

## **Pica as a Predisposing Factor for Traumatic Reticuloperitonitis in Dairy Cattle: Serum Mineral Concentrations and Hematological Findings**

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**Abstract:** In this study, 30 dairy cattle suffering from pica-related symptoms and exhibiting clinical signs of Traumatic Reticuloperitonitis (TRP) and 25 clinically healthy dairy cattle were examined for serum zinc, iron, copper and calcium levels as well as hematological cell counts. No specific clinical signs of mineral deficiency were observed in cattle suffering from pica and TRP were determined. In hematological examination, a mild non-regenerative, normocytic-normochromic anemia detected in cattle suffering from pica and TRP based on the followings: a significant decrease ( $p < 0.001$ ) in erythrocyte count ( $4.88 \times 10^{12} \text{ L}^{-1}$ ), MCV ( $54 \pm 0.53 \text{ fl}$ ) level within the range of the reference value and Hb ( $83.0 \pm 3.64 \text{ g L}^{-1}$ ) and PCV ( $0.266 \pm 0.01 \text{ L L}^{-1}$ ) both of which were near the lower reference limits. A neutrophilia ( $49.33 \pm 2.207\%$ ) ( $p < 0.001$ ) associated with lymphopenia ( $45.0 \pm 2.22\%$ ) was determined in blood cell count. Compared to clinically healthy cattle, dairy cattle suffering from pica-related symptoms and exhibiting clinical signs of TRP (group I) had significantly decreased Zn ( $9.547 \pm 0.51 \mu\text{mol L}^{-1}$ ) ( $p < 0.001$ ), Cu ( $7.193 \pm 0.41 \mu\text{mol L}^{-1}$ ) ( $p < 0.05$ ) and Ca ( $2.41 \pm 0.15 \text{ mmol L}^{-1}$ ) ( $p < 0.01$ ) and mildly elevated Fe ( $50.649 \pm 5.03 \mu\text{mol L}^{-1}$ ) ( $p < 0.05$ ) concentrations. The results suggest that Zn, Cu and Ca deficiencies are associated with pica and may play an important role in its etiology. Taking the status of lactation, pregnancy and milk yield of the dairy cattle into consideration, rations should be supplemented according to mineral deficiency in soil and plants that may prevent the onset of pica, which is an important predisposing factor for TRP.

**Key words:** Dairy cattle, hematological findings, pica, serum minerals, Zinc

### INTRODUCTION

Pica is a condition defined as ingestion of non-food items due to mineral and vitamin deficiencies (Blood and Radostits, 1997). It generally occurs with a complication of the dietary deficiency (Aytug, 1991) and during certain circumstances such as abdominal pain and central nervous system disorders including nervous form of ketosis (Blood and Radostits, 1997).

Ingestion of sharp and perforative foreign bodies including wires, cutter, feed chopper and forage harvester, usually results in traumatic reticuloperitonitis (Aytug, 1991; Blood and Radostits, 1997). In turn, TRP may also lead to serious complications such as peritonitis, peri-reticular abscesses, vagal neuritis, traumatic splenitis,

pericarditis, pleuritis, mediastinitis and pneumonia (Streeter, 1993). Cattle can also ingest, trees, bones, dirt, soil, wool, cadavera and sand (Wallis DeVries, 1996; Smith *et al.*, 2000) that may result in boutilism, lead poisoning, digestive disorders such as rumen atony (rumen stasis), ostium-reticuloomasi obstruction (Okaeme and Akerejola, 1985; Blood and Radostits, 1997; Smith *et al.*, 2000). In humans, pica has been reported to cause lead poisoning and severe digestive system disorders (Ellis and Snoes, 2005).

In cattle, macro-minerals such as Ca, P, Mg and micro-minerals such as Fe, Cu, I, Mn, Se, Zn and Co are the most important essential diet requirements (Aytug, 1991; Blood and Radostits, 1997; Hutcheson, 1993; Leech *et al.*, 1982). Especially, in young and

lactating dairy cattle there is an increased demand for essential minerals. The mineral requirement may increase up to 3 folds during the late pregnancy and the first lactation period (Simons and Hand, 1993). Therefore, the incidence of pica and its possible consequence with TRP is unsurprisingly frequent in dairy cattle (Streeter, 1993).

