Postprint: Effects of Novel Selenium-Enriched and Rumen-Protected Choline Additives on Production Performance and Health Status of Periparturient Dairy Cows

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Abstract

This experiment aimed to investigate the effects of a novel selenium-enriched and rumen-protected choline additive on the production performance and health status of periparturient dairy cows. Using a completely randomized block design, 96 Holstein dairy cows were randomly divided into 4 groups based on parity, body condition score, total milk yield in the previous lactation cycle, and expected calving date: control group, low-dose (LD) group, medium-dose (MD) group, and high-dose (HD) group, with 24 cows in each group. The basal diets of the 4 groups were supplemented with 0, 40, 80, and 120 g/(head · d) of the novel selenium-enriched and rumen-protected choline additive (rumen-protected choline content 95%, selenium content 0.2%), respectively. The preliminary period was from 21 d to 15 d prepartum, and the formal experimental period was from 14 d prepartum to 28 d postpartum. Dry matter intake (DMI), plasma biochemical indices, antioxidant indices in liver tissue, and relative mRNA expression levels of key antioxidant proteins and lipid transport proteins were measured. The results showed: 1) No significant differences were observed among groups in DMI, body weight change, body condition score change, or calving ease score ($P>0.05$). 2) Dietary supplementation with the novel selenium-enriched and rumen-protected choline additive had no significant effect on lactation performance of dairy cows ($P>0.05$), but milk yield in the MD and HD groups was 1.8 and 1.6 kg/d higher than that in the control group, respectively. 3) Postpartum, compared with the control and LD groups, the MD and HD groups showed significantly reduced triglyceride (TG) content and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities ($P<0.05$). 4) Selenium content in plasma, liver tissue, and milk of cows in the MD and HD groups was significantly higher than that in the control group ($P<0.05$). 5) Compared

with the control and LD groups, liver tissue TG content in the MD and HD groups was significantly reduced (P<0.05). 6) Total antioxidant capacity (T-AOC) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in liver tissue of the MD and HD groups were significantly higher than those in the control and LD groups (P<0.05), whereas malondialdehyde (MDA) and hydrogen peroxide (H2O2) contents in liver tissue were significantly lower than those in the control and LD groups (P<0.05). 7) Relative mRNA expression levels of cellular glutathione peroxidase (GPx1), microsomal triglyceride transfer protein (MTTP), and apolipoprotein B100 (ApoB100) in liver tissue of the treatment groups were all significantly higher than those in the control group (P<0.05), but no significant effect was observed on relative mRNA expression level of phospholipid hydroperoxide glutathione peroxidase (GPx4) in liver tissue (P>0.05). The results suggest that the novel selenium-enriched and rumen-protected choline additive has a tendency to increase milk yield and effectively maintains liver function, with the optimal supplementation level being 80 g/(head · d).

Full Text

Abstract

This study investigated the effects of a novel selenium-enriched, rumen-protected choline additive on the production performance and health status of dairy cows during the transition period. Ninety-six Holstein dairy cows were randomly assigned to four groups using a complete randomized block design based on parity, body condition score, total milk yield from the previous lactation, and expected calving date: control, low dose (LD), medium dose (MD), and high dose (HD) groups, with 24 cows per group. The basal diets were supplemented with 0, 40, 80, and 120 g/(head · d) of the selenium-enriched, rumen-protected choline additive (containing 95% rumen-protected choline and 0.2% selenium) for the control, LD, MD, and HD groups, respectively. The pre-trial period ran from 21 to 15 days before calving, and the formal trial period extended from 14 days prepartum to 28 days postpartum. Measurements included dry matter intake (DMI), plasma biochemical indices, liver antioxidant markers, and the mRNA expression levels of key antioxidant and lipid transport proteins. The results showed: (1) No significant differences among groups in DMI, body weight change, body condition score change, or calving difficulty score (P>0.05). (2) Dietary supplementation with the additive did not significantly affect lactation performance (P>0.05), though milk yield in the MD and HD groups was 1.8 and 1.6 kg/d higher than the control group, respectively. (3) During the postpartum period, plasma triglyceride (TG) concentrations and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly lower in the MD and HD groups compared to the control and LD groups (P<0.05). (4) Selenium content in plasma, liver tissue, and milk was significantly higher in the MD and HD groups than in the control group (P<0.05). (5) Liver TG content was

significantly lower in the MD and HD groups compared to the control and LD groups (P<0.05). (6) Liver total antioxidant capacity (T-AOC) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were significantly higher in the MD and HD groups than in the control and LD groups (P<0.05), while malondialdehyde (MDA) and hydrogen peroxide (H O) contents were significantly lower (P<0.05). (7) The mRNA expression levels of cellular glutathione peroxidase (GPx1), microsomal triglyceride transfer protein (MTTP), and apolipoprotein B100 (ApoB100) in liver tissue were significantly upregulated in all treatment groups compared to the control (P<0.05), whereas phospholipid hydroperoxide glutathione peroxidase (GPx4) mRNA expression remained unaffected (P>0.05). These findings indicate that the selenium-enriched, rumen-protected choline additive tends to increase milk yield, effectively maintains liver function, and has an optimal supplementation rate of 80 g/(head · d).

