Production responses of high producing Holstein cows to ruminally protected phenylalanine and tyrosine supplemented to diets containing high levels of canola meal

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Highlights

- Early lactation cows fed a diet containing 170 g/kg DM of canola meal were supplemented with Phe or Phe and Tyr.
- Phe supplementation alone appeared to cause an AA imbalance with negative consequences on animal performance.
- Tyr + Phe supplementation exacerbated negative effects of Phe supplementation.
- Tyr is more bioactive than Phe, with high downside supplementation risks on animal performance.

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Abstract

Phenylalanine (Phe) was first identified as a potentially limiting amino acid (AA) in lactating dairy cows in 1974. There is the possibility that conversion of Phe to tyrosine (Tyr) is not efficient enough to supply all Tyr requirements for milk production in high producing dairy cows, thereby suggesting that Tyr itself could become a functionally limiting AA when it, and/or Phe, is not supplied to the mammary gland in adequate quantities. Our objective was to determine if previous positive responses of lactating cows to Phe supplementation when fed a diet containing high levels of canola meal (CM) could be improved by supplying Tyr in place of some Phe. The experimental design was a 4×4 Latin square using 4 pens of ~315 early lactation multiparous cows/pen with four 21 d periods. Diets were formulated to include a low CM treatment (LCM) containing CM at ~120 g CM/kg dry matter (DM) and a high CM treatment (HCM) with CM at ~170 g CM/kg DM. The other two treatments were the HCM diet, but with Phe (HCM+P) or Phe plus Tyr (HCM+PT) added in a ruminally protected (RP) form. The DM intake tended (P=0.10) to decline, but milk yield did not differ, between the LCM and HCM treatments, while milk fat, lactose and energy output was lower (P<0.02) with the HCM treatment, and body condition score (BCS) gain was enhanced (P<0.01). Adding Phe to the HCM diet had little impact on milk and component yields, but a positive BCS gain of 0.056 units/28 d decreased (P<0.01) to a loss of 0.025 units. Substitution of half the added Phe with Tyr lowered (P<0.05) milk and milk lactose yields while further lowering (P<0.01) the BCS loss to 0.080 units/28 d. While differences among diets in plasma AA concentrations were small, adding Phe to the HCM diet increased (P=0.05) plasma Phe and Ala (P=0.02), but plasma Phe only tended (P=0.09) to decline when Tyr partially replaced Phe to create the HCM+PT diet. Contrary to expectations based upon prior studies, increased CM inclusion from 120 to 170 g/kg DM only modestly decreased overall animal performance. While it is clear that supplementation of Phe, or Phe plus Tyr, to the HCM diet was not necessary, the

unexpected and substantive negative effects of Phe or Phe plus Tyr on overall animal performance suggests that the bioactivity of these AA is highly important, while highlighting the risks of supplementing AA that are not required.

Keywords: Plasma amino acids; Phenylalanine; Tyrosine; Body condition change; digestibility.

Abbreviations: AA, amino acid; ADF, acid detergent fiber; ADICP, acid detergent insoluble CP; AP, absorbable protein; BCS, body condition score; CM, canola meal; CP, crude protein; DDGS, dried distillers grains with solubles; DIM, days in milk; DM, dry matter; EAA, essential AA; HCM, high CM diet; LCM, low CM diet; aNDF, neutral detergent fiber; NE, Net energy; NEAA, non-essential AA; NE_L, NE for lactation; OM, organic matter; PUN, plasma urea N; RP, rumen protected; RDP, rumen degradable CP; RUP, rumen undegradable CP; SCC, somatic cell count; TMR, total mixed ration. List of AA: Threonine (Thr), Serine (Ser), Glutamine (Gln); Glutamic acid (Glu), Glycine (Gly), Alanine (Ala), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Lysine (Lys); Tyrosine (Tyr), Phenylalanine (Phe), Tryptophan (Trp), Histidine (His), Arginine (Arg), Proline (Pro).

1. Introduction

Phenylalanine (Phe) was first identified as a potentially limiting amino acid (AA) in lactating dairy cows by Derrig et al. (1974) based on estimated uptake *versus* theoretical utilization by the mammary gland to support milk protein synthesis. However this finding stimulated very little research to determine effects of Phe supplementation to dairy cow diets on their productive performance, even though Phe was later identified as potentially limiting in corn silage based diets (Piepenbrink et al., 1998; Mulrooney et al., 2009; Christen et al., 2010). In addition, the mammary

gland has been reported to have specific requirements for Phe in milk protein synthesis (Guinard and Rulquin, 1994) while the absence of Phe in AA infusions negatively affected milk and milk protein production (Iroshan et al., 2013).

Since canola meal (CM) is relatively low in Phe compared to other protein meals, we previously completed two studies to determine if Phe supplementation, at two levels, to early lactation dairy cow diets containing high levels of CM would be beneficial to performance (Swanepoel et al., 2015, 2016a). In both studies, supplementation of Phe resulted in a change of energy partitioning with redirection of net energy (NE) towards body condition score (BCS) gain. At a supplementation level of ~15 g/d of intestinally available Phe (Swanepoel et al., 2015), this NE repartitioning was not at the expense of milk protein and fat synthesis, as was the case with a lower Phe supplementation level of ~7.5 g/d in Swanepoel et al. (2016a). The resultant hypothesis; that supplemental Phe does not support milk production until it fulfils its apparent 1st priority of restoring peptides to regenerate previously mobilized muscle protein, is supported by *in vivo* studies which evaluated the ability of the mammary gland to utilize AA in peptides (Backwell et al., 1994; 1996). These studies confirmed use of Phe in peptides for milk protein synthesis, while showing that the contribution of peptide Phe to total Phe supply decreased when Phe was supplemented, while uptake and utilization of supplemental Phe from blood increased.

Even though Tyr is considered a non-essential AA (NEAA), its synthesis depends largely on its conversion from Phe through Phe hydroxylase activity in the liver and mammary gland of lactating bovines (Jorgensen and Larson, 1968; Guinard and Rulquin, 1994). When Tyr is provided in the diet, conversion of Phe to Tyr is reduced. However, additional Tyr cannot fully replace Phe (Womack and Rose, 1946) with conversion of Phe to Tyr continuing even at high levels of Tyr supplementation (Grau and Steele, 1954). There is also a possibility that this conversion is not efficient enough to supply all Tyr requirements for milk production in high

producing dairy cows (Jorgensen and Larson, 1968), suggesting that Tyr itself could become limiting when it, or Phe, is not supplied in adequate quantities to the mammary gland. Thus it may be more accurate to classify Tyr as a 'conditionally essential AA' making it desirable to investigate lactation responses to Phe supplementation potentially enhanced by partial Tyr substitution.

Our objective was to determine if previous positive responses of lactating cows to Phe supplementation when fed a diet containing high levels of CM, and levels of protein sufficient to allow the cows to express their full production potential, could be recreated by RP Phe supplementation, and/or improved by substituting half of the RP Phe with RP Tyr.

