

## Effects of Different Dietary Compositions on Milk Production Performance, Blood Biochemical Parameters, and Nitrogen and Phosphorus Excretion in Lactating Holstein Dairy Cows: Postprint

**Authors:** ZHAO Ruohan, Li Lian, Han Zhaoyu, Yang Fangxiao, Wang Genlin

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### Abstract

This experiment investigated the effects of reducing dietary nitrogen and phosphorus levels or using compound additives on milk production performance, blood biochemical parameters, and nitrogen and phosphorus excretion in lactating Holstein dairy cows, aiming to reduce nitrogen and phosphorus emissions in cow feces and urine without affecting production performance. A paired experimental design was adopted, and 36 lactating Holstein dairy cows were divided into 3 groups with 12 cows each, namely the control (CR) group (original dairy farm formula diet), the low nitrogen and phosphorus (LPP) group [original dairy farm formula diet with 0.70% nitrogen (crude protein) and 0.03% phosphorus reduced], and the compound additive (CA) group [original dairy farm formula diet + compound additive (composed of active yeast, enzyme preparation, and organic zinc)]. The preliminary period was 7 d, and the formal experimental period was 30 d. Milk yield, milk composition, blood biochemical parameters, and nitrogen and phosphorus excretion were measured. The results showed that there were no significant differences in milk yield, dry matter intake, or milk composition among groups ( $P > 0.05$ ); compared with the CR group, the LPP group exhibited increased apparent digestibility of acid detergent fiber and phosphorus by 73.12% and 42.94%, respectively ( $P < 0.05$ ); compared with the CR group, total nitrogen and total phosphorus excretion in the LPP group were reduced by 9.11% and 26.34%, respectively ( $P < 0.05$ ), and in the CA group by 16.35% and 16.10%, respectively ( $P < 0.05$ ); there were no significant differences in blood biochemical parameters between the LPP and CR groups ( $P > 0.05$ ), while blood alanine aminotransferase (ALT) activity and phosphorus content in the CA group were significantly higher than those in the CR group ( $P < 0.05$ ). The results suggest that, under the condition of meeting normal nutritional requirements, both appropriately controlling nitrogen and phosphorus intake in

dairy cows and using compound additives can reduce nitrogen and phosphorus excretion.

## Full Text

### Effects of Different Dietary Compositions on Production Performance, Blood Biochemical Parameters, and Nitrogen and Phosphorus Emissions of Lactating Holstein Cows

ZHAO Ruohan, LI Lian, HAN Zhaoyu, YANG Fangxiao, WANG Genlin College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China\*

#### Abstract

This study investigated the effects of reducing dietary nitrogen and phosphorus levels or using compound additives on milk production performance, blood biochemical parameters, and nitrogen and phosphorus emissions in lactating Holstein cows, aiming to reduce nitrogen and phosphorus excretion in feces and urine without affecting production performance. Using a paired experimental design, 36 lactating Holstein cows were divided into 3 groups with 12 cows per group: control (CR) group (original farm diet), low protein and phosphorus (LPP) group [original farm diet with 0.70% reduced nitrogen (crude protein) and 0.03% reduced phosphorus], and compound additive (CA) group [original farm diet + compound additive (composed of active yeast, enzyme preparation, and organic zinc)]. The pre-test period lasted 7 days, and the formal test period lasted 30 days. Milk yield, milk composition, blood biochemical parameters, and nitrogen and phosphorus emissions were measured. The results showed that there were no significant differences in milk yield, dry matter intake, or milk composition among groups ( $P > 0.05$ ). Compared with the CR group, the LPP group showed increased apparent digestibility of acid detergent fiber and phosphorus by 73.12% and 42.94%, respectively ( $P < 0.05$ ). Compared with the CR group, total nitrogen and total phosphorus emissions in the LPP group were reduced by 9.11% and 26.34% ( $P < 0.05$ ), respectively, while those in the CA group were reduced by 16.35% and 16.10% ( $P < 0.05$ ), respectively. There were no significant differences in blood biochemical parameters between the LPP and CR groups ( $P > 0.05$ ), while blood alanine aminotransferase (ALT) activity and phosphorus content in the CA group were significantly higher than those in the CR group ( $P < 0.05$ ). These results suggest that, under conditions where normal nutritional requirements are satisfied, appropriately controlling nitrogen and phosphorus intake in cows and using compound additives can effectively reduce nitrogen and phosphorus emissions.

