Effects of Precision Essential Amino Acid Formulation on a Metabolizable Energy Basis for Lactating Dairy Cows

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Introduction

Accurate description of both nutrient supply and requirements in the dairy cow continues to be a focus of ongoing research as we work to improve the efficiency of nutrient use in high producing cattle and reduce the environmental impact of milk production. In addition, producers feel the need to optimize their cattle's performance, improving profitability through feed cost savings while complying with nutrient management. As such, areas of opportunity exist in cattle nutrition that can accomplish these objectives, particularly involving protein feeds and nitrogen (N) metabolism. Current diet formulations rely on crude protein as the metric when evaluating N supply (NRC, 2001); however, the aggregation of all N containing nutrients into one metric creates variability in predicting supply, particularly when evaluating animal performance (Ipharraguerre & Clark, 2005). Considerable progress has been made in understanding lysine and methionine requirements of lactating cattle (Rulquin et al., 1993; Schwab, 1996), and providing recommendations and demonstrating improvements in performance when animals are properly supplied with these amino acids (Armentano et al., 1997; Noftsger & St-Pierre, 2003). The same efforts that went into the Met and Lys requirements and supply should be applied to other essential amino acids (EAA), calling for the abandonment of crude protein and the move towards a more accurate representation of N supply on an amino acid basis.

In an effort to address this approach, changes have been made to the most recent research version of the CNCPS v.7 (Higgs et al., 2014), disaggregating crude protein into its constituents and accounting for these on a N basis. The implications of the changes allow for more accurate predictions of rumen N and amino acid supplies to the cow, particularly when coupled with the estimation of endogenous protein flows (Ouellet et al., 2007; Marini et al., 2008) and updated estimations of amino acid requirements and efficiency of use (Lapierre et al., 2007; Lapierre et al., 2012). Work has been conducted to evaluate CNCPS v.7 performance when balancing diets for both rumen N requirements and EAA supply (Higgs et al., 2014). Findings from that study indicated that notwithstanding lower levels of crude protein (< 14% DM) in the diet, cattle maintained a high level of performance when supplied with adequate rumen N and balanced for EAA. Further investigation eluded to a potential relationship between the supply of digestible EAA and the supply of metabolizable energy (ME) in the diets fed. This loglogistic relationship (Figure 1) was demonstrated when the ratio of AA required (AAR) to AA supplied (AAS) was regressed against digestible AA supply relative to Mcals of ME. To further expand on these relationships, optimum points of digestible AAS relative to ME can be estimated by regressing predicted AAR on the digested AAS (figure not shown).

Solving for the upper critical level of the second order derivative of that regression (Doepel et al., 2004), determined the efficiency of use of each EAA , and interpolating that efficiency provides a solution for the supply of EAA per unit of ME at the optimum efficiency of use for productivity (Figure 1). This technique can be applied to calculate the requirements for all EAA and the supply of each AA relative to ME, on a gram basis, can be calculated.

The recognition of protein and energy's interrelationship is not a novel idea, particularly when discussing mammalian metabolism. Metabolic flexibility, particularly in the mammary gland, allows dairy cattle to meet their energetic needs through either the use of high yielding energy substrates or N containing compounds (i.e. amino acids) when other substrates are lacking (Lobley, 2007). Studies have demonstrated that the supplementation of both propionate and casein have a greater, additive effect on milk yield in both cattle (Raggio et al., 2006) and lactating sows (Dunshea et al., 2005) than if either one was solely supplemented. In spite of the collinearity of these two types of nutrients, their relationship seems more prevalent when exploring the relationship between digestible EAA and metabolizable energy (Higgs et al., 2014). Further, nearly all of AA supply can be related energy when swine diets are formulated (NRC, 2012). Depending upon the stage of life, the weight of animal, and it's production (meat or milk), the Swine NRC provides specific tables containing ideal amino acid profiles for a given animal which can then be related to a recommended energy content of the diet.

With this in mind, the objective of this study was to evaluate the approach in lactating cattle using CNCPS v.7 to formulate diets adequate in rumen N and balanced for EAA relative to the ME supply. Our hypothesis was that the efficiencies of use for each EAA determined by Higgs (2014) and Higgs and Van Amburgh (2016) are the optimum efficiencies and we when related to energy, the requirements can be calculated on a gram basis and that pending upon the results of this study, will either be modified or reinforced. The hypothesis involved testing the ranges in the grams of digestible AA required per unit of ME (Figure 1). Those ranges represent the upper and lower limits of each EAA observed in the data sets and the hypothesis involved evaluating the limits as a sensitivity test of the concept.

