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The Effect of Using Different Zinc Sources on Performance, Blood Parameters, and Some Enzymes Related to the Immune System in Holstein Calves

Research Article

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ABSTRACT

This study aimed to evaluate the effects of different zinc sources on performance, blood parameters, and key immune system enzymes in calves. The experiment was conducted over a period of 70 days using 20 calves divided into four treatment groups. The treatments included: (1) a basal diet without zinc supplementation, (2) a basal diet supplemented with 40 ppm zinc in the form of nano zinc oxide, (3) a basal diet supplemented with 40 ppm zinc in the form of zinc oxide, and (4) a basal diet supplemented with 40 ppm zinc in the form of zinc glycine. The results indicated that starter intake, total feed intake, daily weight gain, and final weight in calves fed diets containing different zinc forms showed significant improvement compared to the control group during the last month of the study ($P<0.05$). A significant difference was also observed between the control group and the groups receiving nano zinc oxide and zinc glycine during the second period of the experiment ($P<0.05$). Blood parameter analysis revealed that zinc supplementation had no significant effect. However, the zinc levels in the nano zinc oxide and zinc glycine groups were significantly higher compared to the control ($P<0.05$). Additionally, the inclusion of different zinc forms markedly influenced total antioxidant activity and superoxide dismutase concentration in calves ($P<0.05$). Overall, these findings suggest that dietary supplementation with various forms of zinc.

KEY WORDS blood parameters, Holstein calf, immune system, organic and inorganic forms of zinc.

INTRODUCTION

With the advancement of nutritional technologies, new mineral supplements have been produced that require research and comparison with previous products, including nano-supplements. Currently, nanotechnology is the most advanced and newest human technology, enabling the production of new materials, tools, and systems at the atomic level and creating structures with entirely new molecular arrangements (Altemimi *et al*. 2024). Thus, nanobiotechnology converges basic sciences, agriculture, food industries, medical sciences, and biotechnology, having numerous applications. Nanoparticles are a class of materials with dimensions ranging from 1 to 100 nanometers, possessing unique properties. Given recent advancements in nanotechnology, it is expected that metal oxide nanoparticles will be used in various fields, including catalysis, environmental remediation, agriculture, and biomedicine (Seyedalipour *et al*. 2014; Nile *et al*. 2020). The most recognized methods include microemulsion, colloidal synthesis, precipitation, sol-gel methods, and thermal synthesis. Due to the unique properties of nano-oxide, this material is used in various industries, including pharmaceuticals, food, and different sciences such as agriculture, and even as feed additives

(Song *et al*. 2010; Parashar *et al*. 2020). Among the mineral nutrients that can be used in various forms with nanotechnology and whose role in animal health and growth has been proven is zinc. Zinc is a trace mineral essential for the health, immunity, and optimal performance of animals (Abdelnour *et al*. 2021). Zinc deficiency can lead to reduced production and health disorders in livestock (Case and Carlson, 2002). Zinc is one of the minerals involved in many vital body functions, such as growth, DNA synthesis, and the structure of hormones and enzymes, making it essential in animal diets (Suttle, 2010). This element is one of the most limiting factors in the nutrition of domestic animals, and since the body cannot store large amounts of this element, it must be provided daily in the diet (Pal *et al*. 2010; Silva *et al*. 2024). Any increase or deficiency of this element can cause adverse effects and reduce animal performance (Zaboli and Arabi, 2013). Besides its positive effect on insulin secretion and release, zinc is also effective in the structure and activity of glycolysis pathway enzymes, thus influencing glucose oxidation and uptake by body cells, ultimately reducing blood glucose concentration (Najafzadeh *et al*. 2013; Poudel *et al*. 2017).

This element plays a role in protein breakdown and ammonia production in the rumen, affecting blood urea levels. Zinc may also play a role in the expression of the appetite-controlling hormone (cholecystokinin), and its absence in the diet can reduce appetite (Petrič *et al*. 2024). Higher concentrations of zinc in the diet may also negatively affect appetite (Rajendran *et al*. 2013). Given the above, the present study aims to investigate the effect of using different sources of zinc on performance, blood parameters, and the expression of some enzymes related to the immune system in Holstein calves. The objectives of this study were to examine the impact of using different forms of zinc sources in the diet on the performance of suckling calves to achieve optimal management indicators for calf rearing, such as feed intake, weight gain, and to investigate changes in some blood parameters and the immune system of the calves.

MATERIALS AND METHODS

Experimental animals and housing

For this experiment, 20 newborn female Holstein calves, approximately the same age and with an average body weight of 36 ± 8 kg, were selected from the dairy farm. The calves were divided into four experimental groups of five calves each, based on body weight and age at birth. Each group was randomly assigned to one of the experimental diets. The calves in the four groups were housed individually, with each pen equipped with separate feeding and watering facilities. The pens were roofed and had concrete floors. Before transferring the calves to the pens, the floors and surrounding areas were washed and limed. After drying, the floors of each pen were covered with bedding. Manure was removed once daily before the morning feeding, and straw was spread over the pen surface after each cleaning.

Calf feeding and water consumption

Immediately after birth, each calf was fed 2 liters of colostrum in two consecutive feedings, 6 hours apart. Colostrum feeding continued for another 2 days at 10% of body weight. During the milk-feeding period, the calves were fed 2.5 kg of milk twice daily, at 7 a.m. and 5 p.m. Drinking water was provided twice daily, in the morning and afternoon.

Preparation of zinc

Sources Zinc oxide and nano zinc oxide were obtained from Pishgaman Nano Materials Iranian Company (Mashhad, Iran). Zinc glycine was sourced from Zowareh Livestock and Poultry Supplements Company (Isfahan, Iran) for this project.

