Effects of Different Zinc Sources on Growth Performance, Serum Hormones, and Immune Indices in Newborn Calves (Postprint)

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Abstract

This study aimed to investigate the effects of different zinc sources on growth performance, serum hormones, and immune indices in newborn calves. Thirty-six newborn Holstein heifer calves were selected and randomly divided into 3 groups with 12 calves per group. The control group received no supplementation, while the experimental groups were supplemented with 457 mg zinc methionine and 104 mg zinc oxide (equivalent to 80 mg of zinc) per calf per day, respectively; the zinc methionine and zinc oxide were mixed into milk for feeding. The experiment lasted until 14 days of age. Starter feed was introduced at 4 days of age. Daily feed intake and diarrhea incidence were recorded, and withers height, body length, chest circumference, and body weight were measured at the beginning and end of the experiment; blood samples were collected at the end of the experiment to determine serum immunoglobulin and hormone concentrations. The results showed: 1) Compared with the control group, zinc methionine supplementation significantly increased average daily gain ($P<0.05$) and significantly decreased diarrhea rate ($P<0.05$) in calves. Different zinc sources had no significant effects on average daily feed intake, total increase in withers height, total increase in body length, and total increase in chest circumference ($P>0.05$). 2) Compared with the control group, both zinc oxide and zinc methionine supplementation significantly decreased serum insulin concentration ($P<0.05$). 3) Compared with the control group, zinc oxide supplementation significantly increased serum immunoglobulin G and immunoglobulin M concentrations ($P<0.05$). There was no significant difference in serum immunoglobulin A concentration among groups ($P>0.05$). In conclusion, supplementing newborn calves with zinc methionine can effectively promote growth and reduce diarrhea rate, while supplementing with zinc oxide helps improve immune function.

Full Text

Effects of Different Zinc Sources on Growth Performance, Serum Hormone, and Immune Indices of Newborn Calves

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Abstract

This study investigated the effects of different zinc sources on growth performance, serum hormone levels, and immune indices in newborn calves. Thirty-six newborn Holstein heifer calves were randomly divided into three groups of 12 calves each. The control group received no supplementation, while the experimental groups received daily supplementation of 457 mg zinc methionine or 104 mg zinc oxide per calf (equivalent to 80 mg zinc). Zinc methionine and zinc oxide were mixed into milk for feeding. The trial lasted until 14 days after birth. Starter feed was introduced on day 4, with daily recording of feed intake and diarrhea incidence. Body height, slant length, chest circumference, and weight were measured at the beginning and end of the trial. Blood samples were collected at the end of the trial to determine serum immunoglobulin and hormone concentrations. The results showed that: (1) Compared with the control group, zinc methionine supplementation significantly increased average daily gain ($P<0.05$) and significantly reduced diarrhea rate ($P<0.05$). Different zinc sources had no significant effects on average daily feed intake, total body height gain, total body slant length gain, or total chest circumference gain ($P>0.05$). (2) Both zinc oxide and zinc methionine supplementation significantly reduced serum insulin content compared with the control group ($P<0.05$). (3) Zinc oxide supplementation significantly increased serum immunoglobulin G and immunoglobulin M contents compared with the control group ($P<0.05$). No significant differences were observed in serum immunoglobulin A content among groups ($P>0.05$). In conclusion, supplementing newborn calves with zinc methionine effectively promotes growth and reduces diarrhea rate, while zinc oxide supplementation helps improve immune function.

Keywords: newborn calves; zinc methionine; zinc oxide; growth performance; diarrhea; serum hormone; immune function

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Introduction

Calves represent the future of dairy operations, and their growth and health status directly affect the production performance of adult cows. Therefore, improving calf growth performance and reducing disease incidence are crucial for enhancing economic efficiency in dairy farming. Zinc is an essential trace element widely distributed in the cytoplasm and most organelles of animal cells. Its primary functions include maintaining normal morphology of epithelial cells and normal structure and function of biological membranes, preventing oxidative damage to membranes, and protecting normal receptor function. Research has shown that zinc can improve animal growth performance, enhance immunity, and effectively alleviate diarrhea. Zinc is recognized globally as the most effective agent for treating and preventing infant diarrhea, having saved millions of lives, and the World Health Organization (WHO) has implemented its use for diarrhea treatment in over 70 countries. Previous studies have demonstrated that adding high-dose zinc oxide (2,250 mg/kg) to weaned piglet diets promotes growth, improves immunity, and effectively reduces post-weaning diarrhea. However, due to concerns about animal excretion and environmental pollution, China has explicitly banned the use of high-dose zinc. The revised 2017 "Feed Additive Safety Use Standards" stipulate that zinc supplementation in calf milk replacer should not exceed 180 mg/kg. Current feeding standards do not clearly define the appropriate zinc supplementation level for calf diets. The NRC (2001) reported that calves fed semi-synthetic diets showed no zinc deficiency symptoms with zinc supplementation as low as 8 mg/kg, while calves fed natural diets still exhibited zinc deficiency symptoms even with 8–30 mg/kg zinc supplementation. Glover et al. found that feeding 80 mg zinc oxide or zinc methionine daily to diarrheic newborn calves aged 1-8 days alleviated diarrhea to varying degrees, indicating that 80 mg zinc has therapeutic effects on newborn calf diarrhea. However, whether dietary supplementation of 80 mg zinc has preventive effects on diarrhea in newborn calves remains unreported. Therefore, this trial aimed to investigate the effects of zinc methionine and zinc oxide on growth performance, diarrhea incidence, serum hormone levels, and immune indices in newborn Holstein heifer calves, providing a theoretical basis for the scientific application of inorganic and organic zinc in early calf rearing.

