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# Concentration of Calcium and Phosphate Serum Following Administration of Zinc in Friesian Holstein Bulls

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### ABSTRACT

Minerals play an important role in bulls as they function to form muscles of and maintain the quality of sperm. Zn mineral in the diet will also affect the levels of calcium and phosphorus in the blood. The objective of this study was to determine the effect of Zn supplementation on the levels of calcium and phosphate serum in Friesian Holstein bulls. This study used ten Friesian Holstein bulls, 16-18 months old and divided into two groups, consisting of five bulls each, i.e. with no added Zn (control) and 60 ppm of Zn supplementation, respectively. Zinc was administered daily for four months. Blood samples were taken before treatment and after the four-month treatment for the examination of calcium and phosphate levels. The result showed that 60 ppm Zn supplementation was not significantly different ( $p > 0.05$ ) on the blood calcium and phosphate levels. However, the calcium level tends to increase while that of phosphate tends to decrease.

**Keyword:** calcium, Friesian Holstein bulls, phosphorus, Zn

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## INTRODUCTION

Zn mineral is important for the body to maintain health, production and reproduction. In bulls this mineral is needed as a source of protein and sperm-producing. This mineral cannot be converted from other nutrients, therefore must absolutely be present in the feed, although the amount needed is relatively small. Zn need in dairy cows is between 40 to 60 ppm [10]. Zn insufficiency will affect in various physiological functions of the body, such as the metabolism, hormonal synthesis and actions of various enzymes. Severe Zn deficiency is characterized by decreasing immune cell function and increasing the incidence of infection.

In livestock, Zn mineral is used to assist metabolism and provide rumen microbial requirements. In case of Zn mineral deficiency, the rumen microbial fermentation activity is not optimum, thus reducing the productivity [11]. Therefore Zn supplementation is often done to improve the health status, immune response and livestock productivity. Zn deficiency studies and its health impacts have been reported.

Excessive administration of Zn can depress other minerals such as calcium and phosphate. On the other hand, zinc insufficiency may result in hypocalcemia. This mineral is essential for the formation of collagen. Collagen is the site where calcium phosphate deposits inside the bone. Without the formation of collagen and the presence of Zn, calcium cannot be deposited in the bones so it will be easy to remove again [5]. The information about the effect of Zn supplementation on serum calcium (Ca) and phosphate (P) levels in bulls is relatively limited, so this research needs to be done.

## MATERIALS AND METHODS

This research was carried out at a dairy farm in Ciawi Bogor, and at the Laboratory of Clinical Pathology, Department of Clinical, Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agriculture University. Ten healthy Holstein bulls, 16-18

months, were divided into two groups (control, and treatment). The dietary, environmental, and husbandry factors were similar in both groups. Zinc (60 ppm) was added to concentrate of treatment group, but no zinc sulfate was added to the diet of the control group. Feed was given in the form of grass and concentrate. The feed was provided daily for four months. Drinking water was given ad libitum.

Blood samples were collected from the jugular veins before Zn supplementation (Pre Zn) and four months after the supplementation (Post Zn).

Blood samples were allowed to clot at room temperature and were centrifuged at 1500 rpm for 30 minutes to separate serum. The harvested blood sera were dispensed into plastic tubes and stored at  $-20^{\circ}\text{C}$  before being analysed. Then the sera were analyzed by using spectrophotometer to find out the concentration of calcium and phosphate by commercial kits.

## Data Analysis

Data were analyzed by using Analysis of Variance (ANOVA), and if there were differences, the analysis was continued with Duncan's Multiple Range Test (Duncan's Multiple Range Test).

## Results and Discussion

The minerals are used for a variety of physiological processes such as building blood and bone, making hormones, and regulating heartbeat. There are two types of minerals. Macrominerals are needed in large amounts. Trace minerals are needed in very small amounts. Calcium is the most common mineral found in the body, every day approximately 1% of calcium is exchanged between bone and soft tissue. Calcium as an essential mineral has an important role in metabolism. The results of observed calcium and phosphate levels in male FH cows before and after feeding with Zn mineral supplementation are presented in Table 1 and Figure 1.