Methodology

Accurate description of feed chemistry for all ingredients included in this experiment was of the utmost importance when considering this study's validity. Protein feed samples obtained from a commercial feed mill (Purina Animal Nutrition, Caledonia, NY) and forage samples from the Cornell University Ruminant Center were screened for chemical composition. Of particular interest was the quantification of total N, N digestibility, and amino acid profiles for all feeds. Quantification of total N was obtained via the Leco total combustion method (Leco, St. Joseph, MI). Amino acid profiles from parallel laboratory experiments (Van Amburgh et al., 2017) were adapted and matched to fit the analyzed feeds. Upon completion, both feed chemistry results and animal inputs were implemented within CNCPS v.7 for diet formulation.

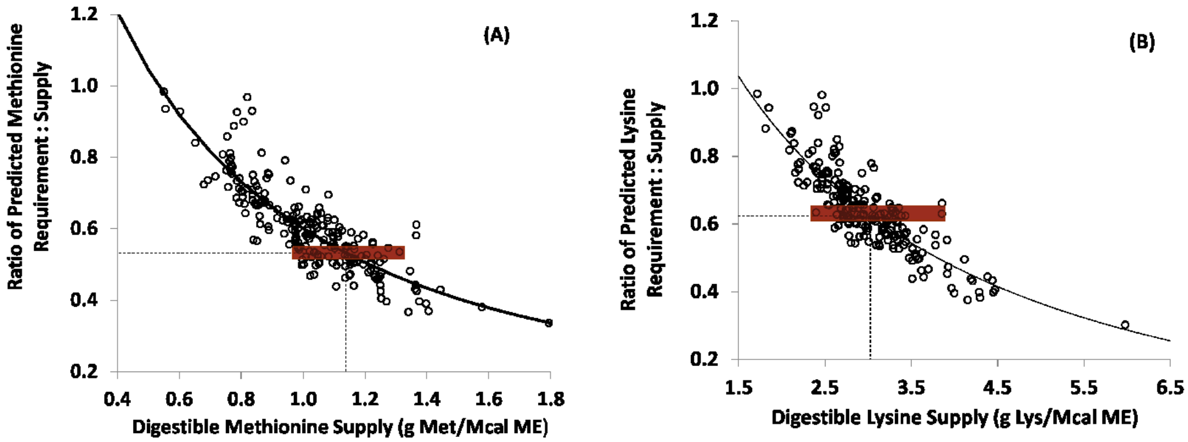

Figure 1. Relationship between model predicted EAA requirement: supply and EAA supply relative to ME for Met (A) and Lys (B). The dashed line in (A) & (B) represents the Met or Lys supply at the optimum ratio of model predicted Met or Lys requirement and supply. The red bar represents ± 1 standard deviation of AA supply relative to ME supply.

Dietary treatments within this experiment were based on previous results exploring amino acid balancing in lactating dairy cattle (Higgs, 2014; Higgs and Van Amburgh, 2016). Findings from this study suggest an optimal requirement of each EAA at a given level of metabolizable energy (Figure 1; shaded rectangles); however, variation exists around data, creating ambiguity about their accuracy. In an effort to confirm the values, three diets were formulated to be isocaloric and excess in energy as a means to prevent a first-limiting effect on animal performance. The only differences in these diets were in the level of EAA fed, creating differences in the ratios of EAA to metabolizable energy. The Neutral diet (NEU) was formulated to match the optimal ratios determined by Higgs (2014) and Higgs and Van Amburgh (2016), whereas the Positive (POS) and Negative (NEG) control diets were formulated to be one standard deviation above and below the optimal ratio for each EAA (Table 1).

Thus, the three diets were isocaloric, varied only in the level of EAA fed and the rumen N balance was positive for all diets through the use of additional urea when need to ensure the rumen N balance was always positive. Protein feeds were evaluated for intestinal digestibility using the assay of Ross et al. (2013) to ensure EAA availability in formulation and this information was considered to ensure the grams of each EAA met the formulation criteria. Cattle on the Neutral diet (14.5% crude protein [CP]) were fed according to previously calculated optimal grams of AAS to ME (Higgs, 2014), whereas the Positive (16% CP) and Negative diets (13.5% CP) were formulated for ± 1 standard deviation relative to the Neutral diet, respectively for all EAA (Figure 1; Table 1).