2. Materials and methods

The experimental design was a 4 x 4 Latin square utilizing a William's design (Williams, 1949) which balances for potential carryover effects, and used 4 pens of ~315 early lactation multiparous cows/pen in 4 experimental periods of 21 d each. Pens were essentially the same structurally, and cows in all pens were provided the same number of free stalls, bedded with the same dried manure solids, had access to similar outside dry lots, were milked in the same milking parlour and had fresh water available *ad libitum* from the same number of water troughs. Lactation characteristics of the cows at the start of the study were also very similar among pens with average days in milk (DIM) of the 4 pens being 62, 79, 77 and 68, while average lactation numbers were 2.9, 2.8, 2.8 and 3.1 and average BCS's were 2.74, 2.72, 2.74 and 2.77. The study took place during winter from November 2015 to February 2016 inclusive, to span the winter solstice in order to maintain similar day lengths when ambient temperatures were -1.6 to 23.7^{o}C and humidity was 26 to 100%, with the highest humidities coinciding with the lowest temperatures at night. All cows were cared for relative to applicable laws of the State of California and the USA.

2.1. Farm and management

The commercial dairy farm used, selected for its consistent and highly organized management systems, is located near Hanford (CA, USA), and milked ~5000 Holstein cows three times a day in shifts starting at 04:00, 12:00 and 20:00 h. Once a week multiparity cows were randomly and equally allocated from a single fresh pen to all of the four early lactation pens with an equal number of late lactation cows removed to low production pens. Normal cow movement in (from the fresh cow pen) and out (to the low cow pens as well as to sick pens) of the 4 lactation pens was minimally restricted by the study. Treatments were randomly allocated to one of the 4 early lactation pens at the start of the 1st period and rotated after each 21 d period.

2.2. Diets

Mixing of total mixed ration (TMR), and all other farm practices, were as outlined in Swanepoel et al. (2014). Diets based on the same lots of alfalfa hays, the same silos of whole crop winter wheat and corn silages, and multiple deliveries of corn grain were mixed on a daily basis using the same ingredients and batches for all treatments with a premix, prepared daily on-site, containing most dry ingredients (*i.e.*, almond hulls, CM, cracked Pima cottonseed, corn gluten meal, wheat hay, liquid (sugar beet) molasses, mineral premix, corn dried distillers grains (DDGS), all from multiple deliveries except the wheat hay which was a single lot). All hays were added to the ration (alfalfa hays) or premix (wheat hay) in a long (baled) form early in the mixing sequence to ensure complete breakdown of long fibers in the two screw vertical mixer. As mixed rations were adjusted weekly, if necessary, to reflect measured dry matter (DM) determinations of the wheat and corn silages but changes between periods were minor and similar for all treatments. Chemical analysis of ingredients used in the total mixed rations are listed in Table 1.

Table 1. Chemical analysis of ingredients used in the total mixed rations (g/kg dry matter) fed to the treatment groups[1].

	Dry matter	Organic matter	Crude protein	ADF[2]	aNDF[3]	Lignin(sa)[4]	Starch
Alfalfa, hay (High quality)	914	874	227	286	332	59.9	12.7
Alfalfa, hay (Medium quality)	909	880	216	330	386	77.6	12.3
Almond, hulls	953	895	56.8	239	242	118	85.0
Canola, pellets (solvent)	891	919	457	223	256	87.8	4.50
Corn, silage	320	928	109	267	396	25.3	266
Corn distillers grains, dry	899	939	325	121	268	13.3	74.7
Cottonseed, Pima cracked	899	949	231	330	421	162	0.90
Corn, grain flaked	854	987	84.1	38.9	77.1	2.50	724
Wheat, silage	361	862	133	333	424	43.4	92.9
Wheat, hay	920	839	109	345	515	33.2	56.6
Corn gluten feed	893	930	214	126	321	25.8	174

[1] $n = 4$. One sample/period, all combined to support a single analysis.
[2] Acid detergent fiber, expressed inclusive of residual ash.
[3] Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.
[4] Lignin treated with sulphuric acid and expressed exclusive of residual ash.

The low CM (LCM) and high CM (HCM) diets were formulated using NRC (2001) tabular guidelines to be similar relative to ingredient profiles, including canola meal, to the diet used in Swanepoel et al. (2016a) with the LCM treatment containing CM at ~120 g CM/kg DM and the HCM treatment with CM included at ~170 g CM/kg DM (Table 2). The increase in CM with the HCM (*versus* LCM) diet was compensated by reductions in levels of cottonseed, corn gluten feed and DDGS such that similar proximate nutrient profiles were maintained between the LCM and HCM diets. The other two treatments were the HCM diet, but with Phe (HCM+P) or Phe plus Tyr (HCM+PT) added in a ruminally protected (RP) form. Cows were fed twice daily, receiving a total of ~15,500 kg of as mixed TMR/d, with the 1st feeding being (~10,800 kg) between 04:30 and 07:30 h which included the full daily allocation of RPAA products for all three HCM treatments. Between 11:00 and 12:30 h a final load of ~4,700 kg was fed which contained no RPAA products. The "TMR Tracker" system (Digi-Star LLC, Fort Atkinson, WI, USA) kept a record of the actual ingredient profiles of each load of TMR mixed as well as weights of each load of TMR fed. The amount of TMR fed during the final 7 days of each experimental period were used together with weighed daily refusals by pen during this period to calculate DM intake/cow/pen. As visual inspection of refusals suggested they were very similar to the TMR, because refusals were < 2.5% of offered feed, and because of thorough and complete mixing of the TMR, the refusals were not sampled or chemically analyzed.

2.3. The rumen protected AA products

The RP Met product (Smartamine M; Adisseo USA Inc., Alpharetta, GA, USA) and the RP Phe product (QualiTech Inc., Chaska, MN, USA) were fully described in Swanepoel et al. (2015), which used very similar RP products to those evaluated by Sakkers et al. (2013). Based upon Sakkers et al. (2013), as in Swanepoel et al. (2015), a whole tract stability of 600 g/kg was assumed for the RP Phe. The RP Tyr product, also manufactured by QualiTech Inc., was prepared according

Table 2. Ingredient profile and chemical composition (g/kg dry matter) of total mixed rations (TMR) with low canola meal (CM), high CM and high CM supplemented with ruminally protected Phe and/or Tyr.

