Project Final Report

Title of Project:

Update on the knowledge related to the feeding value of hydrolyzed poultry feathers for lactating dairy cattle

Principal Investigator:

P.J. Kononoff
Associate Professor of Dairy Nutrition/Extension Dairy Specialist,
University of Nebraska-Lincoln,
Department of Animal Science,
C220j Animal Science Bldg.,
Lincoln, NE 68583-0908,
Phone: (402) 472-6442,
Email: pkononoff2@unl.edu

Submitted To:

Rafael E. Rivera, M.S.
Manager Food Safety & Production Programs
U.S. Poultry & Egg Association
Phone: 678-514-1978
rrivera@uspoultry.org

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EXECUTIVE SUMMARY

A study utilizing lactating dairy cows was conducted to evaluate the effects of increasing the inclusion of hydrolyzed poultry feathers (hydrolyzed feather meal: **HFM**) in a mixed ration was conducted. The overall objective of this study was to test the effects on feed intake, milk production, total tract digestibility of protein, and energy utilization. Two driving effects of increasing the proportion of feather meal in the diet (0, 3.3, 6.6, and 10 % of the diet DM) of this study explain many key observations. Firstly, a quadratic effect on feed intake was observed (increased at low inclusion but decreased at the highest inclusion). Secondly, total tract digestibility of both fiber and starch was stimulated with the inclusion of feather meal while the digestibility of protein was moderately decreased. The production of milk protein was reduced with increasing the proportion of feather meal and this may have been a result of reductions in the supply of metabolizable protein or amino acids. Increasing feather meal in the diet resulted in diets with a greater concentration of energy and maintained the production of fat correct milk. Overall, feather meal appears to be a highly palatably feed ingredient for dairy cattle and can be used to replace more expensive feed ingredients while also maintaining milk production. Future work should focus in the digestibility of protein and the potential overcome challenges associated with limiting amino acids.

INTRODUCTION

Each year there is over 350 million metric tons of feather meal produced in the United States (Becker, 2005). Feather meal is high on protein (~85 %) and when fed to dairy cattle, a high proportion (~65%) of this protein bypasses rumen fermentation and directly supplies protein to the small intestine. The digestibility of this bypass protein in the small intestine is also high (~65%) (National Research Council (U.S.), 2001) making this feed a good source of protein for the Nation's 9 million dairy cattle which consume consuming over 55 million metric tons of feed each year (USDA ERS - Dairy Data). The digestibility is feather meal is enhanced through by heating the byproduct in pressure vessels resulting in hydrolysis (Garcia et al., 2011). Surprisingly, despite the high digestibility of protein, very few studies have sought to test the inclusion of feather meal on nutrient utilization in dairy cattle. It is also important to note that methods used to process and hydrolyzed feather meal have improved the nutritional value of HFM. For example the industry has advanced the use of continuous cooking with variable temperatures. As a consequence of this there is an urgent need to conduct studies that evaluate the feeding value of HFM currently available for lactating dairy cattle. *The objectives of this research was to evaluate the overall feeding value of hydrolyzed feather meal when fed to lactating dairy cattle and to test the effects on total tract digestibility of protein and energy utilization*.

MATERIALS AND METHODS

Experimental Design, Cows, Treatments.

The experimental design was three times replicated 4 × 4 Latin square and used 12 lactating Jersey dairy cows (88.4 ± 27.7 DIM at the first collection; 445.3 ± 45.2 kg BW) and 35 d periods. Cows were milked three times daily at 0700, 1400, and 2100 hr representing an AM, mid-day and PM milking. A total of 4 treatments be used, namely 1) Control diet; no feather meal, 2) low feather meal (**LFM**): 3.29 % diet DM 3) medium feather meal (**MFM**): 6.59 % diet DM, 4) high feather meal (**HFM**): 10 % diet DM (Table 1). Source of feather meal included in the diets were agreed upon and identified through discussion our research group and The Poultry Protein & Fat Council (Tucker, GA) to ensure ingredients tested are indicative of field availability and application. Animals were blocked into each square by milk production (kg/d). Treatments alternated 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 days in duration. Each period included 28 days for ab libitum diet adaptation, targeting about 5% refusals during that time, followed by 4 days of collection with 4 days of 95% ad libitum feeding to reduce the amount of refusals. The 4 diets were formulated with treatments containing different concentrations of feather meal (Table 1). Inclusion of this ingredient was achieved by replacing nonenzymatically browned soybean meal and blood meal. All dietary treatments contained corn silage, alfalfa hay and a concentrate mixture that was combined as a TMR. The TMR was mixed in a Calan Data Ranger (American Calan, Inc, Northwood, NH) and fed once daily at 0900 hr to the cows.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and froze 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), NFC (100 – (% NDF + % CP + % Fat + % Ash)), sugar (DuBois et al., 1956), starch (Hall, 2009),

