Impact of Starch and Energy on Amino Acid Requirements of Lactating Cows

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Introduction

The recognition of numerous metabolic interactions between energy and amino acid (AA) supply has become relevant regarding the efficiency of milk protein production (Lobley, 2007). Although these interactions at the post-absorptive levels can be complex, understanding the net supply of these nutrients relative the cows requirement can improve precision feeding, which in turn improves animal performance while reducing excessive nitrogen and AA supply fed (Lapierre et al., 2006). Improvements in the overall nitrogen (N) and AA efficiency of use is a metric widely used in the assessment of milk and milk component production given its implications in reducing both N inputs to cattle and a reduction in nutrient excretion into the environment. Previous calculations indicate that the efficiency of use for N averages around 25% of feed N intake and is highly variable, with ranges between 10% and 40% (Huhtanen and Hristov, 2009, Calsamiglia et al., 2010). Dijkstra et al. (2013) suggest that the upper limit for N efficiency of use for lactating cattle is above 40% and can be achieved through accurate predictions of AA requirements as well as a proper understanding of their biochemical interactions with energy and energy signaling mechanisms.

The disaggregation of metabolizable protein (MP) into the supply of each AA has become a precision feeding method to improve AA efficiency of use. Currently, the CNCPS v.7 predicts the requirements of each essential amino acid (EAA) and calculates the net supply of these EAA to determine animal productivity and assess first limiting nutrients. Further, the model expresses the requirements of each EAA relative to metabolizable energy (ME) to account for the energy demands for milk yield (Higgs and Van Amburgh, 2016). Application of this approach allows for a reduction in the MP supply, inherently dropping dietary crude protein (CP) and improving nitrogen use efficiency and allowing for the calculation of N requirements in two compartments, the rumen N requirement and the post-absorptive EAA requirement. Previous studies have formulated diets that are targeted for an optimum supply of EAA relative to ME and are lower in CP (13.5-14.5% DM) without compromising milk volume or protein yield relative to diets that have higher supply of EAA relative to ME and are consequently elevated in CP (Higgs et al., 2014, LaPierre et al., 2019). As a result, the nitrogen efficiency of use has improved for these diets which are targeted for the optimum supply of EAA; however, this improvement in efficiency is still not at the upper level described by Dijkstra et al. (2013).

To improve N and EAA efficiency of use in dairy cattle, it is important evaluate the level of glucogenic nutrients available to the animal. Literature has shown that increasing levels of glucogenic nutrients, including ruminally produced propionate and intestinally available glucose, has improved the post-absorptive transfer efficiency of AA from the

gastrointestinal tract to the mammary gland and allowed for greater yields of milk and milk protein (Lemosquet et al., 2010, Rius et al., 2010, Cantalapiedra-Hijar et al., 2016). Further, glucogenic nutrients might support N retention in peripheral tissue in the presence of elevated AA supply, improving the efficiency of productive use for N beyond the amount needed for milk protein production (Nichols et al., 2016, Curtis, 2018). Given the current understanding of glucogenic nutrients on AA utilization in dairy cattle, our objective was to evaluate the efficiency of use for EAA and N when supplying two levels of glucogenic nutrients in the form of ruminally produced propionate via differences in dietary starch, in addition to two levels of EAA supply. The EAA were formulated relative to ME (g digestible AA/Mcal ME) to assess if the optimum ratio of each EAA supply relative to ME changed with the addition of glucogenic nutrients. Our hypothesis was that cattle fed the higher level of glucogenic nutrients without the larger supply of EAA would have increased N efficiency through improved milk protein yield over cows with lower levels of glucogenic nutrients.