	Treatments					*P*		
	LCM[1]	HCM[2]	HCM +P[3]	HCM +PT[4]	SEM	LCM *vs.* HCM[5]	HCM[5] *vs.* HCM+P	HCM+P *vs.* HCM+PT
Ingredient profile, g/kg DM [6]								
Alfalfa, hay (MQ)[7]	61.3	66.2	66.1	65.1	0.69	<0.01	0.91	0.28
Premix								
Almond, hulls	150	154	154	154	2.8	0.22	0.96	0.94
Cottonseed, Pima cracked	29.2	13.2	13.2	13.2	0.14	<0.01	0.95	0.97
Corn gluten feed	21.0	0.0	0.0	0.0	3.23	<0.01	1.00	1.00
Mineral, premix	31.2	31.2	31.2	31.2	0.20	0.98	0.88	0.81
Canola, pellets (solvent)	118	171	171	171	2.0	<0.01	0.94	0.90
Wheat, hay	19.6	19.9	19.9	19.9	0.19	0.28	0.93	0.88
Molasses, beet liquid	15.4	15.6	15.5	15.6	0.15	0.45	0.93	0.90
Corn distillers grains, dry	77.0	26.7	26.7	26.7	2.98	<0.01	0.99	0.99
RPM Product [8]	0.00	0.17	0.17	0.17	0.002	<0.01	1.00	0.34
RPP Product [9]	0.00	0.00	1.93	0.97	0.016	1.00	<0.01	<0.01
RPT Product [10]	0.00	0.00	0.00	0.97	0.006	1.00	1.00	<0.01
Alfalfa, hay (HQ)[11]	69.5	68.9	69.2	70.2	0.82	0.52	0.77	0.35
Corn, grain flaked	193	205	204	204	3.1	<0.01	0.86	0.87
Wheat, silage	143	148	147	147	2.4	0.10	0.82	0.98
Citrus, wet/pulp	31.3	31.6	31.3	31.3	5.65	0.97	0.96	0.99
Corn, silage	55.7	55.6	55.6	55.4	2.56	0.97	0.99	0.95
Nutrient profile, g/kg DM [12]								
Dry matter	600	593	585	586	3.9	0.46	0.35	0.93
Organic matter	901	904	906	900	1.02	0.20	0.32	0.03
Crude protein (CP)	176	178	176	174	2.2	0.63	0.55	0.76
ADICP[13]	38.2	37.2	39.2	37.6	0.69	0.53	0.23	0.33
NDICP[14]	105	104	106	106	1.0	0.73	0.45	0.79
ADF[15]	232	232	230	222	0.9	0.80	0.33	0.01
aNDF[16]	291	290	290	277	3.0	0.87	0.98	0.08
Fat	36.2	34.7	33.4	33.8	0.43	0.15	0.20	0.64
Lignin(sa)[17]	54.4	52.7	53.7	56.3	1.93	0.70	0.82	0.54
Starch	201	201	200	202	1.9	0.98	0.81	0.69
Calcium	7.0	7.6	7.8	7.5	0.06	<0.01	0.33	0.08
Phosphorus	3.7	3.6	3.3	3.5	0.22	0.87	0.47	0.68
Potassium	15	15	16	16	0.20	0.45	0.17	0.81
Magnesium	3.0	3.0	3.0	3.1	0.03	0.28	1.00	0.46
Sulphur	3.0	3.1	3.1	3.4	0.41	0.98	0.98	0.76

[1] Low canola meal ration: Canola meal included at 120 g/kg dry matter.
[2] High canola meal ration: Canola meal included at 170 g/kg dry matter, with 2 g of intestinally absorbable Met as part of the base ration.
[3] High canola meal ration (with added Met as above) and 14 g of intestinally delivered Phe.

[4] High canola meal ration (with added Met as above) and 7 g for each of intestinally delivered Phe and Tyr.
[5] HCM treatment without Phe or Tyr supplementation.
[6] Based on average ingredient composition during sampling week for each pen, each period, using TMR tracker system.
[7] Alfalfa hay, Medium quality.
[8] Ruminally protected Met (Smartamine M, Adisseo USA Inc., Alpharetta, GA, USA). Fed at 3.4 g/cow/d to deliver 2 g intestinally absorbable Met.
[9] Ruminally protected Phe (QualiTech Inc., Chaska, MN, USA). Fed at 38.9 or 19.4 g/cow/d to deliver 14 g or 7g intestinally absorbable Phe, depending on treatment.
[10] Ruminally protected Tyr (QualiTech Inc., Chaska, MN, USA). Fed at 19.4 g/cow/d to deliver 7 g intestinally absorbable Phe.
[11] Alfalfa hay, High quality.
[12] Total mixed ration samples collected twice during sampling week for each pen, each period (*i.e.*, 16 total samples).
[13] Acid detergent insoluble CP (g/kg of CP).
[14] Neutral detergent insoluble CP (g/kg of CP).
[15] Acid detergent fiber, expressed inclusive of residual ash.
[16] Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.
[17] Lignin treated with sulphuric acid and expressed excusive of residual ash.

to the same specifications using the same production method as the RP Phe and contained 600 g/kg Tyr with 400 g/kg of a fat matrix. All HCM treatments received 3.4 g/cow/d of RP Met (~0.126 g/kg DM) in order to eliminate the possibility of a deficiency of Met (estimated to deliver ~2.0 g of intestinally absorbable Met/cow/d). The HCM+P treatment received 38.9 g/cow/d (~1.45 g/kg DM) of RP Phe (estimated to deliver ~14 g of intestinally absorbable Phe/cow/d), while the HCM+PT treatment received 19.4 g/cow/d (~1.23 g/kg DM) of both RP Phe and RP Tyr, substituting half of the Phe in the HCM+P treatment for Tyr.

All RPAA products were weighed into plastic bags appropriate for the treatments based upon 315 cows being in each pen. These bags were added to the first (main) load of TMR delivery of the day by placing it on the bucket of the loader used to add ingredients during TMR mixing. Actually achieved RPAA deliveries were calculated based upon the actual number of cows in each pen during the 7 day period of DM intake measurement.

2.4. Sample collection, preparation and analytical methods

2.4.1. Total mixed diets and ingredients

Feed ingredients and TMR were sampled twice during the last 7 d (*i.e.,* the sampling week) of each experimental period. Ten handfuls (~200 g each) of each TMR were collected (Robinson and Meyer, 2010) at pre-marked posts and evenly spaced intervals along the bunk line immediately after feeding and before the cows had access. Ingredient samples from all 4 periods were pooled (n = 4 samples/ingredient), while TMR samples were pooled within period and pen (n = 16 TMR samples) for chemical analysis.

Samples of TMR and most feeds were dried for 48 h at 55^{o}C and allowed to air equilibrate at ambient temperature for 24 h to ensure moisture stability. All samples were ground (model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1 mm screen. The TMR samples were assayed for crude protein (CP), fat, ash, neutral detergent fiber assayed with heat stable amylase

(aNDF) and starch (Schalla et al. 2012). Acid detergent fiber (ADF), lignin(sa), acid- and neutral-detergent insoluble CP (ADICP and NDICP) were assayed as described by Swanepoel et al. (2015). Samples were digested in acid and diluted to analyse Ca, P, K, Mg and S via inductively coupled plasma spectroscopy. Feed samples were only assayed for ash, CP, ADF, aNDF, lignin(sa) and starch by the same methods.

2.4.2. Animal measurements

Backups of the electronic herd record system DairyComp 305 (Valley Agricultural Software, Tulare, CA, USA) were made daily to crosscheck normal cow movements in and out of the pens as described in Section 2.1. Cow sampling and BCS scoring was during normal morning lockup (*i.e.*, ~50 min/pen/d for normal health and reproductive checks immediately after the morning milking) on the last two days of each experimental period.

2.4.2.1. Milk production and composition

Milk yields were recorded by Dairy Herd Improvement Association personnel for each cow on day 21 of each experimental period. A small representative sub-sample was collected from the sample collection flask (after a short period of mixing) and preserved with 2-Bromo-nitropropane-1, 3-diol for subsequent analytical testing. Optical infrared analysis was used to determine fat, true protein, lactose and somatic cell count (SCC) using the Bentley Combi at the Dairy Herd Improvement Association laboratory in Hanford (CA, USA).

2.4.2.2. Body condition score

A group of the same ~120 cows/pen were scored for BCS. This was completed by the same trained scorer on the 1st day of Period 1 and at the end of each experimental period. An adapted 5 point BCS system (Ferguson et al. 1994) was used (Swanepoel et al., 2014), with intermediate points between the ¼ point scores when cows could not be clearly allocated to a ¼ point BCS.

2.4.2.3. Blood plasma

Blood was collected from the tail vein of the same group of 12 cows/pen (selected from the base group of ~120 cows/pen scored for BCS) with collection, treatment and analysis for free AA and plasma urea N (PUN) following methods in Swanepoel et al. (2014).