The experiment was conducted at the Cornell University Ruminant Center (Harford, NY) from July - December 2018. All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. One hundred and forty-four (n=144) Holstein cows [26 primiparous and 118 multiparous; 2.9 ± 1.4 lactations; 92 ± 24 DIM at enrollment] were enrolled in a 114 day longitudinal study. Two enrollments (96 cattle in enrollment 1 [July – November 2018] and 48 cattle in enrollment 2 [August – December 2018) periods were necessary to maintain the relevant period of lactation for observation. Cattle were blocked into 16 cow pens and balanced for parity, DIM, previous lactation performance, and current body weight. Cattle were housed in a freestall setting at stocking density of 100%. Each pen was fed TMR once daily at approximately 0600 h and pens were targeted for 5% refusal rate. All nine pens were fed the POS diet during a 14 day covariate period and randomly assigned to one of three diets described above for the remaining 100 d. Covariate samples were taken in the second week of the period to allow animals to acclimate to their new environment and diet.

Table 1. The predicted AA supply for each diet compared with the calculated optimum supply (g digested AA/Mcal ME)

	Negative[1]		Neutral		Positive	
g AA/Mcal ME	Formulated	Target	Formulated	Target	Formulated	Target
Arg	2.01	2.03	2.25	2.04	2.30	2.32
His	0.88	0.79	0.98	0.91	1.17	1.03
Ile	2.08	1.86	2.27	2.16	2.24	2.45
Leu	3.24	2.95	3.54	3.42	4.00	3.89
Lys	2.84	2.62	3.00	3.03	3.49	3.44
Met	1.01	0.98	1.09	1.14	1.29	1.30
Phe	2.15	1.86	2.30	2.15	2.54	2.44
Thr	2.03	1.85	2.26	2.14	2.40	2.43
Trp	0.65	0.51	0.68	0.59	0.62	0.67
Val	2.27	2.14	2.51	2.48	2.76	2.82
Lys:Met	2.65	2.67	2.75	2.66	2.70	2.65

[1] Negative = All EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2014); Neutral = All EAA scaled to ideal EAA ratio according to Higgs (2014); Positive = All EAA scaled one standard deviation above EAA ratio according to Higgs (2014). All diets balanced and in excess of ME.

Body weight and body condition score (1-5 scale) were measured and recorded weekly for all cattle. Cattle were milked three times daily (0600, 1400, and 2200 h) with milk weights recorded at every session (Del Pro Farm Manager; De Laval). Milk samples were collected weekly during three consecutive milkings and analyzed for fat, true protein, lactose, total solids, and MUN (Dairy One, Ithaca, NY). Milk component yield was calculated as the sum-product of daily milk yields at each session throughout a given week and the analyzed component values of the same week. Energy corrected milk was calculated according to Tyrrell and Reid (1965). Dry matter intake was determined daily for each pen as the difference between feed offered and refused (FeedWatch; Valley Ag Software). Samples of TMR and refusals were sampled twice each week, composited, and analyzed for nutrient composition using near infrared reflectance spectroscopy. Forage samples were collected weekly and sent for wet chemistry analysis of chemical components. Additionally, mix ingredients included in the grain mix were collected whenever new batches of grain were delivered to the farm and analyzed by wet chemistry for chemical composition.

Blood samples were taken from cattle every other week throughout the experiment. Cattle were bled at least 4 hours following feeding from the coccygeal vein into heparinized Vacutainers (Becton Dickinson, Rutherford, NJ). Plasma samples were subjected to plasma urea N (PUN) analysis via enzymatic colorimetric assay.

A sub-sample of six cows per pen were chosen for fecal spot sampling twice throughout the experiment. Eight samplings over a 3-day period (Day 1: 1300 h, 1900 h, Day 2: 0100 h, 0700 h, 1600 h, 2200 h, Day 3: 0400 h, 1000 h) were performed, compositing the six cows into a single pen sample for each time point. Samples were processed and used to determine fecal N and estimate total tract NDF digestion using uNDF as an internal marker (Huhtanen et al., 1994; Raffrenato et al., 2018).

All statistical analysis was performed using SAS (v.9.4, SAS Institute Inc., Cary, NC). Feed chemistry results were produced via PROC MEANS to provide mean, standard deviation, and standard error of all feed components analyzed. Continuous measurements which were not repeated over time were subjected to ANOVA (PROC MIXED) with fixed effects including enrollment and dietary treatment. Measurements taken over time were subjected to repeated measures ANOVA (PROC MIXED) and included fixed effects of enrollment, dietary treatments, time, and the interaction of dietary treatment and time. Cow within pen was considered random in both instances and any measurements taken within the covariate period of the experiment were utilized as a covariate measure within the models, where applicable. Values generated from CNCPS outputs are raw means.