Experimental treatments

The feed ingredients and components of the experimental diets are reported in Table 1. The experimental diets were as follows:

1. Basal diet (without zinc supplement, as control diet).
2. Basal diet + 40 ppm zinc in the form of nano zinc oxide.
3. Basal diet + 40 ppm zinc in the form of zinc oxide.
4. Basal diet + 40 ppm zinc in the form of zinc glycine.

Table 1 Percentage of feed components in the starter used for calves in experimental treatments

Feed ingredients	Percentage
Barley	29.5%
Corn	22%
Soybean meal	31.5%
Wheat bran	13%
Vitamin-mineral supplement[1]	1%
Calcium carbonate	1%
Enzymit	1%
Salt	0.5%
Baking soda	0.5%

The experimental diets were formulated using NRC (2001) software for dairy cows, based on the nutritional requirements of a 36 kg calf and considering the chemical composition of the available feed ingredients. The percentage of the starter diet components is provided in Table 1.

The chemical composition of the feed ingredients consumed by the calves (moisture, crude protein, crude fiber, ash, calcium, and phosphorus) was determined in the Animal Nutrition Laboratory of Mohaghegh Ardabili University (Table 2).

Calf feeding method

Before the main experimental period began, a short adaptation period was implemented to accustom the calves to the experimental diet and individual pens. After a few days, the calves adapted to the conditions of using the experimental diets. Various forms of zinc were added to the morning milk feed and provided to the calves. The forage portion was also added to the calves' concentrated diet after one month of milk feeding. The daily feed intake was given in a single meal at 9:30 a.m. as a separate diet and provided to the calves *ad libitum*, ensuring that the feed residue each day (on a dry matter basis) was about 10% of the previous day's feed.

Sampling and recording experimental data

Performance traits

Given that the calves were fed individually, the amount of feed consumed by each calf was recorded daily. The amount of feed placed in each calf's bucket was recorded daily, and the leftover feed was collected and weighed the next morning. Fresh feed was then placed in the bucket. The calves were weighed individually every two weeks using a digital scale with an accuracy of 300 grams. The feed conversion ratio was calculated by dividing the average feed intake of each calf by its weight gain.

Calf health assessment

The health of the calves (eye discharge, nasal discharge, respiration, and stool consistency) was monitored daily based on the method provided by the University of Wisconsin. The best health status was scored as zero, and the worst health status was scored as three. The scoring method for calf health is shown in Table 3.

Blood sampling

Blood samples were collected from the calves at the end of the rearing period from the jugular vein. The samples were centrifuged at 3500 rpm for 15 minutes at 4 °C. The obtained serum was collected using a sampler and placed into 2 mL microtubes. The samples were stored at -20 °C until laboratory analysis.

Chemical analysis of blood samples

After thawing the frozen blood samples at room temperature, the concentrations of glucose, cholesterol, triglycerides, and total protein were measured in the Nutrition Laboratory of the Department of Animal Science, Faculty of Agricultural Sciences and Natural Resources, Mohaghegh Ardabili University, as follows:

Total cholesterol measurement

Total cholesterol was measured using an enzymatic-colorimetric method with a kit from Pars Azmoon Company (catalog number 010-500-1, Iran) and a spectrophotometer at a wavelength of 500-546 nm, according to the kit instructions, with two repetitions. A control sample was included every 10 samples (20 tubes). The intra-assay coefficient of variation was 4.7%.

Triglyceride measurement

To measure the serum triglyceride concentration, 10 µL of serum was mixed with 1000 µL of reagent, incubated in a 37 °C water bath for 10 minutes, and then the optical absorbance was read against the reagent blank at 546 nm. Then, 10 µL of standard reagent was mixed with 1000 µL of reagent, and its optical absorbance was also recorded at 546 nm. The triglyceride concentration was calculated using the following formula:

Triglyceride concentration= (absorbance of serum/absorbance of standard) × standard concentration

Glucose measurement

The glucose concentration was measured using an enzymatic-colorimetric method with a Pars Azmoon kit (catalog number 017-500-1, made in Iran) and a spectrophotometer at a wavelength of 500-546 nm, according to the kit instructions and with two repetitions. To evaluate accuracy and calculate the coefficient of variation, a control sample was placed every 10 test samples (20 tubes), and the obtained concentrations for the control serum were compared with the concentrations listed on the control serum bottles to ensure the obtained concentration was within the range listed on the control serum bottles. The intra-assay coefficient of variation was 7.9%.

Total protein

In this test, protein forms a blue complex with copper ions in an alkaline environment. The intensity of the color produced is proportional to the amount of protein in the sample. The procedure is as follows: 20 µL of serum is mixed with 1000 µL of reagent, incubated in a 37 °C water bath for 10 minutes, and then the optical density of the test is read against the reagent blank at 546 nm. Then, 20 µL of standard reagent is mixed with 1000 µL of reagent, and its optical density is also recorded at 546 nm. The total protein

concentration is calculated using the formula:

Total protein concentration= (serum optical density/standard optical density) × standard concentration

Measurement of serum malondialdehyde (MDA)

The basis of the MDA serum measurement method is the reaction with thiobarbituric acid (TBA), extraction with n-butanol, spectrophotometric measurement, and comparison of absorption with the standard curve.

Preparation of solutions

1% orthophosphoric acid solution

This solution was prepared using commercial phosphoric acid from Merck in a 250 mL flask by dissolving 85% phosphoric acid and bringing it to volume with deionized water.

0.67% thiobarbituric acid

The thiobarbituric acid used in this test was obtained from Merck. 1.675 g of thiobarbituric acid (C2H4N2O2S) was dissolved in a 250 mL flask with deionized water and used freshly prepared.