2.4.2.4. Fecal

Fecal collection was by rectal sampling the same group of 12 cows/pen described above for blood sampling. At the end of the study, the 12 cows/pen were combined into 2 groups/pen of 6 cows/group (the same 6 cows sampled in each period in each group) to create an analytical set of 32 fecal samples (*i.e.,* 2 groups/pen/period) for analysis. The creation of these pooled group samples was to support the assumption that DM intake, and nature of that intake, of the group was equal to that of the pen average so that whole tract apparent digestibility could be calculated based upon group. Samples were frozen at -20^{o}C and subsequently analyzed for DM, ash, aNDF, ADF, lignin(sa) and N after drying and grinding as described earlier for feed and TMR samples.

2.5. Calculations

Air equilibrated DM was calculated for all TMR, silage and wet ingredient samples prior to chemical analysis as the weight of the 48 h air equilibrated sample dried at 55^{o}C divided by the wet weight of the sample dried. Final oven DM of TMR and feeds were calculated as the air equilibrated DM multiplied by the 105^{o}C oven DM. The milk energy content (MJ/kg) and output (MJ/d), net energy (NE) output/input represented by change in BCS (MJ/d), NE for lactation (NE_L; MJ/d) and diet NE_L density (MJ/kg DM) was as described by Swanepoel et al. (2015). Whole tract apparent digestibility (g/kg DM) was calculated for organic matter (OM), CP, aNDF and ADF as:

$$1 - [((\text{Lignin(sa)}_{TMR} \times 0.95)/\text{Nutrient}_{TMR}) / (\text{Lignin(sa)}_{Feces} / \text{Nutrient}_{Feces})]$$

assuming that lignin(sa) in the TMR was 950 g/kg indigestible (Stensig and Robinson, 1997).

2.6. Statistical analysis

Cows were excluded from statistical analysis if they were not in their originally assigned pen for the duration of the study, regardless of reason or length of absence. On this basis the number of cows for potential statistical analysis of milk production was 572, with 494 for BCS – all of which were in the milk production dataset. Outlier analysis, completed blind to treatments by identifying values deemed to be biologically implausible, excluded 6 cows from the milk production dataset (*i.e.*, 3 cows for low milk yields and 3 cows for high SCC), and 3 cows were removed from the BCS dataset due to abnormally high BCS changes between two experimental periods. Thus the final statistical analysis was 566 cows for milk production parameters and 491 for BCS.

A final group of 16 (*i.e.*, 4 cows/pen) were randomly selected from the eligible cows for plasma AA and statistical analysis.

Dry matter intake (n = 4 pens, calculated on a pen basis with 4 pens/period), TMR component and ingredient composition and NE balance (n = 4 pens) used pen as the experimental unit in the GLM option of SAS (2016) with period, pen and treatment as fixed effects.

Production, BCS, apparent digestibility and plasma AA were analysed using the MIXED procedure of SAS (2016) for a 4 x 4 Latin square design, with cow nested within pen in the random statement, because cows were recognized to be metabolically independent units, with period, pen and treatment as fixed effects. Pre-planned contrasts compared the LCM to the HCM treatment, as well as the HCM to the HCM+P, and HCM+P to the HCM+PT treatments.

All reported values are least squares means with differences accepted as significant if $P \leq 0.05$ and trends accepted if $P \leq 0.10$.

3. Results

3.1. Diet evaluation

The chemical composition of the ingredients used in the TMR (Table 1) were generally consistent with NRC (2001) values. However, alfalfa hay, Pima cottonseed, wheat and corn silage all had slightly higher CP levels than in previous studies (Swanepoel et al., 2014, 2015, 2016a).

As per design, the HCM diet had a higher inclusion (P<0.01) of CM compared to the LCM diet (171 *versus* 118 g/kg DM; Table 2). This was accompanied by reduced inclusion (P<0.01) of Pima cottonseed (13.2 *versus* 29.2 g/kg DM), corn gluten feed (0.0 *versus* 21.0 g/kg DM) and DDGS (26.7 *versus* 77.0 g/kg DM), and increased inclusion (P<0.01) of corn grain (205 *versus* 193 g/kg DM) in order to obtain minimal differences in the chemical profiles of the LCM and all three HCM diets.

There were no differences amongst the three HCM diets except for inclusion of 3.7 and 38.1 g/cow/d of RP Met (all HCM diets) and RP Phe (HCM+P treatment) respectively, or 19.1 g/cow/d each of RP Phe and RP Tyr (HCM+PT treatment) – very close to the targeted 3.4, 38.9 and 19.4 g/cow/d. The TMR met all nutrient requirements of lactating dairy cows producing 45 to 50 liters of milk/d (NRC, 2001). There were no differences in nutrient profiles amongst the treatment diets that were deemed biologically important enough to have an effect on animal performance, although Ca levels in all three HCM diets were slightly higher than that of the LCM diet.

3.2. Animal measurements

3.2.1. Dry matter intake and apparent whole tract digestibility

The DM intake (Table 3) was little affected by treatment, even though LCM fed cows tended (P=0.10) to have higher DM intake compared to all the HCM fed cows. Apparent total tract digestibility of CP was not affected by treatment (Table 3), but apparent OM (P=0.02), aNDF

(*P*=0.01) and ADF (*P*=0.05) digestibility was lower for LCM *versus* HCM fed cows. There was a tendency for lower apparent ADF (*P*=0.09) digestibility for HCM+PT *versus* HCM+P fed cows.

Table 3. Dry matter (DM) intakes (kg/d) and apparent total tract digestibility (g/kg DM) of total mixed rations (TMR) of cows fed TMR with low canola meal (CM), high CM and high CM supplemented with ruminally protected Phe and/or Tyr.

	Treatments (Trt)					*P*			
	LCM[1]	HCM[2]	HCM +P[3]	HCM +PT[4]	SEM	LCM *vs.* HCM[5]	HCM[5] *vs.* HCM+P	HCM+P *vs.* HCM+PT	Trt
Dry matter intakes (kg/d)[6]	28.4	27.0	26.9	27.2	0.34	0.10	0.85	0.73	0.22
TMR Digestibility (g/kg DM)[6]	596	650	635	631	14.5	0.02	0.47	0.87	0.09
Organic matter	476	546	538	518	30.5	0.12	0.86	0.66	0.40
Crude protein	367	455	417	391	22.0	0.01	0.24	0.40	0.06
aNDF[8]	329	399	383	323	23.4	0.05	0.65	0.09	0.08
Acid detergent fiber									

[1] Low canola meal ration: Canola meal included at 120 g/kg dry matter.
[2] High canola meal ration: Canola meal included at 170 g/kg dry matter, with 2 g of intestinally absorbable Met as part of the base ration.
[3] High canola meal ration (with added Met as above) and 14 g of intestinally delivered Phe.
[4] High canola meal ration (with added Met as above) and 7 g for each of intestinally delivered Phe and Tyr.
[5] HCM treatment without Phe or Tyr supplementation.
[6] *n*=4 Pens
[7] Based on 32 final fecal samples of 6 cows/pen combined into 2 groups/pen/period and 16 final TMR samples, collected twice/pen/period.
[8] Neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash.

3.2.2. Milk production and its composition

Milk yield (Table 4) did not differ between LCM and HCM fed cows, or between HCM and HCM+P fed cows, but replacing part of the Phe with Tyr (HCM+PT) reduced milk yield (45.9 *versus* 46.5 kg/d; *P*=0.05). The HCM fed cows had lower milk fat (*P*=0.02) and lactose (*P*<0.01) yields compared to LCM fed cows, with substitution of some Phe with Tyr reducing (*P*=0.02) lactose yields (2.17 *versus* 2.21 kg/d). Milk protein yields tended to be lower (*P*=0.08) for HCM+P

fed cows *versus* HCM fed cows, but replacement of some Phe with Tyr (HCM+PT) was not impactful.

