Effects of Pasteurized -Lactam-Positive Milk on Growth, Development and Serum Immune Indices of Calves (Postprint)

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Abstract

This study aimed to investigate the effects of feeding pasteurized -lactam-positive milk on growth performance and serum immune indices in calves. Eighteen healthy 3-day-old Holstein bull calves with similar body weight were selected and randomly divided into 2 groups (9 calves per group). Calves in the control group were fed -lactam-positive milk, while those in the experimental group were fed pasteurized -lactam-positive milk. Pasteurization conditions were heating at 63–65 °C for 30 min. Calves were weaned at 61 days of age, and the experimental period lasted for 180 days. The results showed that: 1) Compared with -lactam-positive milk, the counts of total bacteria, Escherichia coli, and Salmonella in pasteurized -lactam-positive milk were extremely significantly reduced ($P < 0.01$); 2) Compared with the control group, the average daily gain of calves in the experimental group during the period of 3–60 days of age was significantly increased ($P < 0.05$); 3) Compared with the control group, the serum immunoglobulin A (IgA) content at 30 days of age, and serum immunoglobulin M (IgM) content at 15 and 30 days of age of calves in the experimental group were significantly increased ($P < 0.05$), the serum immunoglobulin G (IgG) content at 30 days of age was significantly decreased ($P < 0.05$), the serum interleukin-1 (IL-1) content at 15 days of age was significantly increased ($P < 0.05$), and the serum interleukin-6 (IL-6) content at 7 days of age was significantly decreased ($P < 0.05$). These results suggest that feeding pasteurized -lactam-positive milk improved the growth and development of calves during the nursing period and affected the immune system of calves to a certain extent, but it could not be determined whether there was an enhancing or suppressive effect.

Full Text

Effects of Pasteurized -Lactam Antibiotic Milk on Growth, Development and Serum Immune Parameters of Calves

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Abstract

This study was conducted to investigate the effects of feeding pasteurized -lactam antibiotic milk on growth, development and serum immune parameters of calves. Eighteen healthy 3-day-old Holstein male calves with similar body weight [(42.82±0.35) kg] were randomly divided into two groups of nine calves each. Calves in the control group were fed -lactam antibiotic milk, while those in the experimental group were fed pasteurized -lactam antibiotic milk. Pasteurization was performed at 63–65 ℃ for 30 min. Calves were weaned at 61 days of age, and the experimental period lasted 180 days. The results showed that: 1) compared with -lactam antibiotic milk, pasteurized -lactam antibiotic milk exhibited extremely significant reductions in total bacterial count, Escherichia coli count, and Salmonella count ($P<0.01$); 2) compared with the control group, the experimental group showed significantly higher average daily gain during the 3–60 day period ($P<0.05$); 3) compared with the control group, serum immunoglobulin A (IgA) content at 30 days of age and serum immunoglobulin M (IgM) content at 15 and 30 days of age were significantly increased ($P<0.05$), serum immunoglobulin G (IgG) content at 30 days of age was significantly decreased ($P<0.05$), serum interleukin-1 (IL-1) content at 15 days of age was significantly increased ($P<0.05$), and serum interleukin-6 (IL-6) content at 7 days of age was significantly decreased ($P<0.05$). These results indicate that feeding pasteurized -lactam antibiotic milk improved the growth and development of suckling calves and affected their immune system to some extent, though it cannot be determined whether these effects represent enhancement or suppression.

Keywords: Holstein male calves; antibiotic milk; pasteurization; growth and development; serum immune parameters

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Dairy cows undergoing clinical treatment are typically administered -lactam antibiotics such as penicillin via injection, which results in antibiotic residues in the milk produced during and after treatment. Milk containing antibiotic residues and high somatic cell counts is collectively referred to as "antibiotic milk," also known as waste milk abroad. In Germany, annual production of antibiotic milk accounts for 1%–4% of total milk output, sufficient to feed all calves on dairy farms. As a zero-cost feed resource, producers often feed antibiotic milk to calves to reduce rearing expenses. According to a 2006 survey of over 300 dairy farms in Heilongjiang Province conducted by our research group, approximately 95% of farms fed antibiotic milk to calves, with 66% performing no treatment whatsoever. However, milk quality plays a crucial role in calf growth and health, necessitating research into the effects of antibiotic milk on calf development.