Keywords: rumen-protected choline; selenium; novel additive; transition dairy cows; production performance; triglyceride

Introduction

Transition period dairy cows experience decreased dry matter intake (DMI) while facing increased energy demands for fetal development in late gestation and milk production after calving. This energy deficit enhances adipose tissue mobilization, producing non-esterified fatty acids (NEFA) that cannot be completely oxidized for energy in the liver. Consequently, NEFA are re-esterified into triglycerides (TG) in the liver, leading to fatty liver syndrome and other metabolic disorders that impair liver function, compromise health status, and reduce production performance [1-3]. Choline (which requires protection for effective absorption and utilization) synthesizes phosphatidylcholine in the body, which promotes very low-density lipoprotein (VLDL) synthesis. This facilitates TG utilization in liver tissue, effectively preventing TG deposition, protecting liver function, and improving lactation performance [4-7]. Zheng et al. [8] demonstrated that dietary supplementation with rumen-protected choline during the transition period delayed the decline in plasma glucose (Glu) concentration and significantly reduced plasma -hydroxybutyrate (BHBA), NEFA, and total cholesterol levels. Similarly, Cooke et al. [9] showed that rumen-protected choline decreased liver TG deposition, while Elek et al. [10] confirmed that 60 g/d of rumen-protected choline effectively reduced liver fat and TG content in periparturient dairy cows. Furthermore, the oxidation of NEFA for energy in liver tissue during the transition period generates oxidative byproducts that cause oxidative stress, further damaging liver function [11-12]. Selenium promotes the synthesis of antioxidant enzymes that scavenge these oxidative products, thereby exerting antioxidant effects and maintaining cow health [13-14]. However, dairy farming practices often overlook the concurrent oxidative stress experienced by transition cows suffering from energy metabolic diseases.

Our research team developed a novel selenium-enriched, rumen-protected choline additive specifically for liver protection. Theoretically, this additive provides dual functions of choline and selenium, preventing energy metabolic diseases while simultaneously scavenging free radicals and oxidative byproducts to maintain liver function and promote cow health. This trial evaluated the effects of this novel additive on production performance and health status when supplemented to transition dairy cows, providing theoretical basis and data support for its practical application.

Materials and Methods

1.1 Additive Composition

The selenium-enriched, rumen-protected choline additive was manufactured by Bright Farming Co., Ltd., containing 95% rumen-protected choline (25% choline content with 75% effective utilization rate) and 0.2% selenium.

1.2 Experimental Animals and Management

1.2.1 Location and Animals

The trial was conducted at Xinghuo Dairy Farm No. 2 in Shanghai. Ninety-six healthy Holstein dairy cows were selected at approximately 21 days prepartum, with (2.17±0.21) parities, body condition score of 3.47±0.11, and previous lactation total milk yield of (10.82±0.56) t.

1.2.2 Basal Diet and Feeding Management

Cows were housed in a double-row tail-to-tail tie-stall system with ad libitum access to water. The basal diet was formulated according to NRC (2001) dairy cattle nutrient requirements combined with production practices, and fed as total mixed ration (TMR) [15]. Diet composition and nutrient levels are presented in Table 1 . Feed was delivered three times daily at 06:00, 13:30, and 19:00 using a mechanical feed delivery vehicle, with 5% refusals maintained daily for DMI measurement. Milking occurred once at each feeding time.

1.3 Experimental Design

A complete randomized block design was employed, allocating 96 cows to four groups (control, LD, MD, and HD; n=24 each) based on parity, previous lactation milk yield, body condition score, and expected calving date (Table 2). The basal diets were supplemented with 0, 40, 80, and 120 g/(head · d) of the selenium-enriched, rumen-protected choline additive for the control, LD, MD, and HD groups, respectively. The additive was mixed with one-third of the basal diet before morning feeding. The pre-trial period was from 21 to 15 days prepartum, and the formal trial period was from 14 days prepartum to 28 days postpartum, with weekly sampling intervals.

1.4 Sample Collection and Processing

1.4.1 Milk Samples

Vacuum pipeline milking equipment was used to record daily morning, midday, and evening milk yields after calving. During each postpartum sampling period, milk samples were collected on three consecutive days, mixed at a 4:3:3 ratio, and 50 mL was preserved with 5% potassium dichromate and stored at 4°C for analysis.