**Keywords:** lactating Holstein cows; milk production performance; blood biochemical parameters; nitrogen and phosphorus emissions

## 1 Introduction

With the rapid development of large-scale livestock farming, the environmental hazards caused by livestock waste have attracted increasing attention, particularly pollution resulting from nitrogen and phosphorus emissions [1]. In 2015, for example, China's dairy cattle inventory was approximately 15.072 million head, generating about 1.2 billion tons of waste including manure, wastewater, bedding, and feed residues. The negative environmental impact of dairy cow feces and urine is growing increasingly severe, with nitrogen and phosphorus emissions from these sources being of particular concern. As nitrogen and phosphorus are essential nutrients required for animal growth, development, and production, appropriate amounts play crucial roles in maintaining dairy cow production and reproductive performance [2]. However, if dietary protein is blindly increased and phosphorus utilization efficiency is ignored in feed formulations to improve milk yield, the proteins and phosphorus in feed cannot be maximally utilized, thereby increasing nitrogen and phosphorus excretion in animal manure.

Research indicates that the average utilization efficiency of nitrogen and phosphorus in Chinese dairy cows is only 10%-22%, significantly lower than the average level of 19%-35% in the United States, Australia, New Zealand, and other countries [3]. Milk urea nitrogen (MUN) accounts for only 25%-35% of nitrogen intake, with the remaining nitrogen excreted through urine and feces [4]. Phosphorus is primarily excreted via feces, with fecal phosphorus comprising 68.7% of total phosphorus emissions. Urinary phosphorus excretion is relatively low, accounting for only 1% of total phosphorus emissions, while 30.3% of phosphorus is utilized by dairy cows [5]. These data demonstrate that phosphorus emissions in feces are substantial. The discharge of excessive nitrogen and phosphorus into the environment not only wastes feed resources but also severely pollutes the environment and water sources. Therefore, this experiment aimed to reduce nitrogen and phosphorus emissions through two nutritional measures—reducing dietary nitrogen and phosphorus levels and feeding compound additives—without affecting dairy cow production performance, thereby providing technical references for the healthy and sustainable development of the dairy industry.

### 1.1 Experimental Period and Location

The experiment was conducted from February to April 2017 at the Shuyang Dairy Source Base of Weigang Dairy in Suqian City, Jiangsu Province.

### 1.2 Experimental Materials and Design

Based on the principle of similar age, parity, lactation stage, and milk yield, 36 healthy Chinese Holstein cows were randomly divided into 3 groups with 12 cows per group using a paired experimental design. The experimental period lasted 37 days, including a 7-day pre-test period and a 30-day formal test period. The

control (CR) group was fed the original farm diet. The low protein and phosphorus (LPP) group received a diet with 0.70% reduced nitrogen (crude protein) and 0.03% reduced phosphorus compared to the original farm diet, achieved by partially replacing dried distillers grains with solubles (DDGS) with beet pulp. The compound additive (CA) group was fed the CR diet supplemented with compound additives (composed of active yeast, enzyme preparation, and organic zinc). The compound additive was administered by mixing 10 g of the additive with 90 g of cornmeal daily and adding it to the diet.

### 1.3 Experimental Diets and Management

The composition and nutrient levels of the experimental diets are shown in Table 1. The experimental cows were housed in tie-stall barns and fed three times daily at 06:00, 13:00, and 20:00. Daily feed refusal was controlled below 5%. Cows were milked three times daily using a pipeline milking system, had free access to water, were exposed to conventional lighting, and were kept in clean and dry pens.

