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

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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. A completely randomized block design was adopted, and 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 prepartum 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 mRNA relative 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 the milk yield of the MD and HD groups was 1.8 and 1.6 kg/d higher than that of the control group, respectively. 3) Postpartum, compared with the control and LD groups, the MD and HD groups showed significantly decreased triglyceride (TG) content and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities ($P<0.05$). 4) The 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, the TG content in liver tissue of the MD and HD groups was significantly decreased (P<0.05). 6) The 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), while the contents of malondialdehyde (MDA) and hydrogen peroxide (H2O2) in liver tissue were significantly lower than those in the control and LD groups (P<0.05). 7) The mRNA relative 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 had no significant effect on the mRNA relative expression level of phospholipid hydroperoxide glutathione peroxidase (GPx4) in liver tissue (P>0.05). These 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 and 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: a control group, low-dose (LD) group, medium-dose (MD) group, and high-dose (HD) group, with 24 cows per group. The basal diets were supplemented with 0, 40, 80, and 120 g/(head · d) of the selenium-enriched and 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 prepartum, and the formal trial period extended from 14 days prepartum to 28 days postpartum. Measurements included dry matter intake (DMI), plasma biochemical parameters, liver tissue antioxidant indices, and mRNA relative 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) Postpartum plasma triglyceride (TG) content 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 tissue TG content was significantly lower in the MD and HD groups

compared to the control and LD groups (P<0.05). (6) The MD and HD groups exhibited significantly higher total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in liver tissue (P<0.05), while malondialdehyde (MDA) and hydrogen peroxide (H O) contents were significantly lower (P<0.05). (7) The mRNA relative expression levels of cellular glutathione peroxidase (GPx1), microsomal triglyceride transfer protein (MTTP), and apolipoprotein B100 (ApoB100) in liver tissue were significantly higher in the treatment groups than in the control group (P<0.05), while phospholipid hydroperoxide glutathione peroxidase (GPx4) mRNA expression showed no significant difference (P>0.05). These results indicate that the selenium-enriched and 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 dairy cows experience decreased dry matter intake (DMI) combined with increased energy demands for fetal growth in late gestation and milk production after calving, leading to enhanced body fat mobilization. The resulting non-esterified fatty acids (NEFA) cannot be completely oxidized for energy in the liver and are subsequently re-esterified into triglycerides (TG), ultimately causing fatty liver and other energy metabolic diseases that impair liver function, compromise health status, and reduce production performance [1-3]. Choline (which requires protection for effective absorption and utilization) can synthesize phosphatidylcholine in the body, which promotes very low-density lipoprotein (VLDL) synthesis and facilitates TG utilization in liver tissue, thereby preventing TG deposition, protecting liver function, and improving lactation performance [4-7]. Zheng et al. [8] reported that dietary supplementation with rumen-protected choline in transition dairy cows delayed the decline in plasma glucose (Glu) content and significantly reduced plasma -hydroxybutyrate (BHBA), NEFA, and total cholesterol concentrations. Cooke et al. [9] also demonstrated that rumen-protected choline decreased TG deposition in liver tissue. Elek et al. [10] confirmed that dietary supplementation with 60 g/d of rumen-protected choline effectively reduced liver fat and TG content in dairy cows during the transition period. Additionally, the oxidation of NEFA for energy in liver tissue during the transition period generates oxidative products that cause oxidative stress, further damaging liver function [11-12]. Selenium promotes the synthesis of antioxidant enzymes to scavenge these oxidative products, thereby exerting antioxidant effects and maintaining cow health [13-14]. However, dairy farming practices often overlook the fact that transition cows suffering from energy metabolic diseases also experience oxidative stress. Our research team developed a novel liver-protective additive combining choline and selenium, which theoretically provides dual functions of choline and selenium—preventing en-

ergy metabolic diseases while scavenging free radicals and oxidative products to maintain liver function and promote cow health. This trial supplemented transition cow diets with this selenium-enriched and rumen-protected choline additive to investigate its effects on production performance and health status, providing theoretical basis and data support for the additive' s application in dairy production.

Materials and Methods

1.1 Reagents

Selenium-enriched and rumen-protected choline additive: Produced by Bright Farming Co., Ltd., containing 95% rumen-protected choline (25% choline content, 75% effective utilization rate) and 0.2% selenium.

1.2 Experimental Animals and Management

1.2.1 Location and Animals **Location:** Xinghuo Dairy Farm No. 2, Shanghai.
Animals: Ninety-six healthy Holstein dairy cows at approximately 21 days prepartum, with (2.17±0.21) parities, body condition score of 3.47±0.11, and total milk yield of (10.82±0.56) t from the previous lactation.

1.2.2 Basal Diet and Feeding Management Cows were housed in a double-row tail-to-tail tie-stall system with free 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 shown in . Feed was provided three times daily (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.