crude fat (2003.05; AOAC international 2006), ash (943.05; AOAC international 2000) and minerals (985.01; AOAC international 2000) and a subsample measured gross energy (GE) (Parr 6400 Calorimeter, Moline, IL) at the University of Nebraska – Lincoln lab. Total mixed rations were sampled (500 g) on each day of each collection period and were froze at -20°C. The samples were then composited by period and treatment. A subsample was sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for complete nutrient analysis with the same lab processes as the individual feed ingredients (Table 2). The TMR was used to determine particle size according to Heinrichs and Kononoff (2002) using the Penn State Particle Separator (Table 3 a, b). 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. (Hagerstown, MD) for nutrient analysis of 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), starch (Hall, 2009) and ash (943.05; AOAC international 2000). A subsample was also used to measure GE (Parr 6400 Calorimeter, Moline, IL) at the University of Nebraska – Lincoln lab.

Total fecal and urine output was collected from each individual cow during the collection period for 4 consecutive days. Personnel were assigned and present during all times of sample collections and when possible, manually collected feces with a 3 quart little giant 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 208 L (55 gallon) garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce nitrogen losses prior to subsampling. The feces were subsampled (500 g) every day for 4 consecutive days and dried at 60°C in a forced air oven for 48 hours and then composited by cow and period prior to being ground to pass through a 1 mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces sample were sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for nutrient analysis of DM (AOAC international, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF with sodium sulfide (Van Soest et al., 1991), starch (Hall, 2009) and ash (943.05; AOAC international 2000). Total urine was collected by inserting a 30 French foley catheter into each cow's bladder with a stylus (Tamura et al., 2014). The balloon was inflated to 55 mL with physiological saline and tygon tubing drained into a plastic carboy (15 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 HCl targeting a pH below 5.0 prior to subsampling (500 mL) and freezing at -20°C every day of the collection period. Prior to analysis urine was thawed and boiled to remove the water content. To boil the urine, 2 thawed 250 mL bottles of urine were poured into a 600 mL beaker. Fourteen urine filled beakers were placed into a boiling water bath (Ankom Technology, Macedon, NY) underneath a hood. The water bath was turned on in the morning and off in the afternoon, for approximately 6 hours each day, to reduce the chance of the sample being overheated and burned. After water was boiled away, the remaining dark brown paste was then composited by cow and period. The brown paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) and analyzed. Once lyophilized, sample size was reduced using mortar and pestle and then analyzed. Urine samples were analyzed at the University of Nebraska – Lincoln for lab corrected DM (100°C oven for 24 hr), N (Leco FP-528, Leco Corp.) and GE (Parr 6400 Calorimeter, Moline, IL).

Milk production was measured daily and milk samples were collected during the AM, mid-day and PM milking times for 4 consecutive days or days 29 to 32 of the entire period. Two tubes were collected 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, SNF, MUN and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). The milk contained inside the conical tube was lyophilized and then composited by cow and period for nutrient analysis. Milk samples were analyzed at the University of Nebraska – Lincoln for lab corrected DM, N and GE. To determine the DM content of individual feed ingredients, TMRs, refusals, feces and urine samples were dried at 60 °C in a forced air oven for 48 hours and then composited by treatment or cow and period. Milk samples were lyophilized to determine DM. Feed ingredients, refusals and feces were ground as previously described with the feces and for lab corrected DM and GE. 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 described by Foth et al. (2015) and Freetly et al. (2006) that were built at the University of Nebraska - Lincoln. Prior to collections, 5 headboxes were used to test the rate of recovery of gas by burning 100% ethyl alcohol in the sealed headbox and comparing this measure to calculated gas concentrations. These calculations were based on weight of alcohol burned and a measured volume of gas sample. For each cow, a collection period of 1 consecutive 23-hr interval measured O_2 consumption, and CO_2 and CH_4 production. The design of the headboxes allowed for feed to be placed in the bottom of the box and ad libitum access to water was available for the cows from a water bowl placed inside the headbox. Within the headbox, temperature and dew point were recorded every minute for a 23 hr interval using a probe (Model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (Model XR440, Pace Scientific Inc., Moorseville, NC). Fifteen minutes before the start of the collection, the doors were closed and motor was turned on. Line pressure was measured using a manometer (Item # 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recoded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas in the headbox was measured using a gas meter (Model AL425, American Meter, Horsham, PA). From the headbox, continuous amounts of outgoing and incoming air were diverted to 2 different collection bags (61 × 61 cm LAM-JAPCON-NSE, 44 L; PMC, Oak Park, IL) using glass tube rotameters (Model 1350E Sho-Rate “50”, Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed (Emerson X-stream 3-channel analyzer, Solon, OH) at U.S. Meat Animal Research Center (MARC) according to Nienaber and Maddy (1985). Heat production was estimated through calculation of O_2 consumption, and CO_2 and CH_4 production with correction for urinary N loss according to Brouwer (1965; Equation 1). The gaseous products were reported in liters and the mass of urinary N in grams. Respiratory quotient was calculated using the ratio of CO^2 produced to the O_2 consumed and was not corrected for nitrogen. Volume of CH_4 produced was multiplied by a constant of 9.45 kcal/L to estimate the amount of energy formed from the gaseous products. Energy balance was calculated for each cow and adjusted for excess N intake according to Freetly et al. (2006) using the following equations:

HP (Mcal/d) = $3.866 \times O_2$ L + $1.200 \times CO_2$ L – $0.518 \times CH_4$ L– $1.431 \times$ N g [1]

Metabolizable energy (**ME**) (Mcal/d) = gross energy intake Mcal/d – fecal energy Mcal/d – urinary energy Mcal/d – methane energy Mcal/d [2]

Retained energy (**RE**) (Mcal/d) = ME – HP [3]

Tissue energy (**TE**) (Mcal/d) = RE – milk energy Mcal/d [4]
Tissue energy in protein (g/d) = (N balance g/d) × (5.88 kg of protein/kg of N) × (5.7 Mcal/kg of protein)/1F000 [5]
Metabolizable energy for maintenance was found by regression of RE on ME and is the ME at zero RE as shown in Figure 1.

Statistical Analysis
Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2013, Cary, NC). Treatment was considered a fixed effect. Cow within square, was considered as a random effect. The mean observation of each treatment was generated by using the LSMEANS option. Linear and quadratic effects were tested using the CONTRAST statement of SAS. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

The feeding trial was successfully completed and the Jersey dairy cows consumed feed and produced milk similar to highly successful commercial dairy farms (Table 4). Increasing the inclusion of feather meal resulted in a quadratic effect on DMI ($P = 0.041$) with the highest DMI observed in cows consuming LFM and MFM treatments. Practically it is useful to know that feather meal actually had a stimulatory effect on DMI when included up to 6.6 % of the diet DM as this is contrary to some field suggestions that feather meal has a negative effect on DMI. The inclusion of feather meal resulted in similar milk yield when included up to 6.6 % of the diet but when included at 10.0 % of the diet DM milk yield was negatively affected ($P = 0.086$). Increasing the inclusion of feather meal did not affect ($P = 0.454$) the yield of milk fat averaging 1.68 ± 0.052 kg/d across treatments however the inclusion did ($P = 0.020$) reduce the yield of milk protein from 1.05 to 0.96 ± 0.030 kg/d. Interestingly the yield of energy correct milk was not affected by treatment averaging 39.3 ±1.089 kg/d across treatments.

The effect of feeding hydrolyzed feather meal on total tract digestibility of DM, OM, CP, NDF, and starch is listed in Table 6. The digestibility of fiber and starch was modestly increased ($P = 0.011$ and 0.034) with the inclusion of feather meal while the digestibility of CP was reduced ($P < 0.001$). The reduction of digestibility of CP may have resulted in reduced metabolizable protein and may in part explain why milk protein yield was negatively affected when feeding feather meal. Further research should investigate if the effect was a result of total protein or perhaps a deficiency in histidine which is low in feathermeal. In either case, it is likely that manipulations in formation may aid in mitigating this negative effect when feeding feathermeal. The effects of treatment in energy use is listed in Table 7. The response of total intake of GE, DE, ME and NEL generally followed that of DMI. Specifically, that increasing the inclusion of hydrolyzed feather meal resulted in quadratic responses with highest intakes of energy being observed in intermediate treatments. One interesting observation of the current study is that increasing the inclusion of feather meal increased the energy content of the diet with NEL being observed to increase from 1.18 to 1.26 Mcal/kg when feather meal was increased from 0 to 10 % of the diet DM. This was not expected the formulated energy content was actually reduced (Table 1) when increasing feather meal. The driving reason for this observation is not apparent but may be due to positive effect feather meal on total tract digestibility of fiber and starch digestion as well as modest increase in the concentration of crude fat in diets containing feather meal. Tissue energy was not affected by treatment and was slightly negative across treatments and typical of high producing cows in early lactation. Maintenance energy and efficiency of energy use for lactation. Estimated maintenance

energy requirement is illustrated in Figure1 and was determined through regression of ME intake and RE and then solving for ME intake when RE equals zero. Estimated maintenance requirement was calculated to be 184 kcal/MBW with efficiency of ME use for lactation (k1) of 0.78.

The response of total intake and excretion of nitrogen generally followed that of DMI. Specifically, that increasing the inclusion of hydrolyzed feather meal resulted in quadratic response ($P = 0.028$) with highest intakes of nitrogen being observed in cows consuming the intermediate treatments. Although total nitrogen balance was not affected, increasing the inclusion of feather meal resulted in the proportion of N excreted in the feces to increase linearly ($P < 0.001$) while that excreted in the urine decreased linearly ($P = 0.001$). This observation is likely a result of the reducing effect of feather meal on total tract digestibility of CP. Despite this effect treatment did not affect total nitrogen balance.

