

Effects of Milk Replacer Protein Sources on Nutrient Digestion and Rumen Fermentation in Early-Weaned Calves: Postprint

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Abstract

This experiment aimed to investigate the effects of different protein sources in milk replacer on nutrient digestion and rumen fermentation in early-weaned calves. Fifty Chinese Holstein bull calves with similar body weight and aged (21±5) days were selected and randomly divided into 5 groups with 10 calves per group. The control group (MP group) received milk replacer containing milk-derived protein, while the experimental groups received milk replacer containing plant-derived protein and milk-derived protein [crude protein (CP) ratio of 70:30]. The plant-derived proteins were soy protein concentrate (SP group), modified wheat protein (WP group), peanut protein concentrate (PP group), and rice protein isolate (RP group). The experimental period lasted 42 days. Rumen fluid was collected at 28, 42, and 56 days of age to determine rumen fermentation parameters; digestion and metabolism trials were conducted at 29-35 days and 57-63 days of age, respectively. The results showed: 1) There was no significant difference in average dry matter intake (DMI) among groups from 22 to 63 days of age ($P > 0.05$); DMI in SP and RP groups was significantly higher than that in MP, WP, and PP groups at 57-63 days of age ($P < 0.05$). 2) At 29-35 days of age, organic matter (OM) digestibility in the MP group was significantly higher than that in WP, PP, and RP groups ($P < 0.05$); crude protein (CP) digestibility in the MP group was significantly higher than that in the other four groups ($P < 0.05$), while SP and RP groups were significantly higher than WP and PP groups ($P < 0.05$), and WP group was significantly higher than PP group ($P < 0.05$); ether extract (EE) digestibility in MP, SP, and RP groups was significantly higher than that in WP and PP groups ($P < 0.05$); calcium (Ca) digestibility in MP and RP groups was significantly higher than that in WP and PP groups ($P < 0.05$); phosphorus (P) digestibility in the MP group was significantly higher than that in SP, WP, and PP groups ($P < 0.05$). At 57-63 days of age, OM digestibility in the MP group was significantly

higher than that in WP and PP groups ($P < 0.05$); CP digestibility in the MP group was significantly higher than that in WP, PP, and RP groups ($P < 0.05$); Ca digestibility in the MP group was significantly higher than that in SP and WP groups ($P < 0.05$); there was no significant difference in EE digestibility between the MP group and the four groups SP, WP, PP, and RP ($P > 0.05$), and there was no significant difference in P digestibility among all groups ($P > 0.05$). 3) At 28 and 42 days of age, there were no significant differences in any rumen fermentation parameters among groups ($P > 0.05$); at 56 days of age, rumen fluid pH in SP and RP groups was significantly lower than that in WP and PP groups ($P < 0.05$), total volatile fatty acid (TVFA) concentration showed no significant difference between the MP group and the other four groups ($P > 0.05$), but PP group was significantly lower than SP and RP groups ($P < 0.05$); propionate and butyrate concentrations in SP and RP groups were significantly higher and lower than those in MP, WP, and PP groups, respectively ($P < 0.05$), isovalerate and valerate concentrations in the MP group were significantly lower than those in all other groups ($P < 0.05$). In conclusion, under the present nutritional regulation conditions, digestibility of plant-derived protein was lower than that of milk-derived protein in calves, and the difference in digestibility decreased with increasing age; among plant-derived proteins, soy protein had relatively high digestibility; compared with milk-derived protein, plant-derived protein could promote earlier rumen development in calves.

