

Carryover effects of 4 daily rumen drenches of maize gluten meal and rumen protected essential amino acids initiated immediately after calving

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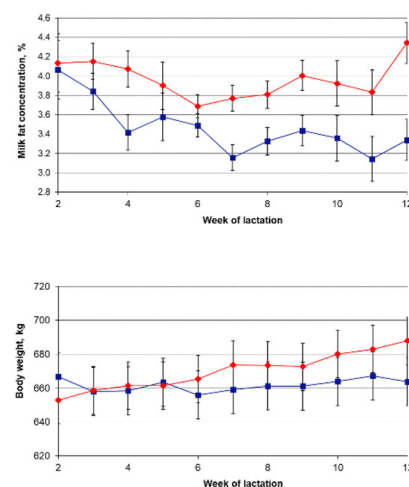
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Carryover effects of 4 daily rumen drenches of maize gluten meal and rumen-protected essential amino acids initiated immediately after calving

C. K. Reynolds,^{1*}  L. A. Crompton,¹  A. K. Jones,¹ and C. G. Bartram²

Graphical Abstract

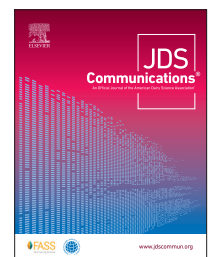


Summary

Cows experience their greatest deficit of metabolizable protein relative to requirements in very early lactation. Previously, abomasal infusion of casein starting immediately after calving markedly increased milk yield with positive carryover effects. In this study, a rumen drench of protein and essential amino acids for the first 4 days in milk (DIM) given within 8 hours of calving did not affect the milk yield of Holstein cows, but increased milk fat and protein concentration for the subsequent 12 weeks of lactation. Treated cows gained body weight (BW) over the course of the study, whereas the BW of controls remained constant. These results suggest homeorhetic effects of protein supplementation very early in lactation that warrant further investigation.

Highlights

- Four daily rumen protein drenches increased milk fat concentration for 12 weeks.
- Milk protein concentration tended to be increased by rumen protein drenches.
- The rumen protein drench did not affect milk yield or DMI.
- Treated cow BW increased with DIM, but BW was unchanged for control cows.



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Carryover effects of 4 daily rumen drenches of maize gluten meal and rumen-protected essential amino acids initiated immediately after calving

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Abstract: The objective was to determine the effects of a daily rumen drench of maize gluten meal, as a source of RUP, and rumen-protected Met, Lys, and His for the first 4 d of lactation on DMI and milk production and composition of dairy cows during their first 12 wk of lactation. Twenty multiparous Holstein cows were randomly assigned at calving to a control rumen drench of 30 L of warm water or a 30-L aqueous suspension of maize gluten meal and rumen-protected Met, Lys, and His calculated to provide Met, Lys, His, and Leu for absorption equal to their amounts in 600 g of casein. Cows received their first drench within 8 h of calving and then again 24, 48, and 72 h later. Cows were fed a TMR for ad libitum consumption and at 7 DIM cows were moved from calving boxes to a cubicle yard, and measurements of daily DMI and milk yield and weekly milk composition, BW, and BCS were obtained through wk 12 postpartum. A blood plasma sample obtained at 7 DIM was analyzed for metabolite, protein, and albumin concentration. There was no effect of treatment on DMI or milk yield, but milk fat concentration was higher and milk protein concentration tended to be higher for treated cows during wk 2 to 12. While BCS was not affected, treated cows gained BW over the course of the study (+32 kg), whereas control cows maintained a similar BW (−3 kg). Plasma metabolite and protein concentrations were not affected by treatment. Four daily rumen doses of supplemental RUP and rumen-protected EAA, initiated within hours of calving, had sustained positive effects on milk fat and protein concentration and BW of lactating Holstein cows, suggesting homeorhetic effects that warrant further investigation.