CONCLUSIONS

A study utilizing lactating dairy cows was conducted to evaluate the effects of increasing the inclusion of hydrolyzed feather meal in a mixed ration was conducted. The overall objective of this study was to test the effects on feed intake, milk production, total tract digestibility of protein, and energy utilization. This research indicated that feed intake was increased when feeding feather meal up to 6.6% of the diet DM inducting this it is a palatable feedstuff. Total tract digestibility of both fiber and starch was stimulated with the inclusion of feather meal while the digestibility of protein was moderately decreased. The production of milk protein was reduced with increasing the proportion of feather meal and this may have been a result of reductions in metabolizable protein. Increasing feather meal in the diet resulted in diets with a great concentration of energy and maintained the production of fat correct milk. Overall feather meal appears to be a highly palatably feed ingredient for dairy cattle and can be used to replace more expensive feed ingredients while also maintaining milk yield.

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Table 1. Chemical composition and analysis of treatments formulated to contain hydrolyzed feather meal.

	Treatment[1]			
Item	Control	LFM	MFM	HFM
Ingredient, %DM				
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
Calcium carbonate	1.11	1.11	1.11	1.11
Tallow	0.89	0.89	0.89	0.89
Ca-salts of LCFA[3]	0.89	0.89	0.89	0.89
Sodium bicarbonate	0.56	0.56	0.56	0.56
Urea	0.56	0.44	0.33	0.22
Calcium PhosDi	0.44	0.44	0.44	0.44
Magnesium oxide	0.31	0.31	0.31	0.31
Salt	0.24	0.24	0.24	0.24
Agipro-L	0.22	0.22	0.22	0.22
SmartamineM	0.13	0.13	0.13	0.13
Vitamin premix[4]	0.05	0.05	0.05	0.05
Trace mineral premix[5]	0.04	0.04	0.04	0.04
Chemical Composition[6]				
DM, %	61.4 (0.40)	61.6 (0.27)	61.6 (0.30)	61.3 (0.82)
CP, % DM	17.3 (0.65)	17.2 (0.50)	17.2 (0.45)	17.2 (0.55)
Crude fat, % DM	3.54 (0.38)	3.80 (0.41)	4.06 (0.46)	4.33 (0.53)
ADF, % DM	20.5 (1.55)	21.2 (1.08)	22.0 (1.46)	22.7 (1.85)
NDF, % DM	32.8 (1.55)	33.1 (1.56)	33.3 (1.58)	33.6 (1.62)
Lignin, % DM	3.23 (0.29)	3.98 (0.39)	4.73 (0.50)	5.49 (0.61)
Ash, % DM	7.64 (0.18)	7.56 (0.07)	7.47 (0.09)	7.39 (0.20)
Starch, % DM	27.0 (1.04)	27.1 (0.81)	27.1 (0.77)	27.1 (0.94)
Gross energy, cal/g[7]	4205.7 (57.6)	4233.5 (35.9)	4261.4 (36.9)	4289.2 (59.4)
ME milk, kg/d[8]	32.5	32.3	32.1	31.9
MP milk, kg/d[8]	35.3	34.5	33.8	33.0
NE_L, Mcal/k[8]	1.76	1.75	1.74	1.73

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2] Soypass, LignoTech, Overland Park, KS.

[3]Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. Princeton, NJ.

[4] Formulated to supply approximately 148,500 IU/d vitamin A, 38,500 IU/d vitamin D and 902 IU/d vitamin E in total rations.

[5] Formulated to supply approximately 2,300 mg/kg Co, 25,000 mg/kg Cu, 2,600 mg/kg I, 1,000 mg/kg Fe, 150,000 mg/kg Mn, 820 mg/kg Se and 180,000 mg/kg Zn in total rations.
[6]Values determined by Cumberland Valley Analytical Services, Hagerstown, MD, Mean (SD).
[7]Determined from composite samples from experiment and analyzed at the University of Nebraska-Lincoln, mean (SD).
[8]Values formulated from Cornell-Penn-Miner dairy model (Boston et al., 2000).