Full Text

Effects of Protein Source in Milk Replacer on Nutrient Digestion and Rumen Fermentation of Early-Weaner Calves

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Abstract: This study investigated the effects of different protein sources in milk replacer on nutrient digestion and rumen fermentation in early-weaner calves. Fifty Chinese Holstein bull calves with similar body weight at (21±5) days of age were randomly allocated to 5 groups (n=10). The control group (MP group) received milk replacer containing milk-derived protein, while experimental groups received milk replacer with a 70:30 ratio of plant-derived protein to milk-derived protein [crude protein (CP) basis]. Plant protein sources included soybean protein concentrate (SP group), modified wheat protein (WP group), peanut protein concentrate (PP group), and rice protein isolate (RP group). The 42-day trial included rumen fluid collection at 28, 42, and 56 days of age, and digestion-metabolism trials during 29-35 and 57-63 days of age. Results showed: 1) No significant differences in average dry matter intake (DMI) from

22–63 days of age among groups ($P>0.05$), though DMI during 57–63 days was significantly higher in SP and RP groups compared to MP, WP, and PP groups ($P<0.05$). 2) During 29–35 days of age, OM digestibility in the MP group was significantly higher than WP, PP, and RP groups ($P<0.05$); CP digestibility in the MP group was significantly higher than all other groups ($P<0.05$), with SP and RP groups significantly higher than WP and PP groups ($P<0.05$), and WP significantly higher than PP ($P<0.05$); EE digestibility in MP, SP, and RP groups was significantly higher than WP and PP groups ($P<0.05$); Ca digestibility in MP and RP groups was significantly higher than WP and PP groups ($P<0.05$); P digestibility in the MP group was significantly higher than SP, WP, and PP groups ($P<0.05$). During 57–63 days of age, OM digestibility in the MP group was significantly higher than WP and PP groups ($P<0.05$); CP digestibility in the MP group was significantly higher than WP, PP, and RP groups ($P<0.05$); Ca digestibility in the MP group was significantly higher than SP and WP groups ($P<0.05$); EE digestibility showed no significant differences between MP and other groups ($P>0.05$); P digestibility did not differ significantly among groups ($P>0.05$). 3) No significant differences in rumen fermentation parameters among groups at 28 and 42 days of age ($P>0.05$). At 56 days of age, rumen fluid pH in SP and RP groups was significantly lower than WP and PP groups ($P<0.05$). Total volatile fatty acids (TVFA) concentration in the MP group did not differ significantly from other groups ($P>0.05$), but PP group was significantly lower than SP and RP groups ($P<0.05$). Propionate and butyrate concentrations in SP and RP groups were significantly higher and lower, respectively, than MP, WP, and PP groups ($P<0.05$), while isovalerate and valerate concentrations in the MP group were significantly lower than all other groups ($P<0.05$). In conclusion, under these nutritional conditions, plant protein digestibility was lower than milk protein in calves, though this difference decreased with age. Soybean protein showed higher digestibility among plant proteins, and plant protein sources promoted earlier rumen development compared to milk protein.

Keywords: calf; plant-derived protein; milk replacer; digestion; metabolism; rumen

The protein source in animal feed directly affects diet quality and rearing costs, making it a critical consideration in animal production [1]. Modern dairy farming commonly practices early weaning of calves using milk replacers. Numerous studies have investigated protein sources for calf milk replacers, with protein ingredients primarily classified into milk protein and non-milk protein categories [citation not found]. Milk-derived protein is considered the “optimal” protein source for calf diets due to its high digestibility, balanced amino acid composition, and low antinutritional factor levels. Recent research on soybean protein products for calf milk replacers has demonstrated comparable effects to milk protein [3–6]. Plant proteins from wheat, peanut, and rice exhibit similar nutritional characteristics to soybean protein and are produced in substantial quantities in

China.

Developing the application potential of these plant protein sources to expand available protein resources for calf milk replacers is of significant importance. This study systematically investigated the effects of different protein sources in milk replacer on nutrient digestion, metabolism, and rumen fermentation in early-weaner calves by formulating five milk replacers using four plant protein sources (soybean, wheat, peanut, rice) and milk protein, with several major limiting amino acids maintained in relative balance, to evaluate the efficacy of various plant protein sources.

1.1 Experimental Animals

The trial was conducted at Beijing Zhuochen Livestock Co., Ltd. Fifty healthy Chinese Holstein bull calves at (21 ± 5) days of age with body weight of (46 ± 6) kg were selected and divided into 5 groups (1 control and 4 experimental groups, $n=10$) based on equivalent body weight and age.