Standard solutions

MDA standards were prepared using 1, 1, 3, 3-tetramethoxypropane (C7H16O4) in six different concentrations (0.5, 1, 2, 4, 8, and 12 nmol/mL) by dissolving in deionized water and used to plot the standard curve. MDA measurement started by dissolving 500 µL of serum in 3 mL of 1% phosphoric acid. After vortexing, 1 mL of 0.67% thiobarbituric acid solution was added to the test tube, vortexed thoroughly, and placed in a boiling water bath for 45 minutes. After cooling the test tubes under cold water, 3 mL of n-butanol was added, vortexed for 1-2 minutes, and centrifuged at 3000 rpm for 10 minutes. The organic phase (supernatant) was separated, and the optical density was measured at 532 nm against n-butanol as a blank. The results were transferred to the standard curve to determine the serum MDA concentrations (Konieczka, 2004).

Measurement of total antioxidant activity

The total antioxidant activity of the serum was measured using an ELISA spectrophotometer at the Faculty of Medicine, Tabriz University of Medical Sciences.

Measurement of superoxide dismutase

The activity of the superoxide dismutase enzyme in whole blood was measured using the RANSEL kit (product of RANDOX, UK) according to the kit manufacturer's instructions and by spectrophotometry at Tabriz University of Medical Sciences.

Experimental design and statistical model

This experiment was conducted in a completely randomized design with 4 treatments and 5 replicates. The experimental period was 70 days. After data collection, statistical analysis was performed using SAS software (SAS, 2003) and the GLM procedure. Normality tests were conducted using Proc univariate, outliers were identified and removed, and normality was tested again. Data collected at a single time point during the study period were analyzed using analysis of variance (ANOVA), while repeated measures data were analyzed using the General Linear Model in (GLM) procedure. Mean comparisons were performed using the general linear model method, and a significance level of 5% was considered. For health score data, even after removing outliers and transforming the data, normality was not achieved, so it was analyzed non-parametrically using the Kruskal-Wallis method.

RESULTS AND DISCUSSION

The results of the effect of different forms of zinc on the feed intake of Holstein calves are presented in Table 4. The findings indicate that adding zinc to the experimental diets significantly affected feed intake compared to the control group. Data from the second period showed a significant increase in concentrate dry matter intake in the experimental treatments compared to the control group ($P<0.05$). The dry matter intake of concentrate for the control, nano zinc oxide, zinc oxide, and zinc glycine treatments in the second period were 1007.11, 1360.06, 1279.93, and 1419.86 grams per day, respectively. The data on dry matter intake of alfalfa in Holstein calves are shown in Table 4. The results indicated that supplementing different forms of zinc had no effect on alfalfa intake. Statistical analysis of total dry matter intake in the second period (Table 4) showed a significant difference between the experimental treatments and the control group ($P<0.05$). The dry matter intake in the second period for the control, nano zinc oxide, zinc oxide, and zinc glycine groups were 1089.97, 1495.09, 1418.63, and 1512.76 grams per day, respectively, with the highest intake reported for the zinc glycine group. Puchala *et al.* (1999) found that 150 mg of zinc oxide significantly increased feed intake in goats.

Elamin *et al.* (2013) observed no significant difference in feed intake between control and zinc-supplemented goat kids (33 mg Zn/kg as zinc sulfate), with both groups recording similar values (478.68±12.39 g/day). Overall, zinc supplementation did not affect the feed intake of Nubian goats.

Yang and Sun (2006) and Mishra *et al.* (2014) reported that nano zinc oxide (nZnO) enhanced growth performance and feed intake in weaned pigs and broiler chickens.

Table 2 Chemical analysis of starter components used in calf nutrition in experimental treatments

Starter components	Moisture %	Crude protein %	Ash %	Crude fiber %	Calcium %	Phosphorus %	Zinc %
Alfalfa	8.41	20.64	15.56	18.29	2.53	0.07	0.59
Concentrate	9.71	31.08	6.38	6.2	0.76	0.1	1.32

Table 3 Calf health scoring based on the method provided by the university of Wisconsin

Score zero	Score one	Score two	Score three
Normal watery discharge	Some discharge from one nostril	Purulent discharge from both nostrils	Very high amount of purulent nasal discharge
Normal eye	Small amount of eye discharge	Moderate amount of eye discharge	Very high amount of eye discharge
Normal ear	Head shaking or ear flicking	Slight unilateral ear drooping	Head tilting or bilateral ear drooping
Normal stool consistency	Semi-formed, dough-like	Loose but remains on bedding	Watery, passes through bedding like a sieve
No cough	Only one cough when stimulated	Frequent coughing when stimulated	Spontaneous, frequent discharge

Table 4 Effect of adding different forms of zinc on feed intake in holstein calves (grams per day)

Concentrate intake (grams per day)	Control	Nano zinc oxide	Zinc oxide	Zinc glycinate	P-value
First period (30 days)	315± 56.23	358.50±58.34	376.13±58.54	366.66±65.13	0.2321
Second period (40 days)	1007.11±113.25^{b}	1360.06±106.59^{a}	1279.93±106.95^{a}	1419.86±118.98^{a}	0.0018
Overall average	751±0.092^{b}	950±0.070^{a}	935±0.070^{a}	936±0.078^{a}	0.0031
Alfalfa intake (grams per day)					
First period (30 days)	42.82±30.96	50.55±19.83	62.19±21.35	31.25±20.62	0.1734
Second period (40 days)	82.99±41.31	133.56±29.54	138.48±28.94	92.52±31.84	0.2995
Total feed intake (grams per day)					
First period (30 days)	357.80±60.12	407.68±59.68	472.02±59.88	396.08±66.62	0.1182
Second period (40 days)	1089.97±113.59^{b}	1495.09±100.70^{a}	1418.63±101.04^{a}	1512.76±112.41^{a}	0.0005