Table 2. Feed Chemical Analysis for alfalfa hay, brome hay, corn silage, and concentrate mixes (DM basis)[1]

	Alfalfa Hay		Brome Hay		Corn Silage		CON Concentrate		LFM Concentrate		MFM Concentrate		HFM Concentrate	
Chemical	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CP, % of DM	16.5	1.36	8.73	0.43	8.05	0.35	25.6	1.17	25.5	1.11	25.4	1.05	25.4	0.99
Soluble Protein, % of DM	5.70	0.52	2.03	0.36	4.73	0.33	4.98	0.90	4.91	0.84	4.84	0.77	4.78	0.70
ADICP[2], % of DM	1.44	0.18	1.43	0.50	0.53	0.02	0.84	0.26	2.09	0.38	3.35	0.51	4.60	0.64
NDICP[2], % of DM	2.62	0.42	4.49	0.67	0.68	0.06	3.26	0.98	4.05	1.20	4.85	1.41	5.65	1.63
ADF, % of DM	41.4	3.57	44.7	2.35	20.1	0.59	13.9	0.66	15.4	1.45	17.0	2.24	18.5	3.02
NDF, % of DM	51.3	4.22	69.2	2.70	34.3	1.50	23.0	1.37	23.5	1.43	24.1	1.49	24.6	1.54
Lignin, % of DM	8.95	1.31	6.25	0.83	2.95	0.45	2.05	0.29	3.63	0.48	5.22	0.68	6.81	0.87
NFC, % of DM	23.8	3.37	14.5	2.45	51.3	1.61	40.6	2.06	39.9	1.50	39.2	0.94	38.5	0.38
Starch, % of DM	1.33	0.38	1.38	1.70	42.7	1.73	17.9	1.31	18.0	1.73	18.0	2.14	18.1	2.56
Sugar, % of DM	4.23	1.81	5.18	2.83	0.78	0.43	14.3	0.90	13.8	0.93	13.3	0.96	12.9	0.98
Crude fat, % of DM	1.48	0.56	1.88	0.26	3.43	0.17	3.93	0.72	4.48	0.85	5.04	0.98	5.60	1.11
Ash, % of DM	9.53	0.49	9.71	0.32	3.71	0.35	10.2	0.45	10.1	0.46	9.90	0.47	9.73	0.48
Ca, % of DM	1.05	0.12	0.42	0.06	0.18	0.01	1.86	0.20	1.89	0.22	1.93	0.24	1.97	0.26
P, % of DM	0.36	0.01	0.29	0.01	0.22	0.02	0.46	0.03	0.45	0.03	0.45	0.03	0.45	0.03
Mg, % of DM	0.23	0.02	0.13	0.02	0.14	0.00	0.52	0.03	0.53	0.03	0.54	0.03	0.55	0.03
K, % of DM	3.62	0.04	2.21	0.15	0.95	0.09	1.30	0.04	1.21	0.03	1.12	0.03	1.03	0.03
S, % of DM	0.20	0.02	0.20	0.02	0.15	0.02	0.32	0.06	0.44	0.06	0.57	0.07	0.69	0.07
Na, % of DM	0.03	0.01	0.03	0.01	0.02	0.00	0.68	0.03	0.70	0.03	0.73	0.04	0.76	0.04
Cl, % of DM	0.11	0.01	0.34	0.05	0.18	0.02	0.63	0.03	0.65	0.03	0.66	0.02	0.68	0.02
Fe, mg/kg	179.3	47.3	170.5	20.9	119.0	21.7	717.8	29.8	663.1	53.5	608.4	77.3	553.8	101.0
Zn, mg/kg	25.3	2.87	24.3	3.77	30.3	3.86	175.8	39.1	181.1	38.6	186.4	38.2	191.8	37.8
Cu, mg/kg	8.00	0.00	7.50	1.00	6.50	0.58	29.5	2.89	30.4	2.49	31.3	2.10	32.3	1.71
Mn, mg/kg	29.3	2.63	49.8	5.68	25.5	1.91	105.3	5.91	111.4	7.19	117.6	8.46	123.8	9.74
DCAD[4]	78.6	1.20	35.8	4.48	10.7	2.78	24.7	5.49	15.4	4.90	6.16	4.31	-3.10	3.72

[1]Mean and SD were calculated based on samples of each feedstuff collected during each period and estimated by a commercial feed testing laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) treatments: CON = Control; LFM = 3.3 % inclusion of feather meal; MFM = 6.6 % inclusion of feather meal; and HFM = 10 % inclusion of feather meal.

[2]ADICP = Acid-detergent-insoluble crude protein; NDICP = Neutral-detergent-insoluble crude protein

[3]NFC = Nonfiber carbohydrate calculated by difference 100-(% NDF + % CP + % Fat + % Ash)

[4]Dietary cation-anion difference (mEq/100g of DM = ((Na + K) – (Cl + S))/100 g of DM)

Table 3a. Particle distribution of treatments formulated to contain hydrolyzed feather meal based on the total mixed ration (DM basis)[1]

	Control		LFM		MFM		HFM	
Particle Size, %[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
> 19.0 mm	4.00	2.33	3.00	1.07	3.75	1.28	5.13	2.03
19.0 -- 8.0 mm	27.5	2.62	29.9	1.96	28.3	4.13	27.6	2.13
8.0 -- 1.18 mm	47.5	2.20	48.0	2.45	48.1	2.42	47.5	2.67
< 1.18 mm	21.0	1.51	19.0	0.93	20.0	4.96	19.9	1.64

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2]Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002).

