds.2019-17762>).

Gas Consumption and Production and Energy Utilization

To assess the effects of placing animals in the head-box-style indirect calorimeter on feeding behavior, we compared DMI for when cows were in the headboxes to when cows were out of the headboxes. When cows were placed in headboxes, DMI decreased ($P = 0.02$) compared with when cows were not in the headboxes (19.3 vs. 20.1 ± 0.73 kg/d; data not shown). However, no interactions between treatment or period with headbox placement were observed ($P > 0.76$; data not shown) and differences between treatment effects for energy partitioning may be interpreted with confidence. De-

spite the fact that we strived to make animals comfortable and familiar with the peculiar experimental setting, the modest reduction in DMI was not unexpected but still within normal ranges, and we are unaware of alternative techniques that would allow us to make the measurements described here.

Oxidation of carbohydrates, lipids, and protein, individually, give rise to a unique and nearly fixed quantity of heat per unit of O_2 consumed and CO_2 produced (McLean and Tobin, 1987). This principle is applied in the Brouwer (1965) equation when measurements of O_2 consumption and CO_2 production, along with correction for CH_4 production and urinary N excretion, are used to indirectly estimate heat production. In the current study, O_2 consumption and CO_2 and CH_4 production quadratically increased ($P < 0.07$) from 0HFM to 6.7HFM but then decreased when cows consumed 10HFM (Table 3). Consequently, a similar quadratic response ($P < 0.07$) was observed for methane energy and heat production. Because increased DMI typically leads to increased heat production (Purwanto et al., 1990; Reynolds et al., 1991), the quadratic relationship for gas variables and heat production were likely due to a similar quadratic relationship in DMI (see later discussion).

The replacement of blood meal and nonenzymatically browned soybean meal with HFM linearly increased (P

< 0.01) the GE content of the diet. Similar to GE content, DE, ME, and NE_L content linearly increased ($P < 0.02$) with increasing HFM. Increased dietary GE, DE, ME, and NE_L content may have been due to increased fatty acid content, which inherently possesses a greater enthalpy (9.4 Mcal/kg of DM) than either CP (5.6) or NDF (4.2; NRC, 2001). In agreement, Andrew et al. (1991) reported increased dietary NE_L concentration when supplementing Ca-salts of fatty acids. Increased dietary energy content supported similar ($P > 0.37$) milk energy output (average of 25.5 ± 1.17 Mcal/d) and tissue energy (-0.50 ± 1.50 Mcal of NE_L/d), whereas DMI quadratically decreased.

Estimated NE_M for the current experiment was not affected ($P > 0.55$) by treatment (average of 97.9 ± 21.2 kcal/MBW; Figure 1). Our estimated NE_M is greater than that used by the current NRC (2001; 80 kcal/MBW). However, our observations fall within range reported by others who have determined NE_M for cows fed at production levels of intake and these range from 74 to 124 kcal/MBW (Moe et al., 1972; Kebreab et al., 2003; Moraes et al., 2015). It has been suggested that

NE_M has increased over time in lactating dairy cows and may at least in part be due to a concurrent increase in genetic merit of cows (Moraes et al., 2015). Interestingly, the large variance that we observed for NE_M (21.7% of mean), in part, demonstrates the challenge of estimating NE_M requirements for cows, particularly for cows at a high plane of production. The k_L linearly increased ($P = 0.07$) with increasing HFM. This increase in efficiency may have occurred due to an increase in the fat content of the diet, which is suggested to have a lower heat increment compared with energy that is derived from the basal diet (NRC, 2001). Andrew et al. (1991) calculated the k_L for Ca-salts of long-chain fatty acids as 0.77, which is greater than the previously reported k_L for basal diets of 0.64 (Moe et al., 1972). An evaluation of historical studies has suggested that k_L has increased since the 1960s and this may in part be explained by increased inclusion of supplemental fat (Moraes et al., 2015). Furthermore, an increase in the ratio of milk fat to milk protein in the current study with increasing HFM inclusion may have increased k_L because synthesis of milk fat is thought to be more

