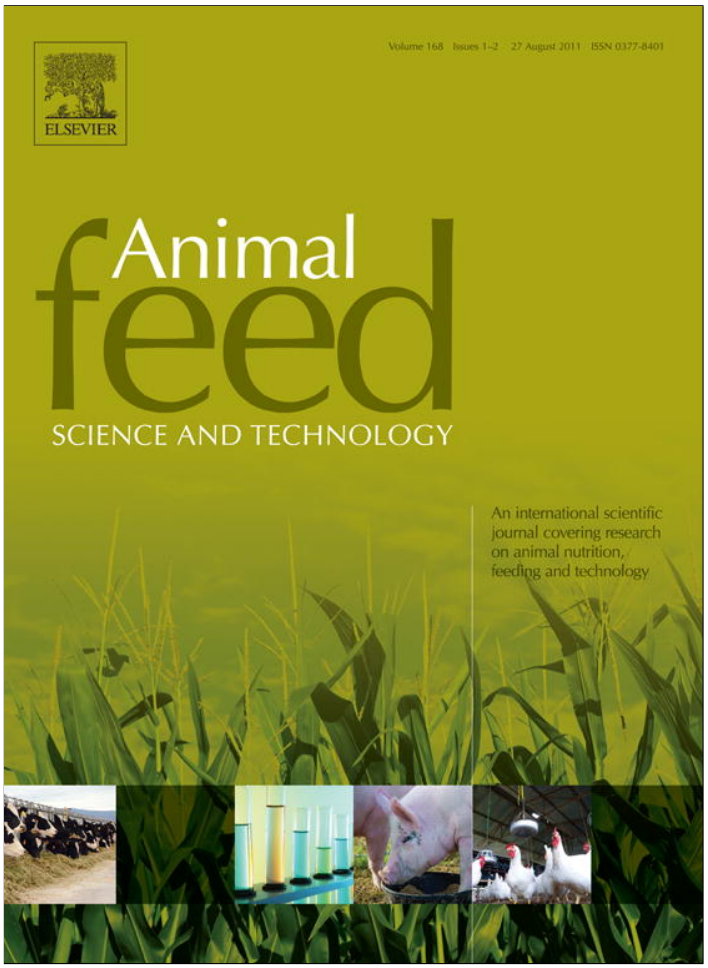

Animal Feed Science and Technology 168 (2011) 30–41

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Productive responses of lactating dairy cattle to supplementing high levels of ruminally protected lysine using a rumen protection technology

P.H. Robinson[a,*], N. Swanepoel[b], I. Shinzato[c], S.O. Juchem[a]

[a] Department of Animal Science, University of California, Davis, CA 95616, USA
[b] Meadow Feeds, Warich Close Office Park, 39 Van Vuuren Street, Roodepoort 1737, South Africa
[c] Ajinomoto Co. Inc., Tokyo, Japan

ARTICLE INFO

Article history:
Received 25 August 2010
Received in revised form 23 March 2011
Accepted 25 March 2011

Keywords:
Milk production
Plasma amino acid imbalance
Milk protein

ABSTRACT

Increased milk production requires high intakes of crude protein in the diet, and/or improved supply and profiles of amino acids (AA) delivered to the duodenum, in order to meet animal needs for milk and milk component synthesis. Our objective was to estimate the rumen escape of a ruminally protected lysine (RPL) product and determine effects of feeding it on dry matter (DM) intake, milk production and plasma AA profiles of high producing dairy cows. The study was two 2 × 2 crossovers (*i.e.*, early and mid-lactation dairy cows) with 28-d experimental periods. All cows were fed the same total mixed ration (TMR), calculated to be first limiting in lysine, with treatment pens receiving 17 kg/pen/d of the RPL supplement (to deliver 41 g of lysine/cow/d) mixed into the TMR. Extensive evaluation of the RPL suggested that this feeding level delivered 15–21 g/d of intestinally absorbable lysine. Control cows were fed the RPL without lysine (*i.e.*, the fat matrix) at the same level as the fat matrix which was fed to the RPL cows. Feeding the RPL did not influence DM intake in early lactation cows (26.8 kg/d), but production of milk (48.0 *versus* 50.0 kg/d), as well as milk fat, true protein and lactose, and energy, were higher (P<0.01) in RPL supplemented cows. In addition, cows supplemented with RPL gained body condition score (BCS) whereas control cows lost BCS during the 28-d period (*i.e.*, 0.020 *versus* −0.069 units/28 d; P=0.056). In mid-lactation cows, DM intake was not influenced, and only milk fat and energy outputs increased (P<0.05) with RPL feeding. BCS change was not influenced. Plasma lysine levels in mid-lactation cows were much higher (P=0.01) with RPL feeding, suggesting that the feeding level of RPL exceeded their lysine needs. However a lack of impact of RPL feeding on plasma lysine levels in early lactation cows suggests that lysine needs may not have been met with RPL feeding. In contrast to an earlier study by our group, with early lactation cows fed a similar diet where milk yield was not impacted by 7–10 g/d of lysine delivered to the intestinal absorptive sites, may support the unconventional hypothesis advanced in that study that body protein turnover is the first limiting AA priority in early lactation cows followed by milk component synthesis.

Abbreviations: AA, amino acid; ADF, acid detergent fiber; aNDF, amylase treated neutral detergent fiber; aNDFom, aNDF exclusive of residual ash; BCS, body condition score; CP, crude protein; DM, dry matter; HP-Arg, highly (ruminally) protected arginine; 3-MH, 3-methylhistidine; RPL, ruminally protected lysine; SG, specific gravity; TMR, total mixed ration.

* Corresponding author. Tel.: +1 530 754 7565; fax: +1 530 752 0175.
E-mail address: phrobinson@ucdavis.edu (P.H. Robinson).

0377-8401/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.anifeedsci.2011.03.019

1. Introduction

Methods to increase milk production through dietary changes have been extensively researched and it is generally accepted that milk protein proportion increases with increased dietary crude protein (CP) level, even though changes are small. There is, however, no single source of rumen undegradable CP which provides an ideal balance of essential amino acids (AA) that matches the AA profile of milk thereby ensuring optimal milk production. It is difficult to formulate rations to provide all required AA concentrations using currently available feed sources and metabolic models. Corn proteins have a relatively low lysine content (~70 g/kg of total essential AA; NRC, 2001) and, with the increasing incorporation of corn byproducts in dairy rations due to the motor fuel ethanol distillation industry, supplementation with ruminally protected lysine (RPL) products, which aim to deliver additional absorbable lysine to the intestinal absorptive sites, has become a major focal point for nutritionists and the feed industry.

