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Assessment of Serum Mineral and Certain Biochemical Variables in Self-Sucking Dairy Cows

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Abstract: Self-sucking, that is, a cow sucking on her own teats is an important and leading cause of economic loss. However, the causes of self-sucking are virtually unknown, although numerous possible influencing factors, such as feeding management, nutrient deficits, genetic factors and housing systems have been suggested. In this study, our main objective was to investigate the possible effects of mineral levels on self-sucking in dairy cows. Biochemical variables of blood serum were also investigated. Cows which self-sucked had significantly lower serum concentrations of Mn, Co, Zn, P, Na, Cl, K and total protein, compared with the control group. Levels of serum cholesterol, HDL-C, LDL-C and ALP activity were significantly increased in the self-sucking group. There were no significant differences in serum levels of Ca, Mg, Cu, Fe, saturated Fe, iron binding capacity, urea, creatinin, uric acid, total bilirubin, amylase, gamaglutmyl transaminase, lactate dehidrogenase, creatin kinase, creatine kinase-MB, alanine transaminase and aspartate transaminase activities between the self-sucking group and the control group. The findings provide novel information about whether macro and micro element deficiency may cause self-sucking in dairy cows. An evaluation of our results supports the hypothesis that energy deficiency is a possible cause of self-sucking. Although the explanation is not clear, it may be related to decreased Co, Mn and P levels, which are important in carbohydrate and energy metabolism.

Key words: Self-sucking, dairy cow, biochemistry, trace element

INTRODUCTION

Dairy cows have been bred over the past centuries to produce more milk and this has resulted in many different health problems in dairy herds. Among these are behavioral problems such as inter-sucking, cross-sucking, self-sucking, moving the tongue and chewing on nothing. Self-sucking is when a cow sucks on her own teats and it usually includes the swallowing of milk (Bademkiran *et al*., 2007; Lidfors and Isberg, 2003) (Fig. 1). Milk sucking or galactophagia are other synonyms for intersucking, which are usually used for cows that also succeed in swallowing milk from teat of another cow (Lidfors and Isberg, 2003). Self-sucking is when a cow sucks on her own teats and it usually includes the swallowing of milk (Bademkiran *et al*., 2007; Peterse *et al*., 1978; Boe, 1990), It can lead to udder damage, mastitis, milk loss and loss of breeding animals, which give rise to important economic losses. When it happens during the final term of pregnancy, it may prevent the formation of colostrum, thus depriving the new-born calf.

In previous studies, 1-49% of dairy farmers reported intersucking in cows and 0.5-1% of cows were observed self-sucking. The causes of self-sucking are virtually unknown, but numerous possible influencing factors have been suggested, such as feeding management, nutrient deficits, genetic factors, housing systems and the effect of age (Bademkiran *et al*., 2007; Lidfors and Isberg, 2003; Keil *et al*., 2001; Phillips *et al*., 1999; Wood *et al*., 1967; Kursa and Kroupova, 1976; Peterse *et al*., 1978; Grommers, 1979; Schlüter *et al*., 1981; Suéss and Sebestik, 1982).

Macro and trace elements are necessary for the maintenance of normal metabolic states and productivity in animals (Yokus *et al*., 2004). Several minerals participate

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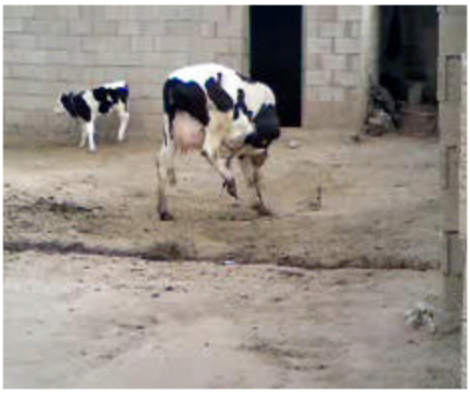

Fig. 1: Self-sucking a dairy cattle

as key components in a multitude of enzymes and play an important role in many biochemical functions. Inadequate intake of these minerals may cause impairment of biological functions or pathological changes. As far as we know, there is no literature on the role of mineral levels in the etiology of self-sucking in cattle.