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

In contrast, Zaboli and Arabi (2013) observed no significant effect of nZnO on feed intake in lambs, and Wright and Spears (2004) similarly found no effect of zinc supplementation on feed intake in Holstein calves. Petrič *et al.* (2024) demonstrated that dietary supplementation with 2.5 ppm nZnO significantly increased feed intake in lambs compared to the control group, whereas higher concentrations (5, 10, 20, and 40 ppm) did not elicit similar effects. Additionally, the 2.5 ppm group exhibited a more favorable feed conversion ratio (FCR). Singh *et al.* (2018) found no significant differences in nutrient intake and digestibility among lambs supplemented with varying levels of nZnO and conventional ZnO; however, zinc retention and absorption were notably higher in the nZnO groups, indicating improved bioavailability. Hussan *et al.* (2022) reported that broilers receiving 2.5 ppm nZnO exhibited significantly increased feed intake, greater body weight gain, and improved FCR compared to both the control and other treatment groups. Similarly, Liu *et al.* (2023) found that zinc supplementation—using either zinc proteinate or zinc oxide—enhanced average daily gain (ADG) and reduced the feed-to-gain ratio (FGR) in Holstein dairy calves, with zinc proteinate producing more pronounced effects and lowering the incidence of diarrhea.

Hou *et al.* (2023) studied the effects of zinc amino acid supplementation on calves. Chelated zinc (Zn-Bon) significantly increased starter intake and tended to improve digestibility of organic matter, neutral detergent fiber, and starch. Zn-Bon and Zn-Sul reduced the frequency of medical treatments, indicating better health. Although not statistically significant, Zn-supplemented calves showed trends towards higher body weight and growth rates. The study highlights the benefits of Zn supplementation, especially with organic chelated forms, for improving feed intake and overall health in dairy calves.

Rajaei-Sharifabadi *et al.* (2024) investigated early-life zinc supplementation in pre-weaned dairy calves. Chelated zinc (Zn-Bon) significantly increased starter intake compared to the control group. While overall body weight gain was not significantly different, Zn-supplemented calves, especially those receiving Zn-Bon and Zn-Sul, showed trends towards higher body weight and required fewer medical treatments. There was also a tendency for improved digestibility of organic matter, neutral detergent fiber, and starch in Zn-supplemented groups. The study suggests that early-life Zn supplementation, particularly with chelated zinc, can improve starter intake, health, and nutrient digestibility in pre-weaned dairy calves.

Kincaid *et al*. (1997) found that adding 300 ppm zinc oxide had no effect on feed intake in weaned calves. Anil *et al*. (2019) reported that nano zinc oxide (nZnO) significantly improved growth performance and feed intake in crossbred calves. Calves receiving nZnO showed better overall health and reduced incidence of diseases compared to the control group. The study highlights the potential benefits of using nZnO in livestock feed to enhance growth and health outcomes.

Khan (1978) reported no effect on daily dry matter intake in lambs when zinc levels were increased from 26.02 to 85.67 mg/kg dry matter. The varying effects of dietary zinc (Zn) on livestock performance can be attributed to several factors: Different forms of zinc (e.g., zinc oxide, zinc sulfate, chelated zinc, nano zinc) have varying bioavailability. Organic and nano forms are generally more bioavailable than inorganic forms, leading to different outcomes in growth and health. The amount of zinc supplemented can significantly impact results. Low, medium, and high doses can have different effects on growth performance, feed intake, and health (Ogbuewu and Mbajiorgu, 2023).

Different species and even different life stages within a species respond differently to zinc supplementation. For example, the effects on weaned piglets may differ from those on adult cattle . The presence of other dietary components, such as phytates, can affect zinc absorption and utilization. Diets high in phytates can reduce zinc bioavailability (Alimohamady *et al*. 2019). The initial zinc status of the animals can influence the effectiveness of supplementation. Animals with zinc deficiency are likely to show more pronounced improvements compared to those with adequate zinc levels (Szuba-Trznadel *et al*. 2021). The overall health and immune status of the animals can influence how they respond to zinc supplementation. Healthier animals might show different growth and performance outcomes compared to those under stress or disease. Conditions such as housing, temperature, and stress levels can also impact the effectiveness of zinc supplementation (Smerchek *et al*. 2023). These factors collectively contribute to the inconsistent results observed in studies on dietary zinc supplementation in livestock.

The results of the effect of using different forms of zinc on the final weight of Holstein calves are shown in Table 5. The results showed that the final weight of the groups that received zinc supplements significantly increased compared to the control group ($P<0.05$). The final weight for the control, nano zinc oxide, zinc oxide, and zinc glycine treatments were 105.31, 112.83, 116.58, and 112.16 kg, respectively.

The results of the effect of using different forms of zinc on daily weight gain and feed conversion ratio of Holstein calves are shown in Table 5. As observed, adding different forms of zinc in the first period increased the daily weight gain of the treatments compared to the control group. The data for this period show that the groups that received supplements had a significant increase compared to the control group ($P<0.05$). The groups that received zinc oxide and zinc glycine had a significant difference ($P<0.05$), but these two did not have a significant difference with the group that received nano zinc oxide. The daily weight gain in this period for the control, nano zinc oxide, zinc oxide, and zinc glycine groups was 0.548, 0.745, 0.700, and 0.760 grams per day, respectively. Statistical analysis of the data related to daily weight gain in the second period showed that the groups that received nano zinc oxide and zinc oxide had a significant increase compared to the control group ($P<0.05$), and the group that received zinc glycine did not have a significant difference compared to the control group and the other two groups. The daily weight gain in this period for the control, nano zinc oxide, zinc oxide, and zinc glycine treatments was 0.858, 1.044, 1.112, and 0.922 kg per day, respectively.