Lactating dairy cows typically experience a period of negative protein balance (MP intake minus milk protein yield) in the first weeks of lactation, with the nadir occurring within days of calving (Bell et al., 2000). The negative balance of protein during very early lactation suggests that MP supply may limit milk and milk protein yield. Abomasal infusion of casein (Larsen et al., 2014) or an equivalent amount of free AA (Larsen et al., 2013, 2015; Galindo et al., 2015), starting immediately after calving, markedly increased milk yield of Holstein dairy cows. Infusions began within the first 12 h after calving and normally within 6 h (M. Larsen, Aarhus University, Foulum, Denmark, personal communication) and were achieved by inserting an infusion line through a rumen fistula and reticulo-omasal orifice. Infusions were initially equivalent to 678 g (Larsen et al., 2014) or 795 g (Larsen et al., 2015) casein protein daily and then incrementally reduced over 4 wk as feed intake increased. Over the course of these studies, milk yield increased by an average of 7.8 to 8.4 kg/d for cows receiving supplemental casein or AA compared with a control infusion. Estimated MP supply was similar for infused and control cows at 28 DIM, but the milk yield response was immediate and maintained throughout the remainder of both experiments, suggesting carryover effects. This compares to a milk yield response of 3.4 kg/d when cows received supplemental rumen-protected (rp) Met as a feed top-dressing for 3 wk before and 4 wk after calving (Osorio et al., 2013). In other work, supplemental rpMet and Lys for 3 wk before and after calving had no effect on milk yield, but increased milk protein concentration in wk 1 and 2 postpartum (Lee et al.,

2019). The greater milk yield response to casein or a mixture of AA based on casein suggests a requirement for AA in addition to Met and Lys for the milk yield response observed in the studies of Larsen et al. (2014, 2015). In this regard, abomasal infusion studies of Haque et al. (2012) found that Met, Lys, His, and Leu accounted for virtually all of the milk protein yield response to a mixture of EAA based on an “ideal” mixture of EAA proposed by Rulquin et al. (2007). In the case of the abomasal infusion studies beginning within hours of calving, we hypothesize that casein or AA infusion may have increased milk yield through the provision of limiting AA, but also through mammary cell-signaling effects that could have had carryover effects on subsequent milk composition and yield. Therefore, our objective was to determine if an oral rumen drench of maize gluten meal as a source of RUP and rpMet, rpLys, and rpHis for 4 d immediately after calving would have carryover effects on milk yield or composition during the first 12 wk of lactation, compared with cows receiving a water control treatment.

All animal procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 (<https://www.legislation.gov.uk/ukpga/1986/14/contents>) following approval by the local animal welfare and ethical review board. Based on the replication (n = 4 per treatment) used in the study of Larsen et al. (2014), 20 multiparous Holstein cows beginning their second, third, or fourth lactation (mean parity 2.9 for both treatments) were randomly assigned to one of 2 treatments at calving in pairs with equal parity. Animals experiencing any calving difficulties were excluded. The cows were housed in a straw-bedded group

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close-up pen for 3 wk before expected calving date and moved to a straw-bedded group maternity pen (42 m × 15 m) for 7 d after calving, with continuous access to fresh water. Stocking density of the maternity pen varied according to calving rate, but a typical number would be 7 cows. Before calving, they were fed a transition diet (40% maize silage, 16% grass silage, 20% chopped barley straw, 20% lactation ration concentrates, plus minerals and dietary anions) for ad libitum consumption. After calving, they were fed once daily a TMR ad libitum (at least 5% refusals) formulated to meet nutrient requirements and comprising 15% grass silage, 35% maize silage, 1.5% chopped barley straw, and 48.5% concentrates with supplemental minerals and vitamins. The lactation concentrates included rolled wheat, unmolassed sugar beet pulp, soybean hulls, wheat feed (similar to wheat middlings), soybean meal, and rapeseed meal and the TMR was formulated to contain 16% CP, 24% starch and sugars, and 38% NDF.