Methodology

To test the effect of dietary starch and EAA supply on lactation performance and N use efficiency, an experiment was conducted at the Cornell University Ruminant Center (Harford, NY) form December 2019-April 2020. The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. One hundred and ninety-two Holstein cows (2.68 ± 1.37 lactations; 85 ± 26 days in milk; 672.2 ± 82.5 kg BW) were blocked in pens of 16 (n=12) by parity, days in milk, body weight, and previous lactation performance as part of randomized block design. Two enrollment periods, 96 cattle in Enrollment 1 [December 2019 – February 2020] and 96 cattle in Enrollment 2 [February 2020 – April 2020], were necessary to maintain the relevant period of lactation for observation. Each pen was fed TMR once daily at approximately 0630 h where pens were fed in the same sequence and targeted for a 5% refusal rate. All cattle were fed a common diet for a one-week acclimation period followed by a one-week covariate period in which baseline samples were taken to be used in the statistical analysis. Immediately following the covariate period, pens were randomly assigned one of four dietary treatments and fed for 7 weeks as part of the treatment period.

Dietary treatments included a 2 x 2 factorial design with two levels of dietary starch (23% [**LS]**and 29% [**HS**] DM) and two levels of essential amino acid supply (100% **[100]** and 105% **[105]** of the optimum grams of EAA per Mcal/ME requirement according to Higgs and Van Amburgh (2016). Diets were formulated using CNCPS v7 which predicts EAA requirements similar to Doepel et al. (2004) and Lapierre et al. (2007) but expresses requirements relative to ME (Higgs and Van Amburgh, 2016). Given the emphasis towards the evaluation of N and EAA efficiency of use, all diets were formulated to be isocaloric; however, diets did vary in the ingredients that supply energy and EAA. High starch (HS 100 and HS 105) diets were formulated with higher levels of starch containing ingredients, with a majority being a highly digestible steam flake corn, allowing for an increased pool size of fermentable starch in the rumen. To match the caloric density of the HS diets, the low starch diets (LS 100 and LS 105) were supplemented with a high palmitic form of Energy Booster (MSC Company, Dundee, IL), which did increase the

level of fatty acids consumed by those cattle (Table 1). Rumen unsaturated fatty acid load (RUFAL) was formulated to be similar in all four diets. Protein feeds were evaluated for intestinal digestibility using the Ross et al. (2013) assay to predict intestinally digestible N for more accurate predictions of EAA supply. Further, updated EAA profiles for commonly fed feeds determined within our lab (Van Amburgh et al., 2017) were implemented within the model to improve EAA supply predictions.

Table 1. Formulated EAA supply relative to megacalories of metabolizable energy

	Grams EAA:Mcal ME				
Essential Amino Acid	Higgs (2016)[1]	LS 100[2]	LS 105	HS 100	HS 105
Arginine	2.04	2.79	2.94	2.72	2.84
Histidine	0.91	1.12	1.16	1.10	1.19
Isoleucine	2.16	2.15	2.25	2.11	2.16
Leucine	3.42	3.18	3.37	3.20	3.32
Lysine	3.03	2.95	3.09	2.95	3.09
Methionine	1.14	1.11	1.18	1.11	1.18
Phenylalanine	2.15	2.09	2.21	2.06	2.12
Threonine	2.14	2.01	2.08	1.99	2.07
Tryptophan	0.59	0.60	0.62	0.59	0.61
Valine	2.48	2.34	2.43	2.30	2.39

[1] Optimum supply of EAA per Mcal ME according to Higgs et al. (2014)
[2]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements

Body weight and body condition score (1-5 scale) were measured and recorded weekly for all cattle. Milk samples were collected weekly during three consecutive milkings and analyzed for fat, true protein, lactose, total solids, and MUN (Dairy One, Ithaca, NY). A subset of cattle had milk samples taken at each milking to be analyzed for fatty acids (Barbano et al., 2014, Woolpert et al., 2016). Dry matter intake was determined daily for each pen as the difference between feed offered and refused (FeedWatch; Valley Ag Software). Samples of forages, TMR and refusals were sampled three times each week, composited, and analyzed for nutrient composition using near infrared reflectance spectroscopy. Additionally, feed ingredients included in the grain mixes were collected whenever new batches were delivered to the farm and analyzed by wet chemistry for chemical composition. A sub-sample of eight 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 eight 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 and TMR chemistry results were produced via PROC TABULATE to provide mean, standard deviation, and standard error of all feed components and diets analyzed. Continuous measurements which were not repeated over time were subjected to ANOVA (PROC MIXED) with fixed effects including pen, level of starch, and level of protein.

