ARTICLE

EFFECTS OF CALCIUM BOROGLUCONATE INJECTIONS ON HEALTH, PRODUCTION, AND REPRODUCTION CAPACITY OF NEWLY CALVED COWS

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ABSTRACT

Most metabolic abnormalities in dairy cows including milk fever, ketosis, retained placenta, and displaced abomasums occur during the first two weeks of lactation and cause the metabolic abnormalities that happen during the early part of the lactation period. This research was conducted on 48 head of Holstein dairy cows in the Govaver Dairy Complex in Kermanshah. It was the first calving of two, the second calving of 12, and the third calving of 22 of the cows. The cows in each group were randomly placed into two groups each with 24 members. The protocol was applied on the second group but not on the first group (the control). The cows in the control group did not receive any protocol after calving, but those in the second group received 250 cc of 40% calcium Borogluconate immediately after calving. The calcium Borogluconate solution was first warmed to reach 38-40˚C and then injected slowly into the jugular veins of the cows. Data related on production of dry matter, milk, milk products, and blood metabolites, and on metabolic abnormalities was analyzed, and comparison of the means of the treatments showed the injections had significant effects on dry matter and average milk production, fat content of milk, calcium concentration in blood serum, and on concentrations of phosphorous and magnesium.

KEY WORDS
Newly calved cow, calcium Borogluconate, metabolic abnormalities, hypocalcaemia, Govaver Dairy

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INTRODUCTION

Most metabolic abnormalities including lameness reflect events that happen in the early part of the lactation period. Three physiological functions must be maintained in the periparturient period to prevent these metabolic abnormalities. These include having a strong immune system, keeping blood calcium content at its normal level, and increasing feed consumption in the days prior to and following parturition. Each of these three factors increases the risk for the development of most common abnormalities during the periparturient period [1]. Kimura et al. (1999) showed that activity of neutrophils in cows mastectomized after parturition returned more rapidly to the normal level compared to healthy cows. These results indicated that the start of the lactation period might result in changes in the composition and duties of peripheral blood mononuclear cells. One of the most important effects of mastectomy is the resolution of hypocalcemia, which is one of the main metabolic factors in the occurrence of metabolic abnormalities [2]. Moreover, hypocalcemia, as a stress, leads to a 5-7 fold increase in blood cortisol level; and the potential role played by cortisol in suppressing immune system is well-known [2] Furthermore, calcium is necessary for muscle contraction, which decreases by a drop in blood calcium level. However, this drop is intensified before the downer cow complication is observed [3].

Daniel et al. (1983) proved that the speed and power of smooth muscle contractions in the intestinal system are directly proportional to blood calcium concentration, and low blood calcium levels led to reduced contraction of abomasum and, finally, to its filling up with gas and to abomasal displacement. Since hypocalcemia reduces feed consumption, it may result in greater tissue mobilization and, eventually, lead to increased risk for ketosis and fatty liver. These two abnormalities have potential effects on the immune system [4]. Therefore, keeping blood calcium in the range of 8.5-10 mg/dL can be an effective factor in improving the performance of the immune system during the periparturient period. Many studies have been carried out on reducing incidence of subclinical and clinical hypocalcemia. Clinical hypocalcemia has substantially decreased through reducing calcium content of feed prior to parturition, by using anionic salts in the ration rations of cows nearing parturition, and through utilizing feed complements and adding calcium to drinking water immediately after parturition. However, subclinical hypocalcemia continues to have a high incidence in herds of dairy cows [5].

Calcium concentrations in blood, milk, and colostrum are 8.5, 80-120, and 255-470 mg/dL, respectively, and, hence, calcium concentrations in milk and colostrum are at least 10 and 30 times greater compared to that in blood, respectively. A recently calved cow produces 2.7-26.6 kilograms of colostrum on the first milking day [6]. Calcium excretion via urine and feces will be equal to 21 g/day, and calcium received in the ration will be 100 grams, or 50 grams considering an absorption rate of 50% [5]. On parturition day, 15-20 grams of calcium is removed from the bones [7]. This research intended to study the effects of intravenous injection of 40% calcium borogluconate immediately after parturition on production capacity, metabolic abnormalities, and other health indices during the first 21 days of the lactation period.