1.2 Experimental Design and Diets

A single-factor randomized design was employed. Five milk replacers were formulated using four plant protein sources (soybean protein concentrate, modified wheat protein, peanut protein concentrate, rice protein isolate) and milk protein (whole milk powder and skim milk powder) as primary protein sources, containing 22% CP, 19.66 MJ/kg gross energy (GE), Lys:Met:Thr:Trp=100.0:29.50:65.0:20.5, and Lys=1.84%. Amino acid levels were adjusted by adding crystalline amino acids to the basal diet.

The control group (MP group) received milk protein-based milk replacer, while experimental groups received milk replacer with protein provided by a 70:30 ratio of plant protein to milk protein based on total CP content. The four plant protein sources were soybean protein concentrate (CP=65.2%), modified wheat protein (CP=77.8%), peanut protein concentrate (CP=54.7%), and rice protein isolate (CP=82.0%), designated as MP, SP, WP, PP, and RP groups, respectively. All groups received the same starter feed. Nutrient composition of milk replacer and starter feed is shown in Table 1 .

The trial lasted 42 days. Digestion-metabolism trials were conducted during 29-35 and 57-63 days of age, with 4 calves selected per group for each period, including a 4-day preliminary period and 3-day collection period.

1.3 Animal Management

Selected calves were fed 3 kg of colostrum within 12 h after birth, followed by colostrum and fresh milk at 10% of body weight. A transition period from 15-20 days of age gradually increased the milk replacer to milk ratio from 1:3 to 3:1, with complete milk replacer feeding by 21 days of age.

Milk replacer was mixed with boiled water cooled to 40-50°C at a 1:7 ratio (m/m), stirred thoroughly into emulsion, and fed twice daily (08:00, 18:30). Calves had ad libitum access to water 30 min after feeding. Daily milk replacer emulsion was provided at 10% of body weight, adjusted weekly according to weight gain.

Starter feed was introduced at 3 weeks of age, with daily allowances of 400 g and 800 g per calf during 4-5 and 6 weeks of age, respectively, followed by ad libitum feeding during 7-9 weeks. Daily feed refusals were recorded. Calves were individually housed in pens (2.25 m² each) by group. Calf barns were disinfected weekly with quicklime. Nutrient levels and composition of milk replacer and starter feed are shown in Table 1.

1.4 Sample Collection and Analysis

1.4.1 Feed and Fecal Sample Collection

Feed samples were collected daily before morning feeding during the collection period, mixed, and stored in sealed bags at -20°C. Fecal samples were collected using the total collection method. Daily fecal output per calf was recorded, and 10% of total feces was collected as composite samples. Fresh feces (100 g) were mixed with 10 mL of 10% HCl for nitrogen fixation and stored at -20°C.

1.4.2 Rumen Fluid Collection

At 28, 42, and 56 days of age, rumen fluid was collected from 4 calves per group before morning feeding using a sterilized oral tube. After filtration through 4 layers of gauze, pH was immediately measured using a portable pH meter (testo-206-pH2). The remaining filtrate was aliquoted into 10 mL centrifuge tubes (autoclaved) and stored at -20°C.

1.4.3 Chemical Analysis

Feed and fecal samples were analyzed for dry matter (DM), organic matter (OM), GE, crude protein (CP), ether extract (EE), calcium (Ca), and phosphorus (P) content using methods described by Zhang Liying [citation not found]. Rumen fluid NH₃-N concentration was determined by the phenol-hypochlorite colorimetric method. Volatile fatty acid (VFA) concentrations were measured by gas chromatography, and total VFA (TVFA) concentration was calculated.

1.4 Statistical Analysis

Raw data were initially processed using Excel 2013. Nutrient digestibility data were analyzed using the GLM procedure of SAS 9.2, while other data were analyzed using the Mixed procedure for P-values and SEM. Significance was declared at P<0.05.