Over the last few years, a series of studies were completed by our group to examine the AA needs of modern high producing dairy cows. An initial study was completed to determine effects of feeding a RPL product on feed intake, digestibility and milk production of high producing dairy cows (Swanepoel et al., 2010a). However, this study, calculated to deliver 7–10 g/d of intestinally absorbable lysine, resulted in substantive declines in milk fat yield and concentrations in both early and mid-lactation cows, although production of milk, milk true protein and lactose yields were not impacted. A decrease in plasma concentrations of 3-methylhistidine (3-MH) suggested that lysine had a direct or indirect enhancing effect on muscle protein turnover thereby diverting energetic precursors from milk fat synthesis. This negative fat response (Swanepoel et al., 2010a), as well earlier one in Trináctý et al. (2009) when feeding RPL at ~10 and ~12 g/d respectively of intestinally absorbable lysine in two studies, are not inconsistent with the literature of post-ruminal lysine supplementation (Robinson, 2010), which generally shows small negative production responses to supplemental intestinally absorbable lysine, possibly due to an AA imbalance that is created at the intestinal absorptive sites when lysine delivery is increased. A subsequent survey (Swanepoel et al., 2010b) identified other AA, particularly isoleucine, valine and histidine, which could become co-limiting if supplies of intestinally absorbable lysine were increased, thereby suggesting that supplementation of a combination of co-limiting AA in addition to lysine could alleviate the AA imbalance and provide cows with a more 'ideal' intestinally delivered absorbable AA profile with the potential to improve animal production levels and efficiency. Thus another RPL study (Robinson et al., 2010) was completed to determine effects of feeding an RPL product, with or without added isoleucine, valine and histidine, at levels and proportions as suggested by Swanepoel et al. (2010b), on feed intake, milk production and composition, as well as plasma AA profiles of high producing multiparity dairy cows. Supplementation of ~10 g/d of intestinally absorbable lysine in this study tended to reduce productive performance, but also reduced loss of body condition score (BCS), while addition of isoleucine, histidine and valine to the RPL increased productive performance of the cows while maintaining the reduced loss of BCS. Robinson et al. (2010) suggested that the general lack of positive impacts of RPL in lactating cows, as reported in the literature, may either have been due to lysine being first limiting but not supplied in sufficient quantity to have a positive milk response, or to supplementation of lysine alone resulting in an imbalance/deficiency in another essential AA (*i.e.*, isoleucine, histidine and/or valine), which supplementation of an AA complex could alleviate, thereby resulting in benefits beyond supplementation of lysine. In contrast, Swanepoel et al. (2010a) speculated that a lack of increase in plasma lysine concentrations, as well as the decreased plasma levels of 3-MH in that study, and the consistency in reduced loss in BCS with RPL supplementation (Robinson et al., 2010), supported lysine as being the first limiting AA, but that the levels of lysine fed were sufficient to only improve body protein turnover and BCS, with no impact on productive response.

To examine this hypothesis, lysine would have to be delivered to the intestinal absorptive sites at higher levels than previously achieved. Thus the objective of this study was to repeat Swanepoel et al. (2010a) as closely as possible, but with an estimated intestinal delivery level of lysine of ~20 g/d, on performance, BCS changes and plasma AA profiles of early and mid-lactation dairy cows.

2. Materials and methods

The study design was two (*i.e.*, early and mid-lactation dairy cows) 2 × 2 crossovers of two periods with the two treatments being reversed (*i.e.*, switched back) after 28 d. One pen in each crossover was fed the control ration containing only the RPL fat carrier, fed at the same level as it was fed in the RPL treatment, while the other pen received the control ration supplemented with RPL. All cows were cared for relative to applicable laws of the state of California and the USA.

2.1. Farm, animals and management

The study was completed on the same commercial dairy located near Tulare (CA, USA) as used in Swanepoel et al. (2010a) which milked ~1400 Holstein cows three times a day with control rations and a statistical design as similar as possible to those used by Swanepoel et al. (2010a). Cows were grouped according to days in milk and pregnancy status in one of two pairs of early and mid-lactation pens, and treatments were randomly allocated to pens. Each pen held ~180 cows, with cows averaging 79 ± 3.4 days in milk with 48.8 ± 0.86 kg/d milk production and cows in mid-lactation pens averaging 256 ± 6.3 days in milk with 41.3 ± 0.59 kg/d of milk at the start of the study. Only cows which were in their initially assigned pen at the end of the 8 wk study were utilized in statistical analysis.

Table 1
Chemical composition, specific gravity and other characteristics of the RPL[a] product and the fat carrier fed to the dairy cows.[b]

	RPL	Fat carrier
Chemical composition		
Dry matter, g/kg	965.3	997.2
Ash, g/kg DM[c]	10.2	<0.0
Lysine[d], g/kg DM	436.0	<0.0
Fatty acids, g/kg DM	424.7	958.2
<C16:0, g/kg fatty acids	2.0	3.3
C16:0, g/kg fatty acids	112.0	104.9
C17:0, g/kg fatty acids	2.2	2.6
C18:0, g/kg fatty acids	846.0	855.9
C20:0, g/kg fatty acids	7.6	5.8
Unidentified, g/kg fatty acids	31.2	27.5
Specific gravity, g/cm^3	1.10	ND[e]
In situ rumen stability (g/kg N)		
Solubility (*i.e.*, 0 h residue)	941	ND
24 h residue	866	ND
36 h residue	845	ND
48 h residue	779	ND
HP-Arg[f] rumen stability (g/kg lysine)		
Sample 1	719	ND
Sample 2	725	ND
Post-ruminal release (g/kg lysine)		
Sample 1	474	ND
Sample 2	538	ND

[a] Ruminally protected lysine product.
[b] Values represent duplicate assays of replicate samples collected near the end of each experimental period.
[c] Dry matter.
[d] Calculated as N × 5.219.
[e] Not determined.
[f] Lysine escape as determined against a highly protected (HP), completely undegradable L-arginine (Arg) product (see Swanepoel et al., 2010a for procedure details).

2.2. Diets

Cows were fed twice daily at 5:00 and 10:30 h to appetite and had free access to water. The total mixed rations (TMR) were mixed before each feeding, after which it was loaded into trucks to be unloaded at the pens. One load was mixed and divided evenly between the two control pens for every feeding. The same was done for the treatment pens. The 'Feed Watch' system (Valley Ag Software, Tulare, CA, USA) recorded all feeding activities, keeping daily records of the total amount of TMR fed to each pen and the actual ingredient profile of each batch of TMR that was mixed. Feed refusals were weighed individually by pen every morning thereby allowing calculation of dry matter (DM) intake per pen. The number of cows in each pen was retrieved from Dairy Comp 305 (Valley Ag Software, Tulare, CA, USA) weekly, allowing group intake expressed on a per cow basis to be calculated.

Cows in all pens were fed the same base ration of alfalfa hay, alfalfa silage, corn silage, steam flaked corn grain, dried distillers grains (corn), almond hulls, canola pellets and cottonseed, with 17 kg RPL/pen/d (*i.e.*, 94.4 g RPL/cow/d or 41 g lysine/cow/d) mixed into the TMR fed to RPL pens and 7.2 kg RPL fat carrier/pen/d (based upon the RPL being 420 g/kg fat; Table 1) mixed into the TMR fed to the control pens.