Measurements taken over time were subjected to repeated measures ANOVA (PROC MIXED) using the same fixed effects with the added fixed effect of 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 and Discussion

Dietary Composition

Dietary ingredients and chemical composition of the four diets fed throughout the experiment are in Table 2. Observed dietary CP was slightly elevated over all four formulated dietary treatments, averaging 15.9% and 16.5% DM for 100% and 105% diets, respectively. Dietary starch observed for the LS diets were similar to the levels formulated for (23.4% formulated vs.23.7% observed) but observed starch levels for the HS diets were lower than formulation (29.1% formulated vs. 27.2% observed). We believe this discrepancy was caused by changes in starch content of the corn silage used throughout the first enrollment (29.5% DM), as this problem was corrected in the second enrollment period with corn silage of higher starch content (33.5% DM). Both LS diets had increased dietary fat over their HS counterparts, (4.5% LS vs 3.6% HS), allowing similar levels of ME intake (~68 Mcals ME/day). The LS diets also had increased levels of palmitic and stearic acid compared to the HS diets, corroborating with the supplementation of the high palmitic Energy Booster.

Daily supply of EAA and MP, as predicted by CNCPS v7 are in Table 3. The supply of most EAA increased from the 100% to 105% EAA requirement diets with the MP supply the 105% diets supplied at nearly five percent over the 100% diets. When evaluated against the optimums as defined by Higgs and Van Amburgh (2016), isoleucine, phenylalanine, tryptophan, and valine were not supplied at a level to maintain a 5% increase over the 100% EAA treatment and averaged about 4 units above the 100% treatment for those EAA. This demonstrates the learning that needs to occur to be able to formulate for each EAA at this precise a level as there are no rumen protected products on the market to simplify the formulation process.

In response to previous work, the supply of histidine was formulated to match or exceed the supply of methionine in these diets, which has been shown to improve lactation performance (Lee et al., 2012, Lapierre et al., 2014). It is also worth noting that although there was separation in the supply of arginine in the 100% and 105% diets, the grams relative to ME were significantly increased over the targeted optimum for this experiment (2.04 grams per Mcal ME). An *in vitro* study on casein and mTOR pathway related regulatory genes has suggested improved expression in the presence of elevated arginine (Wu et al., 2009, Wang et al., 2014), suggesting non-nutritive functionality of this AA which might improve milk protein yield in this experiment. The deviations from the targeted supply of EAA highlight the difficulty in balancing for all EAA in lactating diets,

particularly given the constraints on farm feed inventories, feed ingredient amino acid profiles, and the variability of feed chemistry for the available feeds.