Pasteurization is defined by most national standards as "a broad category of heat treatment processes that inactivate alkaline phosphatase naturally present in raw milk while preserving peroxidase activity," aiming to eliminate pathogenic bacteria while maximizing nutrient preservation and maintaining authentic flavor. Globally, liquid milk is predominantly consumed as pasteurized milk, which commands high market shares in developed dairy nations: 99.9% in Canada, 99.7% in the United States, and over 95% in the United Kingdom, Australia, and New Zealand, though only 30% in China. Liu et al. reported that feeding pasteurized colostrum significantly reduced the ratio of forestomach weight to live weight and improved average daily gain while promoting gastrointestinal development and reducing diarrhea rates in calves. Han et al. found that feeding antibiotic milk significantly decreased average daily gain during early lactation while elevating serum immune parameters before and after weaning. Research findings on antibiotic milk effects on calf performance vary internationally, with few reports from China and even fewer studies examining pasteurized -lactam antibiotic milk impacts on calf growth and serum immune parameters. Therefore, this study investigated the effects of feeding pasteurized -lactam antibiotic milk on calf growth, development and serum immune parameters to provide a theoretical basis for scientific and effective utilization of antibiotic milk on Chinese dairy farms.

1.1 Experimental Animals and Design

Eighteen healthy 3-day-old Holstein male calves with similar body weight [(42.82±0.35) kg] were randomly allocated to control and experimental groups (n=9 per group). The control group received -lactam antibiotic milk, while the experimental group received pasteurized -lactam antibiotic milk. Both types of milk were fed at 37–39 ℃. The experimental period was 180 days.

1.2 Experimental Diet

All milk fed to calves was obtained from lactating cows during and after -lactam antibiotic treatment, containing antibiotic residues. Pasteurization was conducted at 63–65 ℃ for 30 min. The calf diet consisted of pelleted feed and high-quality Chinese wildrye, with formulations optimized using CPM software. Diet composition and nutrient levels are presented in Table 1 .

1.3 Feeding Management

Calves were fed 4 kg of colostrum within 0.5 h after birth. Feeding amounts for -lactam antibiotic milk and pasteurized -lactam antibiotic milk were as follows: 5 kg/d divided into three feedings at 3-7 days of age; 5.5 kg/d divided into three feedings at 8-20 days; 4 kg/d divided into two feedings at 21-40 days; 3 kg/d divided into two feedings at 41-50 days; and 1 kg/d as a single feeding at 51-60 days. Calf starter was introduced at 8 days of age at 0.3 kg/d per calf, increased to 1.0 kg/d after weaning at 61 days. Adequate water was provided, and calf pens were cleaned and disinfected daily to maintain sanitary conditions.

1.4 Milk Sample Collection and Analysis

Milk samples were collected once every 10 days, with three collections per day (morning, noon, and evening) pooled at a 4:3:3 ratio. For composition analysis, 50 mL of the pooled sample was preserved with 5% potassium dichromate at 4 ℃. For microbiological analysis, another 50 mL was collected in sterile bottles and stored at 4 ℃ for analysis within 2 h.

1.4.1 Milk Composition Milk composition was analyzed using a Foss Milkoscan 133B analyzer (Foss Electric, Denmark).

1.4.2 Somatic Cell Count Somatic cell count was determined using a Foss (r) Bentley Somacount CC-5000 analyzer (Foss Electric, Denmark).

1.4.3 Antibiotic Content Antibiotic content was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Agilent Technologies, USA).

1.4.4 Bacterial Counts Milk samples were serially diluted 1:10 to 10 . One milliliter from each dilution was plated on sterile culture media. Total bacteria were cultured on plate count agar (PCA) at 37 ℃ for 48 h, E. coli on violet red bile agar (VRBA) at 37 ℃ for 24 h, and Salmonella on SS agar at 37 ℃ for 24 h before enumeration.