Supplementing different forms of zinc throughout the experimental period showed a significant effect of zinc on the daily weight gain of suckling calves ($P<0.05$). The data related to weight gain throughout the period show that the groups that received zinc forms had a significant increase compared to the control group ($P<0.05$). As observed, zinc oxide with 0.906 kg increase per day had the highest amount compared to other groups. The daily weight gain throughout the experimental period for the control, nano zinc oxide, zinc oxide, and zinc glycine treatments was 0.703, 0.895, 0.906, and 0.840 kg per day, respectively.

Kegley and Spears (1994) in a study on fattening calves by adding 25 mg/kg in the form of zinc oxide and zinc propionate showed that adding zinc increased the average daily weight gain during the growth period. The study by Liu *et al*. (2023) investigates the effects of zinc supplementation on growth, diarrhea, antioxidant capacity, and immune function in Holstein dairy calves. Supplementation with zinc proteinate (ZnPro) and zinc oxide (ZnO) significantly improved the average daily gain (ADG) of the calves. ZnPro showed a greater improvement in growth performance compared to ZnO. Both ZnPro and ZnO decreased the feed:gain ratio (FGR), indicating better feed efficiency. ZnPro was more effective in reducing FGR over the 28-day period. In summary, zinc supplementation, particularly with ZnPro, positively affected growth performance, feed efficiency, diarrhea reduction, antioxidant capacity, and immune function in Holstein dairy calves.

Puchala *et al*. (1999) reported that the use of zinc oxide had a significant effect on the weight gain of goats. The study by Solaiman (2019) investigates the effects of high levels of dietary zinc on various aspects of growth and

health in growing Boer-cross goat kids. High levels of dietary zinc significantly improved the average daily gain (ADG) of the goat kids. This indicates that zinc supplementation can enhance growth rates. The study by Thamizhan *et al*. (2024) investigates the influence of select dietary trace minerals, including zinc (Zn), on growth performance, nutrient utilization, and mineral balance in male goats. Zinc supplementation significantly improved the average daily gain (ADG) in male goats. The goats receiving higher levels of zinc showed better growth rates compared to those with lower or no zinc supplementation. The study found that zinc improved the digestibility of non-fiber carbohydrates, which contributed to better nutrient absorption and utilization. Zinc supplementation helped maintain a better balance of essential minerals in the goats, supporting overall health and growth. In summary, dietary zinc supplementation positively affected weight gain, nutrient utilization, and mineral balance in male goats, leading to enhanced growth performance and overall health.

The study by Yusuf *et al*. (2022) investigates the effects of nano zinc oxide (nZnO) supplementation on the growth performance and health of West African dwarf goats. Supplementation with nZnO significantly improved the average daily gain (ADG) and total weight gain of the goats. The goats receiving higher levels of nZnO (600 mg/kg) showed the greatest improvements in growth performance. The study observed an increase in total dry matter intake (DMI) in goats supplemented with nZnO, particularly at the higher dosage. In summary, nano zinc oxide supplementation in West African dwarf goats led to significant improvements in growth performance, feed intake, health status, and immune function. Sahoo *et al*. (2014) showed that broilers that received nano zinc oxide at 0.06 and 0.03 parts per million had better growth performance compared to the control group. Mandal *et al*. (2007) reported that adding zinc to the diet of calves did not have a significant effect on their body weight. Zaboli and Arabi (2013) observed no significant effect on the weight gain of Angora goats by supplementing zinc oxide and nano zinc oxide.

The effect of adding different forms of zinc on the feed conversion ratio of suckling calves is shown in Table 5. In the results related to the feed conversion ratio for the first period, no significant difference was observed between the groups. The results of the feed conversion ratio for the second period indicated a significant difference between the groups receiving nano zinc oxide and zinc glycine compared to the control group ($P<0.05$). No significant difference was observed in the feed conversion ratio between the groups receiving zinc supplements in any of the periods, while there was a significant difference between the control group and the groups receiving nano zinc oxide and zinc glycine in the second period ($P<0.05$). However, in terms of the overall feed conversion ratio, only the zinc glycine group showed a significant difference ($P<0.05$). Lin *et al*. (2009) stated that nano zinc oxide improved growth performance and feed conversion ratio.

Kachhadia *et al*. (2023) found that supplementary zinc, especially in the form of zinc proteinate (ZnPro), significantly improves the average daily gain (ADG) of calves. ZnPro showed better results compared to zinc oxide (ZnO). Both ZnPro and ZnO reduced the feed: gain ratio (FGR), indicating improved feed efficiency. ZnPro had a more pronounced effect over a longer period. In the study of Hou *et al*. (2023), supplementary zinc proteinate (ZnPro) significantly improved the average daily gain (ADG) of calves over 28 days, while zinc oxide (ZnO) showed improvements only in the first 14 days. ZnPro consistently decreased the feed: gain ratio (FGR) throughout the study, indicating better feed efficiency compared to ZnO.

The study by Liu *et al*. (2023) investigates the effects of zinc supplementation on growth performance, diarrhea, antioxidant capacity, and immune function in Holstein dairy calves. Supplementation with zinc proteinate (ZnPro) and zinc oxide (ZnO) significantly improved the average daily gain (ADG) of the calves. ZnPro showed a greater improvement in growth performance compared to ZnO. Both ZnPro and ZnO decreased the feed: gain ratio (FGR), indicating better feed efficiency. ZnPro was more effective in reducing FGR over the 28-day period. In summary, zinc supplementation, particularly with ZnPro, positively affected growth performance, feed efficiency, diarrhea reduction, antioxidant capacity, and immune function in Holstein dairy calves.

Fadayifar *et al*. (2012) reported that the use of organic zinc supplements improved the feed conversion ratio compared to the control group. Garg and Mudgal (2008) also reported an improvement in the feed conversion ratio and average daily weight gain of lambs supplemented with 20 mg of zinc per kg of dry matter in the form of zinc methionine compared to the control group.