**Table. Calcium and phosphate levels (mg/dL) in male Friesian Holstein cows before and after feeding with Zn supplementation**

		Groups	
	Parameters	Pre Zn	Post Zn
Control	Calcium ( mg/dL)	8.84±0.13	8.34±1.42
	Phosphate ( mg/dL)	6.95±0.28	8.04±2.31
Zn 60 ppm	Calcium ( mg/dL)	8.75± 0.11	9.10±2.02
	Phosphate ( mg/dL)	6.80± 0.42	5.96±0.76

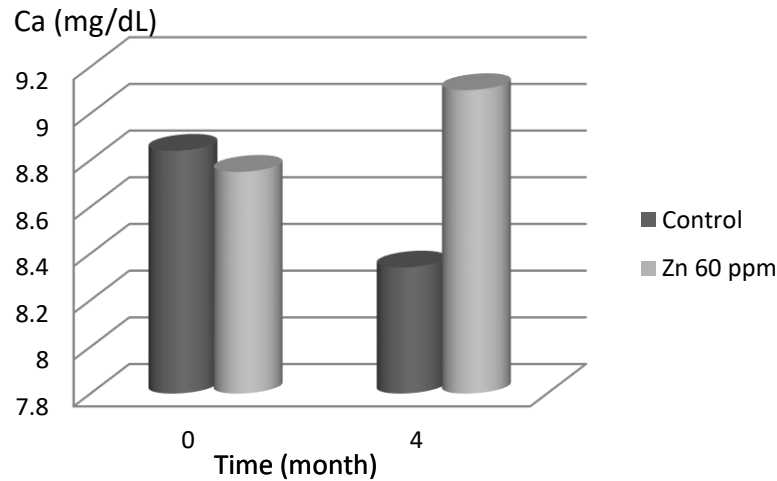
The results showed that the average blood calcium level in male FH cows decreased in the control group, but increased in the treatment group with the supplementation of 60 ppm Zn for four months of observation. The control group's blood calcium was below the normal range, whereas in the group with 60 ppm Zn supplementation was still within the normal range. Reference value of blood calcium level in cattle is between 9-11 mg/dL [8]. The result of statistical analysis showed that blood calcium levels before and after Zn supplementation in the treatment group did not differ significantly ( $P > 0.05$ ). The level of blood calcium in the control group was lower than that in the treatment group. Factors that cause low levels of calcium in the blood are the lack of calcium intake in the diet, low absorption of calcium in the intestine, low levels of calcium feed, and the increasing need of calcium for bone formation during the growth process [12].

Supplementation Zn 60 ppm can increase the blood calcium level; this may be influenced by the increasing level of albumin in the blood due to Zn in the feed, thus increasing blood calcium. The protein-bound calcium (albumin) is an important source for the provision of ready-to-use calcium so that the plasma albumin level can affect total blood calcium. The absorption of Zn depends on the source of Zn, content Zn in the feed and age of the cow [1].

Zn supplementation affects the metabolism of carbohydrates, proteins, fats, appetite, and protein synthesis. Zn supplementation is also

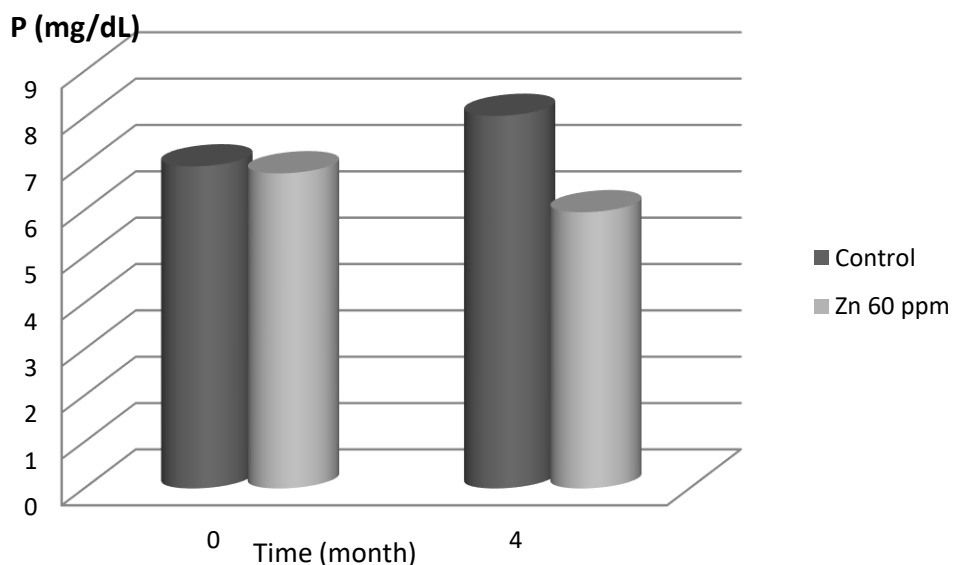
able to improve dry matter digestibility and feed efficiency [11]. Homeostasis calcium levels are regulated by a balance between blood and bone calcium. Calcium plays a role in increasing bone density. Zn mineral serves in the activation of amino acyl-tRNA synthetase in osteoblastic cells and bone tissue [6]. Calcium also plays a role in the ion transport system in the plasma membrane until fertilization occurs [16]. An adequate supply of calcium is required in the cow physiological function of the stud bull as a producer of high cement and muscle mass. Research [14] reported that calcium in cement was higher than in blood plasma, but the mechanism of regulation in cement was unclear. Calcium plays an important role in regulating the process of spermatogenesis, fertilization as well as the main regulator for the capacitation and trigger acrosome reactions in spermatozoa [13]. Calcium levels of bulls are also influenced by age factors, where younger cattle have higher blood calcium levels than adult cows, ie cattle aged three years have higher levels of calcium than eight-year-old cattle.