Table 3. Effects of treatments containing hydrolyzed feather meal fed to lactating Jersey cows on O₂ consumption, CO₂ and CH₄ production, respiratory quotient, and energy utilization

Item ¹	Treatment ^{2,3}				SEM	P-value ⁴		
	0HFM	3.3HFM	6.7HFM	10HFM		L	Q	C
Gas								
O ₂ consumption, L/d	4,761	4,822	4,826	4,616	163	0.11	0.03	0.56
CO ₂ production, L/d	4,884	4,954	4,959	4,707	193	0.11	0.03	0.55
CH ₄ production, L/d	420	434	433	403	24	0.30	0.06	0.75
RQ	1.025	1.025	1.027	1.019	0.012	0.50	0.48	0.56
Component, Mcal/d								
Feces	28.2	29.4	29.3	28.0	1.46	0.85	0.06	0.96
Methane	4.12	4.25	4.24	3.94	0.235	0.29	0.07	0.75
Urine	2.08	2.15	2.21	1.95	0.131	0.46	0.13	0.55
Heat ⁵	23.8	24.1	24.2	23.1	0.84	0.13	0.03	0.58
Milk	25.3	25.2	26.6	25.0	1.17	0.90	0.37	0.21
Tissue ⁶	-1.57	0.27	-0.27	-0.43	1.50	0.62	0.44	0.63
Fraction, Mcal/d								
GE	82.2	85.7	86.4	81.9	3.42	0.95	0.02	0.75
DE	54.1	56.3	57.1	53.9	2.12	0.98	0.03	0.64
ME	47.9	49.9	50.7	48.0	1.87	0.84	0.04	0.67
NE _L ⁷	31.4	33.2	34.2	32.2	1.47	0.45	0.07	0.61
Fraction, Mcal/kg of DM								
GE	4.21	4.23	4.26	4.29	0.019	<0.01	1.00	0.99
DE	2.77	2.79	2.82	2.84	0.039	0.02	0.87	0.73
ME	2.45	2.47	2.50	2.53	0.040	0.02	0.97	0.86
NE _L ⁷	1.61	1.64	1.69	1.70	0.042	0.02	0.57	0.64

¹GE = gross energy; DE = digestible energy; RQ = respiratory quotient, CO₂ production/O₂ consumption, L/L.

²Treatments: 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal.

³Least squares means; largest standard error of treatment mean is shown.

⁴L = linear; Q = quadratic; C = cubic.

⁵Heat = $3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N}$ (Brouwer, 1965).

⁶Tissue = positive residual energy $\times 0.82$ or negative residual energy/1.12 (NE_L basis).

⁷NE_L = $0.080 \times \text{BW}^{0.75} + \text{milk energy} + \text{tissue energy corrected for efficiency of conversion to milk energy}$ (NRC, 2001).

Figure 1. Regression of lactation energy corrected for tissue mobilization on ME intake corrected for tissue retention (see Materials and Methods). Both variables are expressed per unit of metabolic body weight (MBW). The effect of treatment, which represents treatment differences in NE_M , was not significant ($P = 0.55$) and was thus removed. The NE_M was 97.9 ± 21.2 kcal/MBW. The slope of each regression represents k_L (efficiency of utilizing ME intake for milk production). 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal; RMSE = root mean square error.

efficient compared with milk protein. Approximately 50% of milk fat is preformed fatty acid (Bauman and Griinari, 2003), which has a theoretical energetic efficiency of 94 to 97% (Baldwin et al., 1985). Consequently, the incorporation of preformed fat into milk will decrease the overall heat load associated with milk fat synthesis compared with milk protein synthesis. In this experiment, we demonstrate that k_L can differ by dietary treatments, as has been suggested for changes in dietary chemical composition (VandeHaar, 1998; NRC, 2001; Moraes et al., 2015). Therefore, the use of static k_L values may misrepresent the biology underlying the transformation of ME to NE_L in lactating dairy cows.