Table 2. Ingredients and chemical composition of experimental diets

Ingredient, % DM	LS 100[1]	LS 105	HS 100	HS 105
Corn silage	52.61	50.09	42.37	40.03
Mixed grass/Legume silage	8.01	9.94	9.54	7.24
Steam flaked corn	4.19	4.40	12.41	12.20
Corn meal	2.10	3.14	5.34	7.89
Beet pulp	6.86	4.55	1.91	---
Wheat midds	4.29	4.32	7.25	3.81
Canola	3.62	1.15	1.91	7.62
Soybean meal	7.24	9.56	10.88	6.86
SoyPLUS[2]	5.53	7.27	0.95	3.05
Soybean hulls	0.67	1.34	3.63	7.43
Energy Booster HP	1.33	0.96	---	---
Dextrose	0.19	---	0.38	0.38
Urea	0.23	0.19	0.19	0.17
Smartamine M[3]	0.09	0.10	0.08	0.08
Smartamine ML[4]	---	0.04	0.04	0.08
Minerals and Vitamins	3.04	2.96	3.12	3.15
Observed Chemical Composition[6], % DM ± standard deviation				
DM	36.4 ± 1.1	36.5 ± 1.3	38.9 ± 1.7	42.4 ± 3.8
CP	15.9 ± 0.4	16.6 ± 0.6	15.9 ± 0.8	16.4 ± 0.5
NDICP, % CP	16.0 ± 0.2	15.1 ± 0.2	13.7 ± 0.3	14.3 ± 0.4
ADICP, % CP	6.1 ± 0.4	5.5 ± 0.4	5.4 ± 0.8	5.5 ± 0.3
Soluble protein, % CP	40.1 ± 1.5	39.5 ± 1.4	38.7 ± 3.1	37.3 ± 1.2
RUP, % CP	30.0 ± 0.7	30.3 ± 0.7	30.7 ± 1.5	31.4 ± 0.6
Sugar	4.3 ± 0.4	4.5 ± 0.4	4.5 ± 0.6	4.6 ± 0.4
Starch	23.9 ± 1.4	23.4 ± 1.6	27.0 ± 2.3	27.4 ± 2.6
Starch digestion 7hr, % Starch	76.7 ± 2.1	75.2 ± 2.1	77.4 ± 2.9	77.4 ± 1.4
NFC	42.4 ± 0.3	41.9 ± 0.4	44.3 ± 0.5	44.8 ± 0.4
aNDFom	32.2 ± 1.2	31.9 ± 1.6	31.3 ± 2.2	30.6 ± 1.6
uNDF240, % NDF	27.0 ± 1.4	24.9 ± 1.5	23.6 ± 3.5	25.2 ± 1.5
Ether Extract	4.5 ± 0.2	4.5 ± 0.1	3.6 ± 0.3	3.6 ± 0.2
TFA	3.1 ± 0.3	3.0 ± 0.1	2.4 ± 0.2	2.5 ± 0.2
C16:0, TFA	24.2 ± 2.4	22.0 ± 2.3	16.5 ± 3.4	17.3 ± 2.0
C18:0, TFA	5.5 ± 0.4	4.9 ± 0.5	3.5 ± 0.7	4.0 ± 0.7
Ash	7.7 ± 0.4	7.8 ± 0.5	7.6 ± 0.5	7.5 ± 0.5
ME, Mcal/kg	2.61	2.61	2.63	2.64
Pool Size Based on Intake				
Sugar, kg/day	1.12	1.17	1.26	1.31
Starch, kg/day	6.27	6.02	7.55	7.75
aNDFom, kg/day	8.44	8.24	8.74	8.65
Total Fatty Acids, g/day	964.6	894.2	641.4	669.5
RUFAL Load, g/day	507.6	517.9	491.9	523.0

[1]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements

[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. ADICP = CP insoluble in acid detergent; NDICP = CP insoluble in neutral detergent; RUP = Rumen undegraded protein (model predicted), NFC = non-fiber carbohydrates, aNDFom =- amylase and sodium sulfite treated NDF corrected for ash residue, uNDF240 = undigested NDF after 240 hours of in vitro fermentation, ADL = acid detergent lignin, EE = ether extract, TFA = total fatty acids.

Animal Performance and Efficiency

Differences were observed in dry matter intake (DMI) between the cows on the LS and HS diets (P = 0.01) as cattle receiving the HS diets consumed over 2 kg more DM relative to the cattle on LS diets. Differences in these DMI might be attributed to a larger proportion of forage dry matter and NDF fed to the cows on the LS diets (not shown). This could have attributed to increased levels of dietary aNDFom in the LS compared to HS diets (Table 2) and it is likely that these cattle reached a physical fill limitation over their HS counterparts (Cotanch et al., 2014). Also, depression of DMI has been observed in studies where fat was infused post-ruminally (Bremmer et al., 1998, Drackley et al., 2007), although the level of palm fatty acid infusion in these studies was far above the dietary supplemented levels observed in the current study. Additionally, to maintain appropriate intake levels without elevating the caloric density, the cows fed the HS diets were fed more fibrous non-forage ingredients, including soyhulls. Although the rate of degradation of soyhulls is reasonably slow (~0.05/h), their extent of digestion is high (~90% of aNDFom) and added to the increased DMI as they contributed less to an aNDFom physical fill limitation. Rumination time of cattle supports this as the cows fed the LS diets tended to ruminate more with an average of 30 more minutes per day (650 vs. 622 minutes per day; P = 0.09). Cattle consuming a higher supply of EAA had significantly high milk volume and energy corrected milk (P = 0.01), with the HS 105 diet yielding greater volume and components compared to other treatments and this follows both the higher starch and greater intake.

