Prepartum Zinc Supplementation in Dairy Cows and Its Effect on Plasma Calcium, Titers Antibodies and Milk Zinc Content in Postpartum Dairy Cow

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ABSTRACT
The plane of energy nutrient and supplementation of mineral fed to cows in the prepartum period has been shown to influence energy status, immune response, and metabolic health. However, the effect of supplementation of essential mineral like Zn in the diet on plasma titers antibodies and mineral status in postpartum dairy cows is less known. This study investigated the effect of prepartum Zn supplementation in dairy cows on calcium (Ca) status, titer antibodies in plasma and milk Zn content in dairy cows at week 1 after calving. Lipopolysaccharide (LPS) were used as an antigen. Twenty dairy cows (FH) were randomly assigned to 2 groups: dry cow basal diet (control group; CON) and basal diet with supplementation of Zn 40 ppm (CON+Zn). Both Ca in plasma and Zn milk content were determined by atomic absorption spectrophotometry. Blood and milk sample were taken at week 1 after calving. Results showed there were no significant differences on Ca plasma, titers antibodies (IgG and IgM) and milk Zn content among dietary treatments. Numerically, plasma Ca, milk Zn content and IgG binding LPS were higher in cows fed Zn supplementation compared with control group. No significant differences in the status of Ca and Zn and titers IgG binding LPS at week 1 after calving were observed between the 2 groups maybe due to high immune status of these cows. It seems feeding Zn above the requirements before calving maintain immune status, better milk nutrient dense and reduce the incidence of milk fever.

Keywords: Antibodies, Calcium, Early lactation, Milk fever, Zinc

1. INTRODUCTION
Due to high energy output (milk production and maintenance) but limited energy intake (low feed intake), dairy cows frequently experienced severe negative energy balance (NEB) in early lactation. NEB was associated with metabolic disorder [1], metabolic diseases (fatty liver, ketosis and milk fever due to mineral imbalance), immune suppression (low titers antibodies) [2] and reproduction disorder in transition period. Immune suppression is connected to a severe negative energy balance (EB) during the transition phase, as well as a high prevalence of infectious diseases and metabolic problems.

Many feeding strategies has been introduced to modulate immunity and minimize NEB in dairy cows during early lactation. Mineral supplementation in the diet is one strategy to meet the requirement of mineral in dairy cows during transition period. Mineral imbalance causes diseases in cows. The most common macro mineral diseases that afflict transition dairy cows are milk fever and subclinical hypocalcaemia (total blood calcium 2.0 mmol/l). It's vital to remember that milk fever is linked to the onset of a slew of additional issues in dairy animals [3]. Hypocalcaemia is a frequent metabolic disease in which the body's homeostatic mechanisms fail to keep blood calcium levels normal at the start of lactation [4]. Subclinical hypocalcaemia has been documented in up to 50% of periparturient dairy cows [5,
6], with total blood Ca values ranging from 1.38 to 2.0 mmol/L (5.5 mmol/L) [5, 7].

The intake of key minerals like zinc (Zn) was balanced, which improved immunological function and reduced the occurrence of health concerns like milk fever. Cows are reported to be predisposed to milk fever depending on their age, milk output, breed, body condition, dry period length, and feeding [8]. 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]. The lethal doses of Zn is important due to their interaction with other mineral and other metabolic mechanism. Previous studies reported that in ruminants, calcium and zinc are antagonistic to some extent [9].

Zn supplementation to boost cow immunity has been the subject of a lot of 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 in milk, plasma Ca and natural antibodies in plasma. The objective of this study were to evaluate the effect of prepartum Zn supplementation in dairy cows on calcium (Ca) status, titer antibodies and milk Zn content in postpartum dairy cows.

2. MATERIALS AND METHODS

2.1. Experimental Design and Animal

From Breeding and Artificial Insemination (Balai Pembibitan dan Inseminasi Buatan Ternak Sapi Perah (BPPIP-TSP) Bunakasi, Cianjur, Indonesia, a total of 20 Holstein Friesian dairy cows were chosen. Cows were randomly assigned to either a dry cow feed with or without Zn supplementation (group control (CON) vs group (CON+Zn), approximately 4 weeks before calving. Cows in group 2 received 40 ppm Zn in the diet [10]. 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.

Analysis of feed ingredients was carried out at Ruminant Nutrition and Feed Chemistry Laboratory, Department of Animal Nutrition and Feed Technology. Faculty of Animal Husbandry, Universitas Padjadjaran, Jatinangor, Indonesia using proximate analysis. Mineral calcium and zinc analysis is carried out at Central Laboratory of Universitas Padjadjaran.