TABLE:1 shows the composition and nutrient levels of the basal diets (dry matter basis), including ingredients (corn silage, alfalfa hay, oat hay, Chinese wildrye, commercial prepartum and postpartum concentrates, cottonseed without lint, beet meal, and *Flammulina velutipes* residuals) and nutrient levels (dry matter, net energy for lactation, crude protein, neutral detergent fiber, and acid detergent fiber). The commercial concentrates were purchased from Bright Farming Co., Ltd., consisting primarily of corn, barley, soybean meal, dried distillers grains with solubles, minerals, and premix. Net energy for lactation was calculated based on NRC (2001) ingredient values; other nutrients were measured values determined by the Test Center of Shanghai Bright Holstein Co., Ltd. using GB/T methods for crude protein, NDF, ADF, calcium, and phosphorus.

1.3 Experimental Design

A complete randomized block design was employed, dividing 96 cows into four groups based on parity, total milk yield from the previous lactation, body condition score, and expected calving date: control, LD, MD, and HD groups, each with 24 cows (grouping information in). The control, LD, MD, and HD groups received 0, 40, 80, and 120 g/(head · d) of the selenium-enriched and rumen-protected choline additive, respectively, 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 one sampling period per week.

TABLE:2 presents the grouping information, including cow numbers, total milk yield of the last lactation period, initial body condition score, and parity. Two cows were removed due to health problems unrelated to the experimental treatment.

1.4 Sample Collection and Processing

1.4.1 Milk Sample Collection and Processing Vacuum pipeline milking equipment was used to record daily morning, noon, and evening milk yields after calving. During each sampling period postpartum, milk samples were collected continuously for the last 3 days from morning, noon, and evening milkings, mixed in a 4:3:3 ratio, and 50 mL was taken with 5% potassium dichromate added as preservative and stored at 4°C for analysis.

1.4.2 Plasma Sample Collection and Processing Blood was collected from the tail vein before morning feeding at 14 and 7 days prepartum, on calving day, and at 7, 14, 21, and 28 days postpartum, using sodium heparin as anticoagulant. Plasma was separated by centrifugation at 3,500 r/min for 15 min at 4°C and stored at -20°C until analysis.

1.4.3 Liver Tissue Sample Collection and Processing 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 from the hip tubercle to the right forelimb elbow joint and the intercostal space between the 10th and 11th ribs (or between the 11th and 12th ribs for shorter-bodied 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 relative expression levels of key antioxidant and lipid transport proteins.

1.5 Measurements

1.5.1 Dry Matter Intake During the formal trial period, feed was collected daily to record feed offered and refused for each group. Daily intake was calcu-

lated as feed offered minus refused. At the end of the trial, collected feed samples were thawed, thoroughly mixed, sampled using the quartering method, dried to constant weight at 55°C, and analyzed for dry matter content to calculate DMI.

1.5.2 Body Weight, Body Condition Score, and Calving Difficulty Score Body weight was measured before morning feeding on day 1 and the final day 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 calving; 2 = slight difficulty; 3 = requiring assistance; 4 = considerable difficulty; 5 = requiring cesarean section [18].

1.5.3 Milk Composition Milk fat percentage, protein percentage, lactose percentage, total solids content, urea nitrogen content, and somatic cell count were determined using a FOSS automatic milk composition analyzer. Four percent fat-corrected milk yield was calculated as:
4% FCM yield = 0.4 × milk yield (kg/d) + 0.15 × milk fat percentage (%) × milk yield (kg/d).

1.5.4 Plasma Biochemical Indices and Liver Tissue Antioxidant Indices All analyses used assay kits from Nanjing Jiancheng Bioengineering Institute. Plasma biochemical indices included Glu, NEFA, BHBA, TG, AST, and ALT (kit numbers F006, A042-1, H169, F001, C010-1, and C009, respectively). Liver tissue antioxidant indices included T-AOC, SOD, GSH-Px, MDA, and H O (kit numbers A015, A001-1, A005, A003-1, and A064, respectively). All samples were analyzed using colorimetric methods with specified wavelengths on a microplate reader (iMark, BIO-RAD, USA).

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

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

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

TABLE:3 lists the primers for real-time qPCR, including gene names, GenBank accession numbers, primer sequences (5 -3), product sizes, and references.

1.6 Statistical Analysis Data were analyzed using the Mixed model in SAS 9.2 with compound symmetry covariance structure. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as mean ± SD. $P<0.05$ indicated significant difference, and 0.05 $P<0.15$ indicated a trend.

Results

2.1 Production Performance

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

TABLE:4 presents the effects of the selenium-enriched and rumen-protected choline additive on production performance of transition dairy cows, including DMI (prepartum, postpartum, and whole period), body weight change, body condition score change, and calving difficulty score for control, LD, MD, and HD groups.

2.2 Lactation Performance

As shown in , no significant differences were found among groups in milk yield, 4% fat-corrected milk yield, milk fat percentage, protein percentage, lactose percentage, total solids content, or urea nitrogen content ($P>0.05$). However, milk yield in the MD and HD groups was 1.8 and 1.6 kg/d higher than the control group, respectively, showing a trend toward increased milk yield, though the difference was not significant ($P>0.05$).

TABLE:5 shows the effects of the additive on lactation performance, including milk yield, 4% FCM yield, milk fat, protein, and lactose percentages, total solids content, urea nitrogen content, and somatic cell count for all groups.