### 1.4 Sample Collection and Analysis

**1.4.1 Feed Sample Collection and Analysis** During the formal test period, feed and ort samples were collected in sealed bags using the method described in “Feed Analysis and Feed Quality Detection Technology” edited by Zhang Liying [6]. Feed samples were dried in an oven at 65°C for 48 hours to prepare air-dried samples, and the contents of dry matter, crude protein, phosphorus, calcium, acid detergent fiber, neutral detergent fiber, and crude ash in the experimental diets were determined. Crude protein content was determined using the Kjeldahl method, neutral detergent fiber and acid detergent fiber contents were determined using the Van Soest detergent fiber method, calcium content was determined using potassium permanganate titration, phosphorus content was determined using the ammonium vanadomolybdate colorimetric method, and crude ash content was determined using the crude ash method. The apparent digestibility of nutrients was determined using the internal indicator method (4 mol/L hydrochloric acid insoluble ash) with the following calculation formula [6]:

$$\text{Apparent digestibility of dietary nutrients (\%)} = \left[ 1 - \frac{b}{a} \times \frac{c}{d} \right] \times 100$$

where:  $a$  is the content of a nutrient in the diet (%);  $b$  is the content of a nutrient in feces (%);  $c$  is the content of hydrochloric acid insoluble ash in the diet (%); and  $d$  is the content of hydrochloric acid insoluble ash in feces (%).

The content of physically effective neutral detergent fiber (peNDF) was determined using the PSPS sieve with the following calculation formula [7]:

$$\text{peNDF (\%)} = \text{pef}_{1.18} \times \text{dietary neutral detergent fiber content}$$

where:  $\text{pef}_{1.18}$  is the physical effectiveness factor of the diet.

**1.4.2 Fecal Sample Collection and Analysis** Fresh fecal samples were collected from each cow on days 10, 20, and 30 of the formal test period. Approximately 100 g of fresh feces was collected from each cow before morning, noon, and evening feeding using a sampling spoon. The fresh feces were then mixed uniformly and divided into two portions. One portion was mixed with 10% sulfuric acid (20 mL per 100 g of feces) for determination of fecal nitrogen emission. The other portion was dried in an oven at 65°C for 48 hours, cooled to room temperature in air, ground to pass through a 40-mesh sieve, mixed uniformly, and stored frozen in a refrigerator for determination of other nutrient components. Daily fecal excretion was estimated using the method described by Li Guolin [8].

**1.4.3 Urine Sample Collection and Analysis** Urine samples were collected simultaneously with fecal samples using collection containers. Impurities in urine samples were removed by filtration through coarse gauze before placing into clean, dry 50 mL tubes. Urine collected from each cow in the morning, noon, and evening was mixed uniformly, and 30 mL was taken from each cow. A 1:1 ratio of 10% sulfuric acid was added to each tube for nitrogen fixation, then sealed and stored at -20°C for later analysis. During analysis, samples were first restored to room temperature before determination of urinary nitrogen and phosphorus emissions. Urinary nitrogen emission was determined using the Kjeldahl method, and urinary phosphorus emission was determined using the ammonium molybdate spectrophotometric method [6]. Daily urine excretion was estimated using the method described by Li Guolin [8].

**1.4.4 Milk Sample Collection and Analysis** Milk yield was measured and noon milk samples were collected every 7 days during the formal test period. Potassium dichromate preservative (0.6 mg/mL) was added to milk samples, mixed uniformly, and refrigerated at 4°C for milk composition analysis. Milk protein percentage, milk fat percentage, lactose percentage, total solids content, MUN content, and somatic cell count were determined using an automatic milk composition analyzer (CombiFoss FT+, FOSS, Denmark). The weighted average method was used to calculate milk composition content during the formal test period. Four percent fat-corrected milk (FCM) yield was calculated.