Dietary mineral deficiencies may arise from either intake of insufficient levels of minerals or inefficient metabolic usage of minerals (Aytug, 1991; Phillips, 1988). Mineral deficiencies may also result from inadequate absorption of minerals due to antagonistic effects of some other minerals and due to chelation (Hutcheson, 1993). Molybdenum and sulphate compounds may decrease the absorption of Cu whereas Cu increases the absorption of iron and usage of its in hemoglobin production. Iron, Cu and Ca adversely affect absorptions of Zn and Mo whereas Zn, Fe and Ca, limit the usage of Cu (Phillips, 1988).

The clinical outcomes of the mineral deficiencies in cattle include growth retardation, alopecia and depigmentation in the hair and skin (William and Miller, 1993), defective cell-mediated immunity (McDowell *et al.*, 1993), non-infectious abortions, diarrhea, anaemia, osteodystrophia, hypocalcemia, infertility, low milk yields and pica (Aytug, 1991; Blood and Radostits, 1997).

Phosphorus deficiency has been recognized as primary etiological factor in pica (Aytug, 1991; Blood and Radostits, 1997). Phosphorus deficiency, and concomitant Zn and Fe (Ellis and Schnoes, 2005), Na deficiencies (Wallisdevries, 1996) have also been reported as causes. McDonald *et al.* (1995) similarly reported that pica is not a disorder solely related to P deficiency.

The aim of this study was to investigate serum zinc, iron, copper and calcium levels as well as hematological cell counts in dairy cattle suffering from pica-related symptoms and exhibiting clinical signs of TRP and to argue preventive measures for pica, an important predisposing factor in aetiology of reticuloperitonitis traumatica.

## MATERIALS AND METHODS

In this study, Brown Swiss Cross dairy cattle ( $n = 55$ ) were used. The mean age and body weight of the cows were 5.31 years and  $266 \pm 40.93$  kg, respectively. Of these cattle, 30 (group I) were suffering from pica-related symptoms and exhibiting clinical signs of traumatic reticuloperitonitis and 25 (group II) were clinically healthy. Prior to clinical examination, anamnesis of each animal was recorded. In clinical examination, ferrosopic and radiologic examinations were conducted using Hauptner Ferroscope 3 (Art-Nr 39500 Herberholz, Germany-

Solingen) and Vetox 110 radiology machine, respectively. Then blood samples were collected from the jugular vein into 5 mL EDTA tubes for complete blood counts and into 10 mL tubes with no additives for determining the serum Ca, Fe, Cu and Zn concentrations. Complete blood cell count analyses were performed immediately after blood collection. Blood samples used for sera analyses were kept at room temperature for 15 min and then centrifuged at 3000 rpm for 10 min. Sera samples were then kept at  $-20^{\circ}\text{C}$  until analyses.

The cows that had clinically TRP signs were forwarded to rumenotomia. Pica disorder was diagnosed based on the anamnesis and then confirmed by the detection of non-food items and foreign bodies in the lumen of forestomach during rumenotomia.

**Chemical analysis:** Serum Zn, Cu, Fe and Ca levels were measured by Atomic Absorption Spectrophotometer (Model Video, Thermo Jarrell A Corporation), as previously described (Emler, 1982).

**Statistical analysis:** Complete blood counts and serum Zn, Cu, Fe and Ca results were statistically evaluated by Mann-Whitney U test. Data are represented as mean  $\pm$  standard error.

## RESULTS

**Clinical:** According to the anamnesis of the group I, animals were fed on graze pasture and rough foods with a tendency of chewing non-food items. Acute to chronic forms of anorexia with varying degrees was detected at clinical examination. The presence of metallic foreign bodies was detected on group I on the basis of ferrosopic and radiologic examinations though some cows did not respond to the pain tests.

In the dairy cows of group I, the body condition was slightly weaker than normal and no significant clinical signs suggesting any mineral deficiency were detected. In control group (group II), during the clinical examinations, there were no similar signs which were detected in group I.