The aim of our research was to investigate variations in serum levels of Ca, Mg, P, Na, K, Cl, Fe, Cu, Zn, Co, Mn in self-sucking in dairy cattle and the eventual role of these changes on the pathophysiology (etiology) of the disorder.

The serum biochemical profiles included the evaluation of urea, creatinin, urea/creatinin ratio, uric acid, total bilirubin, cholesterol, HDL-C, LDL-C, total protein, iron, iron binding capacity, AST, ALT, LDH, GGT, CK, CK-MB, ALP and amylase activities.

MATERIALS AND METHODS

Cows and design: This study was conducted on family farms in the Diyarbakir area of Turkey between February 2005 and May 2007. Ninteen Red home breed South Anatolian Cows and 1 Holstein hybrid, aged 2 to 6, which were under same care and feeding conditions and were identified as self-sucking, were used for the study. The self-sucking cows were identified by the reports of their owners and by observations of the researchers. A cow which had been determined as self- sucking at least 3 times in the course of one lactation was recorded as a self-sucker. Self-sucking cows had exhibited this behavior for between 1 and 5 years.

Twenty clinically healthy animals from the same farms were used as a control group. Thus, each self-sucking animal and its control cow had the same conditions. None of the cattle had any diseases and none had received any medication.

Care and feeding: All the cows in the study were kept tied indoors. The all cows were the mid lactation period. They were fed with wheat straw and bran or wheat straw and barley. No nourishment program was applied according to the physiological condition of the cows. In other words, they were always fed the same rations both in lactation, in pregnancy and in the dry period. All the cows were milked by hand twice a day, in the morning and in the evening.

Sample collection: Blood samples were collected once a time in mid lactation. Blood samples without anticoagulant were obtained by jugular venapuncture, using evacuated tubes (Vacutainer®) and serum was separated from cells by centrifugation at 1800×g for 10 min. Aliquots were stored in polyethylene tubes at -20°C until analysis.

Serum analysis: Serum urea, creatinin, uric acid, total bilirubin, cholesterol, HDL-C, LDL-C, total protein, iron, iron binding capacity, saturated iron, AST, ALT, LDH, GGT, CK, CK-MB, ALP and amylase activities were measured colorimetrically by auto analyzer (Olympus, AU 2700, Germany) and commercially available kits. Ionized Ca, Mg, P, Na, K, C1 ions were determined with an Olympus, AU 2700 (Germany) autoanalyzer provided with an ion-selective electrode (ISE). The coefficients of variation for all variables were < 5%.

The concentrations of Cu and Zn in serum were determined by flame atomic absorption spectrophotometry (Thermo, Solaar AA series) equipped with an air-acetylene flame burner and hollow-cathode lamps (operated at 20 mA for copper and at 15 mA for zinc). Atomic absorption was measured at 324.8 nm for copper and 213.9 nm for zinc. The spectral bandwidth was 0.5 nm. The standard stock solutions for trace elements were obtained from Sigma. Standard solutions and samples were diluted in double-distillate water. All the bottles dishes that were used for digestion and transportation of the filters were initially kept in acid solution (HNO_3, 10%) at least 24 h and then rinsed with Type I de-ionized water. The Cobalt and Manganese contents in the serum were quantified by inductively-coupled plasma optical emission spectrometry (ICP-OES) analysis with a PerkinElmer Inc. Life Sciences Optima 2100DV. The ICP-OES was calibrated daily by using certified solution. Co and Mn were measured at 228.616 nm and 257.61 nm respectively.

Statistical analysis: The independent-samples t test was performed in order to compare the two groups for each of the evaluated parameters. A difference with $p<0.05$ 3 was considered to be significant. All statistical analyses were performed with statistics package SPSS version 15.0 (SPSS Inc., Illinois, USA).

RESULTS

The levels of serum biochemical variables in the self-sucking group (group 1) and the control group (group 2) are summarized in Table 1. The concentrations of all mineral levels measured are given as Table 2. In addition, mean milk production for cows with self-sucking and control groups are given as Table 3.