Spears and Kegley (1991) did not observe a significant effect on the feed conversion ratio by supplementing zinc in the diet of calves. Zinc is a component of numerous enzymes involved in protein, fat, carbohydrate, and nucleic acid metabolism. These enzymes are essential for various metabolic processes that support growth and development.Zinc is vital for the function of growth hormones, pancreatic hormones, and sex hormones. Adequate zinc levels help maintain normal hormone function, which is crucial for growth and reproductive performance (Ogbuewu and Mbajiorgu, 2023).

The results of adding different forms of zinc (nano zinc oxide, zinc oxide, and zinc glycine) on blood parameters are shown in Table 6.

Table 5 Effect of adding different forms of zinc on final weight (kg), daily weight gain, and feed conversion ratio (kg/day)

Treatment	Control	Nano zinc oxide	Zinc oxide	Zinc glycinate	P-value
Final weight	105.31±2.87^{b}	112.83±1.84^{a}	116.58±1.74^{a}	112.16±2.42^{a}	0.0322
Daily weight gain (first period)	0.548±0.033^{c}	0.745±0.025^{ab}	0.700±0.024^{b}	0.760±0.030^{a}	0.0001
Daily weight gain (second period)	0.858±0.084^{b}	1.044±0.066^{a}	1.112±0.063^{a}	0.922±0.077^{ab}	0.0126
Daily weight gain (total period)	0.703±0.045^{b}	0.895±0.035^{a}	0.906±0.033^{a}	0.840±0.041^{a}	0.0001
Feed conversion ratio (first period)	0.662±0.091	0.589±0.079	0.717±0.080	0.615±0.089	0.2013
Feed conversion ratio (second period)	1.171±0.118^{b}	1.470±0.110^{a}	1.318±0.110^{ab}	1.670±0.122^{a}	0.0018
Feed conversion ratio (total period)	0.947±0.076^{b}	1.092±0.075^{ab}	1.060±0.063^{ab}	1.217±0.071^{a}	0.0018

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Effect of adding different forms of zinc on blood parameters in Holstein calves

Parameters	Control	Nano zinc oxide	Zinc oxide	Zinc glycinate	P-value
Glucose	57.61±4.30	67.08±3.35	63.70±3.21	62.89±3.90	0.4340
Triglycerides	27.44±2.66	25.89±2.07	25.89±1.98	23.83±2.41	0.8438
Cholesterol	95.69±5.96	92.86±4.64	92.70±4.45	92.002±5.40	0.9782
Total Protein	5.85±0.77	7.25±0.60	6.99±0.58	6.28±0.70	0.4323

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Data analysis of blood parameters (glucose, triglycerides, cholesterol, and total protein) indicated that supplementing with nano zinc oxide, zinc oxide, and zinc glycine had no significant effect compared to the control group. The study by Ranasinghe *et al*. (2015) systematically reviews and meta-analyzes the effects of zinc supplementation on serum lipids. Zinc supplementation significantly reduced total cholesterol levels. There was a significant reduction in LDL cholesterol levels. Zinc supplementation led to a significant decrease in triglyceride levels. There was a slight, non-significant increase in HDL cholesterol levels. The study also discusses zinc's role in insulin action and carbohydrate metabolism, which can influence blood glucose levels, although specific results on blood glucose were not the primary focus. The article does not specifically address the effects of zinc on total protein levels. Overall, zinc supplementation has favorable effects on lipid parameters, potentially reducing the risk of atherosclerosis-related morbidity and mortality.

The study by Olechnowicz *et al*. (2017) examines the role of zinc in various metabolic processes. Here are the key findings related to glucose, triglycerides, cholesterol, and total protein: Zinc plays a crucial role in insulin synthesis, storage, and release, which helps regulate blood glucose levels.

Zinc supplementation has been found to improve blood glucose control. Zinc supplementation can reduce triglyceride levels by enhancing lipid metabolism and reducing oxidative stress. Zinc has been shown to lower total cholesterol and LDL cholesterol levels, while potentially increasing HDL cholesterol levels. This effect is partly due to zinc's role in reducing inflammation and oxidative stress. The study does not specifically address the effects of zinc on total protein levels.

Overall, zinc supplementation has beneficial effects on lipid and glucose metabolism, which can help manage metabolic disorders.

The study by Nazari *et al*. (2023) systematically reviews and analyzes the effects of zinc supplementation in individuals with prediabetes and type 2 diabetes. Here are the key findings: Zinc supplementation significantly reduced fasting blood glucose (FBG) and hemoglobin A1C (HbA1C) levels, indicating improved glycemic control. There was a significant reduction in triglyceride levels with zinc supplementation. Zinc supplementation led to a significant decrease in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, while high-density lipoprotein cholesterol (HDL-C) levels showed a slight increase. The study does not specifically address the effects of zinc on total protein levels. Overall, zinc supplementation has beneficial effects on glycemic control and lipid profiles, which can help manage cardiovascular disease risk factors in individuals with prediabetes and type 2 diabetes1. The study of Hou *et al*. (2023), focused on the effects of zinc supplementation on cardiovascular disease (CVD) risk factors in patients with type 2 diabetes mellitus (T2DM)1. Zinc supplementation significantly lowers triglycerides (TG), total cholesterol (TC), fasting blood glucose (FBG), hemoglobin A1C (HbA1C), and C-reactive protein (CRP), while increasing high-density cholesterol (HDL). Zinc improves glycemic markers, aiding in better blood sugar management. Zinc reduces inflammation markers, which are crucial in managing diabetes and CVD.

