Effects of Nutritional Compound Supplementation in Milk Replacer on Growth Performance, Rumen Fermentation, and Apparent Nutrient Digestibility in Pre- and Post-Weaning Calves (Postprint)

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Abstract

This experiment was conducted to investigate the effects of adding a nutritional compound to reconstituted milk on growth performance, rumen fermentation, and nutrient apparent digestibility in pre- and post-weaning calves. Twenty-eight healthy newborn Holstein calves with a body weight of (39.99±4.65) kg were selected and allocated to 2 groups using a single-factor completely randomized design, with 14 calves per group (half male and half female). The trial consisted of two phases. In Phase 1 (birth to 70 days of age), calves in the control (CON) group were fed reconstituted milk and starter feed, whereas calves in the nutritional compound (NC) group were fed reconstituted milk supplemented with nutritional compound (the nutritional compound replaced 10% of the pure milk powder amount in the CON group) and starter feed. In Phase 2 (71 to 98 days of age), calves in both groups underwent a 1-week weaning transition, after which milk feeding was ceased and only starter feed was provided until the end of the experiment. The total experimental period was 98 days. The results showed: 1) Compared with calves in the CON group, the nutritional compound significantly increased the overall average daily gain, dry matter intake, starter feed dry matter intake, and body weight at 98 days of age in the NC group ($P<0.05$). 2) At 98 days of age, the pH and acetate/propionate ratio in the NC group were significantly lower than those in the CON group ($P<0.05$). 3) The nutritional compound had no significant effect on the apparent digestibility of any nutrients in calves before and after weaning ($P>0.05$). In conclusion, supplementation of a nutritional compound in reconstituted milk can increase feed intake, improve weight gain, and enhance the rumen fermentation environment

post-weaning, enabling calves to adapt more rapidly to solid diet feeding after weaning.

Full Text

Effects of Nutritional Compound Added to Reconstituted Milk on Growth Performance, Rumen Fermentation, and Nutrient Apparent Digestibility in Pre- and Post-Weaning Calves

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Abstract

This study investigated the effects of adding a nutritional compound to reconstituted milk on growth performance, rumen fermentation, and nutrient apparent digestibility in pre- and post-weaning calves. Twenty-eight healthy newborn Holstein calves with an initial body weight of (39.99±4.65) kg were randomly allocated into two groups (n=14 per group, half male and half female) using a single-factor completely randomized design. The experiment comprised two phases: Phase 1 (birth to 70 days of age) and Phase 2 (71 to 98 days of age). During Phase 1, the control (CON) group received reconstituted milk (milk powder solution) and starter feed, while the nutritional compound (NC) group received reconstituted milk containing the nutritional compound (which replaced 10% of the milk powder in the CON group) plus starter feed. During Phase 2, both groups underwent a one-week weaning transition, after which milk feeding ceased and only starter feed was provided until the end of the 98-day trial period. The results showed that: (1) Compared with the CON group, the NC group exhibited significantly higher average daily gain, dry matter intake, starter dry matter intake, and body weight at 98 days of age across the entire experimental period ($P<0.05$). (2) At 98 days of age, ruminal pH and the acetate-to-propionate ratio were significantly lower in the NC group than in the CON group ($P<0.05$). (3) The nutritional compound had no significant effect on the apparent digestibility of any nutrients during either the pre- or post-weaning periods ($P>0.05$). In conclusion, supplementing reconstituted milk with a nutritional compound can increase feed intake and weight gain in calves, improve the rumen fermentation environment after weaning, and facilitate faster adaptation to solid feed feeding.

Keywords: calves; reconstituted milk; nutritional compound; rumen fermentation; nutrient digestion

Introduction

In recent years, China's dairy industry has experienced rapid intensification and scaling-up, leading to substantial increases in per-cow milk production. However, during peak production seasons, milk surplus often occurs. To avoid resource waste from dumping excess milk, dairy farms typically convert surplus milk into powder for storage. Since this milk powder cannot be sold commercially, farms commonly reconstitute it as milk replacer for early calf rearing. This practice serves the dual purpose of utilizing milk powder resources while employing high-quality dairy products to raise replacement heifers. However, the high-temperature, high-pressure spray-drying process used in milk powder production causes significant nutrient losses, particularly of amino acids and vitamins, resulting in nutritional imbalances that severely limit the expression of calves' growth potential and their future milk production performance. Therefore, effective technical measures are urgently needed to address these practical problems and provide guidance for calf rearing.