Table 4. Production performance and body condition scores (BCS) for cows fed total mixed rations (TMR) with low canola meal (CM), high CM and high CM supplemented with ruminally protected Phe and/or Tyr.

	Treatments (Trt)					*P*			
	LCM[1]	HCM[2]	HCM +P[3]	HCM +PT[4]	SEM	LCM *vs.* HCM[5]	HCM[5] *vs.* HCM+P	HCM+P *vs.* HCM+PT	Trt
n = 566 cows									
Yield (kg/d)									
Milk	47.16	46.85	46.48	45.92	0.349	0.28	0.21	0.05	<0.01
Fat	1.63	1.60	1.60	1.58	0.014	0.02	0.99	0.11	<0.01
True protein	1.36	1.35	1.34	1.33	0.009	0.26	0.08	0.12	<0.01
Lactose	2.27	2.22	2.21	2.17	0.017	<0.01	0.25	0.02	<0.01
Energy output (MJ/d)	135.4	132.9	132.3	130.5	0.94	<0.01	0.50	0.06	<0.01
Components	34.89	34.4	34.7	34.6	0.22	0.04	0.19	0.46	0.18
(g/kg)	29.2	29.2	29.1	29.2	0.10	0.30	0.01	0.04	0.04
Fat	48.2	47.4	47.5	47.4	0.06	<0.01	0.36	0.04	<0.01
True protein	2.88	2.85	2.86	2.86	0.01	<0.01	0.34	0.52	<0.01
Lactose	144	129	138	134	14.9	0.40	0.60	0.81	0.86
Energy density (MJ/kg)	2.66	2.70	2.66	2.64	0.017	<0.01	<0.01	0.05	<0.01
Somatic cell count (‘000)	0.002	0.056	- 0.025	- 0.080	0.0112	<0.01	<0.01	<0.01	<0.01
n = 491 cows									
Body condition score (BCS)									
BCS change (unit/28 d)									

[1] Low canola meal ration: Canola meal included at 120 g/kg dry matter.
[2] High canola meal ration: Canola meal included at 170 g/kg dry matter, with 2 g of intestinally absorbable Met as part of the base ration.
[3] High canola meal ration (with added Met as above) and 14 g of intestinally delivered Phe.
[4] High canola meal ration (with added Met as above) and 7 g for each of intestinally delivered Phe and Tyr.
[5] HCM treatment without Phe or Tyr supplementation.

The HCM fed cows had lower levels of milk fat (P=0.04), lactose (P<0.01) and energy (P<0.01) *versus* LCM fed cows, but only protein (P=0.01) and lactose (P=0.04) were similarly effected in HCM+P fed cows *versus* HCM fed cows. In contrast to these declines, milk protein content increased (P=0.04) when Tyr substituted for half of the supplemented Phe.

In contrast to milk yield, milk energy output was lower (P=0.01) for all HCM diets (133 *versus* 135 MJ/d) with partial substitution of Phe with Tyr (HCM+PT) tending to reduce (P=0.06) energy output even further compared to HCM+P (131 *versus* 132 MJ/d).

3.2.3. Body condition score

The BCS and BCS change (Table 4) was higher (P<0.01) for HCM *versus* LCM fed cows, while addition of Phe to the HCM diet reduced BCS (P<0.01) and BCS change (P<0.01) compared to HCM, turning a BCS gain into a loss. Substituting half of the Phe with Tyr in the HCM+P diet further decreased (P=0.05) BCS, and resulted in a larger BCS loss (P<0.01).

3.2.4. Blood plasma

Differences among diets in plasma AA concentrations (Table 5) were small. Plasma Leu was lower (P=0.01), PUN higher (P=0.02) and Lys/Met ratio higher (P=0.03) in HCM *versus* LCM fed cows, although the latter was mainly due to a numerically higher plasma Lys concentration which was reversed when Phe was added to the HCM diet, mainly due to a numerically higher Met concentration. Adding Phe to the HCM diet increased plasma Phe (P=0.05) and Ala (P=0.02) concentrations, but plasma Phe concentration tended (P=0.09) to be reduced when Tyr partially replaced Phe in the HCM+PT diet.

Table 5. Free amino acid (AA), ammonia concentrations (μg/ml) and urea content (mg/dL) in plasma of cows fed total mixed rations (TMR) with low canola meal (CM), high CM and high CM supplemented with ruminally protected Phe and/or Tyr.

	Treatments (Trt)					*P*			
	LCM[1]	HCM[2]	HCM +P[3]	HCM +PT[4]	SEM	LCM *vs.* HCM[5]	HCM[5] *vs.* HCM+P	HCM+P *vs.* HCM+PT	Trt
n = 16 cows[6]									
Essential amino acids	12.7	12.7	13.6	13.4	0.71	0.96	0.27	0.86	0.57
Threonine	34.4	34.6	35.8	35.3	1.33	0.92	0.40	0.73	0.75
Valine	3.67	3.67	3.98	3.86	0.192	1.00	0.22	0.64	0.53
Methionine	15.8	16.0	16.2	15.6	0.71	0.89	0.76	0.44	0.89
Isoleucine	26.3	23.3	23.8	23.1	0.98	0.01	0.69	0.56	0.03
Leucine	8.72	8.39	9.13	8.49	0.324	0.38	0.05	0.09	0.21
Phenylalanine	15.0	15.7	15.4	15.6	0.48	0.32	0.63	0.67	0.72
Tryptophan	11.7	12.9	12.9	13.1	0.62	0.14	0.99	0.75	0.29
Lysine	8.15	7.60	7.93	8.06	0.321	0.12	0.35	0.70	0.42
Histidine	13.5	14.0	14.7	14.9	0.74	0.54	0.37	0.89	0.30
Arginine	3.28	3.60	3.33	3.55	0.113	0.03	0.06	0.13	0.07
Lys:Met ratio									
Non-essential	0.59	0.67	0.62	0.68	0.053	0.08	0.30	0.22	0.18
amino acids	1.29	1.27	1.27	1.28	0.076	0.76	1.00	0.91	0.99
Homocystine	10.6	9.5	10.5	9.9	0.56	0.07	0.08	0.30	0.21
Aspartic acid	8.97	8.73	9.20	9.39	0.345	0.58	0.29	0.67	0.48
Tyrosine	7.15	6.86	7.27	6.64	0.255	0.28	0.12	0.02	0.09
Serine	38.7	36.8	38.5	39.2	1.63	0.29	0.33	0.69	0.55
Glutamic acid	27.8	27.3	28.7	28.1	1.36	0.75	0.33	0.67	0.79
Glutamine	23.7	22.6	25.2	24.1	0.84	0.33	0.02	0.30	0.13
Glycine	0.61	0.64	0.63	0.64	0.019	0.26	0.52	0.52	0.60
Alanine	5.01	4.70	4.88	4.96	0.245	0.38	0.61	0.81	0.82
3-Methylhistidine	10.5	9.4	10.3	10.1	0.41	0.07	0.14	0.71	0.28
Asparagine	2.45	2.41	2.51	2.46	0.079	0.60	0.22	0.55	0.68
Proline	11.38	12.31	11.81	12.38	0.506	0.02	0.22	0.17	0.05
Ammonia									
Plasma urea N (mg/dL)									

[1] Low canola meal ration: Canola meal included at 120 g/kg dry matter.
[2] High canola meal ration: Canola meal included at 170 g/kg dry matter, with 2 g of intestinally absorbable Met as part of the base ration.
[3] High canola meal ration (with added Met as above) and 14 g of intestinally delivered Phe.
[4] High canola meal ration (with added Met as above) and 7 g for each of intestinally delivered Phe and Tyr.
[5] HCM treatment without Phe or Tyr supplementation.
[6] A randomly selected group of 4 cows/pen/period was used for amino acid analysis as it was clear that additional samples would not change significance of differences.