The RPL product was manufactured by the Ajinomoto Company (Tokyo, Japan), and was a matrix of lysine (L-lysine monohydrochloride) which was ruminally protected by a matrix of saturated fatty acids (Table 1) designed to be rumen stable but degrade in the abomasum. The fat carrier was manufactured by Yokozeki Oil and Fat Industries Co. Ltd. (Ibaragi, Japan).

2.3. Evaluation of the rumen protected lysine product

Duplicate RPL samples, collected at the end of each experimental period, were analyzed for chemical composition, including ash, N and fatty acid profile. These samples were also assayed for specific gravity (SG) as described by Swanepoel et al. (2010a). The RPL product used in the previous RPL study by Swanepoel et al. (2010a) had an estimated particle stability of 490 mg/g during mixing and feeding. However, no durability measurements were completed on the RPL used in our study since visual appraisal of the RPL during TMR mixing indicated that it was completely durable with no broken particles, or even particles with changed shape, detected at any time during the mixing process, or in the feed bunks.

Several methods were used to evaluate the ruminal stability/escape and post-ruminal release of lysine in the RPL as follows.

2.3.1. Ruminal in situ stability procedure

Two samples of the RPL were ruminally incubated in duplicate in two dry cows fed a grass hay based diet for 0 (water wash), 24, 36 and 48 h to determine ruminal loss of N from the RPL. The *in situ* bags were constructed of nylon (Nitex; B. & S.H. Thompson, Montreal, QC, Canada) with an effective size (*i.e.*, after tying the bag closed) of 7 × 9 × 2 cm. Each bag contained 5 g of RPL. N stability from the RPL particles (Table 1) at each *in situ* incubation time was calculated as the proportion of N remaining.

2.3.2. HP-Arg ruminal escape procedure

Two samples of the RPL were evaluated for ruminal escape of lysine by the HP-Arg procedure (Nocek et al., 2010). In this procedure the RPL and a highly protected L-arginine (HP-Arg) were placed in the rumen (100 g of each product containing ~400 g/kg lysine or arginine) through the rumen cannula. Duodenal digesta samples were collected every 3 h for 48 h and lysine and arginine concentrations in duodenal digesta were determined. These concentrations were plotted against time of collection and rumen escaping lysine was calculated as the relative areas under the curve.

2.3.3. Post-ruminal release procedure

To determine post-ruminal release of rumen escape lysine, two samples of the RPL were fed to each of three cows at 100 g/cow and cumulative fecal samples were collected at 12 h intervals for 72 h after RPL ingestion. To extract lysine from RPL in feces, it was homogenized mechanically (Super Masscolloider MKZA10-15J, Masuko Sangyo Co. Ltd., Kawaguchi, Japan) after adding hot water (60–70 °C). Lysine extracted from the RPL in each fecal sample was calculated by analyzing free lysine concentrations in fecal slurries. Feces from the cows collected in the same way when not fed the RPL were also analyzed for lysine in the same manner to obtain the basal concentration of fecal lysine. Total grams of lysine excreted were calculated as the sum of lysine in the six 12 h fecal samples, after subtracting basal lysine in feces. Post-ruminal release of RPL lysine was expressed as:

$$1 - \frac{\text{fecal excreted}}{\text{HPArg rumen stable lysine in the RPL}}$$

to calculate post-ruminal release of rumen escaping lysine in the RPL.

2.4. Sample collection, preparation and analytical methods

2.4.1. Total mixed ration and feeds

The TMR and feed ingredients were sampled twice during the last week of each experimental period. Six handfuls of each TMR was collected at evenly spaced locations along the bunk-line, pooled and the entire sample quartered, keeping two opposite quarters for analysis. Commodity feeds were sampled by taking 4–5 handfuls of each. A 'golf club' hay probe (Seifert Analytical, Lodi, CA, USA) was used to take 12–16 core samples of the alfalfa hays. All TMR samples, silages and other wet ingredients were weighed and dried at 55 °C for 48 h, to determine initial DM content, before chemical analysis.

All samples were ground to pass a 1 mm screen on a model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). The DM was determined as gravimetric loss of free water when dried at 105 °C for 2 h in a forced air oven. Ash determination was based on gravimetric loss by heating samples to 550 °C for 8 h. Total N and N in acid detergent fiber (ADF) was determined by the Dumas method (#990.03; AOAC, 2006) utilizing a Leco N analyzer. The ADF and lignin treated with sulphuric acid (lignin(sa)) were determined according to method 973.18 of AOAC (2006). Neutral detergent fiber (aNDF) analysis included sodium sulfite and a heat stable amylase (Van Soest et al., 1991), and is expressed exclusive (*i.e.*, aNDFom) of residual ash. Starch was determined by enzymatic hydrolysis (Smith, 1981) and free sugars as the sum of glucose, fructose and sucrose determined by high-performance liquid chromatography as described by Johansen et al. (1996). Minerals were determined using methods of Johnson and Ulrich (1959), Tracy and Moeller (1990) and Meyer and Keliher (1992). Ether extract was quantified using a standard Soxhlet extraction (#2003.05, AOAC, 2006).

2.4.2. Blood plasma

Blood was sampled from the tail (coccygeal) vessels using two 7 ml evacuated tubes containing K_2 EDTA (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) on day 25 of each experimental period. A sample group of 20 early lactation cows (*i.e.*, 10/pen) and 30 mid-lactation cows (*i.e.*, 15/pen) were pre-selected based upon having the lowest days in milk in their pen at the start of the study. Samples were collected for 16 early lactation cows and 27 mid-lactation cows out of the initial sample group for both periods. Samples were stored on ice and plasma was obtained by centrifugation at 2060 × *g* for 15 min at 4 °C about 2 h later, transferred into two replicated plastic microtubes and stored at −20 °C. A set of plasma samples was analyzed at the AESCL Analytical Services (University of Missouri, Columbia, MO, USA), for AA (*i.e.*, free plasma AA) levels and urea.

2.4.3. Milk production

Milk weights were recorded using the Tru-test milk yield proportioning device (Tru-Test Ltd., Auckland, New Zealand). Milk samples were collected from all cows in the experimental pens on day 28 of each period. A small representative sub-sample was drawn from the flask and preserved with a liquid 2-bromo-nitropropane-1,3-diol preservative for subsequent

analytical testing. Fat, true protein, lactose, and somatic cell counts were determined using near infrared spectroscopy. The somatic cell counts were analyzed using a dye binding technique (Somacount 500, Bentley Instruments Inc. Chaska, MN, USA).

Milk samples from the same cows as blood plasma had been collected had sub-samples preserved by freezing for fatty acid analysis (DePeters et al., 2001).

2.4.4. Body condition score

The sample groups were 100 early lactation cows (*i.e.*, 50/pen) and 170 mid-lactation cows (*i.e.*, 85/pen) which were pre-selected based upon having the lowest days in milk in their pen at the start of the study. Out of the initial sample groups, 68 early lactation cows, and 94 mid-lactation cows, were scored at the beginning of the study and at the end of each experimental period by a single trained scorer using the system of Ferguson et al. (1994). Body condition score change was calculated as the difference between the end and beginning period BCS values within cow.

