EFFECT OF ZINC INTAKE DURING TRANSITION PERIOD ON ZINC AND CALCIUM IN MILK OF SUBCLINICAL HYPOCALCEMIA DAIRY COWS

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Abstract

In early lactation, high producing cows frequently suffer from negative energy balance and mineral imbalances. Negative energy balance is associated with immune suppression and some diseases like a hypocalcemia. Zinc (Zn) is known as immune stimulator, able to increase immune response to diseases. The objective of this study was to study the effect of supplemental Zn intake in dairy cows at 4 weeks prior to calving (dry period) on Zn and calcium (Ca) content in milk and subclinical hypocalcemia. Twenty-two dairy cows (Holstein Friesian) were randomly allocated to either a dry cow ration with or without Zn supplementation (40 ppm Zn in the diet). The cows received a roughage mixture ad libitum and concentrates were fed individually. Post-partum, cows were milked twice daily and throughout the experiment animal health was monitored daily. Blood and milk samples were taken at week 1 after calving. Data were statistically analyzed with SAS. A cow was considered a case of subclinical hypocalcemia when blood calcium lowered below 8.0 mg/100 ml. The results showed the percentage incidence of clinical and subclinical hypocalcaemia (1.5% vs. 31%). Zn and Ca content in milk were not affected by dietary Zn content in diet. In conclusion, feeding above Zn requirements during 4 weeks before calving does not seem to reduce the incidence of clinical milk fever nor subclinical milk fever. Zn supplementation may improve the immune system but its form and absorption must be considered.

Key words: subclinical hypocalcemia, transition cow, zinc supplementation

INTRODUCTION

Dairy cows experience substantial metabolic and physiological adaptations during the transition from pregnancy to lactation [1]. High milk production in dairy cows is associated with increased health problems both metabolic and infection problem especially during the transition period [2] [3]. The transition period in dairy cows is defined as 3 weeks before to 3 weeks after calving [4] and is considered as a critical time in the lactation cycle of a dairy cow. In early lactation, high producing dairy cows often suffer negative energy balance (NEB). Negative energy balance is associated with immune suppression and several diseases[1]. In dairy cows, inability to adapt effectively to a new lactation is not only related to metabolic stress, mineral imbalance and increased occurrence of infectious disease but also impaired function of the immune system [5]. Another consequence of the high metabolic rate and milk production in early lactation is an increase in mineral requirement such as calcium. If the requirement of calcium is not met, this result in cows that suffer from hypocalcemia [6]. Hypocalcemia is a case of Ca deficiency that can occur in clinical or subclinical form. The range of normal Ca levels in cow's blood is 9-12 mg/100ml [7]. Clinical hypocalcemia is characterized by a drastic decrease in calcium levels and in the range of 3-5 mg /100ml, clinically cattle will collapse and cannot rise. Dairy cows under subclinical hypocalcemia have calcium levels in the range of 5-8 mg / 100ml. However, the animal do not show clinical symptoms as is the case in clinical hypocalcemia) [7].

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The manuscript was received: 05.09.2019
Accepted for publication: 02.11.2019

Zn is needed in small amounts but absolutely must be in the feed, because Zn cannot be converted from other nutrients. This mineral plays a role in a variety of enzyme activities, cell growth and differentiation, and plays an important role in optimizing the function of the immune system [8].Zn is able to act as an immune stimulator that is able to increase the immune system. In late of pregnancy of dairy cows, low levels of Zn in the blood can adversely affect fetal growth so that it can cause abortion [8]. The recommended requirement for Zn in pregnant dairy cows (dry period) and early lactation is 40 ppm [9]. However, in general, forages contain low levels of Zn around 20 – 35 ppm [8]. Zn content of around 40-60 ppm in feed is needed to be able to maintain the body's immune system remains optimal. Therefore, to improve immune response it is recommended to supplement Zn in the ration [8]. The limit for giving Zn in rations to cattle is 500 ppm [8].

Zn supplementation for increase the immunity system on cattle have been the focus of much research in the past[10][8][11]. However, there has not been much research on Zn supplementation during transition period and its influence on status Zn and Ca in milk and consequently its effect on the incidence of subclinical hypocalcemia. The objective of this study were to evaluate Zn supplementation in ration during transition period for incidence of subclinical hypocalcemia and its effect on Zn and Ca in milk.

MATERIAL AND METHODS

Experimental Design and Animal

A total of 22 Holstein Friesian dairy cows were selected from BPPIB TSP Bunikasih, Cianjur, Indonesia. Approximately 4 weeks before calving, cows were randomly allocated to either a dry cow ration with or without Zn supplementation (group 1(G1) vs group 2(G2), respectively). Cows in group 2 received 40 ppm Zn in the diet. The cows received a roughage mixture ad libitum and concentrates were fed individually. Drinking water was provided *ad libitum*. Nutrient content of feed ingredients and ration composition and chemical content of each dietary treatments are presented in Table 1 and Table 2, respectively.