Table 3b. Particle distribution of treatments formulated to contain hydrolyzed feather meal based on the total mixed ration (as fed basis)1

	Control		LFM		MFM		HFM	
Particle Size, %[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
> 19.0 mm	4.00	2.33	3.63	1.51	3.88	0.99	5.88	2.03
19.0 -- 8.0 mm	29.1	4.61	34.1	2.03	34.0	2.56	31.6	2.07
8.0 -- 1.18 mm	46.8	3.77	45.4	2.56	45.1	2.64	44.6	2.26
< 1.18 mm	20.1	1.25	17.3	0.87	16.9	1.25	17.9	1.25

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2]Determined using the Penn State Particle Separator on wet basis (Heinrichs and Kononoff, 2002).

Table 4. DMI, milk production and composition, body weight and BCS[5], and water intake of treatments formulated to contain hydrolyzed feather meal

	Treatment[1]					*P*-value		
Item	Control	LFM	MFM	HFM	SEM[2]	Trt	Linear	Quadratic
DMI, kg/d	19.6	20.2	20.3	19.1	0.765	0.185	0.499	0.041
Milk yield, kg/d	31.7	32.0	31.9	29.7	1.258	0.086	0.062	0.084
ECM[3], kg/d	39.7	39.6	40.1	37.8	1.415	0.196	0.158	0.174
Fat, %	5.35	5.23	5.45	5.54	0.216	0.044	0.026	0.182
Fat Yield, kg/d	1.68	1.67	1.72	1.64	0.069	0.454	0.694	0.337
FCM kg/d	40.9	40.9	41.7	39.4	1.470	0.299	0.340	0.197
Protein, %	3.34	3.29	3.23	3.23	0.098	0.262	0.063	0.554
Protein Yield, kg/d	1.05	1.05	1.02	0.96	0.041	0.019	0.004	0.181
Lactose, %	4.84	4.84	4.86	4.84	0.035	0.929	0.883	0.812
MUN[4], mg/dl	15.1	14.7	14.7	14.4	0.238	0.098	0.023	0.866
SCC[5], cells/mL	148.7	102.8	128.1	226.6	120.3	0.098	0.110	0.050
Free water intake, L/d	88.9	98.3	93.6	87.4	5.70	0.334	0.663	0.096
Body Weight, kg	444.4	444.9	451.4	440.6	14.31	0.019	0.637	0.018
BCS[6]	3.10	2.98	3.13	3.04	0.103	0.212	0.859	0.691

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2]Lowest standard error of treatment means is shown.

[3]Energy corrected milk = 0.327 × milk yield [kg] + 7.2 × protein [kg] adjusted for 3.5% fat and 3.2% total protein (DHI Glossary, 2014).

[4]MUN = Milk urea nitrogen.

[5]SCC = Somatic cell count.

[6]BCS = Body Condition Score 1-5 scale according to Wildman et al. (1982).

[abc]Means within rows lacking common superscript differ ($P < 0.05$).

Table 5. Methane production, methane efficiencies, and heat production for treatments formulated to contain hydrolyzed feather meal

Item	Treatment[1]				SEM[2]	*P*-Value		
	Control	LFM	MFM	HFM		Trt	Linear	Quadratic
O_2 consumption, L/d	4932.0	4996.1	4993.4	4779.4	172.9	0.078	0.118	0.038
CO_2 production, L/d	5060.0	5133.3	5131.7	4873.5	196.2	0.083	0.117	0.042
CH_4 production, L/d	435.5	449.4	448.3	416.9	25.3	0.236	0.311	0.077
CH_4/MY, L/kg/d	10.7	11.0	10.7	10.7	0.507	0.792	0.811	0.456
CH_4/ECM, L/kg/d	11.0	11.3	11.2	11.1	0.528	0.850	0.936	0.477
RQ[3], L/L	1.03	1.03	1.03	1.02	0.006	0.735	0.509	0.484
CH_4/DMI, L/kg/d	22.3	22.1	22.0	21.8	0.831	0.914	0.482	0.973
HP[4], Mcal/d	24.7	25.0	25.0	23.9	0.885	0.092	0.141	0.041
HP, kcal/$BW^{0.75}$	254.8	258.0	255.2	249.2	4.86	0.309	0.192	0.174

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2]Lowest standard error of treatment means is shown

[3]RQ = Respiratory quotient (CO_2 production/O_2 consumption).

[4]HP = Heat production, calculated with Brouwer's (1965) equation from oxygen consumption (L), carbon dioxide production (L), methane production (L) and urine–N (g) (HP = 3.866 × O_2 + 1.200 × CO_2 – 0.518 × CH_4 – 1.431 × N).

[abc]Means within rows lacking common superscript differ ($P < 0.05$).