Feed efficiency = 4% FCM yield / dry matter intake

Fat-to-protein ratio = milk fat percentage / milk protein percentage

**1.4.5 Blood Sample Collection and Analysis** On day 1 of the formal test period and day 1 after its conclusion, 5 mL of blood was collected from the tail vein. Blood samples were centrifuged at 3,000×g for 15 minutes to separate serum, which was stored at -20°C. Blood biochemical parameters included total protein (TP), albumin (ALB), globulin (GLB), glucose (GLU), urea nitrogen (UN), insulin (INS), prolactin (PRL), total cholesterol (TC), triglycerides (TG), phosphorus content, and alanine aminotransferase (ALT) activity. TP, ALB,

GLB, GLU, UN, TC, TG contents, and ALT activity were determined using an automatic biochemical analyzer (Hitachi 7600, Japan). INS and PRL contents were determined using radioimmunoassay with kits purchased from Nanjing Jiancheng Bioengineering Institute.

### 1.5 Statistical Analysis

All experimental data were analyzed using SPSS 20.0 software for one-way ANOVA and regression analysis. Multiple comparisons were performed using Duncan's method. Results are expressed as "mean  $\pm$  standard error," with  $P < 0.05$  as the criterion for statistical significance.

## 2 Results

### 2.1 Effects of Different Dietary Compositions on Production Performance of Lactating Holstein Cows

As shown in Table 2, the three different dietary compositions had no significant effects on milk yield, dry matter intake, or milk composition ( $P > 0.05$ ). Numerically, compared with the CR group, the LPP and CA groups showed some increase in milk yield and 4% fat-corrected milk yield. Additionally, milk somatic cell count decreased in both the LPP and CA groups.

### 2.2 Effects of Different Dietary Compositions on Apparent Digestibility of Nutrients in Lactating Holstein Cows

As shown in Table 3, different dietary compositions had no significant effects on apparent digestibility of crude protein or neutral detergent fiber ( $P > 0.05$ ). Additionally, there were no significant differences in apparent digestibility of acid detergent fiber and phosphorus between the CA and CR groups ( $P > 0.05$ ). Compared with the CR group, the LPP group showed increased apparent digestibility of acid detergent fiber by 73.12% ( $P < 0.05$ ) and phosphorus by 42.94% ( $P < 0.05$ ).

### 2.3 Effects of Different Dietary Compositions on Nitrogen Emission in Lactating Holstein Cows

As shown in Table 4, nitrogen intake in the LPP group was reduced by 12.88% compared with the CR group ( $P < 0.05$ ), while there was no significant difference between the CA and CR groups ( $P > 0.05$ ). Different dietary compositions had no significant effects on fecal nitrogen emission ( $P > 0.05$ ). Urinary nitrogen emissions in the LPP and CA groups were reduced by 13.24% and 23.60%, respectively, compared with the CR group ( $P < 0.05$ ). There were no significant differences in nitrogen utilization among the three groups ( $P > 0.05$ ). Total nitrogen emissions in the LPP and CA groups were reduced by 9.11% and 16.35%, respectively, compared with the CR group ( $P < 0.05$ ).

## 2.4 Effects of Different Dietary Compositions on Phosphorus Emission in Lactating Holstein Cows

As shown in Table 5 , phosphorus intake in the LPP group was reduced by 15.40% compared with the CR group ( $P < 0.05$ ), while there was no significant difference between the CA and CR groups ( $P > 0.05$ ). Fecal phosphorus emissions in the LPP and CA groups were reduced by 26.40% and 16.19%, respectively, compared with the CR group ( $P < 0.05$ ). There were no significant changes in urinary phosphorus emissions in the other two groups compared with the CR group ( $P > 0.05$ ). There were no significant differences in phosphorus utilization among groups ( $P > 0.05$ ). Total phosphorus emissions in the LPP and CA groups were reduced by 26.34% and 16.10%, respectively, compared with the CR group ( $P < 0.05$ ).

## 2.5 Effects of Different Dietary Compositions on Blood Biochemical Parameters of Lactating Holstein Cows

As shown in Table 6 , there were no significant differences in any blood biochemical parameters between the LPP and CR groups ( $P > 0.05$ ). Blood ALT activity and phosphorus content in the CA group were significantly higher than those in the CR group ( $P < 0.05$ ), while other parameters showed no significant differences ( $P > 0.05$ ).