Analysis of feed ingredients is carried out at Ruminant Nutrition and Feed Chemistry Laboratory using proximate analysis. Mineral calcium analysis is carried out at Central Laboratory of University Padjadjaran. Mineral zinc analysis Badan Tenaga Nuklir Nasional (BATAN).

Table 1 Nutrient content of napier grass, concentrate and *Indigofera zollingeriana*

Item	Napier Grass	Concentrate	*Indigofera zollingeriana*
Water (%)[a]	68.45	9.64	79.23
Ash (%)[a]	15.10	11.30	11.46
Crude Protein (%)[a]	10.99	18.50	28.15
Crude Fat (%)[a]	1.62	10.22	2.22
Crude Fiber (%)[a]	25.13	17.33	21.40
TDN (%)[a]	49.65	69.66	62.06
Gross energy (Kkal/kg)[a]	2916	3429	3022
Calcium (Ca) (%)[b]	0.26	1.01	0.62
Zinc (Zn) (mg/kg)[c]	35.8	54.2	34.2

[a] Proximat Analysis (2018)
[b]Atomic Absorption Spectrometer (2018)
[c] Neutron Activation Analysis (2018)

Table 2 Ration composition and chemical content of each dietary treatments

Feedstuff	**G1[1]**	**G2[2]**
Napier Grass (%)	45.00	45.00
Concentrate (%)	40.00	40.00
Indigofera zollingeriana(%)	15.00	15.00
Zn (ppm DM)	0.00	40.00
Feed substances		
Dry Matter (DM)	5350	53.50
Ash (%)	13.03	13.03
Crude Protein (%)	16.57	16.57
Crude Fat (%)	5.15	5.15
Crude Fiber (%)	21.45	21.45
TDN (%)*	59.52	59.52
Ca (%)	0.61	0.61
Zn (ppm DM)	42.92	82.92

[1] G1 = Group 1 = Treatment without Zn Supplementation
[2] G2 = Group 2 = Treatment with Zn Supplementation
* TDN = Total Digestible Nutrient

Blood and Milk

Blood and milk samples were taken at week 1 after calving. Blood samples were collected via *coccigea* vena from each cow into serum tubes (Vacutainer) for measurement of Ca in blood. Milk samples were collected from each cow into glass tube (30 ml) for measurement of Zn and Ca in milk.

Statistical Analysis

The general linear model (GLM) of SAS (SAS version 9.4.; SAS institute Inc. Cary, NC,) was used to analyze the effect of zinc intake on status on Zn and Ca in milk and Ca in blood of subclinical hypocalcemia dairy cows. Cows were divided into three groups as healthy cows (Ca in blood =9-12 mg/100ml, n=4), subclinical hypocalcemia (Ca in blood=5-8 mg / 100ml, n=12) and clinical hypocalcemia (Ca in blood =3-5 mg /100ml, n=6).A significant difference in the least squared means (LSM) of the treatments.

RESULT AND DISCUSSION

In the present study there were ten cows that treated with Zn supplementation and there was only one cow (10%) that has normal blood levels of Ca (which is higher than 8.0 mg/100 ml). Meanwhile, there were three from twelve cows fed ration without Zn supplementation showed normal blood levels of Ca (25%). The results showed that the effect Zn supplementation has no significant effect on blood Ca levels (*p*-value > 0.05, table 4). There are several other factors affect blood levels of Casuch as protein, vitamin D, and hormonal mechanisms. High protein avaibility in the blood, the higher the amount of Ca bound by protein, thus detected Ca levels in blood will also increase [12]. Moreover, vitamin D plays a role in the formation of Ca-binding proteins in the intestinal wall that facilitate the absorption of Ca.Ca absorption process is controlled by 1,25-*hydrokxycholecalciferol*(active form of vitamin D for metabolism). Regulating the amount of vitamin D absorbed and produced will regulate the amount of Ca absorbed from the feed so that blood Ca levels remain constant [9]. During the transition period, hormonal changes occur that can result in the fluctuation of Ca absorption. One of the hormones associated with regulating Ca in the body is thyrocalcitonin. Thyrocalcitonin hormone inhibits the removal of Ca from the bones and decreases the level of Ca in the blood. The release of these hormones is regulated by high levels of Ca in the blood [13].