2.3 Plasma Biochemical Indices

As shown in , dietary supplementation with the additive had no significant effect on plasma Glu, NEFA, or BHBA concentrations in transition dairy cows ($P>0.05$). During the prepartum period, the additive also did not significantly affect plasma TG content or AST and ALT activities ($P>0.05$). 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 ($P<0.05$). Over the entire trial period, plasma TG content and AST activity were also significantly lower in the MD and HD groups ($P<0.05$).

TABLE:6 presents the effects on plasma biochemical indices, including Glu, NEFA, BHBA, TG, ALT, and AST for prepartum, postpartum, and whole period. Values with different superscript letters within the same row indicate significant difference (P<0.05); different capital letters indicate extremely significant difference (P<0.01).

2.4 Selenium Content in Plasma, Liver Tissue, and Milk

As shown in , 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).

TABLE:7 shows selenium content in plasma, liver tissue, and milk for all groups.

2.5 Triglyceride Content in Liver Tissue

As shown in [Figure 1: see original paper], 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 compared to the LD group (P<0.05), with no significant difference between the MD and HD groups (P>0.05).

FIGURE:1 illustrates the effects of the additive on TG content in liver tissue of transition dairy cows.

2.6 Liver Tissue Antioxidant Indices

As shown in , compared to the control group, the LD, MD, and HD groups exhibited significantly higher T-AOC and SOD and GSH-Px activities (P<0.05), and significantly lower H O and MDA contents (P<0.05). The MD and HD groups also showed significantly higher T-AOC, SOD, and GSH-Px activities compared to the LD group (P<0.05), along with significantly lower H O and MDA contents (P<0.05). No significant differences were observed between the MD and HD groups for any antioxidant indices (P>0.05).

TABLE:8 presents the effects on liver tissue antioxidant capacity, including T-AOC, SOD, GSH-Px, MDA, and H O for all groups.

2.7 mRNA Relative Expression of Key Antioxidant and Lipid Transport Proteins in Liver Tissue

As shown in [Figure 2: see original paper], GPx1 mRNA relative expression in liver tissue was significantly higher in all treatment groups compared to the control group (P<0.05), with no significant differences among treatment groups (P>0.05). No significant differences were observed among groups in

GPx4 mRNA expression (P>0.05). MTTP and ApoB100 mRNA relative expression levels were significantly higher in all treatment groups compared to the control group (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).

FIGURE:2 shows the effects of the additive on mRNA relative expression levels of GPx1 (A), GPx4 (B), MTTP (C), and ApoB100 (D) in liver tissue of transition dairy cows.

Discussion

Transition dairy cows experience negative energy balance due to decreased DMI and increased energy demands for fetal development and milk production, which severely affects their health and causes significant economic losses in dairy farming. Maintaining cow health under negative energy balance is critical for optimal production performance. In this trial, no significant differences were observed among groups in DMI, body weight change, body condition score change, calving difficulty score, or milk composition. Although not statistically significant, supplementation with 80 g/(head·d) of the additive increased milk yield by 1.6 kg/d compared to the control group, indicating a trend toward improved milk production under similar DMI conditions. This may be attributed to the rumen-protected choline component effectively clearing TG from liver tissue 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 demonstrated effective liver function maintenance, while the significant decrease in liver TG content and improvement in antioxidant capacity confirmed the dual functionality of the additive. Sharma et al. [22] reported that postpartum infusion of 50 g/d choline significantly increased milk yield in dairy cows, and Xu et al. [23] found that rumen-protected choline increased milk yield without affecting milk composition, consistent with our results.

GPx1 and GPx4 are selenoproteins regulated by selenium that play crucial roles in scavenging oxidative products. This study showed that the additive significantly increased GPx1 mRNA expression in liver tissue but did not affect GPx4 expression, suggesting that GPx1 reaches its expression plateau at a higher selenium requirement than GPx4, and that the selenium content from the additive satisfied the requirement for GPx1 plateau expression. Christensen et al. [24] found that selenium deficiency decreased GPx1 mRNA expression by 89% in rat liver, while Sunde et al. [25] confirmed that dietary selenium changes did not significantly affect GPx4 mRNA expression. Zhou et al. [26] reported that in ketotic dairy cows, GPx1 mRNA expression increased significantly when dietary selenium reached 0.3 mg/kg, while GPx4 expression remained unchanged, consistent with our findings.

MTTP functions in the endoplasmic reticulum of liver cells 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 hepatic lipid transport in transition cows by altering ApoB100 mRNA expression. In this trial, the significantly higher mRNA expression of ApoB100 and MTTP in treatment groups likely resulted from rumen-protected choline absorption promoting phosphatidylcholine synthesis, which enhanced VLDL synthesis and secretion, thereby increasing ApoB100 and MTTP expression. The significantly lower liver TG content in all treatment groups supports this mechanism.

Conclusion

1. The selenium-enriched and 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 improves liver tissue antioxidant capacity.
3. The optimal supplementation rate of the selenium-enriched and rumen-protected choline additive in the diet is 80 g/(head · d).

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