[3] reported a significant decrease in calcium levels after supplementation of 100 ppm Zn sulfate for 180 days in calf buffalo. Differences of calcium response in the blood may be due to differences on the amount of Zn in feed. Blood phosphate (P) should be also maintained in optimum levels as well as blood calcium levels. Calcium and phosphate are regulated by hormones such as parathyroid hormone and calcitonin.



Phosphate plays an important role in the formation of energy. It also has a role for bone and tooth formation, energy metabolism, milk secretion, fatty acid transport, phospholipid synthesis, amino acid metabolism, and protein synthesis. P mineral is a component of nucleic

acid involved in cell metabolism and enzyme systems [7,9]. The mean of blood P levels in FH bulls before and after the administration of Zn in the feed is presented in Table 1 and Figure 2.



Based on the data in Table 1, P mineral levels in calves FH ranged from 6.70 to 7.17 mg / dL. According to [8], normal blood phosphorus levels ranged from 4-6 mg/dL. The results obtained from this study were slightly higher than the normal reference value. High levels of phosphorus was allegedly due to the influence of hormones that growth hormone in young animals can increase reabsorption of

phosphate in the kidneys. In addition, ruminants also have phytase enzymes in the rumen to hydrolyze phytic acid into inositol, glucose, and organic phosphorus so as to increase phosphorus levels.

Results of the research on supplementation of Zn 60 ppm in FH bulls for four months showed that the average blood phosphate levels tend to

increase in the control group but decrease in 60 ppm Zn treatment group. Blood P levels in both groups are still in the normal range. The result of statistical analysis showed that P mineral did not differ significantly ( $P>0.05$ ) between the two groups.

The mean of serum P levels in bulls tended to decrease after the supplementation of 60 ppm Zn, but it tended to increase in the control. Calcium homeostasis and blood phosphate occur in healthy animal conditions, in which blood phosphate decreases following an increase in blood calcium [2]. The hormone that regulates homeostasis levels of calcium and phosphate is the parathyroid hormone. Parathyroid hormone works to increase the levels of calcium and lower blood phosphate levels. Homeostasis of the body increases blood calcium by increasing the excretion of phosphate in the kidneys and saliva that can interfere with the normal blood phosphate concentration. This condition can cause hypophosphatemia. Parathyroid hormone stimulates the kidneys to produce 1,25-dihydroxyvitamin D which can improve the efficiency of phosphate absorption in the gut. The secretion of the parathyroid hormone will be stimulated by hypocalcemia not under hypophosphatemia [4].

Blood phosphate is mostly found as inorganic phosphate ions ( $\text{HPO}_4^{2-}$  and  $\text{HPO}_4^-$ ) with only 10% bound to proteins and the rest diffuses freely in the balance of phosphate in bone and intracellular. The phosphate homeostasis is regulated by the intake of phosphate, the fibroblast growth factor 23 (FGF 23), PTH as well as the active form of vitamin D [2]. Decreased blood phosphate concentrations due to reduced intake will be compensated by mobilization of phosphate from bone in an effort to maintain normal blood phosphate levels in

normal circumstances [15]. The concentration of phosphate in the blood should be kept in balance by the exchange of phosphate in bone with phosphate stored in the body. The regulation of phosphate balance involves the absorption of phosphate from the intestine, mobilization of bones and salivary secretion [7].

The 60 ppm Zn supplementation group showed elevated levels of calcium followed by decreased phosphate levels. Parathyroid hormone (PTH) works to increase calcium reabsorption and decrease phosphate reabsorption in the kidney so that calcium levels will increase with decreased serum phosphate levels. Increased phosphate levels in the control group may be due to decrease calcium. In addition phosphate levels are also influenced by the phosphate content in the feed. [2] reported phosphate homeostasis regulated by fibroblast growth factor 23 (FGF 23) involvement, a bone-produced substance that acts within the homeostasis of phosphate in the blood. Many factors influence the blood phosphate levels, including the amount of phosphate in the diet, the amount of excreted phosphate, and the phosphate extracted from its bone storage

## Conclusion

Supplementation of 60 ppm Zn in feed for four months in FH bulls tends to increase serum calcium and decrease phosphate, although the value is still within a normal range. Supplementation of Zn can improve the health status of livestock.

## Suggestion

Further research needs to be done on male FH cows that have different age variations to see the effect of age on serum calcium and phosphorus levels and their effect on hormonal functions.

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