Cows in the transition period

Gromer (1995) defined transition period as three weeks before to three weeks after parturition. The closer cows get to parturition and start of the new lactation period, the greater stress they will experience. In this transition period, many biological changes happen during the various stages of parturition and start of lactation. Transition period is a very critical period for preventing the set of problems calving cows face [8]. Three weeks prior to parturition, dry matter intake of calving cows and heifers reaches about 1.7-1.9% of their body weights. Unfortunately, the appetite of cows declines when the feed they feed for fetal growth and preparation of the udder for the next lactation period increases. That is why it seems reasonable to recommend increases in dry matter (feed material) intake. Research in Illinois and Florida Universities has shown feed restriction during transition period is as effective as forced or free feeding, and incidence of fatty liver is lower in cows fed a restricted ration and their dry matter intake increases after parturition. Reexamination of the results of these studies has indicated there is a significant relationship between reduced feed intake during the last three weeks of pregnancy and the quantity of fat accumulated in the liver after parturition [9].

Calcium

Marked reduction in body calcium occurs during lactation because milk production is the main user of the calcium present in the body. When milk is synthesized in the body of a cow, all sources of calcium in its body are utilized. Moreover, calcium reserves in the bones are used and/or kidneys filter a part of the calcium reserve and thus prevent its excessive excretion [10]. It is not clear what percentage of calcium in the ration can be absorbed by ruminants, but reduced calcium concentration in the rumen can lead to decreased quantity of calcium absorption by the body. Parathyroid hormone indirectly stimulates calcium absorption by the digestive system because it regulates production of 1, 25-dihydroxyvitamin D [13]. In mature cows, calcium concentration is maintained at about 8.5-10 mg/dL and, in a cow weighing 600 kg, 3 grams of calcium are in the plasma and only 8-9 grams in all of the extracellular solutions (outside the bones). The liquid in the canal of cow bone may contain 6-15 grams of calcium. To prevent reduction in blood calcium that leads to paresis pueralis, cows must remove calcium from the bones or increase absorption of calcium in the ration to replace calcium that enters into milk [12]. Hypocalcemia (deficiency of calcium in the bloodstream) and milk fever happen when cows do not remove sufficient calcium from their bones, and calcium in the ration does not compensate for that removed via milk [11].

Hypocalcemia and milk fever

Hypocalcemia and milk fever are metabolic diseases that develop before or at parturition, and are characterized by rapid reduction in plasma calcium concentration resulting from the relatively quick loss of calcium in the production of colostrum. For example, a cow producing 10 liters of colostrum loses about 23 grams of calcium at each milking. This is 9 times the quantity of calcium in the plasma calcium reserve of the cow. The calcium removed from the plasma reserve must be replaced by increased intestinal absorption of calcium, by calcium released from the bones, or by both. At parturition, cows must put more than 30 grams of calcium per day into the calcium reserve and, hence, most cows show signs of hypocalcemia at parturition and from the first day after parturition until the time their intestines and bones get used to satisfying calcium demand for milk production. In some cows, calcium used in milk production causes extracellular plasma calcium concentrations to drop so severely that the nervous and muscular systems cannot function properly, and clinical signs of milk fever appear. Intravenous injection of calcium is used until cows get used to the mechanism of calcium transfer from the intestines and bones. If this treatment is not carried out, about 60-70% of cows die [15].