# 3 Discussion

## 3.1 Effects of Different Dietary Compositions on Production Performance of Lactating Holstein Cows

Livestock excreta represent a major source of environmental nitrogen and phosphorus pollution. This experiment utilized two nutritional measures—reducing dietary nitrogen and phosphorus levels and adding compound additives—to explore the potential for reducing nitrogen and phosphorus emissions in dairy farming. Diet formulation is a critical factor affecting dairy cow production efficiency, particularly as dietary nitrogen level and utilization efficiency directly reflect animal production performance. However, in practical production, dietary nitrogen and phosphorus supplies often exceed animal requirements, leading to resource waste and environmental pollution, which ultimately proves counter-productive.

Zhai Shaowei [9] reported that for cows with milk yields of 20.5-25.0 kg/d, milk yield changed minimally when dietary crude protein levels exceeded 14%. Colmenero [10] found that when feeding diets with crude protein levels of 13.5%, 15.0%, 16.5%, 17.9%, and 19.4% to cows producing 38 kg/d of milk, milk yield no longer increased when crude protein levels exceeded 16.5%. Studies have reported that excessively high dietary nitrogen levels do not increase milk yield and recommended using lower nitrogen levels in diets to meet production needs while reducing nitrogen and phosphorus emissions [11].

Poppy et al. [12] demonstrated that feeding yeast culture increased average daily milk yield by 1.18 kg (range 0.55-1.81 kg). Wang Ling et al. [13] reported that adding 0.8%, 1.0%, and 1.2% compound yeast culture to diets improved dairy cow milk production performance by 8.48%, 10.05%, and 8.97%, respectively. Yeast culture can increase milk yield, improve milk quality, and benefit milk production persistence and feed efficiency. Feeding organic zinc to dairy cows can improve feed conversion efficiency by more than 4% and increase milk yield by 5%-10% [14]. In this experiment, reducing dietary crude protein level from 16.20% to 15.48% in the LPP group actually increased milk yield by 7.04% and feed conversion efficiency by 11.11%. This may be attributed to two factors: first, the replacement of some DDGS with beet pulp, as beet pulp has higher peNDF content than DDGS, and appropriate dietary peNDF content can provide substantial energy for ruminants and maintain high milk yield; second, the dietary crude protein level in the CR group may have already exceeded requirements. In this experiment, supplementing the diet with compound additives also increased milk yield by 10.30% and feed conversion efficiency by 13.89%, likely due to the yeast and organic zinc contained in the compound additives. The bioactive peptides and amino acids in yeast contributed to increased milk yield, while organic zinc participates in numerous enzymatic reactions in the body.

Milk somatic cell count reflects udder health and can predict mastitis incidence. In this experiment, milk somatic cell count decreased in both the LPP and CA groups, indicating that increasing beet pulp and adding compound additives can improve udder health. In the LPP group, beet pulp replaced some DDGS, and due to its high peNDF content, it stimulated chewing and rumination, promoting increased saliva secretion and indirectly improving rumen buffering capacity to maintain pH, thereby preventing rumen acidosis and maintaining rumen health, which also affected udder health. The yeast in the CA group has immune-enhancing effects, including anti-inflammatory capacity and enhanced natural killer cell activity [15]. Organic zinc supplementation promotes proliferation of teat canal epithelial cells, and a greater number of epithelial cell layers provides an effective barrier against pathogen invasion [16]. Therefore, increasing beet pulp content and feeding compound additives in this experiment helped reduce milk somatic cell count and consequently decrease mastitis incidence.

### **3.2 Effects of Different Dietary Compositions on Nitrogen Apparent Digestibility and Emissions in Lactating Holstein Cows**

Fecal nitrogen primarily consists of endogenous protein, undigested microbial protein from the small intestine, undigested dietary protein, and sloughed small intestinal epithelial cells. Fecal nitrogen emissions typically show little variation under different dietary nitrogen levels, mainly because increasing dietary nitrogen level leads to rapid increases in urinary nitrogen emissions, with almost all nitrogen exceeding animal requirements excreted via urine, while fecal nitrogen emissions remain relatively stable. Hynes et al. [17] found that when dietary

crude protein levels were 14.1%, 16.1%, and 18.1%, urinary nitrogen emissions were 193, 208, and 231 g/d, respectively, with significant differences among the three groups, but no significant effects on fecal nitrogen emissions. With similar dietary intake, crude protein level differences of 1.0% and 1.6% could significantly affect urinary nitrogen emissions but not fecal nitrogen emissions [18-19].