1.1 Experimental Materials

Zinc oxide (96.64% purity) was purchased from Hengyang Zhongbao Feed Technology Co., Ltd., Hunan Province. Zinc methionine (98.20% purity) was purchased from Shanghai Huating Chemical Co., Ltd.

1.2 Experimental Animals and Diets

The trial was conducted at Beijing Shunyi Zhongdi Animal Husbandry Technology Co., Ltd. Thirty-six healthy newborn Holstein heifer calves with similar birth weights were selected and fed 4 L of colostrum within 2 h after birth. On days 2–3, calves were fed whole milk using a nursing bottle twice daily (08:00 and 14:00), 2 L each time. From days 4 to 14, calves were fed whole milk using a bucket twice daily (07:00 and 14:00), 4 L each time. Starter feed was introduced on day 4 for ad libitum consumption, with daily intake recorded. The milk was produced by Beijing Shunyi Zhongdi Animal Husbandry Technology

Co., Ltd. The starter feed was calf starter 641p, produced by Beijing Shounong Animal Husbandry Technology Development Co., Ltd. Feed Branch. The starter composition included corn, wheat bran, extruded soybean, soybean meal, cottonseed meal, dicalcium phosphate, vitamins, mineral elements, limestone, sodium chloride, wheat middlings, etc., in pelleted form. Analysis revealed a zinc content of 172 mg/kg. The nutrient levels of milk and starter feed are shown in Table 1.

Table 1 Nutrient levels of milk and starter feed (air-dry basis)

Items	Milk	Items	Starter
Density (g/L)		DM	
Milk protein		CP	
Milk fat		EE	
Total solid		Ash	
DM		NDF	
Lactose		ADF	

Nutrient levels are analyzed values.

1.3 Experimental Design and Management

Calves were randomly divided into three groups of 12 based on similar body weight. Starting from day 1 after birth, the control group received no supplementation, while the experimental groups received daily supplementation of 457 mg zinc methionine or 104 mg zinc oxide per calf (equivalent to 80 mg zinc). Zinc methionine and zinc oxide were mixed into milk for feeding. The trial lasted until 14 days after birth. Calves were housed individually in calf hutches (approximately 3 m^2 per calf) with maintained sanitation. Feeding followed the "four fixed" principle: fixed timing, fixed quantity, fixed temperature, and fixed personnel. Vaccination protocols followed the farm' s standard procedures.

1.4 Sample Collection and Analysis

1.4.1 Milk and Starter Feed Sampling Milk and starter feed samples were collected weekly and stored at -20°C. Milk dry matter, protein, fat, lactose, and total solid contents were determined using a MilkoScan™ FT6000 milk composition analyzer. Zinc content in starter feed was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) according to GB5009.268–2016. Starter feed dry matter (AOAC, 2005; method 930.15), crude protein (AOAC, 2000; method 976.05), crude fat (AOAC, 2003; method 4.5.05), and ash (GB/T 6438-1992) contents were determined. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to Van Soest et al.

1.4.2 Growth Performance and Diarrhea Rate Calves were weighed at birth and on day 15 before morning feeding (fasting) using an electronic scale (capacity 200 kg, precision 0.5 kg). Body measurements (height, slant length, and chest circumference) were taken using a tape measure. Average daily gain (ADG), total body height gain, total body slant length gain, and total chest circumference gain were calculated. Daily milk and starter feed intake were recorded to calculate average daily feed intake (ADFI). Fecal consistency was scored daily according to Teixeira et al. Calves with fecal fluidity and viscosity scores exceeding 3 were recorded as having diarrhea. Each diarrheic day per calf was counted as one incidence day. Diarrhea days and number of diarrheic calves were recorded to calculate diarrhea rate:

$$\text{Diarrhea rate (\%)} = \frac{\text{Total diarrheic calves} \times \text{Diarrhea days}}{(\text{Total calves} \times \text{Total trial days})} \times 100$$