2.5. Statistical analysis

Each experiment was statistically analyzed as a 2×2 crossover design with pen as the experimental unit for DM intake and cow as the experimental unit for milk production. Statistical analysis of the ingredient and chemical composition of TMR and DM intake included period and treatment as factors using the GLM procedure of SAS (2004) by experiment. Animal production and plasma AA were statistically analyzed using the MIXED option of SAS by experiment. Cow within pen was included as a random statement with period, pen and treatment as fixed effects. The BCS was analyzed in the same way, but only for those cows that had BCS scored at the beginning and end of each experimental period.

Cows that moved from their original pen during the study, for health or any reason, were excluded from statistical analysis. This resulted in a total of 170 cows included in the statistical analysis for the early lactation data set and 201 in the mid-lactation data set.

Treatment differences were accepted as significant if $P<0.05$ and tendencies to significance accepted if $0.05 \leq P \leq 0.10$.

3. Results

3.1. Product evaluation

The RPL product (Table 1) had a SG of 1.10 g/cm^3 and the matrix was comprised of 440 mg/g lysine based on N assay, 420 mg/g fatty acids and a negligible ash content. The majority of the fatty acids in the RPL were C18:0 (850 mg/g) and C16:0 (110 mg/g). The RPL fat carrier had the same fatty acid composition as in the RPL.

Results from the *in situ* nylon bag study of the RPL indicated that 60 mg/g of the N was rumen soluble, while 870, 850 and 780 mg/g of N remained in the bags after 24, 36 and 48 h in incubation respectively (Table 1). In contrast, the 24 h HP-Arg rumen stability of the lysine was lower at ~720 mg/g. Post-ruminal evaluation of the RPL suggested that release of the rumen escaping lysine was ~510 mg/g.

3.2. Ration evaluation

The chemical composition of the ingredients used in the TMR (Table 2) were generally similar to the nutrient composition of feeds in NRC (2001).

There were no substantive differences in ingredient composition of the TMR fed to the two treatment groups (Table 3), except for the RPL product which was only fed to the RPL group and the RPL fat carrier which was only fed to the control group. The TMR met all minimum NRC (2001) nutrient requirements, except Se, and their ingredient profiles are generally consistent with typical California dairy rations. The Se concentration on the TMR were slightly lower than NRC (2001) recommendations due to the lack of inclusion of Se in the trace mineral premix.

The chemical composition of feeds, as well as the TMR, fed during this study are almost identical to those fed during Swanepoel et al. (2010a), which was the objective, except for a lower CP level in the almond hulls (39 *versus* 70 g/kg) and a slightly lower CP level in the TMR (169 *versus* 181 g/kg).

3.3. Milk yield and body condition score

The DM intake was not affected by RPL in either the early (Table 4) or mid-lactation (Table 5) cows, but RPL increased ($P<0.01$) yields for milk (50.0 *versus* 48.0 kg/d), fat (1.82 *versus* 1.72 kg/d), true protein (1.43 *versus* 1.35 kg/d) and lactose (2.36 *versus* 2.24 kg/d) as well as milk concentrations of protein (28.8 *versus* 28.2 g/kg) and lactose (47.1 *versus* 46.7 g/kg) in early lactation cows (Table 4). In mid-lactation cows, milk ($P=0.07$), true protein ($P=0.10$) and lactose ($P=0.12$) yields tended, or were numerically, higher, while fat yield and concentrations were higher (1.50 *versus* 1.38 kg/d and 38.2 *versus* 36.1 g/kg; $P < 0.01$) in RPL supplemented cows (Table 5).

Table 2
Chemical analysis of the ingredients used in the total mixed ration (g/kg 105 °C DM[a]) fed to the dairy cows.[b]

	Corn silage	Alfalfa silage	Alfalfa hay	Corn flaked	CS[c]	DDGS[d]	Canola pellets	Almond hulls
Dry matter (g/kg)	286	275	915	865	925	923	901	961
aNDF[e]	498	440	366	88	424	319	269	304
aNDFom[f]	484	387	352	88	401	311	234	296
ADF[g]	324	402	286	31	309	133	189	229
Lignin(sa)[h]	31	71	54	<10	109	32	71	74
Crude protein	72	185	205	77	223	270	408	39
ADICP[i]	61	91	46	<10	73	110	53	264
Ash	71	193	122	13	50	42	81	77
Crude fat	34	50	22	30	220	101	36	25

[a] Dry matter.
[b] $n = 4$ except alfalfa hay $n = 3$.
[c] A 0.5/0.5 mixture of whole linted cottonseed and cracked pima cottonseed.
[d] Dried distillers grains with solubles.
[e] Neutral detergent fiber assayed with a heat stable amylase, expressed inclusive of residual ash.
[f] Neutral detergent fiber assayed with a heat stable amylase, expressed exclusive of residual ash.
[g] Acid detergent fiber, expressed inclusive of residual ash.
[h] Lignin assayed with sulphuric acid.
[i] Acid detergent insoluble crude protein (CP), an estimate of indigestible CP, expressed as g/kg CP.

Feeding RPL increased (P=0.03) the BCS (3.14 *versus* 3.07), changing BCS change from a loss (−0.069 units/28 d) for control cows to a gain (0.020) in early lactation cows (P=0.056). Body condition score and BCS change was not affected by RPL feeding in mid-lactation cows.

3.4. Blood plasma

No plasma AA concentration, including lysine, was affected when RPL was fed to early lactation cows (Table 6). However, in mid-lactation cows (Table 7), the AA which had a tendency, or were numerically, increased with RPL included leucine (P=0.10), phenylalanine (P=0.06), tryptophan (P=0.06) and arginine (P=0.06), while lysine increased 24% (P=0.01).

3.5. Fatty acids

Milk fatty acids (Table 8) which tended to increase in the early lactation group with RPL supplementation included C16:1 *trans* and C20:4 (P=0.10) while C18:1 *trans*-11 fatty acids tended to decrease (P=0.14). C18:2 *cis*-11 *cis*-13 (P=0.05) and C18:3 (P=0.03) were reduced when RPL was fed. No other milk fatty acids were affected by RPL treatment and all levels were within accepted ranges (Dhiman et al., 1999).

4. Discussion

4.1. Product evaluation

The *in situ* rumen stability of the RPL product was much higher than our previously used ruminally protected products, averaging 866 and 845 mg/g retention after 24 and 36 h of incubation respectively compared to 24 h stabilities of 541 and 421 mg/g reported by Robinson et al. (2010) and 584 mg/g reported by Swanepoel et al. (2010a) for their RPL products. Using the 24 h *in sacco* stabilities as the index of rumen stability, and assuming no loss of particle structure during mixing and feeding of the RPL, the amount of lysine which was delivered to the intestinal absorptive sites from the 41 g/d of RPL (as lysine) fed was about 35 g/d.