Treatments were a control rumen drench of 30 L of warm (36°C) tap water or a suspension of 1,765 g of maize gluten meal (Mole Valley Farmers, UK), 5.5 g of MetaSmart (Kemin Industries, South Port, UK; provided by Mole Valley Farmers, UK), 196 g of AjiPro-L (Ajinomoto Co. Inc., Japan; provided by Mole Valley Farmers, UK), and 52 g of a rpHis product (provided by Ajinomoto Co. Inc., Japan) in 30 L of 36°C tap water. The maize gluten meal used contained (as-fed basis) 54.0% CP, 8.83% ether extract, 1.4% ash, 0.5% crude fiber, and 89.9% DM. The amounts provided were based on providing (exceeding in the case of Leu) the Met, Lys, His, and Leu in 600 g of casein (17.6, 49.3, 17.8, and 58.1 g, respectively, reaching the small intestine for absorption), estimates of the RUP (39%) and Met, Lys, His, and Leu composition of maize gluten meal protein (2.4%, 1.7%, 2.1%, and 16.1%, respectively), and estimates of the absorbable AA concentration of the rumen-protected AA products provided by their manufacturer. Drenches were mixed by hand throughout the addition of maize gluten meal followed by AA products and used immediately. Drenching was completed within 3 min and there was no residue. Rumen drenches were given using a commercial veterinary rehydration apparatus (SELEKT Pump-Drencher, Nimrod Veterinary Products Ltd., Moreton-in-Marsh, UK). Rumen drenches were administered as soon as feasible and within 8 h after calving and were then repeated 24, 48, and 72 h later. Rumen drenches were used to be sure cows received the treatments in full, as preliminary attempts to deliver the treatment supplements to freshly calved cows as a TMR top-dressing were not successful.

On d 7 postpartum, cows were moved to loose housing with sand-bedded cubicles and continuous access to fresh water and individually fed at 0900 h using a Calan Broadbent (American Calan, Northwood, NH) gate system to measure DMI as described previously (Hammond et al., 2016). Cows were milked twice daily (approximately 0600 and 1600 h) on a 50-point rotary parlor (Dairy Master UK Ltd., Bromsgrove, UK) with electronic milk yield recording. Milk samples were collected weekly at 2 consecutive milkings beginning at the beginning of wk 2 postpartum. Samples were preserved with potassium dichromate and stored refrigerated before submission for analysis for concentrations of fat, CP, lactose, casein, MUN, and somatic cells by infrared spectrometry (Foss Electric Ltd., York, UK; National Milk Laboratories, Wolverhampton, UK). Daily 3.5% FCM and ECM yields were calculated as described by Gaines (1928) and Gaillard et al. (2016), respectively. Measurements of BW were obtained weekly

using an electronic scale as cows exited the milking parlor for 4 consecutive milkings and BCS was determined weekly by a trained assessor using a 9-point (1 to 5 in 0.5 increments) scale (Mulvaney, 1977). Measurements were obtained through wk 12 after calving.

A blood plasma sample was taken at 1100 h from the tail vein (or artery) using an EDTA-treated Vacutainer on d 7 postpartum and analyzed using an iLab Aries clinical analyzer (Instrumentation Laboratory, Italy) for concentrations of BHB (Williamson and Mellanby, 1974), nonesterified fatty acids (NEFA-HR, Wako Diagnostics, USA), urea (IL Test Urea Nitrogen, Instrumentation Laboratory, Italy), glucose (IL Test Glucose Oxidase, Instrumentation Laboratory, Italy), gamma-glutamyltransferase (IL Test Gamma-GT Liquid, Instrumentation Laboratory, Italy), albumin (bromocresol green, IL Test Albumin, Instrumentation Laboratory, Italy), and total protein (modified biuret, IL Test Total Protein, Instrumentation Laboratory, Italy).

Weekly averages for response variables were statistically analyzed using mixed models procedures of SAS testing fixed effects of parity, treatment, week, parity by treatment interaction, and treatment by week interaction and random effects of cow within treatment using the Kenward-Rogers option for determining degrees of freedom, with week as a repeated effect. Previous 305-d milk yield and genetic index (PIN: a profit index based on production traits) were included in statistical models as covariates. For plasma metabolites and the change in BW from wk 2 to 12, the data were analyzed using a model that included treatment, parity, and treatment by parity interaction, with previous 305-d milk yield and PIN included as covariates. The covariance structure for the repeated effect of week was chosen based on Akaike information criterion corrected for small sample size (AICC) goodness-of-fit criteria. The covariance structures used were compound symmetry (FCM, ECM, milk casein concentration, milk protein yield, milk fat yield), compound symmetry heterogeneous (milk protein percentage, BCS), autoregressive (DMI, milk lactose yield, milk urea concentration, BW), or autoregressive heterogeneous (milk yield, milk fat concentration, milk lactose concentration, SCC). The variance components structure was not chosen for any variables. In addition, the slice option was used to test for significant effects of treatment on a weekly basis. When the animal phase of the study was completed, only 2 cows included were third parity, and the third-parity cow assigned to the control treatment had the lowest genetic index of cows on the study, while the third parity cow assigned to the rumen-dose treatment had the highest genetic index and had the highest milk yield of cows on the study. Because of this lack of third-parity replication and treatment bias these 2 third-parity cows were excluded from the statistical analyses for the results reported, leaving 5 second-lactation cows and 4 fourth-lactation cows assigned to each treatment in the final analysis. Least squares means from the mixed models analysis are presented.