1.4.3 Serum Hormone and Immune Indices On day 15, 10 mL blood samples were collected via jugular venipuncture before morning feeding. Serum was prepared after standing at room temperature for 30 min and centrifugation at 3,000×g for 15 min at 4°C, then stored at -20°C. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined by enzyme-linked immunosorbent assay (ELISA) using kits from Bethyl Corporation, USA, following the manufacturer’ s instructions. Serum somatostatin (SS), insulin-like growth factor-1 (IGF-1), ghrelin (GHRL), insulin (INS), gastrin (GAS), and cholecystokinin (CCK) contents were determined by radioimmunoassay using kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Data were initially processed using Excel 2007 and analyzed by one-way ANOVA using SAS 9.4 software. Duncan’ s multiple range test was used for post-hoc comparisons. $P<0.05$ was considered statistically significant, and 0.05 $P<0.10$ indicated a significant trend.

Results

2.1 Effects of Different Zinc Sources on Growth Performance and Diarrhea Rate in Newborn Calves

As shown in Table 2 , compared with the control group, zinc methionine supplementation significantly increased total weight gain and ADG in newborn calves ($P<0.05$). Throughout the trial, total weight gain and ADG in the zinc oxide group were intermediate between the control and zinc methionine groups, but did not differ significantly from either group ($P>0.05$). Different zinc sources had no significant effects on ADFI or body measurement indices ($P>0.05$). Additionally, different zinc sources showed some alleviating effects on diarrhea incidence. The diarrhea rate in the zinc methionine group was significantly lower than in the control group (P<0.05), while the zinc oxide group did not differ significantly from the other two groups (P>0.05).

Table 2 Effects of different zinc sources on growth performance and diarrhea rate of newborn calves

Items	Control	ZnO	Met-Zn	P-value
Initial weight (kg)				
Final weight (kg)				
Total weight gain (kg)	7.27b	8.18ab	9.05a	
ADG (g/d)	519.16b	584.42ab	646.75a	
Starter ADFI (g/d DM)				
ADFI (g/d DM)				
Total body height gain (cm)				
Total body slant gain (cm)				
Total bust gain (cm)				
Diarrhea rate (%)	26.49b	22.92ab	18.45a	<0.05

In the same row, values with different small letter superscripts differ significantly (P<0.05), while those with the same or no superscripts do not differ significantly (P>0.05). The same applies below.

2.2 Effects of Different Zinc Sources on Serum Hormone Indices in Newborn Calves

As shown in Table 3 , compared with the control group, both zinc oxide and zinc methionine supplementation significantly reduced serum INS content in newborn calves (P<0.05). Different zinc sources had no significant effects on serum SS, GHRL, GAS, or CCK contents (P>0.05). Compared with the zinc oxide group, zinc methionine supplementation showed a trend toward increasing serum IGF-1 content (0.05 P<0.10).

Table 3 Effects of different zinc sources on serum hormone indices of newborn calves

Items	Control	ZnO	Met-Zn	P-value
SS (ng/L)				
IGF-1 (ng/L)	109.97			
GHRL (ng/L)				
INS (IU/L)	61.11a	50.32b	36.77c	<0.01
GAS (ng/L)				
CCK (ng/L)				

2.3 Effects of Different Zinc Sources on Serum Immune Indices in Newborn Calves

As shown in Table 4 , different zinc sources had no significant effect on serum IgA content in newborn calves (P>0.05). Compared with the control group, zinc oxide supplementation significantly increased serum IgG and IgM contents (P<0.05), while zinc methionine supplementation showed no significant differences in these parameters (P>0.05).

Table 4 Effects of different zinc sources on serum immune indices of newborn calves

Items	Control	ZnO	Met-Zn	P-value
IgA (μg/mL)	0.94b	2.17b	1.28a	4.79a
IgG (mg/mL)				
IgM (mg/mL)				

Discussion

3.1 Effects of Different Zinc Sources on Growth Performance in Newborn Calves

Improving growth performance is a primary objective in calf rearing management. Feed intake, daily gain, and body measurement increments are important indicators for evaluating growth performance. Glover et al. found that daily supplementation of 80 mg zinc methionine to diarrheic newborn calves resulted in 40 g higher ADG compared with a placebo group. Zhou et al. reported that adding 60 mg/kg zinc bacitracin to calf diets increased ADG by 30.81%. Cai et al. demonstrated that adding 200 mg zinc to calf diets increased ADG by 12.50%. To minimize environmental pollution while considering diarrhea alleviation, we selected a supplementation level of 80 mg zinc per calf daily based on existing research. The initial body weights of calves in this trial showed no significant differences. This study indicated that dietary supplementation with 80 mg zinc oxide or zinc methionine had no significant effect on ADFI, but 80 mg zinc methionine significantly increased ADG. Previous studies have shown that zinc methionine promotes growth in piglets, laying hens, and meat rabbits, consistent with our findings. The growth-promoting effect of zinc methionine may be attributed to its being a trace element supplement close to the natural form in animals, with good chemical and biochemical stability, high bioavailability, strong anti-interference ability, and low toxicity. No significant differences were observed in body measurement indices among groups, although zinc methionine and zinc oxide tended to increase total body height and slant length gains while slightly decreasing total chest circumference gain, suggesting they may promote skeletal growth.