Results

Dietary ingredients and chemical composition of the three diets fed throughout the experiment are presented in Table 2. Small differences between the formulated and observed levels of CP were observed, specifically for cattle fed the NEG diet (13.5% formulated; 14.0% observed); however, the relative differences in N and EAA supply among treatments was still maintained throughout the experiment. Diets did maintain their isocaloric formulation among treatments and throughout the experiment. All other chemical components in the diet remained relatively similar among treatments and throughout the experiment. As indicated earlier, urea was formulated into all three diets to maintain adequate rumen N levels. One of the structural differences in CNCPS v7 is the ability to separate the rumen N requirements from the post-ruminal EAA requirements and in doing so, makes the process of formulating for EAA more accurate as the N can be partitioned more effectively.

Daily supply of EAA as predicted by CNCPS v.7 are shown in Table 4. Since the objective of the study involved creating isocaloric diets while varying the ratio of EAA to ME, the supply of EAA delivered to cattle had to move in a stepwise fashion. As shown in the table, most EAA (Arg, His, Leu, Lys, Met, Phe, Thr, and Val) increased in supply as the N supply increased in the three diets; however, two EAA (Ile and Trp) did not show this same trend. Ideally, the supply of all EAA would increase in such a way to match the objective of this study. Realistically, both the availability of feed ingredients with a given profile of AA and the variability of feed chemistry for the availale feeds make it a difficult process to have the supply increase in all EAA when moving from the negative to the positive treatment. At this point, we do not believe the lack of Ile and Trp supply had a major effect on the results of the study. Isoleucine is classified as a Group 2 AA and the mammary takes up this AA in excess of output, particularly for the creation of non-essential AA (NEAA) or substrates used in the creation of lactose (Lobley, 2007). Little is known about the supply of tryptophan and milk performance of dairy cattle. Within this study, the average supply of His in all three diets is 90% of the supplied level of Met. Previous literature has suggested that the supply of His should match, or exceed (up to 110% of Met supply) to allow for an optimal response in animal performance (Lee et al., 2012). Further work should be done to evaluate the response of His supplementation in the context of a study similar to the one presented here.

Dry matter intake was not different among the treatments and remained relatively stable throughout the experiment (Table 4; Effect of time not shown). Differences were observed in both milk yield and energy correct milk values, where NEG cattle yielded less milk than both the NEU and POS cattle. Whereas cattle fed, the NEU and POS diets were not significantly different between each other. Similar trends were observed for component yields. This suggests that the increased supply of EAA from the NEG to the NEU had a greater impact on milk yield and component output than the difference in supply from NEU and POS, indicating marginal effectiveness. This apparent limit is further demonstrated, as MUN output is significantly different in each diet, increasing from NEG to POS, suggesting that cattle in the POS did not need the additional EAA supplied in that diet. Overall, this demonstrates a plateau effect of EAA supply indicating the profile and amount of EAA supplied to the cattle fed the NEU diet were adequate for the expected milk yield.

Initial and final body weights were not different among treatments and all treatments were able to provide nutrients for weight gain throughout the experiment. Initial body condition score was not different among treatments and even through final condition scores were greater for NEU and POS compared to NEG, the numerical differences are negligible. Evaluation of feed efficiency indicated that NEU and POS animals were more efficient than NEG. These observations are extended to the N use efficiency where the NEG treatment had the lowest efficiency whereas there was no difference between NEU and POS treatments. Not surprisingly, daily metabolizable protein intake, as predicted by the CNCPS, was different for all treatments and increased in a stepwise fashion. What is interesting is that NEU and POS performed in a similar capacity, despite a 200-gram difference in metabolizable protein supply. This strongly suggests that the MP supply does not fully describe the N requirements of lactating cattle and that refining the diets on a digestible EAA basis, provides more accuracy. A figure summarizing these observations is presented to demonstrate animal performance when given varying ratios of digestible EAA/ME and further highlights the waning effect of increasing EAA supply relative to a fixed level of ME, particularly between NEU and POS diets (Figure 2).