2.1 Effects of Protein Source on Dry Matter Intake

During the trial, dry matter was supplied by milk replacer and starter feed. Milk replacer feeding level (as emulsion) was provided at 10% of body weight with no significant differences among groups ($P>0.05$). As shown in Table 2 , average starter DMI from 22-63 days of age did not differ significantly among groups ($P>0.05$). However, during 57-63 days of age, starter DMI in SP and RP groups was significantly higher than MP, WP, and PP groups ($P<0.05$).

2.2 Effects of Protein Source on Digestion and Metabolism

As shown in Table 3 , protein source significantly affected digestibility of OM, CP, EE, Ca, P, and GE at 29-35 days of age ($P<0.05$). OM digestibility in the MP group was significantly higher than WP, PP, and RP groups ($P<0.05$). CP digestibility in the MP group was significantly higher than SP, WP, PP, and RP groups ($P<0.05$), while SP and RP groups were significantly higher than WP and PP groups ($P<0.05$), and WP group was significantly higher than PP group ($P<0.05$). EE digestibility in MP, SP, and RP groups was significantly higher than WP and PP groups ($P<0.05$). Ca digestibility in MP and RP groups was significantly higher than WP and PP groups ($P<0.05$). P digestibility in the MP group was significantly higher than SP, WP, and PP groups ($P<0.05$).

At 57-63 days of age, protein source significantly affected OM, CP, EE, and Ca digestibility ($P<0.05$), but not P digestibility ($P>0.05$). OM digestibility in the MP group was significantly different from WP and PP groups ($P<0.05$) but not from SP and RP groups ($P>0.05$), with no significant differences among SP, WP, PP, and RP groups ($P>0.05$). CP digestibility in the MP group was significantly different from WP, PP, and RP groups ($P<0.05$) but not from SP group ($P>0.05$), with no significant differences among SP, WP, PP, and RP groups ($P>0.05$) except between SP and PP groups ($P<0.05$). GE digestibility in the MP group was significantly higher than WP and PP groups ($P<0.05$). EE digestibility did not differ significantly between MP and other groups ($P>0.05$), while SP and WP groups were significantly lower than RP group ($P<0.05$). Ca digestibility in the MP group was significantly higher than SP and WP groups ($P<0.05$), with no significant differences among other groups ($P>0.05$).

Digestibility of OM, CP, Ca, and P at 61-63 days of age tended to be lower than at 26-28 days, while EE digestibility increased with age.

2.3 Effects of Protein Source on Rumen Fermentation

As shown in Table 4 , rumen fluid pH ranged from 6.57-6.99 at 28 and 42 days of age with no significant differences among groups ($P>0.05$). At 56 days of age, pH did not differ significantly between MP and SP, WP, PP, or RP groups ($P>0.05$), but WP and PP groups had significantly higher pH than SP and RP groups ($P<0.05$). Rumen NH₃-N concentrations ranged from 11.70-19.76 mg/dL at 28, 42, and 56 days of age with no significant differences among

groups ($P>0.05$). Throughout the trial, rumen pH decreased significantly with age ($P<0.01$), while $\text{NH}_3\text{-N}$ concentration increased significantly ($P<0.01$).

As shown in Table 5, no significant differences were observed in VFA concentrations among groups at 28 and 42 days of age ($P>0.05$). At 56 days of age, significant differences appeared in TVFA, propionate, butyrate, isovalerate, and valerate concentrations ($P<0.05$). Propionate and butyrate concentrations in SP and RP groups were significantly higher and lower, respectively, than MP, WP, and PP groups ($P<0.05$). TVFA concentration in PP group was significantly lower than SP and RP groups ($P<0.05$). Isovalerate and valerate concentrations in the MP group were significantly lower than all other groups ($P<0.05$). Age had a highly significant effect on VFA concentration and proportions throughout the trial ($P<0.01$).