3.2 Effects of Different Zinc Sources on Diarrhea Rate in Newborn Calves

Diarrhea is a common disease in newborn calves that not only reflects current health status but also affects future production and reproductive performance, significantly impacting farm economic efficiency. Therefore, preventing calf diarrhea is a critical management issue. Glover et al. found that daily supplementation of 80 mg zinc oxide to diarrheic newborn calves had 1.4 times the cure rate of a placebo. In this study, dietary zinc methionine significantly reduced diarrhea rate, while zinc oxide also showed a trend toward reduction. Shi et al. reported that injecting 60 mg zinc on day 3 significantly reduced calf diarrhea rate. Lei found that zinc methionine and zinc oxide promoted growth and alleviated diarrhea in piglets. This study demonstrates similar effects in preruminant calves. Chen et al. showed that zinc methionine improved intestinal morphology and optimized cecal microbiota in laying hens. Hu et al. found that zinc oxide affected intestinal barrier function and promoted normal intestinal function in piglets. We hypothesize that zinc may alleviate diarrhea by improving intestinal microbiota and promoting intestinal mucosal development. In this trial, zinc oxide prevented calf diarrhea but showed no significant difference from the control group, possibly due to the relatively low dosage.

3.3 Effects of Different Zinc Sources on Serum Hormone Indices in Newborn Calves

Hormones are crucial regulators of growth, development, and metabolism. The hormones examined in this trial are all related to calf growth and development. INS regulates the metabolism and storage of proteins, carbohydrates, and fats. When sufficient free zinc is available in blood, zinc is adequate in pancreatic -cells, reducing INS secretion. Zinc also enhances INS-degrading enzyme activity, decreasing serum INS content. Liu et al. reported that appropriate zinc doses reduced serum INS content in rats. In this study, dietary zinc methionine or zinc oxide significantly reduced serum INS content, likely due to zinc supplementation. Insulin-like growth factors (IGFs), primarily produced by the liver, are peptides with both growth-promoting and insulin-like effects. O' Neill et al. demonstrated that IGF-1 promotes catabolism at the organismal and tissue levels, stimulates cell division and bone growth, enhances protein, fat, and glycogen synthesis, and promotes amino acid and glucose uptake, thereby facilitating animal growth. This study found that zinc methionine supplementation tended to increase serum IGF-1 content compared with zinc oxide, and the ADG of calves in the zinc methionine group was higher than in the control group, possibly because zinc methionine promoted IGF-1 synthesis and secretion, thereby improving growth performance. Li et al. reported that zinc oxide increased IGF-1 content in piglet small intestine but had no significant effect on serum IGF-1, consistent with our results. Dietary zinc oxide or zinc methionine had no significant effects on other serum hormone contents.

3.4 Effects of Different Zinc Sources on Serum Immune Indices in Newborn Calves

Immunoglobulins are important indicators of immune function, mainly including IgG, IgM, and IgA. IgG is the most abundant antibody in serum and the primary antibody of humoral immunity. IgM is the first antibody to appear during immunity and infection, while IgA is the main antibody in external secretions, all being indispensable for immunity. Serum immunoglobulin content is closely related to calf immunity. Lv et al. reported that low-dose zinc supplementation (zinc sulfate) improved both ADG and immune function in calves. Nagalakshmi et al. also demonstrated that low-dose organic zinc supplementation improved growth performance and immune response in calves. This study showed that zinc oxide supplementation significantly increased serum IgG and IgM contents, while zinc methionine supplementation tended to increase these parameters, but neither affected serum IgA content. Other studies have shown that dietary zinc supplementation improves immune function in piglets and laying hens, consistent with our results. We hypothesize that zinc may promote immunoglobulin synthesis by regulating protein and energy metabolism in animals. Enhanced immunity helps resist stress, which may be one reason for the reduced diarrhea rate in zinc-supplemented groups.

Conclusion

1. Supplementing newborn calves with zinc methionine effectively promotes growth and reduces diarrhea rate.

2. Both zinc oxide and zinc methionine supplementation significantly reduce serum INS content in newborn calves.

3. Zinc oxide supplementation improves immune function in newborn calves.

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