There was no effect of treatment on DMI or milk, FCM, or ECM yield (Table 1), but there was a treatment by week interaction ($P < 0.036$) for ECM, which was numerically lower for cows receiving supplemental protein in wk 2 and 3 and numerically higher in wk 7 to 12 (Figure 1). Milk fat concentration was greater ($P < 0.001$) and milk protein and casein concentrations tended to be greater ($P < 0.10$ and $P < 0.08$, respectively) for protein-supplemented cows throughout wk 2 to 12 (Table 1). Although BCS was not affected, protein-supplemented cows gained BW over the course of the

Table 1. Dry matter intake, milk production and composition, BW, BCS, and plasma metabolite concentrations

Item	Control	Treated	SEM ²	<i>P</i> < ¹		
				Trt	Week	Trt × week
DMI, kg/d	26.3	26.7	0.49	0.506	0.001	0.106
Milk yield, kg/d	44.3	42.1	2.02	0.453	0.001	0.354
3.5% FCM, kg/d	44.2	45.6	1.82	0.600	0.165	0.105
ECM, kg/d	43.8	44.4	1.75	0.831	0.016	0.036
Milk fat, %	3.47	3.97	0.097	0.001	0.077	0.314
Milk protein, %	3.07	3.26	0.073	0.091	0.001	0.447
Milk lactose, %	4.51	4.53	0.019	0.582	0.214	0.590
Milk casein, %	2.36	2.51	0.053	0.074	0.001	0.278
Milk fat, g/d	1,535	1,645	61.9	0.226	0.703	0.145
Milk protein, g/d	1,368	1,358	50.3	0.895	0.001	0.193
Milk lactose, g/d	2,002	1,907	97.7	0.496	0.001	0.219
Milk urea, mg/L	329	337	9.8	0.603	0.001	0.957
SCC, × 1,000/mL	64.4	150.1	53.3	0.277	0.568	0.284
BW, kg	661	670	13.3	0.661	0.321	0.098
BW change, ³ kg	−5.1	35.3	11.0	0.022		
BCS (1–5)	2.54	2.51	0.036	0.582	0.061	0.463
Plasma concentration at 7 DIM						
Albumin, g/L	35.9	35.4	0.65	0.614		
BHB, μM	860	672	83.9	0.915		
γ-Glutamyl transferase, U/L	24.8	25.6	1.44	0.710		
Glucose, mM	3.50	3.48	0.124	0.944		
Nonesterified fatty acids, μM	711	565	108.8	0.351		
Total protein, g/L	69.6	71.3	1.33	0.359		
Urea, mM	5.21	4.95	0.321	0.580		
n	9	9				

¹Probability of no effect of treatment (Trt), a rumen drench of protein and EAA for the first 4 d of lactation.

²Standard error of control mean (n = 9).

³Week 12 minus wk 2.

study (+35 kg), and control cows maintained a similar BW (−5 kg; $P < 0.03$; Figure 1). There was a treatment by parity interaction ($P < 0.013$) for weekly BW measurements, with the difference in BW change being greater in fourth- compared with second-parity cows (parity by treatment interaction for BW change, $P = 0.387$). Treatment by parity interaction was not significant ($P > 0.211$) for other variables, but there was a numerical increase in DMI (1.5 kg/d) with treated fourth-parity cows, but a slight decrease on average (−0.38 kg/d) in second-parity cows. Previous 305-d milk yield ($P < 0.05$) and parity ($P < 0.002$) also affected BW, but were not significant covariates for other variables. Genetic index only had covariate effects on DMI ($P < 0.023$) and milk yield ($P < 0.065$). There was no effect of protein supplementation on concentrations of metabolites and proteins in plasma samples obtained on d 7 of lactation (Table 1).