The results of the analysis are given as mean±standard deviation. Differences between the groups are shown in the same tables.

Table 1: Levels of Urea, Creatinin, Urea/creatinin ratio, Uric acid, Total Bilirubin, AST, ALT, LDH, GGT, CK, CK-MB, ALP, Amylase, Cholesterol, HDL-C, LDL-C and total protein values by groups

	Self-sucking (n = 20)	Control group (n = 20)	
	Mean±SD	Mean±SD	p-value
Urea (mg dL^{-1})	20.67±13.56	18.14±8.96	
Creatinine (mg dL^{-1})	1.18±0.17	1.08±0.14	
Urea/Creat. Ratio	17.81±10.91	17.04±8.82	
Uric acid (mg dL^{-1})	1.02±0.24	0.95±0.16	
T.Bilirubine (mg dL^{-1})	0.21±0.07	0.25±0.08	
AST (U L^{-1})	69.50±15.83	82.21±15.13	
ALT (U L^{-1})	26.21±9.22	30.42±7.01	
LDH (U L^{-1})	2214±493	2401±397	
GGT (U L^{-1})	19.46±8.12	22.00±7.25	
CK (U L^{-1})	145.61± 107.61	197.85±102.83	
CK-MB (U L^{-1})	200.30± 121.03	267.00±108.22	
ALP (U L^{-1})	135.28±62.63	94.50±31.25	0.038
Amylase (U L^{-1})	18.07±4.99	17.35±5.93	
Cholesterol (mg dL^{-1})	207.35±70.87	158.00± 47.52	0.040
HDL-C (mg dL^{-1})	105.92±27.49	85.00±22.03	0.035
LDL-C (mg dL^{-1})	85.57±34.03	62.56±22.66	0.045
Total Protein (g/dl)	6.98±0.81	7.89±0.67	0.003

Note: All values are given as mean ±SD. There were twenty samples from each group. The measured variables are compared by groups (Independent sample t-test)

Table 2: The concentrations of Ca, Mg, Ca/Mg ratio, P, Na, K, Cl, Fe, Fe binding capacity, saturated Fe, Zn, Cu, Mn and Co values according to groups

	Self-sucking (n = 20)	Control group (n = 20)	
	Mean±SD	Mean±SD	p-value
Ca (mg dL^{-1})	8.96±0.87	8.99±0.71	
Mg (mg dL^{-1})	2.47±0.33	2.37±0.24	
Ca/Mg Ratio	3.60±0.53	3.83±0.52	
P (mg dL^{-1})	5.37±1.29	6.51±1.93	0.001
Na (mmol L^{-1})	134.57±3.45	140.78±1.92	0.000
K (mmol L^{-1})	4.30±0.55	4.73±0.38	0.024
Cl (mmol L^{-1})	97.64±4.90	101.21±2.75	0.025
Fe (µ dL^{-1})	131.0±37.21	141.5±44.46	
Fe Bind. (µ dL^{-1})	348.5±55.81	352.85±51.95	
Saturated Fe	233.21±70.06	211.35±33.87	
Zn (mg L^{-1})	0.41±0.06	0.66±0.27	0.003
Cu (mg L^{-1})	0.61±0.18	0.61±0.22	
Mn (µg L^{-1})	0.12±0.06	0.19±0.10	0.036
Co (µg L^{-1})	0.66±0.54	2.25±1.04	0.000

Note: All values are given as mean ±SD. Each groups have twenty samples. The measured variables was compared with according to the group (Independent sample ttest)

Table 3: The mean milk production for cows with self-sucking and control groups (The mean values represent the avarage levels of ten days)

	The mean of milk production of self sucking groups (day/lt)			The mean of milk production of control groups (day/lt)			
	n	Mean	S.D.	n	Mean	S.D.	p-value
Milk production	20	7.45	0.87	20	6.68	0.94	p<0.05

The levels of serum cholesterol, HDL-C, LDL-C and ALP activity were significantly increased in the self-sucking group. Cows with self-sucking had significantly lower serum concentrations of P, Na, K, Cl, Zn, Mn, Co and TP, compared with the control group. There were no significant differences in serum Ca, Mg, Cu, Fe, saturated Fe, Fe binding capacity, urea, creatinin, uric acid, total bilirubin, amylase, GGT, LDH, CK, CK-MB, ALT and AST activities between the self-sucking group and the control group.