Strategies for preventing hypocalcemia

Rations containing high levels of sodium and potassium and low levels of chlorine and sulfur increase incidence of milk fever, while those that include low levels of sodium and potassium and high levels of chlorine and sulfur, or rations to which anionic salts are added, reduce incidence of milk fever [14]. When cows receive rations low in calcium, they will face a negative calcium balance that will cause a slight reduction in blood calcium concentration. This will stimulate PTH secretion that will in turn stimulate calcium removal from bone osteoclasts and production of 1, 25-dihydroxyvitamin D, which will increase calcium removal from bones and prepare the intestines to absorb calcium from the ration during lactation period. Calcium therapy and treatment with vitamin D are ineffective. Available data suggests blood magnesium concentrations lower than 1.6 mg/dL in cows during the periparturition period will increase their susceptibility to hypocalcemia and milk fever [19].

Abomasal displacement and retained placenta

Transition period is an important and risky period for left displacement of abomasum (LDA). Low and high intake levels of concentrates prior to parturition may increase the probability of LDA. Since the rumen is not filled due to lower levels of feed consumption, the chances for LDA increase [21] . Physiologically speaking, fetal membranes must usually be removed within 12 hours after parturition, and retention of allantochorion membranes remain inside the womb for a longer time than that will indicate a pathological state. The period of this placental retention may be 4-8 days, or even longer, and signs of decay and infection will appear in the placenta. Therefore, if the placenta is not expelled two days after parturition, treatment operations must be carried out to remove it so that uterine infections and lesions do not ensue.

Fatty liver and negative energy balance

The fatty liver disease occurs more frequently in high yielding dairy cows, results in metabolic and reproductive disorders and infectious diseases, and may even cause death because the reduced number of white blood cells disrupts the immune system and lowers resistance to diseases and stresses [16]. Clinical symptoms in cows affected by fatty liver are accompanied by excessive obesity at parturition. Such a condition is followed by severe energy shortage and substantial loss of body weight (usually more than 1 kg/day). These complications cause many problems at parturition (such as milk fever, mastitis, uterine infections, etc.), reduce response to drug treatments, and slow down the trend of clinical improvement [17].

MATERIALS AND METHODS

This research was conducted at the Govaver Dairy Complex in Kermanshah from late October 2012 to late May 2013. This Complex keeps 2500 cows, 1100 of which are lactating Holstein cows, and has the required buildings and facilities including a milking parlor with four lines of bilateral milking totaling 48 units with three times of milking per day. The Complex has the record of 34 liters of milk per cow, is one of the farms for rearing and keeping Holstein cows, and serves as an important center of milk production in Kermanshah Province.

The experimental protocol

The group of cows close to calving was separated based on parturition signs (filling up of the udder, loosening of the uterine ligament, and mucus discharge from the uterus) and put in calving boxes. After parturition, 250 ml of 40% calcium borogluconate (which contained 340.1 mg/mL of calcium borogluconate and was warmed up to the body temperature of the calves) were slowly injected into each one. Temperatures of the cows were measured before the injections and cows with fever (body temperature higher than 39.4˚C) were not injected. Forty- eight Holstein cows were randomly divided into two equal groups: treatment 1: the control treatment not injected, and treatment 2: intravenously injected with 250 ml of 40% calcium borogluconate at parturition. The cows were monitored for 21 days.

Collecting samples and recording during the experiment
Dry matter intake

Dry matter consumption was measured collectively for each group, and unconsumed feed was collected from the feed bunks and weighed to calculate feed intake.

Recording milk production and sampling milk

Each week, milk production was recorded and milk samples were taken. Every time a cow was milked, a sample was taken after the milking with the help of a valve embedded in the bottom part of each milkometer (the milking machines were digital). Some milk was then poured into the sampling containers. To each container was added some potassium dichromate to prevent it from going bad, and the containers were kept at 4˚C while being transferred to the laboratory. A model CombiFoss 5000 (Foss Electric, Hillerqd, Denmark) Milko Scan was used to analyze milk composition (percentages of fat and protein, and somatic cell count).