Due to the difficulty of predicting absorbability of AA as well as estimating AA supply to cows, discussed in Swanepoel et al. (2016b), the plasma AA concentrations in this study were compared to indices of mean plasma AA values created for cows fed contemporary California dairy rations capturing a large ingredient variation. In general, most AA concentrations, including Phe and Tyr, were within normal ranges for CA dairy cows. Plasma concentrations for Trp was slightly below normal, but still within normal range, while Pro and Gln concentrations were below normal. Plasma concentrations for all the branched-chain AA Leu, Ile and Val, as well as both Met and Lys, were above normal, which suggests that there were no limitations for these important AA. Overall, the concentrations of most AA were within previously determined normal ranges.

3.2.5. Partial net energy balance

Total NE output did not differ between the LCM and HCM fed cows, but addition of Phe (P=0.06) to the HCM diet tended to reduce total NE output *versus* the HCM group. The calculated dietary NE_L densities of the diets suggested that the HCM diet had a higher (P=0.09) NE_L density than did LCM, but that this increase was removed with addition of Phe.

4. Discussion

Our objective was to determine if previous positive responses of lactating cows to Phe supplementation when fed diets with CP levels deemed sufficient to allow cows to express their full production potential while containing high levels of CM (Swanepoel et al., 2015; 2016a) could be improved by dietary supplementation of Tyr in addition to Phe. Previous research suggests that conversion of Phe to Tyr may not be sufficient to fully supply Tyr requirements of lactating dairy cows (Jorgensen and Larson, 1968), thereby indicating that Tyr could limit milk production. However, as Tyr cannot completely replace Phe (Womack and Rose, 1946), both Phe and Tyr were supplemented.

4.1. Low CM versus High CM diets without Phe or Tyr supplementation

The LCM fed cows generally outperformed the HCM fed cows, with increased CM inclusion resulting in reduced milk fat and lactose content and yield, as well as reduced milk energy density (MJ/kg) and output (MJ/d), which is consistent with Swanepoel et al. (2014). However, the HCM diet resulted in a BCS gain compared to LCM (0.056 *versus* 0.002 units/28 d), which is opposite to the BCS change (0.029 *versus* 0.080 and -0.061 *versus* 0.016 units/28 d) for higher CM inclusion in Swanepoel et al. (2014 and 2016a) respectively. Indeed, no other studies have reported a BCS gain that increases with higher CM inclusion.

Mulrooney et al. (2009) showed a quadratic response in BCS (P=0.06) to higher inclusion levels of CM up to 66 g/kg TMR DM when combining different ratios of CM and DDGS, with BCS tending to decrease at the highest CM inclusion level, which is inconsistent with our results. Christen et al. (2010) reported lower BCS gains with CM compared to DDGS and high protein DDGS (although all treatments resulted in a BCS gain), but these differences did not reach statistical significance. Similarly lower BCS gains for cows fed CM *versus* high protein DDGS supplemented diets occurred in Acharya et al. (2015) at diet CP levels of 143 and 163 g/kg DM.

In conjunction with positive BCS changes with CM feeding, all the above noted studies, including Swanepoel et al. (2014 and 2016a), reported lower plasma Leu, Phe and Tyr concentrations when CM was fed. This is consistent with the reduction in plasma concentrations of Leu (P=0.01) and the tendency for Tyr concentrations to decrease (P=0.07) with higher CM inclusion in the current study. When compared to the plasma AA indices of Swanepoel et al. (2016b), Tyr concentrations for the HCM diet was below 20th percentile of measured data, suggesting that it could be potentially limiting, which is consistent with previous findings and our objective. However, even with Phe concentrations in the HCM diet below the 20th percentile, the lack of a reduction in plasma Phe concentrations due to feeding the HCM *versus* LCM diet suggests

that supplementing Phe to the HCM diet in order to regain potentially lost BCS (as suggested in Swanepoel et al., 2016a) was unnecessary. Also, as suggested by Iroshan et al. (2013), the increased PUN concentrations (P=0.02) could be indicative of a general AA oversupply, with AA that were not utilized for milk production (such as the high concentrations of Met, Lys, Leu and Val in the current study compared to the indices) being catabolized to urea. As many AA are involved in gluconeogenic pathways, excess AA not utilized for milk protein production may result in enhanced body protein accretion, utilizing energy for BCS gain at the expense of production of some milk components.

The evaluation of the high CM diet fed in Swanepoel et al. (2014) suggested that it was limiting in rumen undegradable protein (RUP) and intestinally absorbable protein (AP), while supplying adequate amounts of rumen degradable protein (RDP) to support maximum microbial protein production. The main difference between the diets of the current and previous CM feeding studies was the higher CP level (178 g/kg DM *versus* 153, 151, 168 and 163 g/kg DM in Acharya et al. (2015); Christen et al. (2010); Mulrooney et al. (2009); Swanepoel et al. (2014, 2016a) respectively). When a similar post-experiment evaluation was repeated for the current HCM diet, the RUP and AP deficiency did not exist. Thus it is likely that the higher CP diets in the current study supplied more AP, and higher levels of AA, negating deficiencies that may have existed in HCM diets in previous studies. Since CM contributed 171 g/kg to the HCM diet DM, with a CP content at the highest reported level reported since 1991 (*i.e.*, 457 *versus* the range of 367 to 458 g/kg DM; Swanepoel, 2016), this was a main reason for the higher diet CP content. Yet, with 45% of diet CP being from CM, previously reported AA deficiencies could still have been induced. However CM is considered to have an AA profile in its RUP fraction that is advantageous to milk protein synthesis (Piepenbrink and Shingoethe, 1998) and, even though some AA still decreased in the plasma with higher CM inclusion (*i.e.*, Leu and Tyr) the high quantity of favourable AA

delivered may have supplied enough required essential AA (EAA), such as Phe, postruminally to overcome potential deficiencies, as suggested by a meta-analysis where responses in production were attributed to increased absorption of all EAA from CM (Martineau et al., 2014).

Even though the treatment diets were formulated to be very similar in terms of nutrient (and NE) profiles (Table 2), the HCM diet tended to have a higher measured NE density (6.75 *versus* 6.32 MJ/kg DM; P=0.09) compared to LCM (Table 6). This increase in NE density could also be indicative of an AA oversupply, as reported by Robinson et al. (2000) when oversupplying both Lys and Met resulted in repartitioning of energy to increase the NE density of the diet and improve the energetic efficiency of the cows, possibly due to the lack of ability to express the energy in production. This increase in NE density could be a part of the reason for a tendency of DM intake to decrease (P=0.10) with the HCM fed cows, which could explain the increased digestibility of OM, aNDF and ADF (Table 3) with higher CM feeding. Improved fiber digestibility of HCM over that of LCM also supports the theory of more AA being supplied by the higher CP contribution from CM since increased availability of free AA has been shown to stimulate growth of microbes in the rumen (Cotta and Russell, 1982; Argyle and Baldwin, 1989) and increase NDF digestibility (Yang, 2002). It was also suggested by Soto et al., (1994) that cellulolytic bacteria can be stimulated by AA as long as energy is available, which could have been the case with the HCM diet due to the higher NE density of the diet.