If the HP-Arg determined lysine which was released post-ruminally relative to the 24 h HP-Arg rumen stability is assumed to apply to the *in sacco* residues determined at 24 h in dry cows, then the amount of intestinally absorbable lysine which was delivered to the intestinal absorptive sites from the 35 g/d of lysine which escaped the rumen was ∼21 g/d (Table 7). This is considerably higher than the intestinally absorbable lysine delivered in Swanepoel et al. (2010a), of 7–10 g/d, and in Robinson et al. (2010), of 9–12 g/d, with their RPL products. This *in sacco* calculated value of ∼21 g/d in our study is higher than the ∼15 g/d estimated based upon the HP-Arg procedure *per se* (Table 9).

4.2. Milk yield

Previous responses of milk yield to supplementation of ruminally protected lysine have been variable for both early and mid-lactation cows. Watanabe et al. (2006) reported no response in milk yield when 16 g/d of intestinally absorbable lysine was fed as a fat coated RPL to early lactation cows, which is in agreement with Swanepoel et al. (2010a) in which feeding

Table 3
Ingredient and chemical composition (g/kg dry matter) of the total mixed rations fed to the dairy cows.

	Control	RPL[a]	SE	P
Ingredient composition				
n	4	4		
Alfalfa, hay	201.6	203.0		
Corn, silage	185.2	185.7		
Alfalfa, silage	37.6	38.2		
Corn grain, steam flaked	192.4	194.0		
Almond, hulls	34.6	34.6		
Canola, meal pellets (solvent)	92.8	92.9		
Dried distillers grains, corn	107.0	103.9		
Cottonseed, whole upland	96.3	96.8		
Energy II[b]	22.8	21.5		
Yeast[c]	3.8	3.7		
Mineral premix[d]	21.4	21.3		
RPL product	0.0	4.4		
RPL fat carrier[e]	1.5	0.0		
Nutrient composition				
n	7	7		
Dry matter (DM), g/kg	585	592	9.4	0.42
aNDF[f]	328	329	4.8	0.9
aNDFom[g]	314	317	5.8	0.63
ADF[h]	224	220	4.5	0.32
Lignin(sa)[i]	48	45	1.2	<0.01
Fat	69	71	2.7	0.33
Starch	182	185	5.7	0.67
Sugars	24	23	1.9	0.6
Crude protein	168	170	2.9	0.34
ADICP[j]	76	73	2.3	0.23
Ash	81	80	0.34	0.83
Ca	9.2	9.0	0.75	0.79
P	4.4	4.5	0.05	0.09
K	16.0	15.7	0.57	0.63
Mg	3.1	3.1	0.16	0.71
S	3.0	3.0	0.10	0.99
Na	2.9	2.8	0.69	0.87
Cl	4.9	5.2	0.07	0.61
mg/kg DM				
Zn	84	77	16.0	0.63
Mn	55	50	7.1	0.45
Fe	287	291	32.9	0.90
Cu	11	10	1.6	0.57
Co	0.95	0.73	0.263	0.40
Mo	1.42	1.67	0.091	0.01
Se	0.25	0.22	0.033	0.45

[a] Totally mixed ration with the ruminally protected lysine product.
[b] Nutritech Solutions Ltd. (Abbotsford, BC, Canada).
[c] Diamond V Mills Inc. (Cedar Rapids, IA, USA).
[d] Premix (893 g/kg DM) contained 50.9 g/kg N, 7.5 g/kg P, 9.1 g/kg K, 92.2 g/kg S, 48.8 g/kg Ca, 67.0 g/kg Mg, 34.1 g/kg Zn, 17.9 g/kg Mn, 39.3 g/kg Fe and 3.0 g/kg Cu on a DM basis.
[e] The fat matrix used in manufacture of the RPL.
[f] Neutral detergent fiber assayed with a heat stable amylase, expressed inclusive of residual ash.
[g] Neutral detergent fiber assayed with a heat stable amylase, expressed exclusive of residual ash.
[h] Acid detergent fiber expressed inclusive of residual ash.
[i] Lignin assayed with sulphuric acid.
[j] Acid detergent insoluble crude protein (CP), an estimate of indigestible CP, expressed as g/kg CP.

of 7–10 g/d intestinally absorbable lysine had no effect on milk, protein and lactose yields. Blauwiekel et al. (1997) reported higher milk yields in early lactation cows when 15 g/d of lysine was delivered to the intestinal absorptive sites. In contrast, Piepenbrink et al. (1996) attributed a reduction in milk yield to excessive amounts, and/or improper ratios, of absorbable lysine and methionine delivery when an RPL was fed without ruminally protected methionine. However, the increase in milk yield in the early lactation cows in the current study would not appear to support this hypothesis. The higher milk yield together with the increase in milk fat, protein and lactose yields suggests that, even though lysine supplementation during previous studies had little or no effect on early lactation cows, higher lysine levels delivered to the intestinal absorptive sites increased its absorption, as well as utilization of absorbed AA for milk synthesis. This is also reflected in the plasma AA data where plasma lysine concentrations failed to increase with lysine supplementation in the early lactation cows, suggesting that the absorbed lysine was rapidly utilized to supply the limiting AA (*i.e.*, lysine), thereby allowing higher utilization of other AA to facilitate increased milk, and milk component, production. This increased production also suggests that the

Table 4
Dry matter intake, milk production and composition as well as body score of the early lactation cows.

	Control	RPL[a]	SEM	P
Dry matter intake, kg/d (n = 4)	26.83	26.86	0.296	0.92
Yield, kg/d (n = 170)				
Milk	47.99	50.02	0.613	<0.01
Fat	1.72	1.82	0.032	<0.01
Protein	1.35	1.43	0.016	<0.01
Lactose	2.24	2.36	0.030	<0.01
Components, g/kg (n = 170)				
Fat	35.9	36.4	0.47	0.42
Protein	28.2	28.8	0.17	<0.01
Lactose	46.7	47.1	0.14	<0.01
SCC[b] (counts;,000)	319	331	47.4	0.80
Body score, units (n = 68)				
Mean, units	3.07	3.14	0.060	0.03
Change, units/28 d	−0.069	0.020	0.0320	0.06

[a] Totally mixed ration with the ruminally protected lysine product.
[b] Somatic cell count.

Table 5
Dry matter intake, milk production and composition as well as body score of the mid-lactation cows.

	Control	RPL[a]	SEM	P
Dry matter intake, kg/d (n = 4)	26.17	26.08	0.651	0.91
Yield, kg/d (n = 201)				
Milk	38.32	39.14	0.442	0.07
Fat	1.38	1.50	0.030	<0.01
Protein	1.20	1.22	0.013	0.10
Lactose	1.79	1.82	0.021	0.12
Components, g/kg (n = 201)				
Fat	36.1	38.2	0.55	<0.01
Protein	31.6	31.4	0.13	0.28
Lactose	46.7	46.5	0.11	0.18
SCC[b] (counts;,000)	267	281	35.8	0.68
Body score, units (n = 94)				
Mean, units	3.00	3.01	0.043	0.57
Change, units/28 d	0.013	0.026	0.0244	0.72

[a] Totally mixed ration with the ruminally protected lysine product.
[b] Somatic cell count.