Table 2. Ingredients and chemical composition of experimental diets

Ingredient, % DM	Negative[1]	Neutral	Positive
Corn silage	51.49	51.49	50.40
High moisture ear corn	9.43	9.46	9.93
Triticale	7.25	7.25	7.98
Corn grain	6.38	6.42	5.95
Soybean meal	8.16	5.55	2.72
Soybean hulls	9.25	3.84	2.83
SoyPLUS[2]	--	0.91	3.59
Canola	1.81	9.17	6.31
Urea	0.62	0.51	0.51
Smartamine M[3]	--	0.04	0.05
Smartamine ML[4]	--	--	0.07
Blood meal	--	--	3.08
Energy Booster	0.73	0.73	0.91
Dextrose	1.63	1.63	2.18
Minerals and Vitamins	3.26	2.90	3.15
Chemical components[6], % DM			
CP	14.04	14.75	15.95
SP, % CP	42.93	40.29	37.33
Ammonia, % SP	13.53	14.57	12.67
ADICP, % CP	5.68	5.86	5.46
NDICP, % CP	15.01	15.47	18.66
Acetic acid	0.45	0.45	0.46
Propionic acid	0.02	0.02	0.02
Lactic acid	2.57	2.58	2.61
Sugar	3.95	4.06	3.90
Starch	29.82	29.31	29.30
Soluble fiber	6.01	5.55	5.05
ADF	20.79	19.96	19.77
NDF	32.39	31.03	31.36
Lignin, % NDF	8.06	9.65	8.73
uNDF$_{240}$, % NDF	25.50	29.09	28.73
Ash	6.60	6.92	6.57
EE	3.49	3.61	3.78
Metabolizable Energy, Mca/kg	2.58	2.60	2.61

[1] Negative = balanced for ME (assuming 45 kg ECM), all EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2015); Neutral = balanced for, all EAA scaled to ideal EAA ratio according to Higgs (2015) ; Positive = balanced for ME, all EAA scaled one standard deviation above EAA ratio according to Higgs (2015)

[2] SoyPLUS (West Central Cooperative, Ralston, IA) rumen protected soybean meal

[3] Smartamine M (Adisseo USA Inc, Alpharetta, GA) rumen protected Met (100% AANt)

[4] Smartamine ML (Adisseo USA Inc, Alpharetta, GA) rumen protected Lys (75 % AAN) and Met (25% AAN)

[6] Chemical components are expressed as % DM unless stated. SP = soluble protein; ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; WSC = water soluble carbohydrates; uNDF240 = undigested NDF after 240 hours of in vitro fermentation; EE = ether extract.

Table 3. Daily supply of essential amino acids for each treatment diet.

AA, grams	Negative[1]	Neutral	Positive
Arg	143.14	161.04	164.43
His	62.78	70.42	83.81
Ile	147.85	162.37	160.56
Leu	229.92	253.31	286.27
Lys	201.70	222.12	250.07
Met	71.44	78.30	92.67
Phe	153.00	164.71	181.63
Thr	144.43	161.78	171.85
Trp	45.92	48.93	44.66
Val	161.01	179.55	197.46

[1] Negative = All EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2014); Neutral = All EAA scaled to ideal EAA ratio according to Higgs (2014); Positive = All EAA scaled one standard deviation above EAA ratio according to Higgs (2014). All diets balanced and in excess of ME.

Table 4. Effects of treatment diets on milk production, intake, body weight and body condition scores.

	Negative[1]	Neutral	Positive	SEM	Treatment
Intake and milk production, kg/d					
Dry matter intake	27.9	28.2	28.5	0.27	0.98
Energy correct milk yield[2]	40.5^{a}	43.7^{b}	44.8^{b}	0.57	<0.01
Milk yield	36.8^{a}	39.8^{b}	40.8^{b}	0.47	<0.01
True protein yield	1.13^{a}	1.26^{b}	1.28^{b}	0.01	<0.01
Fat yield	1.53^{a}	1.62^{ab}	1.67^{b}	0.03	<0.01
Lactose yield	1.77^{a}	1.91^{b}	1.97^{b}	0.03	<0.01
Milk composition, %					
True protein	3.09^{a}	3.17^{b}	3.14^{b}	0.02	<0.01
Fat	4.20	4.12	4.14	0.06	0.64
Lactose	4.78	4.82	4.81	0.02	0.31
MUN	10.5^{a}	11.4^{b}	13.8^{c}	0.14	<0.01
Body weight and condition					
Initial Body Weight, kg	691.5	692.7	697.5	4.27	0.83
Final Body weight, kg	721.2	718.2	723.3	3.26	0.09
Body weight change, kg/wk	2.26	2.03	2.53	0.33	0.58
Initial BCS, 1-5 Scale	2.90	2.86	2.84	0.02	0.75
BCS, 1-5 scale	2.88^{a}	2.92^{b}	2.93^{b}	0.01	0.01
CNCPS v.7 Parameters					
Feed Efficiency	1.48^{a}	1.55^{b}	1.59^{b}	0.02	<0.01
Metabolizable Protein Intake, g/day	2656.6^{a}	2974.4^{b}	3207.5^{c}	162.4	0.02
Nitrogen Use Efficiency	0.282^{a}	0.300^{b}	0.299^{b}	0.003	<0.01