Kesler and Abuelo (2024) studied the effects of dietary zinc (Zn) on growth and blood parameters in dairy calves. Supplementary zinc, especially zinc proteinate (ZnPro), significantly improves average daily gain (ADG) and reduces the feed: gain ratio (FGR) in calves, enhancing over-

all growth performance. These findings suggest that ZnPro is more effective than ZnO in improving calf health and growth. The study by Mattioli *et al*. (2019) investigates the effects of injectable copper (Cu) and zinc (Zn) supplementation on pre-weaning beef calves. Here are the key findings: Supplemented calves showed significantly higher weight gain compared to the control group. There were improvements in hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) levels in the supplemented group. Overall, the study suggests that Cu and Zn supplementation can improve growth performance and immune function in pre-weaning beef calves.

The study by Liu *et al*. (2023) explores the effects of zinc supplementation on Holstein dairy calves. Zinc proteinate (ZnPro) significantly improved average daily gain (ADG) and reduced the feed: gain ratio (FGR) compared to zinc oxide (ZnO) and the control group. Overall, ZnPro was found to be more effective than ZnO in enhancing growth performance, reducing diarrhea, and boosting antioxidant and immune functions in dairy calves. Mandal *et al*. (2007) stated that adding different forms of zinc to the diet of calves had no significant effect on albumin, globulin, albumin to globulin ratio, and urea concentration. Zaboli and Arabi (2013) reported that using levels of 20 and 40 parts per million of nano zinc oxide had no significant effect on blood parameters (glucose, urea, albumin, total protein, alkaline phosphatase, lactate dehydrogenase) in goats compared to the control group. Kessler *et al*. (2003) observed that feeding zinc methionine had no significant effect on total protein, albumin, and blood urea concentration in heifers. Sahoo *et al*. (2014) reported that using nano zinc oxide improved health by reducing cholesterol and increasing serum alanine aminotransferase in broilers. Puchala *et al*. (1999) stated that using zinc supplements in the diet did not affect blood urea nitrogen levels.

The results regarding the effect of adding different forms of zinc on the total antioxidant capacity, malondialdehyde concentration, and superoxide dismutase in Holstein calves are shown in Table 7. Statistical analysis indicated that supplementing with different forms of zinc had a significant effect compared to the control group ($P<0.05$). The total antioxidant capacity for the control, nano zinc oxide, zinc oxide, and zinc glycine treatments were determined to be 0.386, 0.445, 0.463, and 0.456 micromoles per liter, respectively. The results for blood malondialdehyde concentration showed no significant effect of different zinc forms compared to the control group. However, the group receiving nano zinc oxide showed a significant difference in superoxide dismutase levels compared to the control group ($P<0.05$), while the zinc oxide and zinc glycine groups did not show significant differences compared to the nano zinc oxide and control groups.

Nagalakshmi *et al*. (2009) stated that zinc supplements significantly affected superoxide dismutase levels in calves. Adding zinc sulfate and zinc propionate as zinc supplements to lamb diets increased blood superoxide dismutase concentration (Nagalakshmi *et al*. 2009).

The results for blood zinc levels showed a significant effect compared to the control group, with nano zinc oxide and zinc glycine increasing blood zinc concentration in the treatment groups ($P<0.05$). Consistent with this study, Arabi *et al*. (2011) reported that plasma zinc concentration significantly increased in groups receiving zinc compared to the control group. This indicates that nano zinc oxide and zinc glycine have higher bioavailability than the inorganic form, zinc oxide. The total antioxidant capacity of serum is an indicator that describes the balance between pro-oxidants and antioxidants. An increase in this index can indicate increased resistance of organisms to oxidative stress. It has been reported that organic zinc supplements do not affect the total antioxidant capacity of male Mehraban lambs (Arabi *et al*. 2011).

Liu *et al*. (2023) found that Zinc supplementation enhanced antioxidant capacity and immune function, contributing to better overall health and growth performance in the calves. The incidence of diarrhea was significantly reduced in calves supplemented with ZnPro and ZnO. ZnPro was more effective in reducing diarrhea during the first 14 days. ZnPro improved the antioxidant capacity of the calves to a greater extent than ZnO, as indicated by higher plasma antioxidant levels. Both ZnPro and ZnO enhanced the immune function of the calves, with increased serum immunoglobulin G (IgG) concentrations observed on days 14 and 28.

ZnPro also enhanced antioxidant capacity and immune function, contributing to overall better health and reduced incidence of diarrhea in calves. Organic zinc sources like ZnPro have higher bioavailability compared to inorganic sources like ZnO, leading to better absorption and utilization in the body (Kachhadia *et al*. 2023). ZnPro and ZnO reduced the incidence of diarrhea, but ZnPro was more effective in the early stages. ZnPro enhanced antioxidant capacity and immune function more effectively than ZnO, contributing to overall better health and growth performance (Hou *et al*. 2023).

In the study of Liu *et al*. (2023) the incidence of diarrhea was significantly reduced in calves supplemented with ZnPro and ZnO. ZnPro was more effective in reducing diarrhea during the first 14 days. ZnPro improved the antioxidant capacity of the calves to a greater extent than ZnO, as indicated by higher plasma antioxidant levels. Both ZnPro and ZnO enhanced the immune function of the calves, with increased serum immunoglobulin G (IgG) concentrations observed on days 14 and 28.