Table 6
Free amino acid and urea concentrations (μg/ml) in plasma of early lactation cows (n = 16) as influenced by feeding the RPL.

	Control	RPL[a]	SE	P
Essential amino acids				
Threonine	10.4	10.0	0.77	0.65
Valine	29.1	29.0	1.41	0.95
Methionine	3.22	3.11	0.135	0.56
Isoleucine	13.5	13.2	0.67	0.74
Leucine	23.7	23.1	1.08	0.68
Phenylalanine	6.94	6.60	0.256	0.36
Tryptophan	6.24	6.11	0.291	0.75
Lysine	9.74	10.44	0.592	0.41
Arginine	12.1	12.0	0.58	0.96
Histidine	6.84	6.93	0.352	0.86
Non-essential amino acids				
Cystine	0.04	0.14	0.048	0.16
Aspartic acid	0.53	0.53	0.027	0.97
Serine	7.69	7.59	0.383	0.86
Asparagine	7.17	7.10	0.409	0.89
Glutamic acid	5.09	5.44	0.215	0.25
Glutamine	28.9	28.5	1.32	0.85
Proline	8.79	8.66	0.533	0.82
Glycine	22.1	22.6	1.48	0.76
Alanine	22.2	22.4	1.02	0.86
Tyrosine	9.50	8.80	0.494	0.25
3-MH[b]	0.17	0.22	0.029	0.26
Urea	232	231	11.2	0.95

[a] Totally mixed ration with the ruminally protected lysine product.
[b] 3-Methyl histidine.

Table 7
Free amino acid and urea concentrations (μg/ml) in plasma of mid-lactation cows ($n = 16$[a]) as influenced by feeding the RPL.

	Control	RPL[b]	SE	P
Essential amino acids				
Threonine	11.9	11.8	0.71	0.96
Valine	27.1	29.3	1.59	0.20
Methionine	3.57	3.72	0.168	0.43
Isoleucine	13.5	14.7	0.78	0.18
Leucine	22.5	24.8	1.30	0.10
Phenylalanine	6.93	7.52	0.263	0.06
Tryptophan	6.01	6.72	0.282	0.06
Lysine	9.65	11.93	0.642	0.01
Arginine	12.8	14.2	0.65	0.06
Histidine	6.08	6.04	0.521	0.91
Non-essential amino acids				
Cystine	0.08	0.08	0.053	0.99
Aspartic acid	0.67	0.69	0.043	0.77
Serine	8.15	8.23	0.390	0.84
Asparagine	7.16	7.56	0.424	0.37
Glutamic acid	6.36	6.63	0.368	0.47
Glutamine	26.8	28.5	1.28	0.34
Proline	9.14	9.61	0.488	0.41
Glycine	25.8	25.0	1.88	0.63
Alanine	24.8	27.2	1.59	0.15
Tyrosine	10.1	11.0	0.49	0.22
3-MH[c]	0.23	0.32	0.046	0.11
Urea	208	236	8.9	0.03

[a] Only 16 of the 27 samples collected were submitted for analysis.
[b] Totally mixed ration with the ruminally protected lysine product.
[c] 3-Methyl histidine.

amount of lysine which was supplemented met, without exceeding, the AA requirements of these early lactation cows for increased milk production, while the failure of plasma lysine levels to increase may suggest that the delivery level of lysine to the intestinal absorptive sites did not meet the lysine requirement for maximum production. In direct contrast to these findings with the early lactation cows, the modest increases in milk production of the mid-lactation cows, except milk fat production, as well as the 24% increase in plasma lysine concentrations, suggests that the delivery level of lysine to the intestinal absorptive sites met the lysine requirement for the mid-lactation cows. As the mid-lactation cows were producing ~10 kg/d less milk, while eating only ~0.7 kg/d less DM, this seems biologically sensible.

Piepenbrink et al. (1996) suggested that an oversupply of methionine and lysine (*i.e.*, 1.5 of estimated requirements) increased fat synthesis, while an intermediate supply did not. It is possible that AA limitations divert some excess AA toward fat synthesis and that supplementation of limiting AA rectified the imbalance thereby increasing milk protein synthesis. Indeed the lack of statistical, or even substantive numerical, differences between treatments in the fatty acid profile of milk fat of our early lactation cows is strong evidence that the increase in milk fat synthesis was by *de novo* processes rather than due to increased digestion of dietary fatty acids from the small intestine.

Cant et al. (2001) reported that excess AA can influence milk fat synthesis when histidine is limiting, but with all other AA present in excess. Swanepoel et al. (2010a) suggested that lysine was the limiting AA during their study and that its supplementation, correcting the AA imbalance/deficiency, diverted nutrients away from milk fat synthesis, thereby reducing milk fat concentrations to normal levels, but that the supplementation level was not high enough to allow a milk production response since lysine was first utilized to support body protein turnover. That supplementation of high levels of intestinally absorbable lysine in the current study resulted in only a small increase of milk production in the mid-lactation cows may be consistent with Piepenbrink et al. (1996) who suggested that lysine was supplied well in excess of AA requirements in their mid-lactation cows, thereby diverting excess AA toward milk fat production *via* gluconeogenesis.

In a study to determine responses of cows to AA imbalances and deficiencies (Weekes et al., 2006) reported increased milk fat yields when AA mixtures devoid of lysine and histidine were infused, but milk protein yield increased after the imbalance was corrected. This seems to be consistent with Piepenbrink et al. (1996). Weekes et al. (2006) also suggested that AA imbalances and deficiencies can be characterized by a low protein:fat ratio in the milk and, because milk protein and fat secretion is variable, the protein:fat ratio of milk may be the most sensitive indicator of a plasma AA imbalance (Cant et al., 2001). This supports the hypothesis that the early lactation cows were adequately supplemented with lysine in our study, while the mid-lactation cows developed an AA deficiency/imbalance since the protein:fat ratio in early cows was the same between treatments at 0.79, while the ratio decreased (0.88 *versus* 0.82) in mid-lactation cows when RPL was supplemented.

A decrease in plasma AA concentrations when a single AA is supplemented suggests that the supplemented AA was first limiting and that its supplementation led to improved absorption and utilization of other AA (Clark, 1975). This was not fully the result for our early lactation cows when RPL was fed, as reductions in AA concentrations, except histidine, were

Table 8
Milk fatty acid concentrations (g/kg) in early lactation cows as influenced by feeding the RPL.