Calf rearing with milk replacer to enable early weaning represents a successful practice in developed dairy countries. Appropriate feeding levels of milk replacer can effectively promote the development of digestive organs, facilitate earlier consumption of conventional diets, and thereby enhance calf growth and health. A nutritional compound is a complex nutrient additive formulated for incorporation into whole milk powder, designed to meet calves' requirements for amino acids, vitamins, digestive enzymes, and other nutrients during rapid growth. This additive can mitigate the adverse effects of nutrient destruction, loss, and deficiency in whole milk powder on calf growth, thereby promoting rapid and healthy development.

This study investigated the effects of supplementing reconstituted milk with a nutritional compound on growth performance, rumen fermentation parameters, and nutrient apparent digestibility in calves during the pre- and post-weaning periods. The objective was to determine the impact of the nutritional compound on calf growth and health, providing a theoretical basis for its application in calf rearing.

Materials and Methods

1.1 Experimental Animals and Duration

The experiment utilized 28 healthy newborn Holstein calves (14 males and 14 females) with an average birth weight of (39.99±4.65) kg. The trial was conducted

from August to November 2017 at Chabei Modern Dairy Farm in Zhangjiakou City, Hebei Province.

1.2 Experimental Diets

The nutritional compound was formulated by Beijing Precision Animal Nutrition Research Center. Its composition (by weight percentage) was: whole milk powder 10%-30%, soybean meal 5%-25%, mineral premix 0.2%-4%, vitamin premix 0.2%-3.6%, amino acid premix 5.2%-15%, microecological enzyme complex 0.4%-1.8%, citric acid 0.3%-0.95%, fumaric acid 0.3%-0.95%, calcium lactate 1%-4%, calcium dihydrogen phosphate 1%-3%, taurine 0.01%-0.03%, carnitine 0.01%-0.1%, coated VC 0.1%-0.5%, and salt 0.2%-1.8%. The microecological enzyme complex (per kg) contained: *Enterococcus faecalis* with 20 billion viable CFU/g (100-300 g), *Bacillus licheniformis* with 300 billion viable CFU/g (13-20 g), and protease with 80 U/g (200-400 g). The amino acid premix (per kg) contained: lysine 150-400 g, methionine 60-710 g, threonine 120-570 g, and tryptophan 60-340 g. The milk powder and calf starter were proprietary products used specifically for calves at Chabei Modern Dairy Farm. The nutrient levels of milk powder, nutritional compound, and starter feed are presented in Table 1 .

Table 1 Nutrient levels of milk powder, nutritional compound, and starter feed (air-dry basis) %

Nutrient levels	Milk powder	Nutritional compound	Starter
GE (MJ/kg)			
DM			
CP			
EE			
Ash			

Note: Nutrient levels were all measured values.

1.3 Experimental Design and Management

A single-factor completely randomized design was employed, with 28 Holstein calves divided into two groups (n=14 each, half male and half female). Initial body weight did not differ significantly between groups (P>0.05). The experiment consisted of two phases: Phase 1 (birth to 70 days of age) and Phase 2 (71 to 98 days of age). During Phase 1, the CON group received reconstituted milk (milk powder solution) and starter feed, while the NC group received reconstituted milk containing the nutritional compound (which replaced 10% of the milk powder in the CON group) plus starter feed. During Phase 2, both groups underwent a one-week weaning transition, after which milk feeding ceased and only starter feed was provided until the end of the 98-day trial period.