Body Condition Scoring (BCS)

At the start and end of the experiment, two weeks before calving, and at parturition, the body condition of each cow was given a score on the scale of 1 to 5, and the average score of body condition for each cow was used for statistical analysis of the data [18].

Taking blood samples

To determine blood composition, 10- ml blood samples were taken from the tail vein using vacuum tubes without anticoagulant agents. Blood samples were taken at 10-11 AM, and 3-5 hours after morning feeding on days 1, 2, and 21 after parturition, to determine blood calcium, magnesium, phosphorous, beta- hydroxybutyrate, non-esterified fatty acids, and urea nitrogen. The samples were immediately centrifuged (in a SIGMA 101 model centrifuge made in Germany) at 3000 rpm for 15 minute to separate the serums, which were kept frozen at - 20˚C until they were analyzed in the laboratory. After carrying out the stages on the farm, blood parameters such as calcium, phosphorous, and magnesium were measured in the laboratory using a Perkin Elmer 35 model spectrophotometer, and NEFA and beta-hydroxybutyrate (BHBA) were measured by using kits.

Measuring metabolic abnormalities

Metabolic abnormalities including milk fever, retained placenta, metritis, endometritis, hypocalcemia, abomasal displacement, and ketosis were carefully recorded during the experiment. Incidence of any metabolic abnormality was defined as the number of cows exhibiting the abnormality divided by the total number of the cows in each group. Cows that showed symptoms such as lethargy, signs of staggers, nervous signs, and various degrees of dizziness with cold ears within 72 hours after calving were considered cows suffering from milk fever. Retained placenta referred to cows in which fetal layers in the vulva, vagina, and uterus were visible within the first 12 hours after calving, and the placenta was not expelled from the body 12 hours after parturition. Moreover, the time of calving, the time of placenta expulsion, and the time taken for placenta expulsion in each cow were recorded, and the data related to the time of placenta expulsion was statistically analyzed. Abomasal displacement was defined as reduced milk production and decreased appetite, and hearing a metallic ring when the area between the ninth and twelfth thoracic vertebrae on the right and left sides of the abdominal cavity was tapped [20].

The experimental design

Since hypocalcemia and milk fever are related to the age and number of calving of cows, the randomized complete block design with more than one observation per block-treatment and with two treatments (treatment 1, without injection as the control, and treatment 2 injected immediately after calving) was used in this research. The data related to milk production and composition was analyzed employing the Mixed Procedure and using repeated measures ANOVA, and the milking week was entered into the statistical analysis as the repeated measures. Data related to blood parameters (calcium, magnesium, phosphorous, beta-hydroxybutyrate, non-esterified fatty acids, and urea nitrogen) was analyzed employing Proc Mixed
And using repeated measures ANOVA, and the sampling days were entered into the statistical analysis as the repeated measures.

$$y_{ijk} = \mu + T_i + cow(T_i) + Day_k + P_j + (p * T)_{ij} + (Day * T)_{ki} + e_{ijkl}$$

In the above equation, Y_{ijk} is the observation related to the i^{th} treatment in the j^{th} block, **μ** the mean, T_i the effect of the treatment, and cow (T_i) the random effect of the cow in the treatment. Furthermore, Day_k is the effect of time, P_i the effect of block; $(p*T)_{ij}$ the mutual effect of treatment and block, $(Day*T)_{ki}$ the mutual effect of treatment and day, and e_{ijkl} the effect of random error. Incidence of metabolic abnormalities was analyzed using the logistic regression software SAS9.1 (2000), and Odd Ratio and Confidence Interval were calculated to determine the relationship between the abnormalities (the dependent variable) and the treatments (the independent variable).