Table 7 Effect of adding different forms of zinc on antioxidant status in Holstein calves

Enzymes	Control	Nano zinc oxide	Zinc oxide	Zinc glycinate	P-value
Total antioxidant activity (µmol/L)	0.386±0.018[b]	0.445±0.014[a]	0.463±0.013a	0.456±0.016[a]	0.0019
Malondialdehyde (µmol/L)	1.74±0.08	1.38±0.08	1.30±0.08	1.32±0.08	0.0620
Superoxide dismutase (µmol/L)	1015.5±190.55[b]	1520.2±183.19[a]	1356.68±186.07[ab]	1397.91±195.21[ab]	0.0400
Serum zinc (µmol/L)	0.675±1.012[b]	5.126±0.789[a]	2.758±0.756[b]	5.529±0.918[a]	0.0017

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 8 Effect of adding different forms of zinc on the health status of suckling calves

Parameters	Control	Nano zinc oxide	Zinc oxide	Zinc glycinate	P-value
Eye score	0.312	0.141	0.141	0.141	0.3703
Respiratory score	0.412	0.212	0.241	0.241	0.3223
Nasal score	0.463	0.312	0.341	0.370	0.4872
Fecal score	0.404	0.492	0.264	0.422	0.6179

In the study of Thamizhan *et al*. (2024) the goats supplemented with zinc exhibited improved immune function and antioxidant capacity, which are crucial for maintaining health and preventing diseases. Yusuf *et al*. (2022) found that goats supplemented with nZnO exhibited better overall health, as indicated by improved hematological parameters such as packed cell volume (PCV) and white blood cell (WBC) counts. The study found that nZnO supplementation positively affected serum chemistry, with increased levels of total protein, globulin, and total cholesterol, especially at the 300 mg/kg dosage. Enhanced immune function was observed in goats receiving nZnO, with higher levels of immunoglobulins and other immune markers.

Kesler and Abuelo (2024) studied the effects of dietary zinc (Zn) on growth and blood parameters in dairy calves. Both ZnPro and zinc oxide (ZnO) reduce the incidence of diarrhea, with ZnPro showing more pronounced effects in the first 14 days. Zinc supplementation increases serum immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations, boosting immune responses. ZnPro enhances antioxidant capacity by reducing serum malondialdehyde (MDA) levels and increasing total antioxidant capacity (T-AOC). The study by Mattioli *et al*. (2019) investigates the effects of injectable copper (Cu) and zinc (Zn) supplementation on pre-weaning beef calves. The immune response, measured by antibody titers to bovine herpes virus 1 (BoHV-1), was enhanced in the supplemented calves.

Malondialdehyde is a small but stable product of lipid peroxidation, formed from the breakdown of unstable peroxides of unsaturated fatty acids. As its name suggests, malondialdehyde is an aldehyde compound, active and highly reactive, produced in the human body from the peroxidation of unsaturated fatty acids. Therefore, by measuring the amount of malondialdehyde in various biological samples, the extent of lipid peroxidation can be determined, and it can be used as a marker to measure the level of oxidative stress in an organism.

On the other hand, since malondialdehyde itself is an active and highly reactive compound, it attacks other molecules, forming strong covalent bonds, which ultimately affect the function of molecules and cells. For example, the binding of malondialdehyde to protein molecules creates advanced lipoxidation end-products. Additionally, the binding of malondialdehyde to purine bases in DNA structure causes mutagenic, atherogenic, and carcinogenic properties of malondialdehyde.

The enzyme superoxide dismutase contains zinc in its structure, and a decrease in the activity of this enzyme in cell membranes, such as red blood cells, leads to increased damage from oxidative stress products (Glass and Gershon 1984). This enzyme is considered the first intracellular defense barrier against oxidizing agents, catalyzing the reaction of superoxide anion to hydrogen peroxide. The production of hydrogen peroxide has an antimicrobial effect, and with the increase of zinc oxide, the concentration of produced hydrogen peroxide increases linearly (Sawai *et al*. 1996). The study by Liu *et al*. (2023) explores the effects of zinc supplementation on Holstein dairy calves. Both ZnPro and ZnO reduced the incidence of diarrhea, with ZnPro showing more pronounced effects. ZnPro enhanced antioxidant capacity by lowering serum malondialdehyde (MDA) levels and increasing total antioxidant capacity (T-AOC). Zinc supplementation, particularly ZnPro, increased serum immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations, indicating improved immune responses. The results related to the addition of different forms of zinc (nano zinc oxide, zinc oxide, and zinc glycine) on the health status of suckling calves are shown in Table 8. The obtained results indicated that the addition of different forms of zinc had no significant effect on the health status of the calves compared to the control group. The lowest health score was given to the best health status, so the group that showed the lowest numerical score had the best health status.

Arabi *et al*. (2012) stated that using different levels of zinc had a significant effect on the health status of calves compared to the control group. It has been reported that nano zinc oxide has bactericidal effects on both gram-positive and gram-negative bacteria (Arabi *et al*. 2012). Additionally, nano zinc oxide is effective against spore-forming bacteria that are resistant to high pressure and temperature (Rosi and Mirkin, 2005). Nano zinc oxide inhibits the growth of Pseudomonas and E. coli bacteria. Nano zinc oxide damages the bacterial cell wall, resulting in the leakage of intracellular contents.

CONCLUSION

The results regarding the supplementation of different forms of zinc on dry matter intake in the final period indicated a significant difference between these treatments and the control group. Data on daily weight gain throughout the entire period showed that groups receiving zinc supplements had higher weight gain compared to the control group. The feed conversion ratio for the entire period in groups receiving nano zinc oxide and zinc oxide was significantly different compared to the control group. Supplementing with different forms of zinc had no significant effect on blood parameters (glucose, cholesterol, triglycerides, and total protein) compared to the control group. The group that received nano zinc oxide and zinc glycine had the highest blood zinc levels, indicating that nano zinc oxide and zinc glycine had the highest bioavailability compared to zinc oxide and the control group. The results related to total antioxidant activity showed that the groups receiving zinc had a significant difference compared to the control group. There was no significant difference in malondialdehyde levels among the different groups. The results for superoxide dismutase levels indicated that the group receiving nano zinc oxide had a significant difference compared to the control group. It was concluded that, overall, the use of different forms of zinc improved feed intake and weight gain in suckling calves.

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