During the rumenotomia of dairy cows in group I, the non-nutritive substances such as hair balls, bones and plastic objects were excluded in addition to sharp and penetrative metallic foreign bodies in reticulum and rumen.

**Hematological:** According to the haematological findings, mean erythrocyte level showed significant decrease in group I ( $4.88 \times 10^{12} \text{ L}^{-1}$ ) ( $p < 0.001$ ) in contrast to the group II ( $6.30 \times 10^{12} \text{ L}^{-1}$ ) and mean value of group I was below the reference ranges (Kramer, 2000).

Table 1: Total blood counts in pica-effected (group I) and healthy (group II) dairy cattle

| Parameters                                      | Group I                  | Group II                 |
|---|--------------------------|--------------------------|
| Erythrocyte ( $\times 10^{12} \text{ L}^{-1}$ ) | 4.88 <sup>a</sup> ±0.15  | 6.30 <sup>a</sup> ±0.20  |
| Hemoglobin (g L <sup>-1</sup> )                 | 83.00±3.64               | 94.50±4.31               |
| MCV (fl)  | 54.65±0.53               | 52.38±0.71               |
| PCV (l L <sup>-1</sup> )                        | 0.26 <sup>a</sup> ±0.01  | 0.33 <sup>b</sup> ±0.01  |
| Leukocyte ( $\times 10^9 \text{ L}^{-1}$ )      | 9.73 <sup>a</sup> ±0.66  | 6.66 <sup>a</sup> ±0.27  |
| Neutrophil (%)                                  | 49.33 <sup>a</sup> ±2.20 | 27.00 <sup>b</sup> ±1.86 |
| Lymphocyte (%)                                  | 45.00 <sup>a</sup> ±2.22 | 64.00 <sup>b</sup> ±1.85 |
| Monocyte (%)                                    | 3.56±0.61                | 5.10±0.56                |
| Basophil (%)                                    | 0.44±0.14                | 0.80±0.13                |
| Eosinophil (%)                                  | 1.06 <sup>a</sup> ±0.18  | 2.10 <sup>b</sup> ±0.23  |

Data are expressed as mean±standard error of mean, <sup>a,b</sup>Means within the same row with different superscripts differ significantly (p<0.05)

Table 2: Serum Zn, Fe, Cu and Ca levels in pica-affected (group I) and healthy (group II) dairy cattle

| Parameters | Zn (μmol L <sup>-1</sup> ) | Cu (μmol L <sup>-1</sup> ) | Fe (μmol L <sup>-1</sup> ) | Ca (μmol L <sup>-1</sup> ) |
|------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Group I    | 9.547 <sup>a</sup> ±0.51   | 7.193 <sup>a</sup> ±0.41   | 50.649 <sup>a</sup> ±5.03  | 2.241 <sup>a</sup> ± 0.15  |
| Group II   | 12.699 <sup>b</sup> ±0.52  | 8.893 <sup>b</sup> ±0.44   | 45.383 <sup>b</sup> ±2.74  | 2.904 <sup>b</sup> ±0.11   |

Data are expressed as mean±standard error of mean, <sup>a,b</sup>Means within the same column with different superscripts differ significantly (p<0.05)

Mean hemoglobin levels in group I was lower than group II, however the difference was found insignificant (p>0.05) (Table 1). In addition group I value (83±3.64 g L<sup>-1</sup>) was at the bottom level of the reference ranges (80-150 g L<sup>-1</sup>). Mean MCV levels differences between group I (54.65±0.53 fl) and group II (52.38±0.71 fl) were insignificant (p>0.05) and both values were in reference ranges (40-60 fl) (Kramer, 2000) (Table 1).

Mean PCV value in group I showed significant decrease in contrast to group II (p<0.01) and this value was at the bottom level of the reference ranges (0.24-0.46 l L<sup>-1</sup>). Mean total leukocyte counts (9.73±0.66×10<sup>9</sup> L<sup>-1</sup>) in group I showed significant increase in contrast to group II (p<0.01), however both values were within reference ranges (4-12×10<sup>9</sup> L<sup>-1</sup>). Mean neutrophil percentage in group I showed significant increase in contrast to group II (p<0.001) and group I value (49.33%) (neutrophilia) was higher than the reference ranges (15-45%). Mean lymphocyte percentage in group I showed significant decrease in contrast to group II (p<0.001) and both values were within reference ranges (45-75%), however group I value was at the lowest range (Table 1).