The changes observed in the LPP group in this experiment were consistent with reports by Hynes et al. [17] and Eriksson et al. [18], where small differences in dietary nitrogen level significantly affected urinary nitrogen emissions but not fecal nitrogen emissions. In this experiment, fecal nitrogen emissions were almost identical between the CR and LPP groups, while urinary nitrogen emissions were 211.02 and 183.08 g/d, respectively, showing significant differences. These differences suggest, first, that the dietary nitrogen level in the CR group may have exceeded the nitrogen requirements of the cows, and second, that although the LPP group had reduced dietary nitrogen level, the apparent digestibility of crude protein was 4.74% higher than in the CR group, possibly because beet pulp replacement of some DDGS increased peNDF content, which has a stimulating effect on the intestinal mucosa of ruminants, promotes gastrointestinal motility and fecal excretion, and thereby enhances digestion of various nutrients such as crude protein, neutral detergent fiber, and acid detergent fiber observed in this experiment.

Zhang Xingfu et al. [20] concluded that using nutritional regulators has long been a research hotspot in nutrition and related disciplines for emission reduction. However, practice has proven that using a single nutritional regulator does not achieve optimal results, and mixing several types can exert synergistic effects. Wang Ling et al. [13] reported that adding 0.8%, 1.0%, and 1.2% compound yeast culture to concentrate reduced total nitrogen emissions by 8.47%, 12.01%, and 9.36%, respectively, compared with the control group. Based on these findings, this experiment attempted to use a mixture of several nutritional regulators to explore whether optimal emission reduction effects could be achieved. Urinary nitrogen emissions in the CA and CR groups were 161.21 and 211.02 g/d, respectively, with significant differences between the two groups, while fecal nitrogen emissions showed smaller differences and total nitrogen emissions differed significantly. Nitrogen utilization efficiency increased by 8.70%, reducing nitrogen discharge to the environment. This may be because the yeast in the compound additive contains abundant nutritional active substances that can increase the activity of beneficial microorganisms in the rumen, better maintain rumen fluid pH and anaerobic environment, and improve dietary nitrogen utilization while reducing nitrogen loss. Additionally, organic zinc supplementation can improve protein feed utilization, which not only saves protein feed resources but also has important significance for reducing environmental pollution and dairy cow feeding costs.

### 3.3 Effects of Different Dietary Compositions on Phosphorus Apparent Digestibility and Emissions in Lactating Holstein Cows

Reducing dietary phosphorus levels and utilizing enzyme preparations are effective measures for improving phosphorus utilization efficiency while meeting dairy cow nutritional requirements. Phosphorus intake is primarily affected by dry matter intake, and fecal phosphorus emissions are proportional to dietary phosphorus intake. Zhang Feng et al. [21] reported that when dietary phosphorus increased from 0.36% to 0.54%, fecal phosphorus emissions increased from 98.45 to 152.78 g/d. Liu Jianxin et al. [1] reported that reducing dietary phosphorus level from 0.57% to 0.37% decreased fecal phosphorus emissions by 35.65%-40.8% and urinary phosphorus emissions by 22.73%-41.03% during early, mid, and late lactation. In this experiment, the dietary phosphorus level in the LPP group was 0.39%, reduced by 0.03% compared with the CR group, but fecal phosphorus emissions decreased by 26.40% and urinary phosphorus emissions decreased by 7.69%. The reduction in phosphorus emissions was primarily due to improved apparent phosphorus digestibility, possibly because the high peNDF content in beet pulp has a stimulating effect on the intestinal mucosa of ruminants, promoting gastrointestinal motility and fecal excretion, thereby enhancing digestion of various nutrients.