Review of the component yields suggest that the higher ECM production for both 105% diets was achieved via yields of different components in the milk. To start, milk true protein yield was increased for cows consuming the HS diets (1.45 kg vs. 1.36 kg; P = 0.01), which is in support of our hypothesis. Milk protein output was highest in cattle fed the HS 105 diet, which is in support of previous findings where the supplementation of AA and glucose precursors have stimulated milk protein output (Raggio et al., 2006). Conversely, cows fed the LS diets had significantly greater yields of milk fat throughout the experiment (1.85 kg vs. 1.78 kg; P = 0.01). The improvements in milk fat secretion is undoubtedly due to the supplementation of fat in the diet, contributing to a greater level of lipogenic nutrients in the diet. Milk samples sent for fatty acid analysis suggest that there was a greater proportion of preformed fatty acids in the milk of cattle fed the LS diet (31.2% vs 29.5%; P = 0.01) whereas cattle on the HS diet produced a greater proportion of de novo fatty acids (26.8% vs. 25.2%; P = 0.01). Dietary fat supplementation has shown to influence the milk fat composition by improving the level of preformed fatty acids available for milk fat yield (Stoffel et al., 2015). Milk urea nitrogen (MUN) levels were lowest in LS 100 cows and highest in LS 105 cows. This difference in MUN is likely due to the corn silage starch levels in the first period, which supplied lower levels of rumen fermentable starch, possibly reducing microbial activity compared to what was initially formulated, however other interactions will be explored once the feed chemistry is evaluated via the CNCPS v.7 evaluations.

The initial BW of cows was not different for all treatments (P = 0.90; Table 4); however, cow fed the LS diets tended to have a higher final BW compared to cows on the HS diets (698.4 kg vs. 693.4 kg; P = 0.09). Body condition scores of HS cows were significantly lower than LS cows at the beginning of the experiment but were not different at the final measurement for the experiment. Feed efficiency (Milk yield:DMI) and ECM feed efficiency were significantly higher for cows fed the LS diets, with the HS 105 diet having the lowest feed efficiency. The lower level of feed efficiency can again be attributed to higher levels of DMI as cattle on this diet were those who had the best lactation performance. Alternatively, it has been well documented that an increase in feed efficiency is observed when supplemental fat, in the form of palmitic acid, is fed (Rico et al., 2014, Boerman et al., 2015, Nichols et al., 2018a). This might have influenced our results separate from the efficiency of use for N. Efficiency of use for feed N into milk N was higher for cows fed at 100% EAA requirements, with HS 100 having the highest N efficiency (32.5%; P = 0.04). The current literature has shown that an increase in AA supply improves milk protein output, but at the cost of N use efficiency (Dijkstra et al., 2013, Apelo et al., 2014). This is in support of our current hypothesis as it suggests that the optimum grams of EAA per Mcal of ME is creating better efficiency of use for these EAA over a higher supply of EAA fed in the 105% diets and that supplying more EAA does not always result in greater milk protein synthesis.

Table 3. Daily supply of essential amino acids for each treatment diet as calculate using CNCPS v7 using actual feed chemistry and dry matter intakes.

Essential Amino Acid, grams	LS 100[1]	LS 105	HS 100	HS 105
Arginine	190.9	201.3	186.2	194.5
Histidine	76.6	79.0	75.0	81.2
Isoleucine	147.0	154.2	144.3	147.7
Leucine	217.8	230.3	218.7	226.8
Lysine	202.0	211.5	201.5	211.6
Methionine	76.1	80.6	75.9	80.5
Phenylalanine	142.9	151.1	140.7	145.3
Threonine	137.9	142.3	136.4	141.8
Tryptophan	40.9	42.4	40.7	41.9
Valine	160.2	166.5	157.4	163.7
Total EAA	1392.3	1459.2	1376.8	1435.0
MP Supply	2872	3005	2852	2980