Mean monocyte percentage showed decrease in contrast to group II, however it was insignificant (p>0.05), both values were within reference ranges (2-7%). Mean basophile percentage were insignificant between groups (p>0.05) and both values were within reference ranges (0-2%) (Table 1).

Mean eosinophil values in group I showed significant decrease in contrast to group II (p<0.01) and both values were within reference ranges (0-20%) (Table 1).

**Serum minerals:** Mean serum zinc levels in group I showed significant decrease in contrast to group II (p<0.001) and this value of group I was below the reference ranges (10.71-19.89 μmol L<sup>-1</sup>). Mean serum Cu<sup>2+</sup> levels in group I showed significant decrease in contrast to group II (p<0.05) and this value of group I was below the reference ranges (7.87-39.95 μmol L<sup>-1</sup>) (Karagül *et al.*, 2000). Mean serum Ca<sup>2+</sup> levels in group I showed significant decrease in contrast to group II (p<0.01) and this value of group I was below the reference ranges (2.420-3.093 mmol L<sup>-1</sup>) (Table 2).

Mean serum Fe<sup>2+</sup> levels in group I showed significant increase in contrast to group II (p<0.05) and this value of group I was above the reference ranges (26.865-40.297 μmol L<sup>-1</sup>) (Karagül *et al.*, 2000) (Table 2).

## DISCUSSION

In the present study, clinical examination of the dairy cows in group I revealed that anorexia and pica disorder were the only clinical signs and no other significant clinical sign suggesting any mineral deficiency, was detected. This result was agreed with Doyle and Huston (1993) suggesting that a typical syndrome is not evident in complex mineral deficiencies. Poole (1982) stated that the symptoms in cattle with copper deficiency was only limited with anorexia. According to a survey results, subclinic copper deficiency was determined in 44% of the cows grazed on pasture (Bingley and Anderson, 1972). It is reported that copper and zinc deficiencies may cause immunodeficiency and susceptibility to certain diseases (Mills, 1987).

According to the hematological results, mean erythrocyte counts showed significant decrease in group I in contrast to the group II (p<0.001) and mean value of group I was also below the reference ranges. These results may be an indicative of subclinic anaemia. In dairy cows with TRP, chronic anorexia and inflammation may result in toxemia and stress, which then induces a quite suppression on the immune system (Balıkcı and Gunay, 2004). In the present study, the immunosuppression and low serum copper levels (Thorntén *et al.*, 1972) can be considered as the possible causes of anemia. Besides mean MCV levels in the reference ranges, mean Hb and PCV levels below the reference ranges may all be attributable to Constable (1993) and Warner and Harrus (2000), stating that non-regenerative, normocytic-normochromic and mild to intermediate anemia may develop due to inflammatory diseases. The non-regenerative type anemia can be explained by the compensation capability of the cardiovascular system

and increasing oxygen-carrying capacity to the tissues of the erythrocytes, in slowly developing anemia (Whitney, 1993).

Mean total leukocyte counts in group I showed significant increase in contrast to group II ( $p < 0.01$ ), however both values were within reference ranges. Besides in group I, the other hematological changes were neutrophilia and lymphopenia with normal significant changes in monocyte levels (Table 1). These findings were consistent with previous results by Yoshida (1986b), Sahal *et al.* (1987), Ozba *et al.* (1996) and Balıkcı and Gunay (2004).

Neutrophilia is considered as a consequence of inflammation in TRP. It has been well known that inflammation in TRP tends to occur focally in the periton and has a tendency to become chronic. As well as the neutrophilia in the present case may be explained with Whitney (1993), suggesting that neutrophil counts are elevated in localized suppurative inflammation more than in generalized cases. Besides the decreased lymphocyte levels within no change in monocyte levels detected in this study may be explained with Latimer and Rakich (1989), Whitney (1993) stating that neutrophilia in inflammation generally accompanies lymphopenia and monocyte levels alterations closely depend on the inflammation mediators and endogen steroid status.