1.5 Growth and Development Measurements

Body weight and measurements were recorded before morning feeding at 30, 60, and 180 days of age, and average daily gain was calculated for each period.

1.6 Serum Immune Parameter Measurements

Blood samples were collected via jugular venipuncture before morning feeding at 7, 15, 30, 60, 90, and 180 days of age. Serum was separated by centrifugation (4,000 r/min, 10 min), aliquoted, and stored at -20 ℃. Serum immunoglobulin

A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor- (TNF-) were measured by enzyme-linked immunosorbent assay (ELISA) using kits from R&D Systems (USA).

1.7 Statistical Analysis

Data were analyzed using the GLM procedure of SAS 9.2 software. Results are expressed as "mean ± standard error." Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2 Results

2.1 Composition of -Lactam Antibiotic Milk and Pasteurized -Lactam Antibiotic Milk

As shown in Table 2 , pasteurized -lactam antibiotic milk exhibited extremely significant reductions in total bacterial count, E. coli count, and Salmonella count compared with untreated -lactam antibiotic milk ($P<0.01$). However, no significant differences were observed in lactose percentage, milk protein percentage, milk fat percentage, total solids percentage, urea nitrogen content, or somatic cell count ($P>0.05$).

2.2 Effects of Pasteurized -Lactam Antibiotic Milk on Calf Growth and Development

Table 3 shows that average daily gain during 3–60 days of age was significantly higher in the experimental group than in the control group ($P<0.05$). No significant differences were found in body weight at 30, 60, or 180 days of age, nor in average daily gain during 60–180 days or 3–180 days ($P>0.05$), though values were numerically higher in the experimental group.

Table 4 demonstrates that compared with the control group, the experimental group exhibited significantly greater body height at 60 days of age and heart girth at 90 and 180 days of age ($P<0.05$). No significant differences were observed between groups in body oblique length, body straight length, cannon circumference, leg circumference, or chest depth at any time point ($P>0.05$).

2.3 Effects of Pasteurized -Lactam Antibiotic Milk on Serum Immune Parameters

Table 5 reveals that compared with the control group, the experimental group showed significantly increased serum IgA content at 30 days of age and serum IgM content at 15 and 30 days of age ($P<0.05$), while serum IgG content at 30 days of age was significantly decreased ($P<0.05$). Serum IL-1 content at 15 days of age was significantly higher ($P<0.05$), whereas serum IL-6 content at 7 days of age was significantly lower ($P<0.05$). No significant differences were detected between groups in serum IgA, IgM, IgG, IL-1 , or IL-6 content at other time points, nor in serum IL-2 or TNF- content at any time point (P>0.05).

3 Discussion

3.1 Comparison of Milk Composition

-Lactam drugs are the first choice for treating clinical and subclinical mastitis and endometritis in dairy cows due to their broad spectrum, low cost, convenience, and low toxicity. In this study, no significant difference in antibiotic content was observed between the two milk types, indicating that pasteurization did not affect antibiotic residues, consistent with Jorgensen et al. The milk fat and protein percentages in pasteurized -lactam antibiotic milk met international standards. Although some milk components such as protein are partially lost during pasteurization, no significant differences were observed between the two milk types in this study, aligning with Zang et al. Lactose is relatively stable during pasteurization with minimal content variation, which matches our results. Zhu et al. reported that milk fat and lactose are negatively correlated with somatic cell count, though this relationship was not observed in our study. Somatic cell count is an important indicator of cow health. When the udder is infected by bacteria or mechanically damaged by milking machines, leukocytes are secreted in large quantities to combat infection and repair tissue, resulting in elevated somatic cell counts in milk and potential changes in milk composition. In this study, somatic cell counts exceeded 1×10 cells/mL in both milk types, consistent with the pathological status of lactating cows and indicating that pasteurization does not affect somatic cell count.

Most clinical and subclinical mastitis cases are caused by bacterial infection. Bacteria can adhere to mammary epithelial cells, triggering local immune responses characterized by redness, inflammation, and decreased milk production. Total bacterial count is an indicator of overall microbial contamination and milk quality, with higher counts reflecting poorer cow health and milk quality. Pasteurization extremely significantly reduced total bacteria, E. coli, and Salmonella in -lactam antibiotic milk, consistent with Li et al. and Jorgensen et al., clearly demonstrating that pasteurization effectively kills pathogenic bacteria, reducing disease transmission risk to calves and improving milk safety.