The lack of response of milk and milk protein yield in the present study compared with the studies reported by Larsen et al. (2014, 2015) may be due to several reasons, including the short-term nature of the supplementation employed in our study (4 vs. 28 d) and uncertainty concerning the extent to which the AA provided via rumen drenching were absorbed in the small intestine as the degree of rumen degradability and digestibility in the small intestine used to calculate the amounts provided were estimates. In the abomasal infusion studies of Larsen et al. (2014, 2015), there was a continuous postruminal supply of highly digestible protein, whereas a single daily rumen dose may have resulted in a short-term increase in AA absorption and elevation of plasma EAA over the course of each day. In this regard, it is unlikely that the profile of EAA supply achieved in the present study using a single daily rumen dose of

maize gluten meal and supplemental rpMet, rpLys, and rpHis was comparable to the pattern of EAA supply achieved by continuous abomasal infusion of casein. In addition, as discussed previously, Haque et al. (2012) demonstrated that Met, Lys, His, and Leu were responsible for virtually all the milk protein response to supplemental MP in dairy cows, but it may be that in very early lactation other EAA are also required to elicit a milk and milk protein yield response. In a more recent study in multiparous Holstein cows, Bahloul et al. (2022) used a similar abomasal infusion protocol as that used by Larsen et al. (2015) to infuse either the total amino acids (TAA) or only the EAA contained in 805 g/d of casein, with infusions beginning immediately after calving and then incrementally reduced over 35 successive DIM. Over the course of 50 DIM, milk yield was greater for TAA than for EAA (47.9 vs. 39.3 kg/d, respectively), indicating that the milk response to supplemental EAA at the onset of lactation, when body protein balance is most negative (Bell et al., 2000), is greater with the additional provision of supplemental NEAA.

Whereas milk and milk fat and protein yield were not affected by the rumen protein drench in the present study, there was a positive effect of treatment on milk fat and protein concentration that became more evident as lactation progressed, such that ECM yield was numerically greater at the end of the sampling period (Figure 1). Reasons for this response are not certain, but the present results and those of previous studies (Larsen et al., 2014, 2015) suggest a carryover metabolic effect, perhaps due to cell-signaling (e.g., mTORC1 effects of Leu; Arriola Apelo et al., 2014) or hormonal responses to supplemental AA that resulted in a positive carryover effect on milk composition.