Nutrient Digestibility and Nitrogen Utilization

Increasing inclusion of HFM linearly increased ($P < 0.04$) total-tract digestibility of NDF, and starch (Table 4). Modest improvements in NDF digestibility with increased HFM may have occurred due to an increase in the RDP content from 8.1 to 8.8% of the diet DM

for 0HFM and 10HFM, respectively. Estimated RDP content increased with HFM because compared with blood meal and nonenzymatically browned soybean meal, the ingredient is more degradable in the rumen (NRC, 2001). It is well established that feeding diets low in RDP can decrease NDF digestibility (Colmenero and Broderick, 2006; Lee et al., 2012). Although an increase in starch digestibility with HFM inclusion was not expected, it is also possible that increased supply of RDP may have supported a more favorable rumen environment (Russell et al., 1992) leading to increased ruminal degradation of starch. Apparent total-tract digestibility of CP linearly decreased ($P < 0.01$) with increasing HFM inclusion. This was likely due to a lower digestibility of RUP contained in HFM (65%) compared with blood meal (80%) and nonenzymatically browned soybean meal (93%; NRC, 2001). Decreased CP digestibility with increased HFM inclusion is supported by in vitro estimated total-tract CP digestibility for the 3 RUP sources (50.3 ± 1.98 , 94.3 , and 98.0 for HFM, blood meal, and nonenzymatically browned soybean meal, respectively). Waltz et al. (1989) reported that feeding HFM to replace of blood meal decreased apparent total-tract CP digestibility (63.4 vs. 73.9%) and AA absorption in the small intestine (65.4 vs. 78.5%). Increasing inclusion of HFM linearly increased ($P < 0.04$) digestibility of total fatty acids, 16C fatty acids, and 18C fatty acids. This response indicates that the fat in HFM is highly digestible. An increase in dietary content of *cis*-9 C18:1 with increasing HFM (0.85, 0.90, 0.95, and 1.01% of DM for 0HFM, 3.3HFM, 6.7HFM, and 10HFM, respectively) may have increased fatty acid digestibility. de Souza et al. (2019) reported that increasing *cis*-9 C18:1 from 0.68 to 0.98% of diet DM linearly increased digestibility of total, 16-C, and 18-C fatty acids.

Digested N is partitioned toward productive, maintenance, or metabolic functions and may be influenced by diet. In the current study, N intake quadratically increased ($P < 0.01$) from 0HFM to 6.7HFM and decreased for 10HFM (Table 5). A similar quadratic response ($P < 0.01$) was observed for fecal N excretion. The observed responses in N intake and fecal N excretion can be explained by an increase in dietary CP content with increased HFM inclusion and a similar quadratic relationship for DMI with increasing HFM inclusion (see later discussion). As HFM inclusion increased, linear decreases ($P < 0.01$) were observed for urinary N excretion. Decreased N digestibility in the current study may explain why urinary N excretion decreased with increasing HFM. Because MP supply is a function of intestinal digestibility of protein, decreased N digestibility will lead to decreased MP supply. As

Table 4. Effects of treatments containing hydrolyzed feather meal fed to lactating Jersey cows on total-tract digestibility (%)

Item	Treatment ^{1,2}				SEM	P-value ³		
	0HFM	3.3HFM	6.7HFM	10HFM		L	Q	C
DM	65.9	65.7	66.1	66.0	0.77	0.68	0.94	0.61
OM	68.0	67.8	68.2	68.0	0.77	0.84	0.96	0.54
NDF	45.6	45.6	47.6	48.7	1.38	<0.01	0.63	0.56
CP	63.4	61.1	59.9	58.6	1.46	<0.01	0.45	0.70
Starch	96.3	96.4	97.2	97.1	0.61	0.04	0.80	0.31
Total fatty acids	77.2	77.7	78.5	80.6	1.30	<0.01	0.38	0.75
16C fatty acids	78.3	79.2	80.4	82.4	1.19	<0.01	0.48	0.93
18C fatty acids	79.6	80.0	80.3	82.3	1.32	0.04	0.35	0.63
Energy	65.8	65.8	66.2	66.1	0.838	0.52	0.86	0.67

¹Treatments: 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal.