Calves were weighed at birth after fasting and ear-tagged. Within one hour after birth, each calf received 4 L of colostrum, followed by another 2 L at 12 hours postpartum. Calves were then transferred to individual hutches (approximately 3 m^2 per calf) for single housing, with pens maintained in clean sanitary conditions. Throughout the feeding period, strict adherence to the "four fixed" principle was implemented: fixed timing, fixed quantity, fixed temperature, and fixed personnel. During the first week (days 1-7), all calves received fresh milk. Days 8-13 served as a milk transition period, during which the ratio of experimental milk to fresh milk gradually increased from 1:2 to 2:1, enabling calves to adapt gradually. By day 14, calves were fully transitioned to their respective experimental milk treatments. Calves were fed milk twice daily (06:30 and 16:30) at 4 L per feeding from day 2 postpartum. Starter feed and warm water (approximately 39°C) were provided ad libitum from day 14, with daily starter intake recorded.

Preparation and feeding methods: 1) Reconstituted milk: Milk powder was mixed with pasteurized warm water at approximately 45°C at a weight-to-volume ratio of 1:7.5, with a feeding temperature of approximately 39°C. 2) Milk powder + nutritional compound: The nutritional compound replaced 10% of the milk powder in the CON group to formulate a compound milk powder, which was then mixed with pasteurized warm water at approximately 45°C at a weight-to-volume ratio of 1:7.5, with a feeding temperature of approximately 39°C.

1.4 Measurements and Analytical Methods

1.4.1 Nutrient Levels of Milk Powder, Nutritional Compound, and Starter Feed Samples of milk powder, nutritional compound, and starter feed were collected every 14 days and stored at 4°C. After the experiment, samples were transported to the laboratory for determination of dry matter, crude protein, crude fat, crude ash, calcium, phosphorus, and gross energy content according to standard methods.

1.4.2 Growth Performance Calves were weighed at birth and at 70 and 98 days of age before morning feeding to calculate average daily gain (ADG). Daily intake of milk powder, nutritional compound, and starter feed was recorded to calculate dry matter intake (DMI), starter DMI, and feed conversion efficiency (G/F).

1.4.3 Rumen Fermentation Parameters Six calves from each group with body weights closest to their group mean were selected for rumen content collection at 42 and 98 days of age, 2 hours after morning feeding. Rumen contents (100 mL) were collected using a sterile oral tube, filtered through four layers of gauze, and immediately analyzed for pH using a portable pH meter (testo-206-pH2). Samples were then aliquoted into 10 mL sterile centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. Volatile

fatty acid (VFA) concentrations were determined according to the method of Cao et al., and ammonia nitrogen (NH -N) concentration was measured using the indophenol blue method.

1.4.4 Nutrient Apparent Digestibility Digestibility was determined using the internal marker fecal collection method. Fecal samples were collected from calves during days 49-55 and 84-90 of the trial. Acid-insoluble ash (AIA) was used as an internal marker according to GB/T 23742-2009. Fecal samples were analyzed for dry matter, crude protein, crude fat, crude ash, and gross energy content. Apparent digestibility was calculated as:

Apparent digestibility of a nutrient (%) = 100 - 100 × (AIA in diet / AIA in feces) × (nutrient in feces / nutrient in diet)

1.5 Statistical Analysis Data were initially processed using Excel 2007. Statistical analysis was performed using SPSS 19.0 software with one-way ANOVA and Duncan' s multiple range test. Differences were considered significant at $P<0.05$ and trending at $0.05<P<0.10$.

Results

2.1 Effects of Nutritional Compound on Growth Performance of Pre- and Post-Weaning Calves

As shown in Table 2 , the nutritional compound significantly increased body weight at 98 days of age in the NC group compared with the CON group ($P<0.05$). The NC group also exhibited significantly higher ADG during days 71-98 and across the entire birth-to-98-day period ($P<0.05$). Dry matter intake and starter DMI were significantly higher in the NC group during all periods (birth to 70 days, 71-98 days, and birth to 98 days) ($P<0.05$). However, the nutritional compound had no significant effect on feed conversion efficiency at any stage ($P>0.05$).