DISCUSSION

Both the literature review and inquiries revealed that intersucking in cows occurs on a large number of farms in many different countries (Lidfors and Isberg, 2003). The feeding regime (i.e., daily feeding frequency, the duration of feeding, ad libitum feeding, the amount of roughage, the quality of food) has often been reported to have an effect on the occurrence of pathological sucking behavior such as intersucking, cross-sucking and self-sucking in both heifers and adult cows (Lidfors, 2003). Nutritional deficits and poor food quality have been suggested to have an effect on intersucking and self-sucking (Schlüter *et al*., 1981; Lidfors and Isberg, 2003). Some farmers believe that minerals deficiencies might increase intersucking (Keil *et al*., 2000). Unfortunately, no adequate values have been published. There is only a study related to serum mineral levels, which associates decreased Na concentration with the occurrence of crossucking (Phillips *et al*., 1999). In our study also, serum Na concentration in the self-sucking group was significantly lower than in the control group. Lowered Cl and K concentrations were also observed in the self-sucking group. The reason for the low level of K in the self-sucking group might be increased aldosterone level caused by low levels of Na and Cl (Lippmann, 1995). Furthermore, energy deficiency leads to adrenal stress which causes elevated aldesterone levels and consequently decreased K levels (Lothammer, 1985).

Microorganisms in the rumen are able to synthesis the vitamin B_{12} needs of ruminants if the diet is adequate in Co. Vitamin B_{12} is a cofactor enzyme, methylmalonyl-CoA mutase, that catalyses the conversion of methylmalonyl-CoA to succunyl-CoA, which is a key to

propianate metabolism. Propionate is a volatile fatty acid (VFA), a product of rumen fermentation and an important precursor for gluconeogenesis and consequently energy production in ruminants (Sano *et al*., 1999). Because of the large dependence of ruminants on VFA, inadequate Co for vitamin B_{12} synthesis leads to effective energy starvation (Miller, 1981).

It has been suggested that if cattle have too little energy in their feed it may increase the risk of self-sucking and intersucking (personal communication with Lidfors (2006, 2008). For this reason we speculated that there might be an association between self-sucking etiopathologies and decreased concentrations of Co, which is important in propionate and energy metabolism. Also, Manganese is a key component of the metallo-enzyme pyruvate carboxylase, a critical enzyme in carbohydrate and energy metabolism (Baly *et al*., 1985) (Fig. 1). Therefore, another reason for self-sucking may be low Mn levels.

As pointed out above insufficiency of Co would prevent the conversion of propionate to succinate. Succinate is indispensable both for the tricarboxylic acid cycle (TCA) and in the formation of glucose and lactose via gluconeogenesis. If succinate is not formed from propionate, it can be formed by the catabolism of amino acids produced by the hydrolysis of protein (Fig. 2). We believe that the contribution of amino acids to gluconeogenesis would increase when glucose demand was elevated. Consequently, serum protein levels would decrease because of the increased energy deficiency due to a negative energy balance. These findings are in agreement with Overtan *et al*., (1999).

The finding in this study that milk production was increased in self-sucking cows is consistent with previous observations that milk production in dairy cows is positively related to milking frequency (Stelwagen, 1996; Bar-Peled *et al*., 1995; Hale *et al*., 2003). Thus, another factor in decreased TP concentration in cows that self-sucked compared to the control group is presumably due to the higher demand for protein as a result of increased milk production, itself resulting from increased milking frequency. Also, elevated milk production can be triggered by energy deficiency. This data is in agreement with the hypothesis related to energy deficiency in self-sucking cattle (Lidfors, 2006, 2008).