Table 6. Apparent DM, OM, CP, NDF, Starch and Ash digestibility of treatments

	Treatment[1]					*P*-value		
Component	Control	LFM	MFM	HFM	SEM[2]	Trt	Linear	Quadratic
DM, %	65.9	65.8	66.1	66.0	0.648	0.937	0.692	0.947
OM, %	68.0	67.8	68.2	68.0	0.625	0.942	0.851	0.970
CP, %	63.5	60.2	58.4	57.1	0.812	< 0.001	< 0.001	0.151
NDF, %	45.8	46.8	47.6	48.7	1.06	0.080	0.011	0.920
Starch, %	96.3	96.4	97.2	97.1	0.376	0.129	0.034	0.795
Ash, %	59.9	59.6	59.5	58.8	1.38	0.934	0.543	0.869

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[2]Lowest Standard error of treatment means is shown

Table 7. Partitioning of energy for treatments formulated to contain hydrolyzed feather meal.

	Treatment[2]					*P*-Value		
Item[1]	Control	LFM	MFM	HFM	SEM[3]	Trt	Linear	Quadratic
GE intake, Mcal/d	82.2	85.7	86.4	81.9	3.24	0.197	0.958	0.038
DE, Mcal/d	54.1	56.3	57.1	53.9	1.98	0.249	0.982	0.051
ME, Mcal/d	47.9	49.9	50.7	48.0	1.73	0.295	0.852	0.065
NE_L, Mcal/d	23.2	24.9	25.7	24.1	1.11	0.381	0.482	0.121
Component, Mcal/d								
Feces	28.2	29.4	29.3	28.0	1.41	0.299	0.849	0.062
Methane	4.12	4.25	4.24	3.94	0.239	0.236	0.311	0.077
Urine	2.08	2.15	2.21	1.95	0.118	0.433	0.493	0.166
Heat	24.7	25.0	25.0	23.9	0.885	0.092	0.141	0.041
Retained	23.2	24.9	25.7	24.1	1.11	0.381	0.482	0.121
Milk	25.3	25.2	26.6	25.0	1.26	0.532	0.903	0.389
Tissue	-2.06	-0.25	-0.94	-0.91	1.461	0.814	0.645	0.512
DE, % of GE	65.8	65.8	66.2	66.1	0.652	0.880	0.512	0.854
ME, % of GE	58.2	58.4	58.8	58.9	0.697	0.779	0.316	0.958
Feces, % of GE	34.3	34.2	33.8	33.9	0.652	0.880	0.512	0.854
Methane, % of GE	5.01	4.93	4.88	4.80	0.187	0.591	0.176	0.997
Urine, % of GE	2.55	2.50	2.56	2.39	0.116	0.702	0.409	0.617
Heat, % of GE	30.1	29.3	29.0	29.5	0.518	0.385	0.283	0.179
Milk, % of GE	30.9	29.8	30.9	30.3	1.319	0.920	0.925	0.835
Tissue, % of GE	-2.74	-0.77	-1.05	-0.90	1.748	0.807	0.477	0.579
GE, Mcal/kg of DM	4.21	4.23	4.26	4.29	0.01	< 0.001	< 0.001	0.999
DE, Mcal/kg of DM	2.77	2.79	2.82	2.84	0.03	0.141	0.024	0.873
ME, Mcal/kg of DM	2.45	2.47	2.50	2.53	0.03	0.145	0.023	0.972
NE_L, Mcal/kg of DM	1.18	1.23	1.27	1.26	0.04	0.246	0.068	0.412

[1] GE = gross energy; DE = digestible energy; ME = metabolizable energy; NE_L = net energy lactation

[2]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.

[3]Lowest standard error of treatment means is shown.

Table 8. Partitioning of nitrogen for treatments formulated contain hydrolyzed feather meal

	Treatment[1]					*P*-Value		
Item	Control	LFM	MFM	HFM	SEM[2]	Trt	Linear	Quadratic
Mass, g/d								
N intake	542.0	564.7	563.0	527.5	21.4	0.133	0.423	0.028
Fecal N excretion	199.1	226.6	238.9	236.7	11.1	< 0.001	< 0.001	0.016
Urine N excretion	165.5	159.1	154.9	118.6	9.38	0.008	0.002	0.129
Total N excretion[3]	364.6	385.7	393.7	355.6	17.7	0.163	0.747	0.032
Milk N concentration	198.8	198.0	203.4	185.4	10.5	0.608	0.437	0.386
N balance[4]	-21.3	-19.1	-34.0	-13.6	13.7	0.749	0.895	0.509
TE in protein[5]	-0.72	-0.64	-1.15	-0.45	0.459	0.749	0.895	0.509
N, % of intake								
Fecal N	36.8	40.0	42.3	44.5	0.79	< 0.001	< 0.001	0.463
Urine N	30.4	28.3	27.7	22.5	1.43	0.005	0.001	0.279
Milk N	36.8	36.1	36.3	35.2	1.79	0.933	0.564	0.907
N balance	-3.90	-4.43	-6.19	-2.17	2.59	0.748	0.769	0.389

[1]Treatments: LFM = low feather meal, 3.3 % inclusion of feather meal; MFM = medium feather meal, 6.6 % inclusion of feather meal; and HFM = high feather meal 10 % inclusion of feather meal.
[2]Lowest standard error of treatment means is shown.
[3]Total N excretion = Fecal N + Urine N.
[4]Nitrogen balance = intake N – Fecal N – urine N – milk N.
[5]TE = Tissue energy.