1.4.2 Plasma Samples

Blood was collected from the tail vein before morning feeding at 14 and 7 days prepartum, on the day of calving, and at 7, 14, 21, and 28 days postpartum. Heparin sodium was used as anticoagulant, and plasma was separated by centrifugation at 3,500 r/min for 15 min at 4°C, then stored at -20°C.

1.4.3 Liver Tissue Samples

On day 14 postpartum, five cows were randomly selected from each group. Under fasting conditions and standing restraint, liver tissue was collected via puncture at the intersection point of the line between the tuber coxae and right elbow joint with the intercostal space between the 10th and 11th ribs (or 11th and 12th ribs for shorter cows), moved upward 2.5-3.5 cm [16]. Tissue was rinsed with pre-cooled sterile saline, aliquoted into cryovials, and stored in liquid nitrogen for determination of selenium content, TG content, antioxidant indices, and mRNA expression levels.

1.5 Measurements

1.5.1 Dry Matter Intake

During the formal trial period, feed offered and refused was recorded daily for each group. Daily intake was calculated as offered minus refused. At the end of the trial, feed samples were thawed, mixed thoroughly, and sampled using the quartering method. Samples were dried to constant weight at 55°C to determine dry matter content, which was used to calculate DMI.

1.5.2 Body Weight, Body Condition Score, and Calving Coefficient

Body weight was measured before morning feeding on the first and last days of the formal trial period. Body condition scoring was performed using a 5-point scale according to Wildman et al. [17]. Calving difficulty was scored as: 1=easy, 2=slight difficulty, 3=required assistance, 4=considerable difficulty, 5=cesarean section [18].

1.5.3 Milk Composition

A FOSS automatic milk composition analyzer was used to determine milk fat percentage, protein percentage, lactose percentage, total solids, urea nitrogen content, and somatic cell count. Four percent fat-corrected milk yield was calculated as: 4% FCM = 0.4 × milk yield (kg/d) + 0.15 × milk fat percentage (%) × milk yield (kg/d).

1.5.4 Plasma Biochemistry and Liver Antioxidant Indices

All analyses used kits from Nanjing Jiancheng Bioengineering Institute. Plasma biochemistry kits: Glu (F006), NEFA (A042-1), BHBA (H169), TG (F001), AST (C010-1), and ALT (C009). Liver antioxidant kits: T-AOC (A015), SOD (A001-1), GSH-Px (A005), MDA (A003-1), and H O (A064). Colorimetric methods were used with wavelengths specified by each kit, measured using a microplate reader (iMark, BIO-RAD, USA).

1.5.5 Selenium Content in Plasma, Liver, and Milk
Selenium content was determined according to the method of Wu [19].

1.5.6 Liver Triglyceride Content
Liver TG content was measured using a colorimetric enzyme-linked immunosorbent assay according to Schwartz et al. [20].

1.5.7 mRNA Expression of Key Antioxidant and Lipid Transport Proteins
Total RNA was extracted from liver tissue using RNAiso Plus. Reverse transcription was performed with PrimeScript™ RT Master Mix. Primers were validated using Premix Taq™ (TaKaRa). Real-time quantitative PCR was conducted using UltraSYBR Mixture (Beijing CoWin Biotech Co., Ltd.) to quantify mRNA expression of GPx1, GPx4, MTTP, and ApoB100, with -actin as the internal reference gene. The 2^(-ΔΔCT) method was used for analysis [16]. Primer sequences are listed in Table 3 .

1.6 Statistical Analysis
Data were analyzed using SAS 9.2 with a mixed model and compound symmetry covariance structure. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as mean±SD. Significance was declared at $P<0.05$, and trends were noted at $0.05 P<0.15$.

Results

2.1 Production Performance

As shown in Table 4 , no significant differences were observed among groups in prepartum, postpartum, or overall DMI ($P>0.05$). Similarly, body weight change, body condition score change, and calving difficulty score did not differ significantly among groups ($P>0.05$).

2.2 Lactation Performance

Table 5 shows that milk yield, 4% fat-corrected milk yield, milk fat percentage, protein percentage, lactose percentage, total solids, and urea nitrogen content did not differ significantly among groups ($P>0.05$). However, milk yield in the MD and HD groups was 1.8 and 1.6 kg/d higher than the control group, respectively, indicating a trend toward increased production.

2.3 Plasma Biochemical Indices

Dietary supplementation with the additive did not significantly affect plasma Glu, NEFA, or BHBA concentrations ($P>0.05$) (Table 6). During the prepartum period, plasma TG content and AST and ALT activities were also unaffected ($P>0.05$). However, during the postpartum period, plasma TG content and AST and ALT activities were significantly lower in the MD and HD groups compared to the control and LD groups ($P<0.05$). Across the entire trial period, plasma TG content and AST activity remained significantly lower in the MD and HD groups ($P<0.05$).