Table 2 Effects of nutritional compound on growth performance of pre- and post-weaning calves

Item	CON	NC	P-value
Body weight (kg)			
Birth			
98 days of age	116.97	124.65	
ADG (kg/d)			
Birth to 70 days	0.95	1.14	
71 to 98 days	0.78	0.87	
Birth to 98 days	0.14	0.20	
Starter DMI (kg/d)			

Item	CON	NC	P-value
Birth to 70 days	2.28	2.53	
71 to 98 days	0.75	0.86	
Birth to 98 days	1.04	1.10	
DMI (kg/d)			
Birth to 70 days	2.51	2.76	
71 to 98 days	1.46	1.58	
Birth to 98 days			
G/F			
Birth to 70 days			
71 to 98 days			
Birth to 98 days			

Note: Values in the same row with different superscripts differ significantly (P<0.05). The same applies below.

2.2 Effects of Nutritional Compound on Rumen Fermentation Parameters of Pre- and Post-Weaning Calves

As shown in Table 3 , at 98 days of age, the nutritional compound significantly decreased ruminal pH and the acetate-to-propionate ratio in the NC group compared with the CON group (P<0.05). Additionally, the NC group showed trends toward higher total VFA (TVFA) concentration and molar proportion of propionate, and a lower molar proportion of acetate (0.05<P<0.10). However, the nutritional compound had no significant effect on any rumen fermentation parameters at 42 days of age (P>0.05).

Table 3 Effects of nutritional compound on rumen fermentation indexes of pre- and post-weaning calves

Item	CON	NC	P-value
42 days of age			
NH -N (mg/dL)			
TVFA (mmol/L)			
Molar proportion (%)			
Acetate			
Propionate			
Butyrate			
Acetate/Propionate			
98 days of age			
NH -N (mg/dL)	6.12	5.56	
TVFA (mmol/L)			
Molar proportion (%)			
Acetate			

Item	CON	NC	P-value
Propionate			
Butyrate			
Acetate/Propionate	1.73	1.38	

2.3 Effects of Nutritional Compound on Nutrient Apparent Digestibility of Pre- and Post-Weaning Calves

As shown in Table 4 , the nutritional compound had no significant effect on the apparent digestibility of any nutrients during either the pre- or post-weaning periods (P>0.05).

Table 4 Effects of nutritional compound on nutrient apparent digestibility of pre- and post-weaning calves %

Item	CON	NC	P-value
49-55 days of age			
DM			
OM			
CP			
GE			
EE			
84-90 days of age			
DM			
OM			
CP			
GE			
EE			

Discussion

3.1 Effects of Nutritional Compound on Growth Performance of Pre- and Post-Weaning Calves

Protein is a crucial dietary component for maintaining animal growth, production, and reproduction. Inadequate dietary protein severely impairs nutrient utilization by the digestive system, thereby reducing overall animal productivity. Previous studies have demonstrated that protein-rich concentrate supplements (17%-19% crude protein) significantly increase body weight and ADG in weaned calves, with similar results reported by other researchers. The nutritional value of protein primarily depends on its amino acid composition, particularly essential amino acids, with amino acid balance being the key determinant of protein quality. In the current study, the significantly higher ADG observed in the

NC group during the post-weaning and entire experimental periods may be attributed to the severe amino acid deficiency caused by milk powder processing, which inhibited growth and reduced weight gain in the CON group.

Dietary probiotics can accelerate the establishment of gastrointestinal microbiota and promote digestive organ development, thereby increasing dry matter intake and daily gain. The nutritional compound used in this study contained probiotics, and the NC group exhibited significantly higher DMI and starter DMI throughout the experimental period. Feed conversion efficiency is an important indicator of feed remuneration. The lack of significant effect on feed conversion efficiency in the NC group may be due to the substantial increase in DMI, which could have transiently reduced gastrointestinal digestive and absorptive capacity, resulting in short-term decreases in feed efficiency.