	Control	RPL[a]	SE	P
C4	41.0	40.5	1.32	0.69
C6	20.7	19.8	0.69	0.36
C8	10.7	10.0	2.68	0.27
C9	0.3	0.3	0.04	0.71
C10	22.7	21.7	1.04	0.50
C11	0.4	0.5	0.07	0.54
C12	23.7	23.0	1.03	0.61
C13	1.2	1.2	0.09	0.82
C14	81.8	79.7	2.34	0.42
C14:1 cis	4.8	5.3	0.50	0.35
C15	7.2	7.3	0.36	0.67
C16	273.8	270.1	3.08	0.25
C16:1 trans	2.7	3.2	0.21	0.10
C16:1 cis	9.5	10.4	0.55	0.16
C17	4.8	4.9	0.13	0.40
C17:1 trans	0.3	0.3	0.02	0.47
C18	130.0	131.5	5.71	0.75
C18:1 trans 5	0.4	0.4	0.01	0.40
C18:1 trans 7	0.4	0.4	0.02	0.41
C18:1 trans 6&8	4.5	4.3	0.12	0.18
C18:1 trans 9	4.4	4.5	0.08	0.31
C18:1 trans 10	8.8	8.1	0.77	0.40
C18:1 trans 11	18.7	17.6	0.85	0.14
C18:1 trans 12	6.4	6.5	0.15	0.67
C18:1 trans 13&14	12.7	12.5	0.34	0.57
C18:1 trans 16	5.2	5.2	0.10	0.86
C18:1 cis 9 & 10	206.5	215.0	5.24	0.16
C18:1 cis 11	6.2	6.3	0.25	0.87
C18:1 cis 12	7.3	7.1	0.23	0.36
C18:1 cis 13	1.0	0.9	0.04	0.92
C18:2	36.6	36.3	0.95	0.83
C18:2 cis 9 trans 11	8.8	8.2	0.61	0.35
C18:2 cis 12 trans 10	0.1	0.1	0.02	0.27
C18:2 cis 11 cis 13	0.2	0.1	0.02	0.05
C18:2 all trans	0.3	0.3	0.01	0.51
C18:3	3.1	2.9	0.07	0.03
C18:3 n6	0.4	0.4	0.02	0.94
C20	1.4	1.3	0.06	0.76
C20:3	1.4	1.5	0.07	0.22
C20:4	1.5	1.6	0.06	0.10
C20:5	0.2	0.2	0.02	0.84
C22:4 n6	0.3	0.3	0.02	0.40
C22:5 n3	0.6	0.6	0.14	0.88
Unknown	26.9	27.6	0.67	0.41

Fatty acids below detection levels were: C14:1 trans, C15:1 trans, C15:1 cis, C17:1 cis, C18:1 trans 15, C18:2 trans 8 cis 10, C18:2 cis 11 trans 13, C18:2 cis 9 trans 11, C22:1, C22:2 n6, C22:3 n3, C22:5 n6, C24:1.

[a] Totally mixed ration with the ruminally protected lysine product.

only numerical. But it contrasts to the mid-lactation cows where phenylalanine, tryptophan, arginine and leucine tended ($P<0.10$) to increase and valine, methionine and isoleucine increased numerically, with threonine and histidine unaffected and plasma urea levels increasing sharply. This suggests that another AA became limiting in the mid-lactation cows after lysine was supplemented, thereby preventing utilization of other AA for milk protein synthesis. This further supports the hypothesis of lysine being oversupplied and diverted, at least partly, to support milk fat synthesis with the rest being excreted as urea in the urine at an additional energy cost to the cows. The lack of increased histidine concentrations, while the concentrations of several other AA increased, could be because the diet fed to these cows was limiting in histidine (Cant et al., 2001), causing it to become the next limiting AA after lysine was supplemented, as suggested by Swanepoel et al. (2010b). This is supported by Weekes et al. (2006) who concluded that AA imbalances increased essential AA concentrations in plasma, but attributed it to body protein synthesis being stimulated, or degradation inhibited, siphoning the limiting AA out of plasma.

Since 3-MH concentrations in plasma can be used to assess body protein synthesis and degradation (Blum et al., 1985), and Swanepoel et al. (2010a) suggested, based on the lower plasma 3-MH and leucine concentrations, that supplemented lysine may have increased leucine absorption, thereby stimulating muscle protein turnover in mid-lactation cows. This is consistent with our current study where plasma 3-MH and leucine concentrations in mid-lactation cows tended to increase with RPL supplementation, which could indicate an increase in body protein turnover, possibly due to the histidine limitation and/or deamination and excretion of excess AA. This is also supported by the BCS data, where lysine supplementation led

Table 9
Comparison of estimated intestinal availability of lysine from Swanepoel et al. (2010a) and the current study estimated based upon ruminal *in sacco* incubations and the HP Arg procedure.

Study	Swanepoel et al. (2010a)		Robinson et al. (2010)		Current study	
Technique	*In sacco*	HP Arg	*In sacco*[a]	*In sacco*[b]	*In sacco*	HP Arg
Lysine intake, g/d	41.0	41.0	23.4	24.7	41.2	41.2
Mixing durability[c], proportion	0.49	0.49	0.98	0.98	0.98	0.98
Lysine delivery to the rumen, g/d	20.1	20.1	22.9	24.2	40.4	40.4
In sacco rumen stability[d], proportion	0.58	0.45	0.54	0.42	0.87	0.72
Post-ruminal lysine delivery, g/d	11.7	9.0	12.4	10.2	35.0	29.1
Whole tract indigestibility[e], proportion	0.04	0.04	0.04	0.04	0.36	0.36
Whole tract indigestibility, g/d	1.6	1.6	0.9	1.0	14.4	14.4
Estimated post-ruminal lysine release[f], g/d	10.1	7.4	11.5	9.2	20.6	14.7

[a] This was the RPL product.
[b] This was the RPAA product.
[c] Estimated stability of the RP pellets to mixing and delivery to the feed bunks. For Swanepoel et al. (2010a) this was the value which was determined and reported in the paper, whereas for Robinson et al. (2010) and the current study (where no durability measure was made because of a lack of visual evidence of a loss of durability), these are assumed minimal losses.
[d] 24 h ruminal *in sacco* stability values for '*In sacco*' (all measured in the same way) and the HP-Arg procedure (all measured in the same way) for 'HP-Arg'.
[e] For Swanepoel et al. (2010a) and the current study these were the values which was determined and reported in the papers whereas for Robinson et al. (2010), where whole tract indigestibility of lysine was not measured, the value of Swanepoel et al. (2010a) was assumed due to the similarity of measured 24 h *in sacco* rumen stability.
[f] Calculated as lysine delivery to the intestine (g/d) minus whole tract indigestible lysine (g/d).

to reduced extent of BCS gain for mid-lactation cows. Weekes et al. (2006) showed indirect evidence which suggested that turnover of body protein was stimulated (*i.e.*, degradation inhibited) when some essential AA were absent from infusion, is consistent with results from our early lactation cows in which lysine supplementation increased BCS, and changed a BCS loss to a BCS gain.