3.1 Effects of Protein Source on Digestion and Metabolism

Previous research indicates that replacing milk protein with plant protein reduces nutrient digestibility in calves, with the degree of reduction varying according to plant protein type [citation not found], processing method [citation not found], and replacement proportion [citation not found]. Regarding low plant protein digestibility, Li Hui [citation not found] suggested that inferior amino acid balance in plant proteins affects digestion and absorption. Ternouth et al. [12] proposed that plant proteins coagulate less effectively in the abomasum than milk proteins, reducing retention time and pepsin hydrolysis, allowing more plant protein to pass undigested to the lower intestine. Plant protein digestibility also correlates directly with antinutritional factor levels; Tukur et al. [13] found that higher soy protein immunogenic antigen activity increased soy globulin and β -conglycinin content in ileal digesta, reducing apparent nutrient digestibility, consistent with Sun Zewei et al. [14]. Gastrointestinal tissue damage, hypersensitivity reactions [citation not found], and reduced digestive enzyme activity [citation not found] caused by plant protein antigens and lectins also contribute to low efficiency. Additionally, Montagne et al. [17,18] suggested low apparent digestibility results not from reduced hydrolytic capacity or amino acid absorption differences, but from increased endogenous protein secretion and microbial synthesis stimulated by plant proteins, increasing endogenous protein loss.

This study conducted two digestion trials at 29–35 and 57–63 days of age. At 29–35 days, protein source significantly affected OM, CP, EE, and P digestibility, with milk protein CP digestibility significantly higher than soybean, wheat, peanut, and rice protein groups. Following isonitrogenous and isoenergetic formulation with crystalline amino acid supplementation to balance major limiting amino acids, CP digestibility in soybean and rice protein groups exceeded 80%, significantly higher than reports by Li Hui [citation not found] (78.0%), Yao Lukun et al. [19] (74.7%), and Gao Yanxia [20] (65.3%). Soybean and rice protein groups used soybean protein concentrate and rice protein isolate with low antinutritional factor levels, eliminating their negative effects. This demon-

strates that balancing amino acid nutrition and reducing antinutritional factors can improve plant protein utilization. Wheat and peanut protein groups showed the lowest CP digestibility, likely due to higher antinutritional factor levels, suggesting that 70% replacement of milk protein exceeded the digestive and immune capacity of calves at this age. This also indicates that balancing major limiting amino acids cannot completely offset antinutritional factor effects.

At 57–63 days, differences in CP digestibility between plant and milk protein groups decreased, indicating enhanced rumen digestive function with age. This trial only balanced four amino acids (Lys, Thr, Met, Trp); plant protein groups may have had other unbalanced limiting amino acids important for calf growth. Rumen microbial fermentation improved dietary amino acid composition, balancing limiting amino acid supply and reducing differences in CP digestion between plant and milk protein groups. Although wheat, peanut, and rice protein groups remained significantly lower than the milk protein group, the gap narrowed compared to 29–35 days, while soybean protein group showed no significant difference from the milk protein group, suggesting no nutritional barriers to plant protein digestion at this age.

At 29–35 days, OM digestibility in the milk protein group was significantly higher than wheat, peanut, and rice protein groups, possibly due to fiber content in plant protein milk replacers. By 57–63 days, as rumen function developed, the gap in OM digestibility between plant and milk protein groups decreased, indicating enhanced fiber utilization. At 29–35 days, EE digestibility in wheat and peanut protein groups was significantly lower than milk, soybean, and rice protein groups, possibly because antinutritional factors reduced lipase secretion, activity, and fat absorption, similar to Liener [21] reporting reduced pancreatic lipase activity with raw soybean meal addition. By 57–63 days, EE digestibility in plant protein groups did not differ significantly from the milk protein group, with peanut and rice protein groups even exceeding the milk protein group's 89.02% digestibility. Since starter feed EE content was 2.03% and milk replacer was the main EE source, this indicates that by this age, calves could overcome antinutritional factor barriers to EE digestion. P digestibility exceeded 75% at both 29–35 and 57–63 days, significantly higher than reported by Li Hui [citation not found], Li Yingqiu [22], and Huang Xixia et al. [23]. At 29–35 days, Ca digestibility in wheat and peanut protein groups was lower than other groups, possibly related to protein structure differences or lower EE digestibility, requiring further investigation.