3.2 Effects of Nutritional Compound on Rumen Fermentation Parameters of Pre- and Post-Weaning Calves

Ruminal pH and VFA concentrations are critical indicators that reflect rumen function and internal environment stability. In this study, the nutritional compound supplemented with probiotics significantly increased starter DMI throughout the experimental period. Increased starter DMI in pre-weaning calves can reduce ruminal pH, providing a suitable internal environment for dietary fermentation in the rumen. At 98 days of age, ruminal pH was significantly lower in the NC group than in the CON group, with values remaining within the normal physiological range (5.5-7.5), indicating that the nutritional compound improved the rumen fermentation environment by promoting solid feed intake without harming rumen development.

Volatile fatty acids are a major energy source for ruminants, providing approximately 75% of energy in sheep and 70%-80% of metabolic energy in cattle. Previous research has reported that *Bacillus subtilis natto* supplementation increases TVFA concentration and decreases the acetate-to-propionate ratio in dairy cows, improving rumen fermentation type and enhancing milk production and composition. In the current study, the nutritional compound containing probiotics significantly reduced the acetate-to-propionate ratio and showed a trend toward increased TVFA concentration at 98 days of age. No significant differences were observed between groups at 42 days of age, likely because the pre-weaning diet consisted primarily of liquid milk, which is not conducive to rumen development. Research indicates that increased solid feed intake accelerates rumen fermentation rate and extent, as well as VFA absorption and metabolism. The NC group consistently maintained significantly higher starter DMI throughout the experiment. However, during the pre-weaning phase, high milk intake resulted in low starter consumption in both groups, leading to similar rumen fermentation parameters. Post-weaning, solid feed became the sole diet, stimulating rumen development through feed intake, microbial fermentation system maturation, and coordinated fermentation and absorption mechanisms. The higher proportion of starter intake promoted early rumen development, enabling NC calves to

adapt more quickly to the solid feed environment post-weaning. Additionally, research has shown that the effects of *Bacillus natto* on rumen fermentation parameters persist even after supplementation ceases, which may explain the significant differences observed post-weaning in this study.

3.3 Effects of Nutritional Compound on Nutrient Apparent Digestibility of Pre- and Post-Weaning Calves

Digestibility is a key indicator of dietary digestibility and animal digestive capacity. Probiotics can enhance nutrient digestibility, with studies reporting significant improvements in crude protein digestibility in growing pigs and increased gross energy digestibility in post-weaning calves fed yeast and mulberry flavonoid complexes. Probiotics metabolize organic acids that promote intestinal motility and digestive juice secretion, facilitating nutrient absorption. Yeast also contains abundant digestive enzymes that enhance intestinal digestion of macronutrients. However, other studies have reported that enzyme treatment significantly improved gross energy digestibility in 3- to 7-month-old heifers but had no significant effect on protein or ether extract digestibility. Acidification of milk replacer has also been shown to not affect apparent digestibility of dry matter, crude protein, or ether extract, though pH reduction to 4.5 adversely affected intestinal epithelial growth.

Collectively, these studies indicate that dietary supplementation with probiotics, acidifiers, and exogenous enzymes can improve nutrient apparent digestibility. The nutritional compound in this study contained probiotics, acidifiers, and exogenous enzymes—all non-nutritive feed additives beneficial for improving digestibility. However, no significant differences were observed between groups during either period. This may be because pre-weaning calves consumed primarily reconstituted milk, resulting in similar digestibility, while post-weaning, although NC calves consumed significantly more starter feed, this did not reduce dietary nutrient digestibility. This suggests that the nutritional compound may have promoted rumen development and improved nutrient digestibility in NC calves. Further research is needed to confirm the effects of nutritional compounds on nutrient apparent digestibility in pre- and post-weaning calves.

Conclusion

Supplementing reconstituted milk with a nutritional compound effectively replenishes and completes the nutrients lost during milk powder processing, increases dry matter intake and weight gain in calves, promotes early rumen development, improves the post-weaning rumen fermentation environment, and enables calves to adapt more rapidly to solid feed feeding post-weaning.

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Note: Figure translations are in progress. See original paper for figures.