ASSOCIATION BETWEEN PLASMATIC GLUCOSE AND UREA IN DAIRY COWS WITH SUBCLINICAL HYPOCALCEMIA

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Abstract

During transition period, cells epithelial mammary gland differentiates massively in order to support milk production. The major mineral in milk is calcium, excreted in large amounts, while simultaneously maintaining maternal health and physiology (including energy status). The objective of this study is to evaluate the association between plasma glucose and urea in dairy cows with subclinical hypocalcaemia. In total 22 Holstein were used in this study. To analyze the association between glucose and urea, in total 12 Holstein dairy cows with subclinical hypocalcemia (Ca<0.1 mmol/L at d 2) were used in the experiment. Cows were blocked based on calving date and parity. Blood samples were collected approximately on d 7 postpartum for measurement of plasma glucose and urea nitrogen. Disease occurrence was determined based on individual treatment records. Pearson correlations coefficient were used to analyze the correlation between blood metabolites. Subclinical hypocalcemia cows had normal concentration of glucose and urea on d 7. The results shown that the concentration of glucose as indicator for energy status were negatively correlated with plasma urea. No differences were observed between individual of cows in the occurrence of subclinical and clinical hypocalcemia, clinical mastitis, displaced abomasum, dystocia, retained placenta, metritis. These data suggest that early lactation energy status indicated by glucose and urea differs among cows with subclinical hypocalcemia.

Key words: subclinical hypocalcemia, energy status, transition cow

INTRODUCTION

Dairy cows experience substantial metabolic and physiological adaptations during the transition from pregnancy to lactation [1]. High yielding dairy cows is associated with increased metabolic disorder and infection problems especially during the transition period [2,3]. The transition period in dairy cows is considered as a critical time in the lactation cycle of a dairy cow and is defined as 3 weeks before to 3 weeks after calving [4]. 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]. During the transition period, NEB increased gluconeogenesis and reduced utilization of glucose by peripheral tissue as mechanism of the primary homeorhetic adaptation of glucose metabolism. High demand for glucose, amino acids, and fatty acids is dramatically increased at the end of gestation and in early lactation for maintenance and to produce milk. Additionally the demand for calcium increases at the beginning of lactation as calcium is the main mineral for milk [5].

In early lactation, high metabolic rate and milk production also increased 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/100 ml [7]. Clinical hypocalcemia is

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characterized by a drastic decrease in calcium levels and in the range of 3-5 mg /100 ml, clinically cattle will collapse and cannot rise. Dairy cows under subclinical hypocalcemia have calcium levels in the range of 5-8 mg /100 ml. However, the animal does not show clinical symptoms as is the case in clinical hypocalcemia [7].

Dairy cows may mobilize fat and protein during the transition period if an energy deficit exists due to an imbalance of energy requirements versus intake. However, the degree of fat mobilization and whether the cow goes into a NEB varies from cow to cow [8,4]. The hallmark of the dairy cow's NEB is mobilizing fat from body stores as non-esterified fatty acid to utilize for energy. It is also occur for mineral. Dairy cows may mobilize calcium in early lactation if calcium availability deficit versus intake.

The relationship between multiple diseases showed that cows experiencing milk fever were almost 9 times more likely to exhibit signs of ketosis compared with cows not developing milk fever [9]. To our knowledge, there has not been much discussion about the relationship between glucose and urea in blood with the conditions of subclinical hypocalcemia. Hence, more research focusing on subclinical hypocalcemia and its relationship with glucose and urea is warranted. The objectives of this study were to evaluate potential associations between glucose and blood urea in dairy cows with subclinical hypocalcemia.

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	Consentrate	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
Selenium (Se) (mg/kg)[c]	< 0.11	0.32	< 0.10

a Proximate Analysis (2018)
b Atomic Absorption Spectrometer (2018)
c Neutron Activation Analysis (2018)

Table 2 Ration composition and chemical content of each dietary treatments

Composition	Content
Napier Grass (%)	45.00
Consentrate (%)	40.00
Indigofera zollingeriana (%)	15.00
Zn (mg/kg DM)	40.00
Selenium (Se) (mg/kg)	0.03
Chemical content	
Dry Matter (DM)	53.50
Ash (%)	13.03
Crude Protein (%)	16.57
Crude Fat (%)	5.15
Crude Fiber (%)	21.45
TDN (%)*	59.52
Ca (%)	0.61
Zn (mg/kg DM)	82.92
Se (mg/kg BK)	0.43

* TDN = Total Digestible Nutrient

Blood

Blood samples were taken at week 1 after calving. Blood samples were collected via coccygeal vein from each cow into serum tubes (vacutainer) for measurement of Ca, and into plasma tubes (EDTA vacutainer) for measurement of glucose and urea in blood.

Statistical Analysis

The general linear model (GLM) of SAS (SAS version 9.4.; SAS institute Inc. Cary, NC,) was used to analyze correlation between glucose and urea in blood of subclinical hypocalcemia dairy cows. Cows were divided into three groups as healthy cows (Ca in blood = 9-12 mg/100 ml, n=4), subclinical hypocalcemia (Ca in blood=5-8 mg/100 ml, n=12) and clinical hypocalcemia (Ca in blood =3-5 mg /100 ml, n=6). The significant difference is based on the least squared means (LSM) of the treatments, with $p<0.05$ is considered that there is a significant difference.