3.2 Effects of Protein Source on Rumen Fermentation

Rumen fluid pH is a key comprehensive indicator of fermentation status, and maintaining normal pH range is essential for proper fermentation. In this trial, rumen pH remained within the normal range of 6–7 across all periods, decreasing significantly with age, likely related to increasing DMI of milk replacer and starter feed. Suárez et al. [24] suggested that soluble carbohydrates in calf diets ferment rapidly, producing large amounts of organic acids that reduce rumen

pH. At 56 days, soybean and rice protein groups had lower pH than milk, wheat, and peanut protein groups, significantly different from wheat and peanut protein groups, possibly due to higher starter DMI during 43-56 days providing more fermentation substrate. Lower pH can shift fermentation pathways, producing more propionate and butyrate to promote rumen development [citation not found]. Additionally, lower pH facilitates VFA absorption by rumen epithelium, reducing rumen TVFA concentration, which decreases VFA absorption into blood, creating hunger and stimulating feed intake, consistent with higher starter DMI in soybean and rice protein groups.

Rumen NH₃-N concentration reflects the dynamic balance between microbial protein synthesis and protein degradation. Both excessively high and low NH₃-N concentrations impair rumen development; concentrations below 5 mg/dL limit microbial protein synthesis, while high concentrations correlate with incomplete rumen papillae keratinization. Bürger et al. [26] suggested the optimal NH₃-N range is 6.3-27.5 mg/dL. In this trial, NH₃-N concentrations remained within this optimal range across all periods. No significant differences were observed among groups, though concentrations increased significantly with age, indicating progressive rumen development with increasing DMI. The lack of difference suggests protein source did not affect rumen development from this perspective.

VFAs (acetate, propionate, butyrate) are primary products of carbohydrate fermentation and important indicators of rumen maturity [citation not found]. At 28 and 42 days, no significant differences in TVFA concentrations were observed among groups. At 56 days, soybean and rice protein groups had higher TVFA concentrations than milk, wheat, and peanut protein groups, likely due to DMI differences providing more fermentation substrate. Although pH was lower in soybean and rice protein groups at 56 days, TVFA concentrations did not directly reflect this change, possibly because the diet rich in digestible carbohydrates fermented rapidly, producing acids that lowered pH and shifted microbial fermentation to produce more propionate and lactate [citation not found]. Higher starter DMI in soybean and rice protein groups during 43-56 days may have increased lactate accumulation, lowering pH. High lactate levels can promote proliferation of *Megasphaera elsdenii*, which ferments lactate to propionate via the acrylate pathway [citation not found], consistent with higher propionate and lower acetate/propionate ratios in these groups. Hu Honglian et al. [30] reported that lower rumen pH significantly increased plasma butyrate concentration, indicating lower pH facilitates butyrate absorption, consistent with lower butyrate concentrations in soybean and rice protein groups.

At 56 days, isovalerate and valerate concentrations in plant protein groups were significantly higher than the milk protein group. Branched-chain VFAs like isovalerate and valerate are essential growth factors for rumen fiber-degrading bacteria [31], which can increase fiber-degrading bacterial populations, promote rumen development, and enhance OM digestibility, consistent with improved OM digestibility in plant protein groups at 56 days.

Conclusions

Under the nutritional conditions of this study: 1) At 29-35 days of age, milk protein showed higher OM and CP digestibility than plant proteins; differences diminished by 57-63 days of age; rice protein exhibited higher EE digestibility than milk and other plant proteins. 2) At 56 days of age, calves fed soybean and rice protein had lower rumen pH and butyrate concentration but higher propionate concentration compared to milk, wheat, and peanut proteins. All four plant protein groups showed significantly higher isovalerate and valerate concentrations than the milk protein group.

Comprehensive analysis indicates that plant proteins affect nutrient digestibility in calves compared to milk protein, though differences decrease with age. All four plant protein sources promote rumen development compared to milk protein.

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