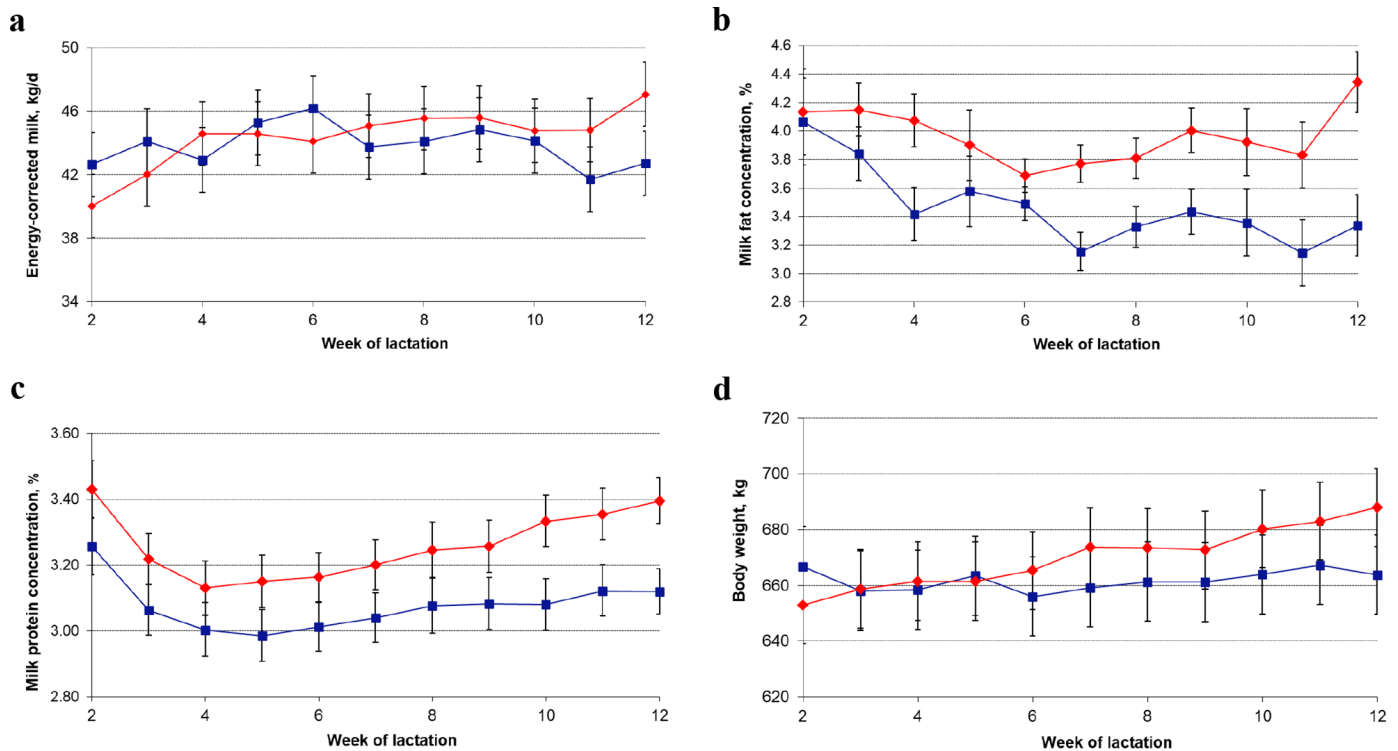


Figure 1. Energy-corrected milk yield (a), milk fat concentration (b), milk protein concentration (c), and BW (d) of cows receiving a control (blue square) or protein and EAA (red diamond) rumen drench for the first 4 d of lactation. Error bars indicate SEM of LSM.

The effect of protein supplementation on BW also suggests a positive homeorhetic effect on metabolism of body tissues and thus BW arising from an increased EAA supply in the first days postpartum. Reasons for the effect on BW are not certain, but in a meta-analysis of published studies showing the effects of dietary composition on lactation performance (Hristov et al., 2005), there were significant positive correlations between BW and diet concentrations of CP, RUP, and RDP, which were associated with positive correlations with DMI. In another study, feeding rpMet and rpHis for 8 wk significantly increased BW gain in early- to mid-lactation dairy cows and this was attributed to a reduced reliance on body protein mobilization to support milk protein synthesis (Giallongo et al., 2015). In the present study, the effect of the rumen drench of protein and EAA on BW was a carryover effect of the 4-d drench treatment that was in part associated with the numerical increase in diet DMI in fourth-lactation cows.

In a recent study, lactating dairy cows were fed supplemental MP, or MP with rpMet and rpLys, from d 3 postpartum for 3 wk (Carder and Weiss, 2017). Supplementation did not affect milk yield, but milk fat concentration was increased by both treatments and dietary supplementation of MP with rpMet and rpLys, but not MP alone, increased milk protein concentration. The effects of MP supplementation on milk fat and protein concentration were maintained for a further 63 d after cessation of MP supplementation (Carder and Weiss, 2017), suggesting the effects were due to more than the supply of EAA for protein synthesis per se, as in the present study. These carryover effects of supplemental EAA in early lactation on milk fat and protein concentration warrant

further investigation to determine the nature of the response, the underlying mechanisms, and the optimum AA composition, dose, and timing for sustained and maximal effects on milk composition and yield and BW.

In conclusion, daily rumen doses of maize gluten meal and rpMet, rpLys, and rpHis provided in the first 4 d of lactation had no effects on milk yield but had positive effects on milk fat and protein concentration and BW that were maintained until wk 12 postpartum. These results suggest positive homeorhetic effects of supplemental metabolizable EAA immediately postpartum in mammary and other body tissues that warrant further investigation.

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Notes

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Nonstandard abbreviations used: PIN = profit index; rp = rumen-protected; TAA = total amino acids.