However, there was no difference in total NE output (MJ/d) for the HCM *versus* LCM diets (Table 6), but rather a re-arrangement of where energy was expressed, either in milk, as was the case for the LCM fed cows, or BCS, as was the case with the HCM fed cows. However, the mechanism responsible for this re-arrangement, and the reason for BCS being favoured over milk composition, is not clear.

Table 6. Partial net energy (NE) balance for cows fed total mixed rations (TMR) with low canola meal (CM), high CM and high CM supplemented with ruminally protected Phe and/or Tyr.

	Treatments (Trt)					*P*			
	LCM[1]	HCM[2]	HCM +P[3]	HCM +PT[4]	SEM	LCM *vs.* HCM[5]	HCM[5] *vs.* HCM+P	HCM+P *vs.* HCM+PT	Trt
Milk energy	135.4	132.9	132.3	130.4	0.52	0.07	0.59	0.14	0.02
output (MJ/d)[6]	0.27	2.47	-1.31	-3.52	0.918	0.30	0.10	0.30	0.10
BCS[7] energy (MJ/d)	179.1	178.8	174.4	170.4	0.90	0.90	0.06	0.08	0.01
Total Net Energy (MJ/d)[8]	6.32	6.75	6.49	6.31	0.100	0.09	0.27	0.42	0.23
NE_L[9] (MJ/kg DM)									

[1] Low canola meal ration: Canola meal included at 120 g/kg dry matter.
[2] High canola meal ration: Canola meal included at 170 g/kg dry matter, with 2 g of intestinally absorbable Met as part of the base ration.
[3] High canola meal ration (with added Met as above) and 14 g of intestinally delivered Phe.
[4] High canola meal ration (with added Met as above) and 7 g for each of intestinally delivered Phe and Tyr.
[5] HCM treatment without Phe or Tyr supplementation.
[6] Milk energy output recalculated with $n = 4$ pens in order to calculate NE_L.
[7] Body condition score.
[8] Total NE calculated as the sum of maintenance, milk and BCS energy. Maintenance energy (MJ/d) calculated using a constant body weight of 657 kg for all treatments.
[9] Net energy available for lactation. $n = 4$ pens.

4.2. High CM diet versus High CM diet with Phe supplementation

Since the Phe concentrations in blood plasma did not decrease for HCM *versus* LCM fed cows, while BCS change was already positive for HCM, previous research discussed above suggests that supplementation of Phe would have resulted in a milk production response. However it also suggests that, due to the lack of loss of BCS and similar plasma Phe concentrations when feeding a higher level of CM, supplementing Phe to the HCM treatment was unnecessary. Indeed, when Phe was supplemented to the HCM diet (HCM+P), both plasma Phe and Tyr concentrations increased, with Phe concentrations approaching the 80th percentile of its AA index (Swanepoel et al., 2016b), suggesting possible oversupply of these AA. That these plasma AA increases were associated with a reduction of milk protein content and yield, in addition to the already lower milk

components for the HCM *versus* LCM fed cows, and with continued higher PUN concentrations, suggests that many AA were not utilized and therefore catabolized. In addition to increased Phe and Tyr concentrations, plasma Ala increased in the HCM+P fed cows to levels above their 80th percentile of the AA index (Swanepoel et al., 2016b). This corresponds with the shift from a BCS gain in HCM to a loss for the HCM+P fed cows, consistent with Ala being abundant in muscle tissue (Bach et al., 2000).

Thus addition of Phe to the HCM diet did not address a Phe and/or Tyr deficiency, but appears to have altered AA metabolism resulting in less synthesis of milk protein as well as stimulating body protein and/or fat mobilization. Indeed it has been demonstrated that supplementing AA to diets in which they are not limiting, as was likely the case for this HCM diet, can lead to unexpected and sometimes negative effects on DM intakes (Robinson et al., 2000; Rulquin and Pisulewski, 2006) and muscle protein synthesis (Appuhamy et al., 2011). That there was a tendency (P=0.06) for a lower NE output (Figure 1) of the HCM+P *versus* HCM fed cows (174 *versus* 179 MJ/d), supports the view that Phe supplementation of a dairy diet without a Phe limitation can impair performance.

Studies investigating effects of Phe on gene expression and milk protein synthesis in incubated bovine mammary epithelial cells have shown that peptide-bound Phe enhanced milk protein synthesis, and are utilized more efficiently than free Phe up to a level of 7% of total Phe supply (Zhou et al., 2015). Thus increasing levels of free Phe in blood through ruminally protected (RP) AA supplementation may be a poor way to meet total Phe needs, suggesting that it may remain limiting and that enhanced Phe availability may require use of an alternate mechanism.

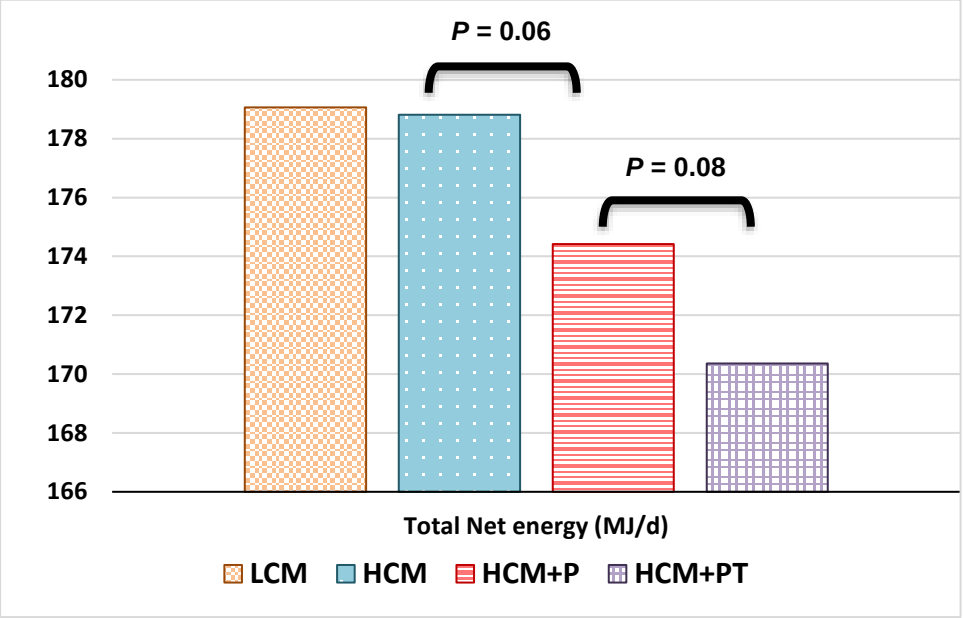

Figure 1. Changes in the Total Net energy (MJ/d) in cows fed the low CM (LCM), high CM (HCM) and high CM ration supplemented with either 14 g of intestinally available Phe (HCM+P) or 7 g each of intestinally available Phe and Tyr (HCM+PT).