Plasma urea and uric acid concentrations are considered to be a significant indicator of dietary protein intake (Schroder *et al*., 2003; Yokus *et al*., 2006). Urea can derive from nonprotein nitrogen sources (NPN) such as protozoan protein synthesis in ruminants. Also, a decreased urea/creatinin ratio is associated with low protein uptake, (Wallach, 2000). However, urea and uric acid concentrations and urea/ creatinin ratio did not vary between the groups. This indicated that the decreased TP concentrations in the self-sucking group could not be associated with lower protein uptake. This confirmed our idea that decreased serum protein level was probably associated to hydrolysated protein used for amino acid catabolism in gluconeogenesis to compensate for energy deficiency.

Some signs of Zn deficiency include emaciate and weakness (Yokus *et al*., 2006). These data are consistent with our clinical observations that there is weight loss in self-sucking cattle compared to control groups, which could be related to decreased Zn levels. In addition, there is some evidence that Co may have a sparing action on Zn deficiency (Davies, 1981). Hence, decreased Zn levels in the self-sucking group could be related to the Co concentration decrease.

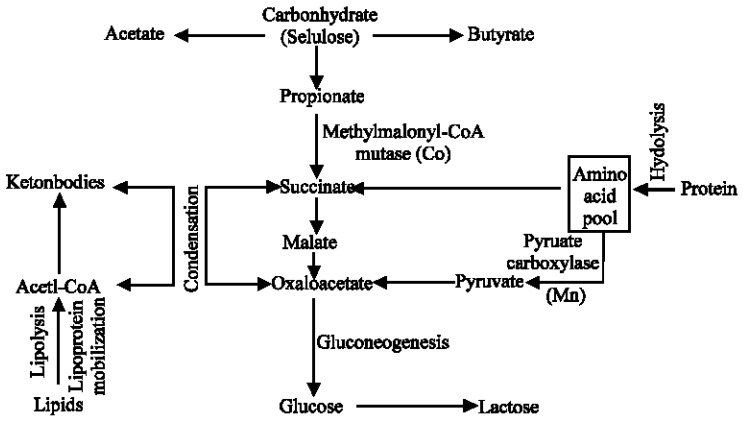

Fig. 2: Diagram showing energy metabolism (VFA, gluconeogenesis, lypolisis and proteolysis) and the importance of the evaluated parameters in this cycle

Increased lipoprotein levels are a reliable indicator of fat mobilization, which increases due to energy deficiency (Seifi *et al*., 2007). In this respect, the high level of serum LDL and HDL is also evidence supporting an imbalance in energy metabolism.

The level of dietary phosphorus intake influences its level in the plasma. One of the signs of P deficiency is abnormal chewing of wood, rocks, bones and soil. Also, phosphorus plays an intimate role in energy metabolism, causing an energy deficiency in phosphorus-deficient animals (Greene *et al*., 1985). Decreased P levels in self-sucking cows supports our other results showing energy deficiency.

It is known that decreased P concentrations stimulate the secretion of ALP indirectly and promote increasing P levels (Yokus *et al*., 2004). Our results showing that ALP activities were elevated as expected with decreased P concentrations.

Not observing a noteworthy alteration in bilirubin, amylase, GGT, LDH, CK, ALT and AST activities proves biochemically conditions of cattle considered as having any disease clinically. The fact that no noteworthy increase in bilirubin, amylase, GGT, LDH, CK, ALT and AST activities was observed proves biochemically that these cattle did not have any other clinical disease). In addition, serum Cu, Ca, Mg, Fe, saturated Fe and Fe binding capacity did not vary between the groups.

The present study provides novel information about macro and micro element deficiency as the cause of self-sucking in dairy cows. An overall evaluation of our findings supports the hypothesis that energy deficiency is a possible factor in the etiopathology of self-sucking (Lidfors, 2006, 2008). Although the explanation is not clear, it may well be related to decreased Co, Mn and P levels, which are important in carbohydrate and energy metabolism. However, other factors may also influence the occurrence of self-sucking.

CONCLUSION

If cows are fed additional trace elements during certain periods, it may be possible to prevent disorders caused by deficiencies of those elements in cattle kept tied indoors. A shortage of these elements may cause a decline on the total performance of cattle and consequently economic loss.

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