²Least squares means; largest standard error of treatment mean is shown.

³L = linear; Q = quadratic; C = cubic.

MP supply decreases, the efficiency of MP use for milk protein synthesis increases (Moraes et al., 2018), and thus catabolism of protein will decrease, leading to decreased urinary N excretion. We observed a cubic response ($P = 0.02$) for N balance ($-27, 9, -24$, and 11 ± 22.5 g/d for 0HFM, 3.3HFM, 6.7HFM, and 10HFM, respectively), which was not expected and is difficult to explain. A variety of methodological issues contribute to error in estimation of N balance, including under-acidification of urine and N volatilization during drying (Spanghero and Kowalski, 1997). Together these errors could contribute to an overestimation of N balance, which, in the current experiment, averaged -8 g/d. Although this is a biologically feasible value, it should be noted that a N-balance of near zero does not necessarily indicate accuracy in the values used to calculate N-balance.

DMI, Milk Yield, and Milk Composition

Decreased DMI has been observed when HFM is fed to lactating dairy cows (Yeo et al., 2003; Stahel et al., 2014; Giallongo et al., 2017); however, this response is not always observed (Harris et al., 1992; Grant and Haddad, 1998). We observed that increasing HFM inclusion quadratically increased ($P = 0.03$) DMI from 0HFM to 6.7HFM, but decreased DMI for 10HFM (Table 6). In the current study, because estimated supply of Lys and His decreased with increasing HFM (see Table 2), a deficiency in one or both of these AA may have caused the decreased DMI for 10HFM. Imbalance in AA supply in nonruminants has been shown to consistently reduce DMI and this response is mediated by appetite centers in the brain (Gietzen, 1993). Giallongo et al. (2017) observed decreased DMI when HFM was

Table 5. Effects of treatments containing hydrolyzed feather meal fed to lactating Jersey cows on nitrogen utilization

Item	Treatment ^{1,2}				SEM	P-value ³		
	0HFM	3.3HFM	6.7HFM	10HFM		L	Q	C
Mass, g/d								
N intake	535	576	577	550	23.8	0.36	<0.01	0.78
Fecal N	199	230	239	237	12.1	<0.01	<0.01	0.64
Urinary N	166	151	155	119	14.8	<0.01	0.22	0.19
Milk N	198	186	207	184	15.0	0.59	0.52	0.06
N balance	-27	9	-24	11	22.5	0.13	0.96	0.02
Proportion of N intake, %								
Fecal N	37.2	39.8	41.3	42.7	1.04	<0.01	0.39	0.71
Urinary N	30.7	26.3	27.0	21.6	2.39	<0.01	0.74	0.12
Milk N	37.0	33.0	36.1	33.7	3.30	0.24	0.54	0.04
N balance	-5.0	1.0	-4.4	2.0	3.96	0.09	0.90	0.02

¹Treatments: 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal.

²Least squares means; largest standard error of treatment mean is shown.

³L = linear; Q = quadratic; C = cubic.

Table 6. Effects of treatments containing hydrolyzed feather meal fed to lactating Jersey cows on DMI, milk production and composition, free water intake, BW, and BCS