[1]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements

Conclusions

It is apparent that the production of milk protein increased as cows were fed the HS diets, supported by an increased supply of EAA; however, those cows also consumed significantly more feed, which would provide for both more glucogenic substrates and greater microbial yield, which would supply even greater EAA. This improvement in milk protein output by the increase in EAA supply in the HS 105 diets occurred while decreasing the efficiency of N utilization compared to the other diets (Table 4). In contrast, cows fed the HS 100 diet had the highest level of N efficiency compared to other treatments and a reasonable but slightly lower milk protein output by approximately 50

g/d. This data supports the hypothesis that greater glucogenic substrates support greater milk protein synthesis and further indicate the optimum EAA values per unit of ME are reasonable but there are some EAA that are required at higher levels to support the energy signaling for greater protein synthesis. Nichols et al. (2018b) recently presented similar findings where the postruminal supplementation of glucogenic precursors improved milk N efficiency at both a low level of MP supply (75% of requirements) and higher level of MP supply (120% of requirements). Given that we were not able to fully meet the balanced requirements for all the EAA at the 105% level, these small deficiencies might explain why the milk protein response was not greater than observed and that milk N efficiency was decreased. Further work to evaluate this interaction between glucogenic supply and milk protein synthesis will have to ensure that all EAA requirements are effectively met. However, this data does suggest the optimum requirements as described by Higgs and Van Amburgh (2016) is a good starting point in formulation of EAA supply relative to ME for lactating dairy cattle.

Improvements in milk fat output and feed efficiency for cattle fed the low starch diets should not be disregarded in light of the improved efficiency of use for N in the HS 100 diet. A body of literature exists that describes similar improvements in feed efficiency when diets are supplemented with lipogenic nutrients and more work is needed to evaluate the effect of fat and fatty acid supplementation when diets are balanced for EAA. Further work, including analysis of plasma samples for urea nitrogen, AA, and insulin content, is also needed to provide data to describe the metabolic signaling and metabolites related to the diets in the current study. Findings from this work will be used for CNCPS v7 model evaluation and allow for refinements in predicted EAA requirements of lactating dairy cattle.

Figure 1. Effect of dietary treatment on milk, energy corrected milk, and component yield for animals fed.

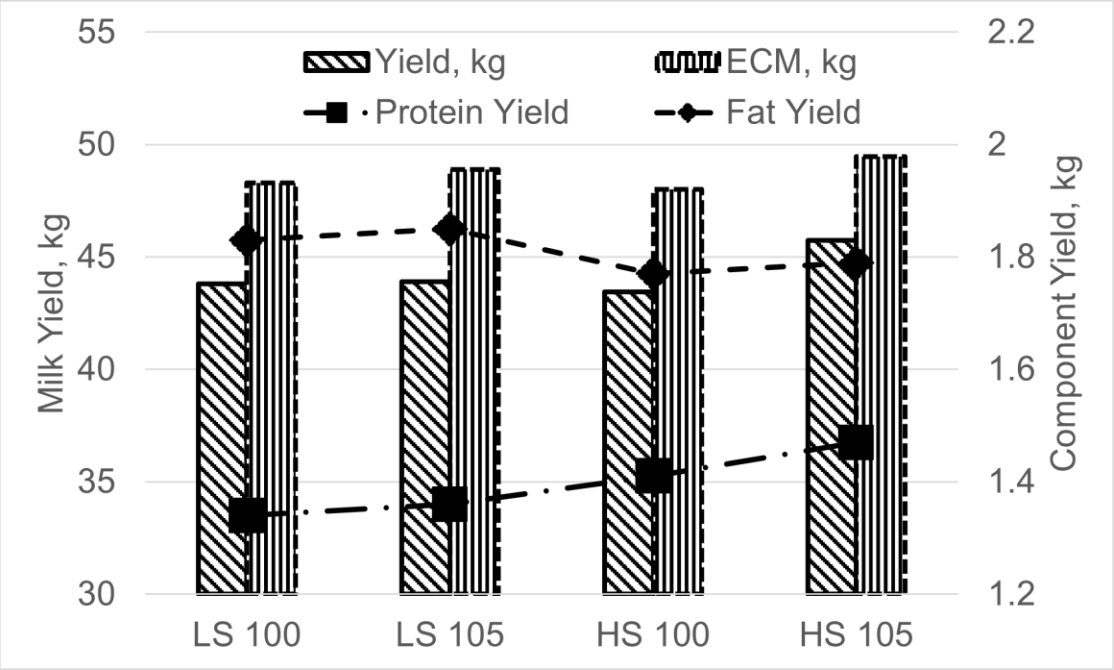

Table 4. Effects of treatment diets on milk production, intake, body measurements, and efficiencies