RESULT AND DISCUSSION

In this study, there were 12 out of a total of 22 (54.5%) dairy cows experiencing subclinical hypocalcemia. This is consistent with the opinions that as many as 50% of periparturient dairy cows may suffer from subclinical hypocalcemia [15,16].

Blood glucose levels are a reflection of the ultimate outcome of metabolism carbohydrates that circulate in the blood [12] and are a source of energy which is important in the maintenance of body cells and muscle tissue [13]. The average blood glucose level in dairy cows is 53.4-72.1 [14]. The average blood glucose levels in dairy cows with subclinical hypocalcemia at 1 week after calving is 59.88 mg/100 ml (Table 3). This indicates that the glucose of cows in this study was in the normal range and showed that the cows in this study most likely did not experience a NEB. Blood glucose entry rate (predominately determined by gluconeogenic rate) [15] relative to the rate of glucose utilization, therefore, does not enable the cow to approach the upper physiological set point.

In the present study, there were no effect of Ca in blood on glucose levels in blood Table 4), which means blood glucose is not influenced by subclinical hypocalcemia condition. Insulin is one of the important hormones that regulates blood glucose levels. Insulin has a key hormone on endocrine regulation, facilitates the movement of glucose across cell membranes, and regulating the concentration of blood glucose [16]. In other studies, with human objects it has been reported that no clear relationship has been found between blood glucose levels with Ca. It is known that Ca does not independently affect blood glucose levels, but is influenced by other nutrients. A previous study showed there were a relationship between magnesium and calcium intake with insulin sensitivity [17]. However, there is no clear association between blood glucose levels and subclinical hypocalcemia.

Normal blood urea nitrogen levels in Holstein cows range from 6-27 mg/100 ml [18]. In the present study showed that the mean of urea level in plasma at week 1 is 21.44 mg/100ml. This indicates that blood urea's cows with subclinical hypocalcemia in this study was in the normal range. Urea levels are a reflection of the urea cycle and is the result of the process of protein metabolism by microbial activity rumen of feed protein and non-protein nitrogen [19]. Blood urea nitrogen is formed in the liver and is the end result of protein catabolism [20]. In the small intestine, amino acids are absorbed into the circulation for metabolism. Blood urea nitrogen is the result of amino acid metabolism in the Krebs-Henseleit cycle.

Table 2 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	$\bar{x}$
Healthy (N), n=4	Ca (mg/100ml)	9.05
	Glucose (mg/100ml)	54.34
	Urea (mg/100ml)	27.98
Subclinical Hypocalcemia (SCH), n=12	Ca (mg/100ml)	6.08
	Glucose (mg/100ml)	59.88
	Urea (mg/100ml)	21.44
Clinical Hypocalcemia (CH), n=6	Ca (mg/100ml)	3.51
	Glucose (mg/100ml)	54.21
	Urea (mg/100ml)	30.03

n = cows
$\bar{x}$ = Mean of whole

In the present study, there were no effect of Ca in blood on urea levels in blood (Table 4), which means that blood urea was not influenced by subclinical hypocalcemia condition. The level of blood urea affected by protein intake and digestibility [21]. Increased blood urea nitrogen levels also affected by an increase of protein metabolism [20] and inefficient use of NH_3 [21]. High urea nitrogen levels indicated a lack of rumen NH_3 utilization to form microbial proteins. Normal urea nitrogen levels indicate that ammonia utilized by microbes in high levels. High undegradable protein content increases urea nitrogen levels. If the rate of formation of NH_3 is greater than its utilization level, then NH_3 will be absorbed into the blood and converted to urea [21].

Table 4 Association between glucose and urea plasma dairy cows with subclinical hypocalcemia

Item	Least Square Means	SEM	P-value
	SCH		SCH
Glucose	59.88	2.27	0.71
Urea	21.44	3.99	0.92

SCH = Subclinical hypocalcemia cows
SEM = Standard Error Means
P-value = Probability value

Table 5 Correlation between glucose and urea in blood plasma

Item	Rho (r)	P-Value
Correlation between glucose and urea in blood plasma	-0.46062	0,0310

The concentration of glucose as an indicator for energy status were negatively correlated with plasma urea (r=-0.46062, Table 5). In other words, if blood glucose is low, then blood urea will be high, and vice versa. This negative correlation seems to be related to protein content in plasma. It is known, there was a link between protein storage in the body with blood glucose levels. It seems high protein content decreased blood glucose. Previous research reported that feeding high protein content and metabolic energy increased total plasma protein levels and reduce blood glucose levels [22].

Blood urea concentrations often fluctuate around calving, influenced by a variety of factors. Glucose availability may be supplemented by increased catabolism of amino acids stored in skeletal muscle and other tissue proteins, thus increasing urea production [8]. In a previous study, degradation of rumen degradable protein (RDP) causes a rise in circulating ammonia concentrations, particularly during energy deficiency, and urea production by the liver also requires energy, and may exacerbate the NEB [23]. Impaired liver function, as commonly occurs after calving, reduces the metabolic clearance of urea [23].

CONCLUSION

In conclusion, blood glucose and urea levels are not directly affected by subclinical hypocalcemia conditions. The results of the study found a negative correlation between blood glucose and blood urea. Furthermore. it might be important to study a relationship between blood glucose and urea with calcium consumption during the transition period.

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