Table 1. Ration composition and chemical content of each dietary treatments

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>CON†</th>
<th>CON+Zn‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Napier Grass (%)</td>
<td>45.00</td>
<td>45.00</td>
</tr>
<tr>
<td>Concentrate (%)</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Indigofera zollingeriana (%)</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Zn (ppm DM)</td>
<td>0.00</td>
<td>40.00</td>
</tr>
</tbody>
</table>

†CON = Treatment without Zn Supplementation
‡CON + Zn = Treatment with Zn Supplementation (40 ppm [10])
* TDN = Total Digestible Nutrient

2.2. Blood and Milk

Blood and milk samples were taken at week 1 after calving. Blood samples were collected via coccygea vena from each cow into serum tubes (Vacutainer) for measurement of Ca and titers antibodies in blood plasma. Milk samples were collected from each cow into glass tube (30 ml) for measurement of Zn and Ca in milk. Both Ca in plasma and Zn milk content were determined by atomic absorption spectrophotometry. Titters of natural antibodies in plasma were measured by an indirect enzyme-linked immunosorbent assay (ELISA) technique [2].

2.3. 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 and titters antibodies in plasma blood A significant difference in the least squared means (LSM) of the treatments. p<0.05 is considered that there is a significant difference.

3. RESULT AND DISCUSSION

In the present study, there were no significant differences on Ca plasma, plasma titers antibodies
(isotype for IgG and IgM) and milk Zn among dietary treatments (Table 2) was found. Numerically, plasma Ca, milk Zn content and IgG binding LPS were higher in cows fed Zn supplementation compared with control group. In agreement with a previous study there were no effect of Zn on Ca in plasma of lactating dairy cows [11]. In the lactating cow, increased dietary calcium had no effect on zinc absorption. Calves fed a low-zinc diet absorb more zinc [11]. In the current study, cows fed Zn supplementation have 2mmol/L higher Zn content in milk compared with control group (Figure 1). In agreement with a previous study that feeding Zn in the cows diet increased Zn and fat content in milk [12].

Table 2. Plasma calcium (Ca), milk zinc (Zn), milk Ca, and plasma natural antibodies (IgG and IgM) binding lipopolysaccharides in of dairy cows after fed (40 ppm) at week 1 after calving (Least square mean (LSM) and standard error mean (SEM)).

<table>
<thead>
<tr>
<th>Variables</th>
<th>(CON)</th>
<th>(CON+Zn)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca milk (mg/kg)</td>
<td>5.18</td>
<td>5.69</td>
<td>0.55</td>
<td>0.53</td>
</tr>
<tr>
<td>Ca in plasma (mmol/L)</td>
<td>117.03</td>
<td>121.04</td>
<td>6.00</td>
<td>0.64</td>
</tr>
<tr>
<td>Zinc in milk (mg/kg)</td>
<td>6.36</td>
<td>8.58</td>
<td>1.50</td>
<td>0.31</td>
</tr>
<tr>
<td>IgG binding LPS</td>
<td>5.19</td>
<td>6.16</td>
<td>0.43</td>
<td>0.14</td>
</tr>
<tr>
<td>IgM binding LPS</td>
<td>6.40</td>
<td>6.52</td>
<td>0.55</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1 CON= Treatment without Zn Supplementation
2 CON+Zn = Treatment with Zn Supplementation (40 ppm)
LPS = lipopolysaccharide
a-b Values within a row with different superscript letters are significantly different (P < 0.05).

No significant differences in the titers IgG binding LPS at week 1 after calving were observed between the 2 groups maybe due to high immune status of these cows. Cows in this study especially have higher titers IgG binding LPS (5.19-6.16) compared with previous study (4.98-5.90) [2]. Zn can help the body combat free radicals and maintain its antioxidant status [13]. In the study, the titers IgG binding LPS was associated with occurrence of clinical mastitis. In previous studies [2], cows with a better energy balance (EB) in early lactation had greater NAb titers in plasma. Other study showed zinc supplementation used to minimize disorders of zinc homeostasis in the body which have an impact on lipid profile and cytokine production. Zn has the ability to increase immunological response in both a non-specific and specific manner. Changes in infection resistance are caused by a faulty immunological response. As a result, the mineral Zn’s sufficiency warrants consideration, considering its function in boosting the immune system and its impact on cattle productivity, as well as its ability to prevent apoptosis, or programmed cell death [14]. It seems Zn supplementation play important role to maintain immune response in dairy cows during transition period.

4. CONCLUSION

Feeding Zn during transition period resulted in high concentration of Zn in milk and increased Ca absorption in cows. Moreover, Zn supplementation plays important role to maintain immune status (IgG binding LPS), better milk nutrient dense (better Zn content in milk) and reduce the incidence of milk fever (maintain Ca in plasma).

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