[1] Negative = All EAA scaled one standard deviation below ideal EAA ratio according to Higgs (2014); Neutral = All EAA scaled to ideal EAA ratio according to Higgs (2014); Positive = All EAA scaled one standard deviation above EAA ratio according to Higgs (2014). All diets balanced and in excess of ME.

[2] Estimated according to Tyrrell and Reid (1965)

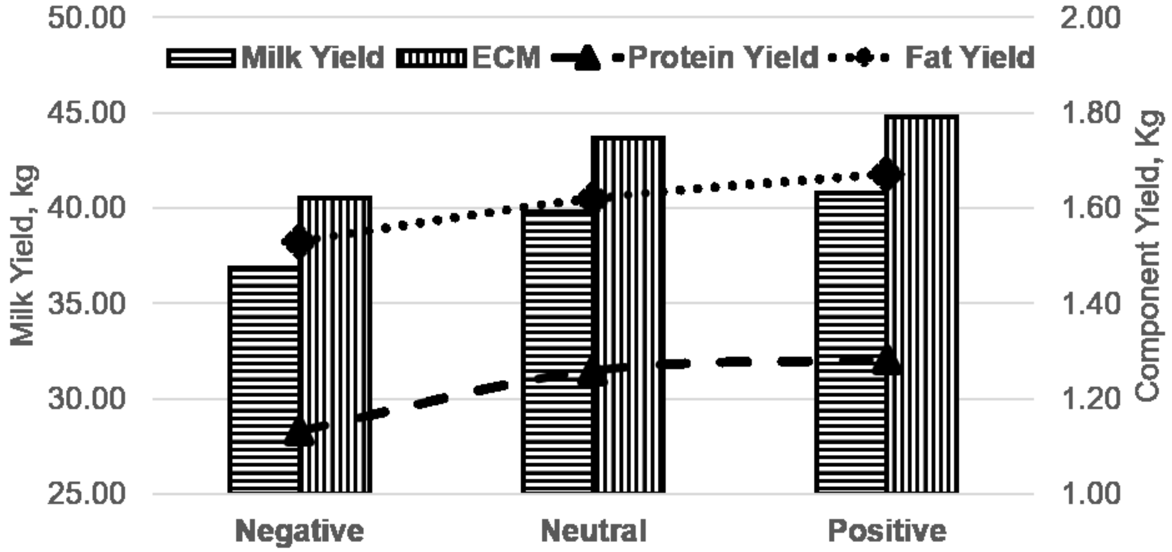

Figure 2. Effect of dietary treatment on milk, energy corrected milk, and component yield for animals fed.

In summary, cattle fed the NEU dietary treatment produced similar levels of energy corrected milk and yield similar production of fat components when compared to cattle fed the POS treatment (Table 4; Figure 2). The productivity of the cattle was similar even though the difference in crude protein of the two diets was over 1 units, suggesting that cattle fed the NEU diet were at least as productive with their N supply as cattle fed the POS diet. Evaluation of MUNs indicate that the excretion of urea nitrogen was higher in the POS diet over the NEU diet, suggesting either that NEU cattle may have had a more balanced profile of EAA or that they were less wasteful with the N given to them. Cattle fed the NEG likely had a deficient supply of EAA as their production and feed efficiency was lower than either the NEU or POS cattle. Further analysis of the data collected from this experiment, coupled with model evaluation through CNCPS v.7, will help to reinforce our hypothesis that the optimum digestible EAA supply relative to ME generated by Higgs (2014) were within the range of true requirements for lactating cattle. The results from this study will be used to formulate diets for a similar study in which cattle performance will be evaluated using a fixed N and AA supply while varying levels of rumen fermentable carbohydrates to stimulate propionate and thus, lactose production.

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