	Dietary Treatments					P-Values				
	LS 100[1]	LS 105	HS 100	HS 105	SEM	Starch	AA	Starch*AA	Time	Starch*AA*Time
Intake and milk production, kg/d										
Dry matter intake	26.65	26.35^{y}	28.95^{x}	28.34	0.66	0.01	0.54	0.83	0.73	0.54
Energy correct milk yield[2]	48.31^{a}	48.90^{ab}	48.02^{a}	49.48^{b}	0.33	0.66	0.01	0.20	0.01	0.65
Milk yield	43.81^{a}	43.92^{a}	43.46^{a}	45.73^{b}	0.26	0.01	0.01	0.01	0.01	0.06
True protein yield	1.35^{a}	1.37^{a}	1.42^{b}	1.47^{c}	0.01	0.01	0.01	0.09	0.01	0.25
Fat yield	1.83^{ab}	1.86^{b}	1.77^{a}	1.79^{ab}	0.02	0.01	0.32	0.76	0.01	0.21
Lactose yield	2.16^{a}	2.17^{a}	2.14^{a}	2.26^{b}	0.01	0.01	0.01	0.01	0.01	0.85
Milk composition, %										
True protein	3.08^{a}	3.13^{a}	3.27^{b}	3.25^{b}	0.02	0.01	0.56	0.02	0.01	0.01
Fat	4.20^{a}	4.26^{a}	4.09^{b}	4.00^{c}	0.04	0.01	0.72	0.07	0.19	0.04
Lactose	4.94	4.93	4.93	4.94	0.007	0.59	0.68	0.79	0.01	0.85
MUN, mg/dL	10.3^{a}	13.9^{b}	11.9^{c}	11.2^{d}	0.15	0.01	0.01	0.01	0.01	0.02
Fatty acid composition, %										
De Novo	25.0	25.3	26.9	26.7	0.27	0.01	0.90	0.38	0.01	0.01
Mixed	43.1	42.6	43.9	42.8	0.37	0.13	0.04	0.42	0.01	0.01
Preformed	31.5^{a}	30.9^{ab}	29.0^{c}	30.0^{bc}	0.38	0.01	0.54	0.03	0.01	0.76
Body Measurements										
Initial Body Weight, kg	676.0	678.9	680.2	686.1	12.5	0.65	0.72	0.90	---	---
Final Body weight, kg	698.5	698.2	696.1	690.7	2.95	0.09	0.34	0.39	---	---
Initial BCS, 1-5 Scale	2.93	2.89	2.83	2.88	0.02	0.02	0.90	0.11	---	---
Final BCS, 1-5 scale	3.00	2.96	3.01	3.00	0.04	0.56	0.50	0.63	---	---
Rumination Time, min/day	655.0	646.3	634.5	610.4	16.2	0.09	0.31	0.64	0.24	0.35
Efficiencies										
Feed Efficiency	1.65^{a}	1.66^{a}	1.59^{b}	1.54^{c}	0.02	0.01	0.03	0.01	0.01	0.01
ECM Feed Efficiency	1.82^{a}	1.85^{a}	1.75^{b}	1.67^{c}	0.02	0.01	0.05	0.01	0.01	0.01
Milk Nitrogen:Feed Nitrogen, %	32.2^{ab}	32.0^{a}	32.5^{b}	31.2^{c}	0.20	0.31	0.01	0.04	0.01	0.01

^{a,b,c} Denotes statistical significance ($P \leq 0.05$) ^{x,y,z} Denotes statistical tendencies ($P \leq 0.10$)

[1]LS 100= Low starch, 100% EAA requirements; LS 100= Low starch, 105% EAA requirements; HS 100= High starch, 100% EAA requirements; HS 105= High starch, 105% requirements [2]Estimated according to Tyrrell and Reid (1965)

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