5. Conclusions

Feeding a ruminally protected lysine to deliver 15–21 g/d of intestinally absorbable lysine increased yields of milk, fat, protein and lactose in early lactation cows, suggesting that it was the first limiting AA and that its supplementation fulfilled an AA need of the cows but, due to the lack of an increase in plasma lysine, that it still may not have been fed at levels high enough to meet their lysine requirement. In contrast, feeding the RPL to mid-lactation cows had little effect on milk yield and, due to a large increase in plasma lysine, the lysine was likely fed to these cows at levels which exceeded their lysine requirement.

Integration of our results with those of our other AA supplementation studies (Swanepoel et al., 2010a; Robinson et al., 2010) suggests that lysine was the limiting AA in all of these studies (as suggested by the SHIELD (Robinson, 2009) metabolic analysis in Swanepoel et al., 2010b), but that low level lysine supplementation is preferentially used to support body protein turnover in early lactation cows, thereby diverting AA from gluconeogenesis and suppressing milk fat synthesis. However if lysine supplementation levels are increased, as in this study, and lysine needs for body protein turnover are met, lysine is then used to support milk protein synthesis thereby freeing energetic precursors to support milk lactose synthesis. Thus the relatively smaller productive benefit of our mid-lactation cows to RPL feeding suggests that another essential AA may have become limiting when lysine needs were met. Since the early and mid-lactation cows received exactly the same ration, and essentially the same level of RPL supplementation, this confirms that AA utilization and requirements are highly dependent on stage of lactation and that separation of lactation stages during AA supplementation studies are essential.

Acknowledgements

This study was conducted on Rib-Arrow farm in Tulare (CA, USA). The authors thank Dave Ribeiro and his farm crew for their cooperation and interest and Dr. Tom Fronk for careful ration formulation, as well as Dr. E.J. DePeters and Mr. S. Taylor for milk fatty acid analysis.

References

AOAC, 2006. Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Arlington, VA, USA.
Blauwiekel, R., Xu, S., Harrison, J.H., Loney, K.A., Riley, R.E., Calhoun, M.C., 1997. Effect of whole cottonseed, gossypol, and ruminally protected lysine supplementation on milk yield and composition. J. Dairy Sci. 80, 1358–1365.
Blum, J.W., Reding, T., Jans, F., Wanner, M., Zemp, M., Bachmann, K., 1985. Variations of 3-methylhistidine in blood of dairy cows. J. Dairy Sci. 68, 2580–2587.
Cant, J.P., Trout, D.R., Qiao, F., McBride, B.W., 2001. Milk composition responses to unilateral arterial infusion of complete and histidine-lacking amino acid mixtures to the mammary glands of cows. J. Dairy Sci. 84, 1192–1200.
Clark, J.H., 1975. Lactational responses to postruminal administration of proteins and amino acids. J. Dairy Sci. 58, 1178–1197.
DePeters, E.J., German, J.B., Taylor, S.J., Essex, S.T., Perez-Monti, H., 2001. Fatty acid and triglyceride composition of milk fat from lactating Holstein cows in response to supplemental canola oil. J. Dairy Sci. 84, 929–936.

Dhiman, T.R., Anand, G.R., Satter, L.D., Pariza, M.W., 1999. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82, 2146–2156.
Ferguson, J.D., Galligan, D.T., Thomsen, N., 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77, 2695–2703.
Johansen, H.N., Glitsø, V., Knudsen, K.E.B., 1996. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. J. Agric. Food Chem. 44, 1470–1474.
Johnson, C.M., Ulrich, A., 1959. Analytical Methods for Use in Plant Analysis. Bulletin 766. University of California, Agricultural Experiment Station, Berkeley, CA, USA, pp. 26–78.
Meyer, G.A., Keliher, P.N., 1992. Overview of analysis by inductively coupled plasma-atomic emission spectrometry. In: Montaser, A., Golightly, D.W. (Eds.), Inductively Coupled Plasmas in Analytical Atomic Spectrometry. VCH Publishers Inc., New York, NY, USA, pp. 473–516.
National Research Council, 2001. Nutrient Requirements of Dairy Cattle, 7th Revised Edition. National Academy Press, Washington, DC, USA.
Nocek, J.E., Miura, M., Shinzato, I., 2010. The effect of feeding a prototype protected lysine (RPL) on production performance and plasma amino acid profile of early lactation dairy cattle. In: ADSA Annual Meeting, Abstract T148.
Piepenbrink, M.S., Overton, T.R., Clark, J.H., 1996. Response of cows fed a low crude protein diet to ruminally protected methionine and lysine. J. Dairy Sci. 79, 1638–1646.
Robinson, P.H., Swanepoel, N., Evans, E., 2010. Effects of feeding a ruminally protected lysine product, with or without isoleucine, valine and histidine, to lactating dairy cows on their productive performance and plasma amino acid profiles. Anim. Feed Sci. Technol. 161, 75–84.
Robinson, P.H., 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: a systematic review of the literature. Livest. Sci. 127, 115–126.
Robinson, P.H., 2009. SHIELD Dairy Ration Evaluator. Department of Animal Science, UC Davis, Davis, CA, USA.
Statistical Analysis Systems, 2004. SAS Institute Inc., SAS/STAT® Software: Changes and Enhancements, Release 8.1, Cary, NC, USA.
Smith, D., 1981. Removing and analyzing total non-structural carbohydrates from plant tissue. Agric. Bull. R2107, Res. Div., Coll. of Agric. and Life Sciences, Univ. of Wisconsin, Madison, WI, USA.
Swanepoel, N., Robinson, P.H., Erasmus, L., 2010b. Amino acid needs of lactating dairy cows: predicting limiting amino acids in contemporary rations fed to high producing dairy cattle in California using metabolic models. Anim. Feed Sci. Technol. 161, 103–120.
Swanepoel, N., Robinson, P.H., Erasmus, L., 2010a. Amino acid needs of lactating dairy cows: impact of feeding lysine in a ruminally protected form on productivity of lactating dairy cows. Anim. Feed Sci. Technol. 157, 79–94.
Tracy, M.L., Moeller, G., 1990. Continuous flow vapour generation for inductively coupled argon plasma spectrometric analysis. Part 1. Selenium. J. Assoc. Off. Anal. Chem. 73, 404–410.
Trináctý, J., Krízová, L., Richter, M., Cerný, V., Ríha, J., 2009. Effect of rumen-protected methionine, lysine or both on milk production and plasma amino acids of high-yielding dairy cows. Czech J. Anim. Sci. 54, 239–248.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Watanabe, K., Fredeen, A.H., Robinson, P.H., Chalupa, W., Julien, W.E., Sato, H., Suzuki, H., Katoh, K., Obara, Y., 2006. Effects of fat coated rumen bypass lysine and methionine on performance of dairy cows fed a diet deficient in lysine and methionine. Anim. Sci. J. 77, 495–502.
Weekes, T.L., Luimes, P.H., Cant, J.P., 2006. Responses to amino acid imbalances and deficiencies in lactating dairy cows. J. Dairy Sci. 89, 2177–2187.