4.3. High CM diet versus High CM diet with Phe and Tyr supplementation

The tendency (*P*=0.08) for a lower total NE output (Figure 1) by the HCM+PT *versus* HCM+P fed cows (170 *versus* 174 MJ/d) suggests that substitution of some RP Tyr in the HCM+P diet further reduced overall cow performance compared to Phe supplementation alone. The tendency (*P*=0.09) for plasma Phe concentrations to decrease in the HCM+PT *versus* HCM+P fed cows (Table 5) is consistent with replacement of half of the supplemented Phe by Tyr. However, the lack of change in plasma Tyr or PUN concentrations, as well as the absence of additional increases in plasma Ala concentrations, even with additional BCS losses, together with a general decrease in AA concentrations for the HCM+PT *versus* HCM+P diet, suggests that available AA may have been utilized elsewhere. A similar lack in change of plasma Tyr concentrations was reported by Rae and Ingalls (1984) when Tyr supplementation increased Tyr concentrations in duodenal fluid of sheep, but not in their plasma, or in the plasma of lactating dairy cows. Rae and Ingalls (1984) also reported that Tyr supplementation resulted in a milk response in lactating dairy cows even

with a diet very high in CP (187 g/kg DM), which is not consistent with our negative response to Tyr. However, this finding was not repeated in a 2nd study by Rae and Ingalls (1984).

Substituting Phe with Tyr seems to have exacerbated negative effects of Phe alone, leading to a further decrease in milk yield and components concomitant with a substantial increase in BCS loss (Figure 2). That such a change occurred is entirely consistent with the tendency (*P*=0.09) for lower apparent total tract digestibility of ADF in the HCM+PT *versus* HCM+P fed cows (Table 3), which suggests the potential for lower milk production based upon a lower digestible energy level of the diet. This supports our conclusion that supplementation of Phe and/or Tyr was not only unnecessary to counter deficiencies, but that their probable unnecessary supply changed metabolic processes associated with milk and body component synthesis.

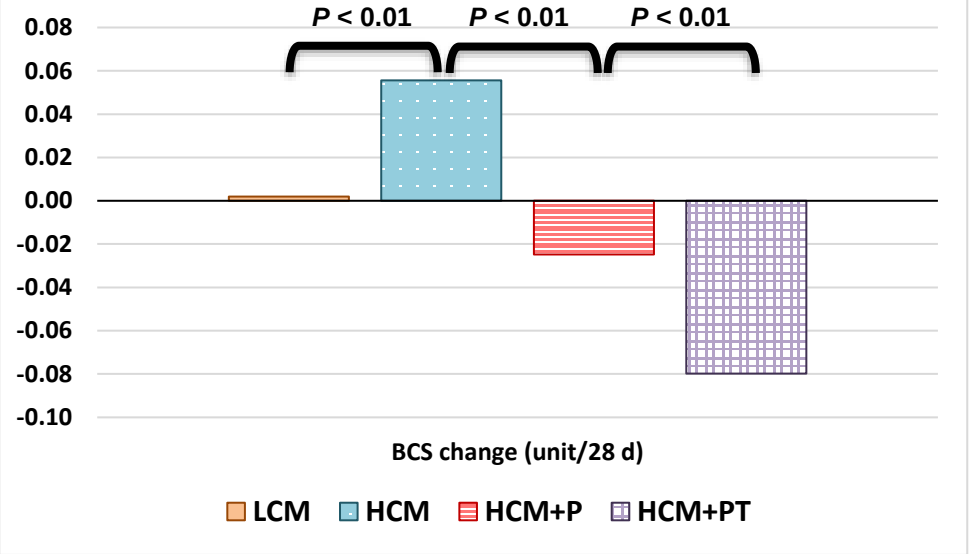

Figure 2. Body condition score (BCS) changes for cows fed the low CM (LCM), high CM (HCM) and high CM ration supplemented with either 14 g of intestinally available Phe (HCM+P) or 7 g each of intestinally available Phe and Tyr (HCM+PT). All changes were *P*<0.01.

That Tyr supplementation can improve production under conditions where it is limiting but when other AA, such as Phe, are adequately supplied, was demonstrated in a number of studies using cultures of mammary secretory cells. These studies have shown that Tyr is not necessarily required to synthesize milk protein as long as adequate Phe is present (Jorgensen and Larson,

1968). However, since Tyr is a NEAA and its synthesis depends on conversion of Phe to Tyr in the liver and bovine mammary gland, Tyr concentrations will decline when Phe is deficient in the diet. Also, when Tyr is supplemented in the diet, conversion of Phe to Tyr is reduced. Two studies conducted by Rae and Ingalls (1984) reported that CM treated with formaldehyde made Tyr postruminally indigestible thereby reducing its availability and uptake by cows which prevented production responses to the formaldehyde treated CM.

Growth studies using rats reported that diets with 8 g/kg Tyr and 1 g/kg Phe did not prevent body weight losses but, by increasing the amount of Phe supplemented, growth was stimulated. This indicates that requirements for Phe were reduced when sufficient Tyr was supplied by the diet (Womack and Rose, 1946), even though Tyr cannot fully replace Phe. In addition, Tyr was only able to stimulate growth when Phe was limiting since addition of Tyr did not provide any benefit when the diet provided adequate amounts of Phe.

All these studies demonstrate that oral supplementation of Tyr can improve production under conditions where Tyr is limiting but when other AA, such as Phe, are adequately supplied. However, responses to Tyr supplementation when Phe was already in excess have not, to our knowledge, been reported. Rae and Ingalls (1984) suggested the involvement of Tyr and Phe in regulation of brain catecholamine synthesis, which can affect and alter energy intakes, blood flow and growth hormone (GH) release. Indeed, since changes in plasma Tyr patterns have an immediate effect on brain neurotransmitter formation, this may support the merit of considering what Tyr supplementation could do under conditions of AA oversupply or imbalance.

4.4. General comments on Phe and Tyr supplementation

While it is clear that supplementation of Phe and/or Tyr to the HCM diet was unnecessary, the unexpected and substantive negative effects on dairy cow performance supports the concept that the bioactivity of these AA is biologically important, while highlighting the downside risks of

supplementing AA when they are not required. The inconsistency and unpredictability of responses of lactating dairy cattle to supplementation of all AA is an ongoing practical problem. As bioactive metabolites, supplementation of Phe and Tyr, and likely other AA, will impact dairy cow performance, but the nature of the responses seem to be highly situationally dependent, including factors such as diet CP level.

Since there is no reliable method to accurately estimate the amounts of absorbable AA delivered to the small intestine, and because a small error in predicting absorbable AA delivery can lead to large animal responses, it appears that formulating diets to specific ranges of absorbable AA assumes much higher feeding precision than is possible for dairy cattle in practice, and that the downside risks of supplementing AA under these conditions is high.

5. Conclusions

Contrary to expectations based on previous studies, increasing the dietary CM inclusion level from 120 to 170 g/kg DM only modestly decreased overall dairy cow performance, possibly due to a dietary protein oversupply (and therefore higher overall AA levels). Thus supplementation of Phe to the HCM diet in order to regain lost BCS was therefore unnecessary. However, supplementing the RP Phe and Tyr to the HCM diet, which did not seem to have an AA limitation, possibly resulted in an oversupply of these AA with unexpected and detrimental consequences, including reductions in milk component outputs and loss of BCS. Even though the efficacy of Tyr *versus* Phe in supporting milk production could not be fully tested, the increased (negative) effects of substitution of half of the supplemented Phe with Tyr suggests that Tyr may be more efficacious and bioactive than Phe alone, and that supplementation of these relatively ‘unimportant’ AA can have substantive impacts on dairy cow performance.

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