Item	Treatment ^{1,2}				SEM	P-value ³		
	0HFM	3.3HFM	6.7HFM	10HFM		L	Q	C
DMI, kg/d	19.6	20.2	20.3	19.1	0.79	0.46	0.03	0.76
Milk yield, kg/d	31.7	32.0	31.9	29.7	1.32	0.04	0.06	0.54
ECM, ⁴ kg/d	39.7	39.6	40.1	37.8	1.54	0.13	0.15	0.31
ECM/DMI	2.03	1.98	1.98	1.98	0.086	0.40	0.51	0.77
Fat, %	5.35	5.23	5.45	5.54	0.209	0.04	0.22	0.23
Fat, kg/d	1.68	1.67	1.72	1.64	0.082	0.69	0.34	0.22
Protein, %	3.34	3.29	3.23	3.23	0.158	0.04	0.52	0.72
Protein, kg/d	1.05	1.05	1.02	0.96	0.040	<0.01	0.14	0.77
Lactose, %	4.84	4.84	4.86	4.84	0.052	0.87	0.80	0.51
Lactose, kg/d	1.54	1.55	1.55	1.44	0.072	0.09	0.098	0.50
MUN, mg/dL	15.1	14.7	14.7	14.4	0.683	0.11	0.91	0.47
Free water intake, L/d	88.9	91.0	93.6	87.4	6.08	0.91	0.19	0.51
BW, kg	444	445	451	441	13	0.61	0.01	0.02
BCS	3.10	2.98	3.13	3.04	0.16	0.86	0.68	0.04

¹Treatments: 0HFM = 0% hydrolyzed feather meal; 3.3HFM = 3.3% hydrolyzed feather meal; 6.7HFM = 6.7% hydrolyzed feather meal; 10HFM = 10% hydrolyzed feather meal.

²Least squares means; largest standard error of treatment mean is shown.

³L = linear; Q = quadratic; C = cubic.

⁴ECM = $0.327 \times \text{milk yield (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{true protein (kg)}$ (Tyrrell and Reid, 1965).

fed at 2.7% of diet DM and fully replaced blood meal; however, supplementation of rumen-protected His to their HFM diet increased DMI.

Feeding increasing HFM quadratically increased ($P = 0.06$) milk yield from 0HFM to 6.7HFM, but decreased milk yield for 10HFM. Milk fat concentration linearly increased ($P = 0.04$) with increasing HFM, which may have occurred due to increased dietary NE_L content. However, milk fat yield was not observed to differ ($P > 0.34$; averaging 1.68 ± 0.082 kg/d). Increasing diet HFM content linearly decreased ($P < 0.04$) milk protein concentration and milk protein yield. Harris et al. (1992) observed that increasing HFM linearly decreased milk protein concentration when feeding HFM at 0, 3, or 6% of diet DM as a replacement for soybean meal. Supply of Lys and His may have an effect on milk protein concentration and yield (NRC, 2001; Giallongo et al., 2016; Zang et al., 2019). For HFM, the Lys (2.57% of CP) and His (1.15% of CP) content are low compared with blood meal (8.98 and 6.36% of CP for Lys and His, respectively) and nonenzymatically browned soybean meal (5.78 and 2.40% of CP for Lys and His, respectively; NRC, 2001). The recommended supplies are 6.6% of MP for Lys (Schwab et al., 2005) and 2.2% of MP for His (Lee et al., 2012). For all diets in the current experiment, estimated Lys supply was less than 6.6% of MP, and for 6.7HFM and 10HFM, estimated His supply was less than 2.2% of MP. Therefore, a deficiency in available Lys, His, or both may have caused

decreased milk protein and research is needed to understand how increased supply of Lys and His in HFM diets affects milk production and composition. Although milk protein yield linearly decreased with increasing HFM, and the lowest feed intake was observed for the 10HFM treatment, ECM was not affected ($P > 0.12$) by HFM (average of 39.3 ± 1.54 kg/d). An increase in dietary energy content with increasing HFM inclusion may have supported sustained milk yield when DMI decreased for 10HFM.

CONCLUSIONS

A study utilizing lactating dairy cows was conducted to evaluate the effects of increasing the inclusion of HFM on energy utilization, nutrient digestibility, N utilization, DMI, and milk production and composition. This research indicated that increasing dietary inclusion of HFM increased diet energy content and efficiency of utilizing energy for milk production. Increasing HFM decreased CP digestibility, leading to increased fecal N excretion. When HFM was fed up to 6.7% of the diet DM, DMI was maintained; however, milk protein content and yield decreased with increasing HFM. Decreased DMI with inclusion of 10% HFM and decreased milk protein with increasing HFM may have been due to deficiency in Lys, His, or both, and research is needed to test the effects of increased Lys and His supply in diets with HFM.

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