3.2 Effects of Pasteurized -Lactam Antibiotic Milk on Calf Growth and Development

Body weight is a key indicator of calf growth status. Aust et al. reported that feeding waste milk did not affect calf growth, while other studies have shown higher body weights before and after weaning in calves fed pasteurized versus unpasteurized antibiotic milk. In our study, the experimental group exhibited significantly higher average daily gain during 3–60 days of age, similar to Song et al., suggesting that pasteurized -lactam antibiotic milk promotes calf growth. This may be attributed to the substantial reduction in total bacteria and harmful pathogens, decreasing potential disease incidence and maintaining calves in a healthy growth state. The lack of significant differences in body weight at individual time points and average daily gain during 60–180 days and 3–180 days suggests that pasteurized -lactam antibiotic milk primarily affects weight gain during the suckling period, with potential for improved overall growth.

Body measurements are important indicators of calf development, reflecting skeletal growth and management level. Hill et al. reported body measurements for Holstein calves: body height 86.4–93.1 cm and body oblique length 82.3–91.0 cm. Our calves' measurements at 60 days fell within these ranges, indicating normal development. The experimental group's significantly greater body height at 60 days and heart girth at 90 and 180 days may be related to their numerically higher body weights and average daily gains. Overall, pasteurized -lactam antibiotic milk promoted calf growth and development.

3.3 Effects of Pasteurized -Lactam Antibiotic Milk on Serum Immune Parameters

Immune function is essential for protecting organisms from bacterial, viral, and microbial invasion. Humoral immunity is mediated by immunoglobulins, with adequate levels required to resist disease and ensure calf health. IgA plays an important role in local mucosal immunity, IgM exhibits bacteriolytic activity and is the earliest immunoglobulin appearing in the digestive tract of newborn animals, and IgG is the most abundant component in humoral immunity with high concentration and long half-life. Daniels et al. reported serum IgG concentrations of 1,385–2,182 mg/dL in newborn Holstein calves, higher than observed in our study. The significantly lower serum IgG content at 30 days in the experimental group may be due to the higher bacterial load in untreated -lactam antibiotic milk, which stimulated greater IgG production. The significantly increased serum IgA at 30 days and IgM at 15 and 30 days in the experimental group may be related to improper storage or feeding practices that allowed microbial recontamination of pasteurized milk. Zou et al. reported that feeding untreated antibiotic milk resulted in high serum IgA and IgM levels, contrary to our findings, indicating that immunoglobulin concentrations are influenced by multiple factors with considerable variation.

Cytokines participate in cellular immune regulation, primarily promoting cell differentiation, proliferation, and antibody production. Kidd reported that IL-1 , IL-2, IL-6, and TNF- are commonly used to evaluate immune function status. Jin et al. demonstrated that IL-6 significantly enhances cellular and humoral immune function in mice, effectively acting as an immune adjuvant. The significantly reduced serum IL-6 content at 7 days in our experimental group may have contributed to the decreased serum IgG at 30 days. The significantly elevated serum IL-1 at 15 days suggests activation of the mononuclear phagocyte system, possibly related to recontamination of pasteurized milk and indicating improved immune function. IL-2 enhances interferon and TNF production while increasing natural killer and cytotoxic T cells, though this was not observed in our study. Overall, changes in serum immune parameters were scattered across time points without clear patterns, and given that the immune system is complex and susceptible to multiple factors, this study cannot conclude whether pasteurized -lactam antibiotic milk enhances or suppresses calf immunity.

4 Conclusions

1. From the perspective of growth and development, feeding pasteurized -lactam antibiotic milk significantly improved average daily gain during the suckling period.
2. Regarding serum immune parameters, feeding pasteurized -lactam antibiotic milk induced immune responses before weaning, but no definitive conclusions can be drawn regarding immune enhancement or suppression, warranting further investigation.

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