The average Zn in milk in cows fed Zn supplementation in healthy conditions, subclinical and clinical hypocalcaemia were 0.71 mg/100 ml. 0.67 mg/100 ml and 0.70 mg/100 ml, respectively. Whereas the average zn in milk in cows fed ration without Zn supplementation in healthy conditions, subclinical and clinical hypocalcaemia were 0.66 mg/100 ml, 0.65 mg/100 ml and 1.26 mg/100 ml, respectively. The Zn content in milk is 0.37 mg/100 ml [14]. Overall, Zn content in milk treated with or without Zn supplementation (Group1 vs Group 2) are higher than 0.37 mg/100 ml (Table 3). There was no significant difference found in the effects of treatment to Zn on Milk. However, this trend indicates that the Zn in milk was increased with Zn supplementation.

In the present study, the average Ca in milk in cows fed ration with Zn supplementation in healthy conditions, subclinical and clinical hypocalcaemia were 123.06 mg/100 ml, 119.13 mg/100 ml 109.24 mg/100 ml, respectively (Table 3). Whereas the average zn in milk of cows fed ration without Zn supplementation in healthy conditions, subclinical and clinical hypocalcaemia were 111.63 mg/100 ml 111.44 mg/100 ml 133.22 mg/100 ml respectively. This trend indicates that the Ca in milk was increased with Zn supplementation. Supplementation of Zn has an indirect effect on milk Ca levels. Better immune system with mineral administration increased absorption of Ca. Zn plays an important role in optimizing the function of the immune system. However, there was no significant difference found in the treatments to the Ca in milk (Table 4).

There is an interrelation between Ca and Zn. Ca is able to directly affect Zn absorption, but there is no reverse arrow, which means Zn has no direct effect on Ca absorption. Prasad (1991) [16]showed that a high level of Ca content could inhibit Zn absorption and increased Zn deficiency in pigs and poultry. Zinc is also known to inhibit the activities of membrane enzymes such as calcium-dependent adenosinetriphosphatase (ATPase) and phospholipase A2 in the maintenance of increased integrity of the membrane structure. Previous study indicated that the intracellular effects of calcium are mediated primarily by calmodulin, a low-molecular-weight calcium-binding protein. Calcium-activated calmodulin has the potential to activate many enzymes and to stimulate many intracellular events.

Table 3 Effects of zinc intake during transition period on calcium in blood (Blood Ca), zinc (Milk Zn), and calcium (Milk Ca) in milk and of subclinical hypocalcemia dairy cows

T	Item	$\bar{x}$ G1	$\bar{x}$ G2
Healthy (N), n=4	Blood Ca (mg/100ml)	8.63	8.16
	Milk Zn (mg/100ml)	0.66	0.71
	Milk Ca (mg/100ml)	111.63	123.06
Subclinical Hypocalcemia (SCH), n=12	Blood Ca (mg/100ml)	6.25	5.96
	Milk Zn (mg/100ml)	0.65	0.67
	Milk Ca (mg/100ml)	111.44	119.13
Clinical Hypocalcemia (CH), n=6	Blood Ca (mg/100ml)	3.92	2.68
	Milk Zn (mg/100ml)	1.26	0.70
	Milk Ca (mg/100ml)	133.22	109.24

T = Treatments
n = cows
[1] $\bar{x}$ G1 = Mean of control (Without Zn Supplementation)
[2] $\bar{x}$ G2 = Mean of Zn supplementation
[3] $\bar{x}$ = Mean of whole

Absorption of Zn is influenced by the amount and balance of other minerals as well as the composition of rations and the chemical form of Zn. Zn absorption in the form of organic is better compared to inorganic form [8]. Organic Zn is related to a protein absorption mechanism compared with ions Zn in the small intestine. Inorganic minerals split in the digestive system forms free ions and absorbed, also forming complexes with molecules in feed and become complex compounds that are difficult to abort, so it is not available for animal [16].

Table 4 The effect Zn intake during transition period on blood Ca, Zn and Ca in milk in the first week after calving (LSM)

Variables	SCH		SEM	*P-Value*
	G1	G2		SCH
Milk Zn	0.65	0.67	0.07	0.81
Milk Ca	111.44	119.13	7.47	0.48
Blood Ca	6.25	5.96	0.26	0.46

SCH = Subclinical hypocalcemia cows
SEM = Standard Error Means
G1 = Group 1/Without Zn supplementation
G2 = Group 2/With Zn supplementation
P-value = Probability value

CONCLUSION

In conclusion, the Zn supplementation did not affect status Ca in blood, nor Zn and Ca in milk. Moreover, it seems Zn was involved during recovery of infection or metabolic problem. Furthermore. it might be important to study the form of its supplementation and absorption in the body. It also needs to be studied further about the relationship between Zn in feed on the absorption of Ca in the body

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