In this experiment, total phosphorus emissions in the CA group were significantly lower than in the CR group, possibly due to the enzyme preparation contained in the compound additive, which improved phosphorus apparent digestibility and utilization efficiency. The results of this experiment also demonstrated that urinary phosphorus emissions were minimal.

### 3.4 Effects of Different Dietary Compositions on Blood Biochemical Parameters of Lactating Holstein Cows

Blood biochemical parameter determination can indirectly reflect livestock health status. Additionally, when using additives and other nutritional regulation measures, blood biochemical parameters are used to reflect animal nutritional status [22] and to discuss whether these measures affect animal production performance and nutritional requirements.

Blood TC and TG are components of blood lipids, and their levels can reflect lipid metabolism status and dietary energy levels. In this experiment, there were no significant differences in these two parameters among the three groups, indicating that reducing nitrogen and phosphorus and using compound additives did not affect energy metabolism levels in cows. Blood ALB and GLB are major components of plasma proteins, and elevated TP content reflects enhanced protein metabolism. Blood TP and GLB in the CA group were higher than in the CR group, suggesting that compound additive supplementation can enhance protein metabolism and improve immune function.

ALT is an important enzyme in protein metabolism, and its activity reflects protein synthesis and catabolism status [22]. Blood ALT activity in dairy cows

ranges from 11.60 to 42.75 U/L [23]. In this experiment, there was no significant difference between the LPP and CR groups, with values within the reported normal range. The CA group showed a significant difference from the CR group, with a 17.58% increase, indicating that compound additives may promote conversion of protein, fat, and carbohydrates. Mineral element content in dairy cow blood is generally stable, with normal phosphorus (P) content ranging from 0.9 to 2.3 mmol/L [24]. All values in this experiment were within the normal range, but the CA group was significantly higher than the CR group, possibly due to the enzyme preparation in the compound additive enhancing phosphorus metabolism in the diet.

Blood UN can indirectly reflect nitrogen utilization from the diet. In this experiment, there was no significant difference in blood UN content between the LPP and CR groups, indicating that reducing dietary nitrogen level by 0.70% did not affect nitrogen metabolism. Blood UN content in the CA group was higher than in the CR group, increasing by 15.54%. Elevated UN content may indicate enhanced protein catabolism or increased hepatic urea recycling due to compound additive use, possibly attributable to dietary nitrogen levels exceeding requirements and compound additives enhancing nitrogen degradation capacity, thereby increasing serum UN content.

#### 4 Conclusions

1. Reducing dietary nitrogen and phosphorus levels by 0.70% and 0.03%, respectively, while increasing beet pulp content, increased dietary peNDF content and ultimately reduced total nitrogen and total phosphorus emissions by 9.11% and 26.34%, respectively.
2. Using compound additives improved feed utilization efficiency and reduced total nitrogen and total phosphorus emissions by 16.35% and 16.10%, respectively.
3. Reducing dietary nitrogen and phosphorus levels and using compound additives can both ensure normal growth and production of lactating dairy cows while reducing nitrogen and phosphorus emissions and decreasing livestock pollution of the environment. These are effective measures for reducing nitrogen and phosphorus emissions during dairy farming and mitigating environmental pollution.

#### References

- [1] 刘建新, 李珊珊, 张彬, 等. 改善奶牛氮磷利用效率的营养策略 [J]. 动物营养学报, 2014, 26(10): 3129-3134.
- [2] 付春丽, 王瑞宁, 傅彤, 等. 降低奶牛氮排放的营养调控措施 [J]. 家畜生态学报, 2012, 33(4): 96-101.
- [3] 柏兆海. 我国主要畜禽养殖体系资源需求、氮磷利用和损失研究 [D]. 博士学位论文. 北京: 中

国农业大学 2015.