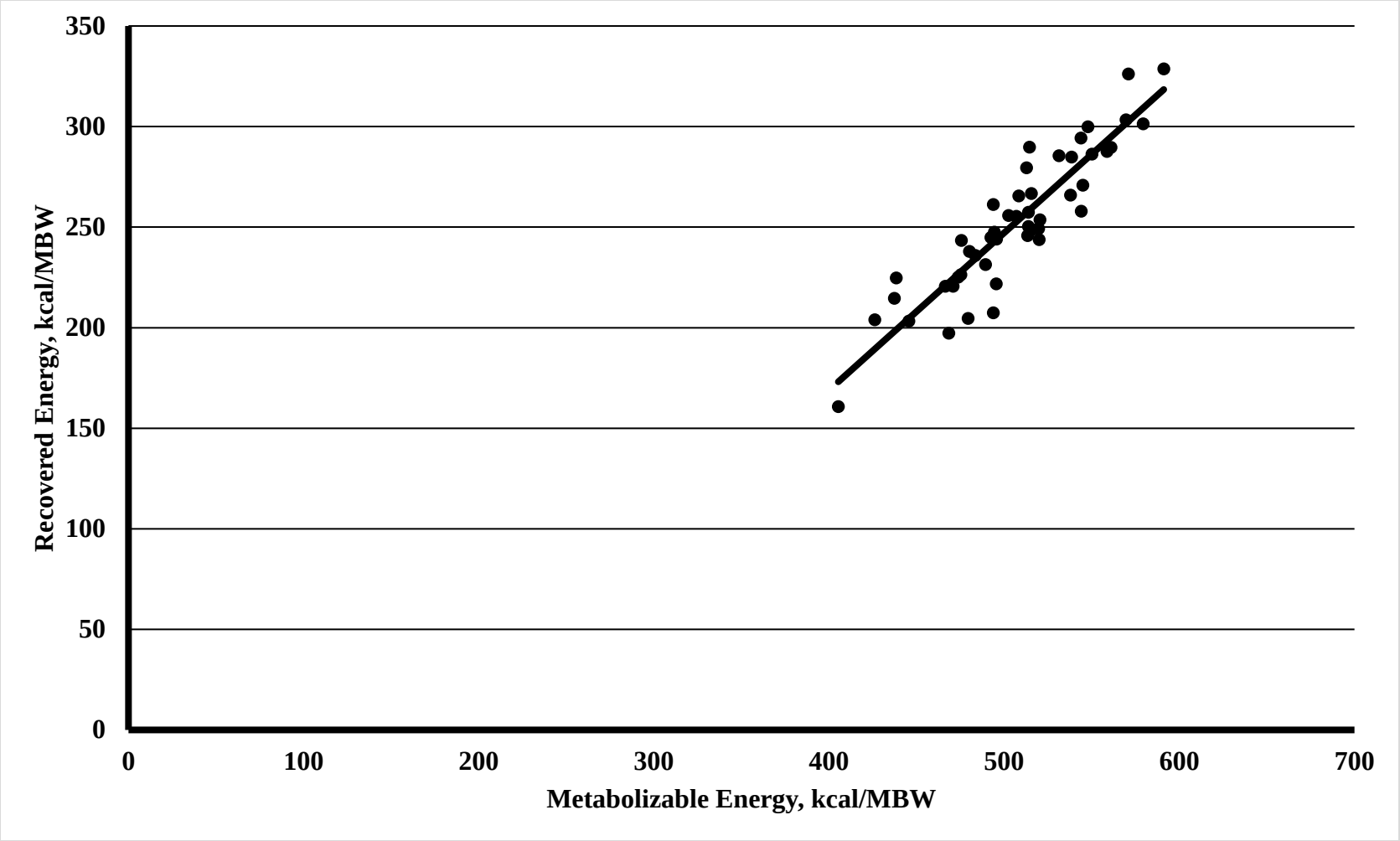

Figure 1. Regression of recovered energy on metabolizable energy intake in kilocalories per metabolic body weight (kcal/MBW; y = 0.7821x – 143.88; R2 = 0.83). Recovered energy = 0 at 184 kcal/MBW and efficiency of converting ME to lactation energy is 78 %