2.4 Selenium Content in Plasma, Liver Tissue, and Milk

As shown in Table 7 , selenium content in plasma, liver tissue, and milk was significantly higher in the MD and HD groups compared to the control group ($P<0.05$), with no significant difference between the MD and HD groups ($P>0.05$). The LD group did not differ significantly from other groups ($P>0.05$).

2.5 Liver Triglyceride Content

Figure 1 [Figure 1: see original paper] demonstrates that liver TG content was significantly lower in the LD, MD, and HD groups compared to the control group ($P<0.05$). The MD and HD groups also showed significantly lower liver TG content than the LD group ($P<0.05$), with no significant difference between the MD and HD groups ($P>0.05$).

2.6 Liver Antioxidant Indices

Table 8 reveals that liver T-AOC and SOD and GSH-Px activities were significantly higher in the LD, MD, and HD groups compared to the control group ($P<0.05$), while MDA and H_2O_2 contents were significantly lower ($P<0.05$). The MD and HD groups showed further improvements compared to the LD group ($P<0.05$), with no significant differences between the MD and HD groups ($P>0.05$).

2.7 mRNA Expression of Key Proteins in Liver Tissue

Figure 2 [Figure 2: see original paper] shows that GPx1 mRNA expression was significantly upregulated in all treatment groups compared to the control ($P<0.05$), with no significant differences among treatment groups ($P>0.05$). GPx4 mRNA expression did not differ significantly among groups ($P>0.05$). MTTP and ApoB100 mRNA expression levels were significantly higher in all treatment groups compared to the control ($P<0.05$), with the MD and HD groups showing significantly higher expression than the LD group ($P<0.05$) but no significant difference between the MD and HD groups ($P>0.05$).

Discussion

The transition period in dairy cows is characterized by decreased DMI due to fetal development and endocrine changes, coupled with increased energy demands for lactation, resulting in negative energy balance that severely impacts cow health and causes significant economic losses. Maintaining cow health under negative energy balance is critical for optimal production performance.

In this trial, no significant differences were observed in DMI, body weight change, body condition score change, calving difficulty score, or milk composition among groups. However, supplementation with 80 g/(head · d) of the additive increased milk yield by 1.6 kg/d compared to the control group despite similar DMI, suggesting a trend toward improved production efficiency. This may be attributed to the rumen-protected choline component effectively clearing liver TG, while the selenium component scavenged free radicals, thereby maintaining liver function, improving health status, and increasing milk yield. The significant reductions in plasma TG content and AST and ALT activities indicate effective liver function maintenance, while the decreased liver TG content and enhanced antioxidant capacity confirm the dual functionality of the additive. Sharma et al. [22] reported that postpartum infusion of 50 g/d choline significantly increased milk yield, and Xu et al. [23] found that rumen-protected choline increased milk yield without affecting milk composition, consistent with our findings.

GPx1 and GPx4 are selenoproteins regulated by selenium that play crucial roles in scavenging oxidative byproducts. This trial demonstrated that the additive significantly increased GPx1 mRNA expression without affecting GPx4, suggesting that GPx1 expression reaches a plateau at higher selenium requirements than GPx4. The supplemented selenium content in the basal diet met the requirement for GPx1 expression plateau. Christensen et al. [24] reported that selenium deficiency decreased rat liver GPx1 mRNA expression by 89%, while Sunde et al. [25] confirmed that dietary selenium changes did not significantly affect GPx4 mRNA expression. Zhou et al. [26] found that selenium supplementation significantly increased GPx1 but not GPx4 mRNA expression in ketotic cows when dietary selenium reached 0.3 mg/kg, consistent with our results.

MTTP functions in the liver endoplasmic reticulum to package TG into VLDL for secretion from the liver, while ApoB100 is a VLDL component involved in VLDL secretion. Sun et al. [7] reported that choline may regulate liver fat transport in transition cows by altering ApoB100 mRNA expression. In this trial, the additive significantly increased ApoB100 and MTTP mRNA expression, likely because absorbed rumen-protected choline promoted phosphatidylcholine synthesis, thereby enhancing VLDL synthesis and secretion and increasing ApoB100 and MTTP expression. The significantly lower liver TG content in all treatment groups supports this mechanism.

Conclusion

1. The selenium-enriched, rumen-protected choline additive significantly reduces liver TG content, effectively maintains liver function, and decreases liver damage.
2. The additive tends to increase milk yield in transition dairy cows and significantly enhances liver antioxidant capacity.
3. The optimal supplementation rate of the selenium-enriched, rumen-protected choline additive is 80 g/(head · d).

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