- [4] 王笑笑, 李若玺, 梅洋, 等. 影响反刍动物氮素利用效率和排放的营养实践 [J]. 动物营养学报, 2016, 28(10): 3042-3050.
- [5] 红敏, 赵瑞霞, 高民, 等. 不同饲养模式下乳牛粪尿中氮磷排放量的研究 [J]. 饲料工业, 2012, 33(11): 48-51.
- [6] 张丽英. 饲料分析及饲料质量检测技术 [M]. 2 版. 北京: 中国农业大学出版社, 2003.
- [7] MERTENS D R. Creating a system for meeting the fiber requirements of dairy cows [J]. *Journal of Dairy Science*, 1997, 80(7): 1463-1481.
- [8] 李国林. 规模化奶牛场产排污系数、污水存贮及土地消纳量相关技术参数研究 [D]. 硕士学位论文. 泰安: 山东农业大学, 2014.
- [9] 翟少伟. 日粮蛋白质摄入量对泌乳奶牛氮排泄量的影响 [J]. *中国奶牛*, 2009(8): 56-59.
- [10] COLMENERO J J O, BRODERICK G A. Effect of dietary crude protein concentration on milk production nitrogen utilization lactating dairy cows [J]. *Journal of Dairy Science*, 2006, 89(5): 1704-1712.
- [11] WANG C, LIU J X, YUAN Z P, et al. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows [J]. *Journal of Dairy Science*, 2007, 90(6): 2960-2965.
- [12] POPPY G D, RABIEE A R, LEAN I J, et al. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows [J]. *Journal of Dairy Science*, 2012, 95(10): 6027-6041.
- [13] 王玲, 吕永艳, 程志伟, 等. 复合酵母培养物对奶牛产奶性能、氮排放及血液生化指标的影响 [J]. *草业学报*, 2015, 24(12): 121-130.
- [14] 吴淑军, 张亚伟, 姚亚军. 有机锌在奶牛上的应用研究 [J]. *饲料广角*, 2017(8): 29-30.
- [15] JENSEN G S, PATTERSON K M, YOON I. Yeast culture has anti-inflammatory effects and specifically activates NK cells [J]. *Comparative Immunology, Microbiology Infectious Diseases*, 2008, 31(6): 487-500.
- [16] GAAFAR H M A, BASIUONI M I, ALI M F E, et al. Effect of zinc methionine supplementation on somatic cell count in milk and mastitis in Friesian cows [J]. *Archiva Zootechnica*, 2010, 13(2): 36-46.
- [17] HYNES D N, STERGIADIS S, GORDON A, et al. Effects of crude protein level in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass [J]. *Journal of Dairy Science*, 2016, 99(10): 8111-8120.
- [18] ERIKSSON T, MURPHY M, CISZUK P, et al. Nitrogen balance, microbial protein production, and milk production in dairy cows fed fodder beets and potatoes, or barley [J]. *Journal of Dairy Science*, 2004, 87(4): 1057-1070.

- [19] REYNAL S M, BRODERICK G A. Effect of dietary level of rumen-degraded protein on production nitrogen metabolism in lactating dairy cows [J]. *Journal of Dairy Science*, 2005, 88(11):4045-4064.
- [20] 张兴夫, 高民. 降低奶牛养殖对环境污染的营养调控关键技术 [J]. *中国畜牧杂志*, 2016(20):35-39.
- [21] 张峰, 邱伟, 刘小静, 等. 日粮磷水平对泌乳奶牛生产性能及磷表观消化率的影响 [J]. *天津农业科学*, 2013, 19(3):38-42.
- [22] 周玉香, 吕玉玲, 王洁, 等. 血液生化指标在动物生产与营养调控研究中的应用概况 [J]. *畜牧与饲料科学*, 2012, 33(Z1):72-74.
- [23] 肖玲, 傅小平, 季节, 等. 上海地区荷斯坦成乳牛部分血清生化指标常值研究 [J]. *乳业科学与技术*, 2003, 26(3):132-134.
- [24] 李新萍, 陶岳, 张孝恩, 等. 新疆石河子地区奶牛血液生化指标正常参考值范围的建立 [J]. *中国奶牛*, 2011(18):47-50.

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

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