The decreases in mean basophil and eosinophile levels in pica-affected cattle with traumatic reticuloperitonitis in contrast to the control group may be related to the effects of the inflammation mediators and endogen steroid release (Latimer and Rakich, 1989; Whitney, 1993).

In the present study sera levels of micro minerals such as Zn and Cu and macro minerals as Ca were decreased in group I in contrast to group II and reference ranges and mean serum Fe levels showed significant increase in group I in contrast to group II. In a different province in Turkey, a study in sheep by Kargin *et al.* (2004) suggested that mean serum Cu, Ca, P and Mg levels closely related to the reference ranges within decreased Zn levels. In another study performed in 5 different provinces in Turkey, plasma Cu levels were detected between  $0.33-1.28 \mu\text{g mL}^{-1}$  (Oncuer *et al.*, 1996). Rowland and Pocock (1976) reported plasma Cu levels in cows as  $0.48-1.03 \mu\text{g mL}^{-1}$  and values equal to  $0.5 \mu\text{g mL}^{-1}$  and below, as critical value and deficiency. In the present study, serum Cu level was  $0.457 \pm 0.261 \mu\text{g mL}^{-1}$  in pica-affected cows with TRP, indicating mineral deficiency. Similarly, Claypool *et al.* (1975) stated  $0.5 \mu\text{g mL}^{-1}$  serum Cu level reflecting hepatic copper level.

In cases of inflammation, serum Zn and Cu levels may be elevated whereas Fe level decreases (Waner and Harrus, 2000). However the results of the present study were contrary to this suggestion. Thus the low levels of serum Zn and Cu may be related to a nutritional disorder as the cows were fed up with pasture and rough foods, as was previously described (Ammerman *et al.*, 1974). It is possible that the elevated serum Fe level may be related to ingestion of metallic substances in pica-affected cows. Normochromic anaemia found in this study has also been supported this condition. Besides primary Fe deficiency is not a common condition in adult cows (Phillips, 1988).

Mean serum Ca level in pica-affected cows with TRP (group I) showed significant decrease in contrast to group II ( $p < 0.01$ ) (Table 2). This was also reported by Yoshida (1986 a) and Sahal *et al.* (1993) in agreement with the present authors results. However low levels of Ca ( $2.241 \pm 0.15 \text{ mmol L}^{-1} = 8.94 \pm 0.641 \text{ mg dL}^{-1}$ ) in group I was not found in a level to cause clinical hypocalcaemia ( $5.6 \text{ mg dL}^{-1}$ , Blood and Radostits, 1997;  $6 \text{ mg dL}^{-1}$  and below, Allen and Sansom, 1993). This might be related to a chronically induced Ca deficiency resulted from Ca mobilization as a compensatoric response (Allen and Sansom, 1993; Rosol and Capen, 1997).

In the present study, Ca deficiency may be associated with fed up of grazing pasture and rough food, inappetence (hypoproteinemia) and increased urinary excretion due to stress induced by TRP.

## CONCLUSION

In pica-affected cattle with TRP, no accompanying symptoms specific to any mineral nutrient deficiency was observed other than inappetence and pica signs.

On the basis of hematology marked reduction in erythrocyte counts, MCV level in reference ranges, Hb and PCV values at the bottom border of the reference ranges in group I, a mild non-regenerative, normocytic-normochromic anemia was detected. Besides as a reflection of inflammatory response, neutrophilia accompanied by low levels of lymphocyte counts was detected.

According to the serum mineral levels, decreased Zn, Cu and Ca levels, mildly increased Fe level above the reference ranges, all suggested Zn, Cu and Ca deficiencies plays an important role on pica disorder.

The diagnostic research work-up in an attempt to put forward the etiology dairy cattle should be determined. Besides diet supplementation in dairy cattle

was reconsidered, referring to National Research Council (NRC) (2001) recommendations on lactation, pregnancy and milk yield and giving a priority to an appropriate protein and energy balance as well as essential mineral nutrients. With these preventive of mineral nutrient deficiencies, the mineral composition of soil-plant interaction in relation to the every era of large-scale probably affecting measures, the incidence of pica and related complications such as TRP can be minimized.

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