Dry matter intake

As shown in [Fig. 1], dry matter consumption in the ration after calving in treatments 1 and 2 were 15.63 and 16.43 kg/day, respectively. Therefore, the treatment had a significant effect on dry matter consumption (p<0.05). The effect of time on dry matter intake was also significant (p<0.05). Results showed that when the cows were fed calcium and phosphorous rich rations, dry matter consumption was higher compared to when they were fed rations low in calcium and phosphorous. This difference does not seem to be related to DM digestion because DM percentage was different in both rations with low and high calcium and phosphorous content that had the same fiber content.

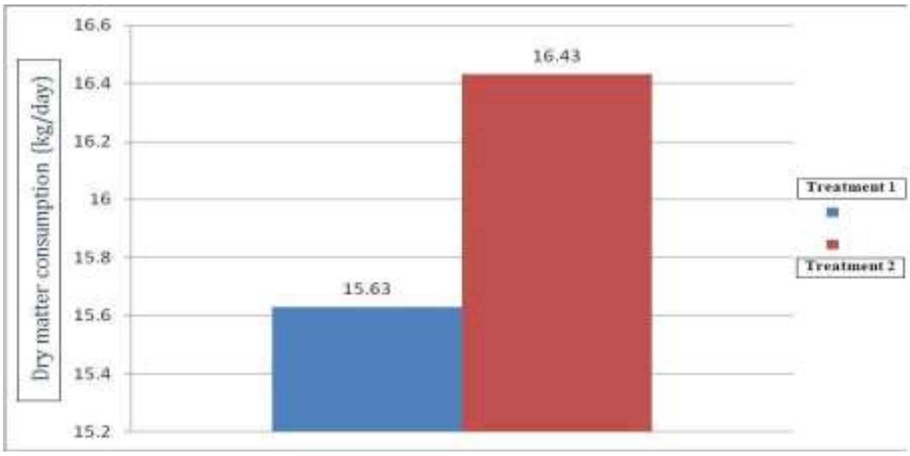

Fig. 1: Changes in dry matter intake in the experimental treatments 1 (not injected) and 2 (injected immediately after calving)

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Milk Production

[Fig. 2] indicates daily milk production in the first 21 days of the lactation period in treatments 1 and 2 was 24.60 and 25.24 kg/day, and the treatments had significant effects on daily milk production. Treatment 2 had significantly higher milk production compared to treatment 1 ($p<0.05$). The effects of blocks on milk production were significant ($p<0.05$), so were the effects of time or weeks of lactation ($p<0.05$). Pregnancy and lactation reduced blood calcium levels because they caused calcium to be transferred from blood to the fetus and to milk. The most important time when calcium is at its lowest level in a cow's blood is the time of calving.

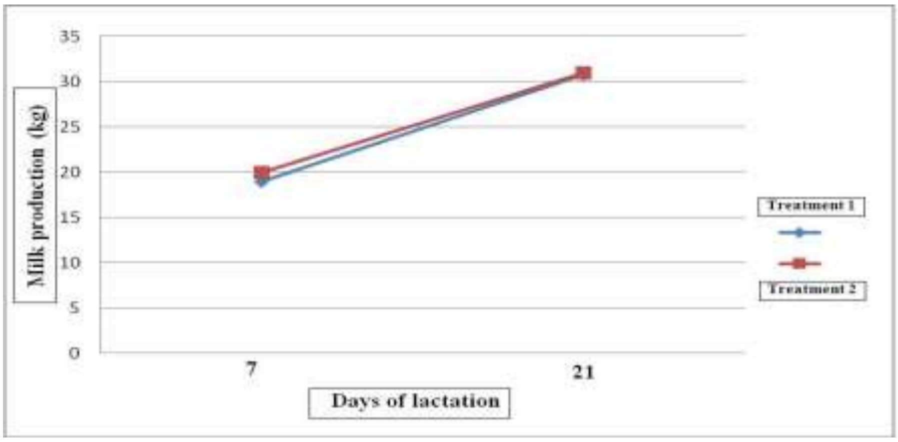

Fig. 2: Changes in milk production on lactation days in treatment 1 (not injected) and treatment 2 (injected immediately after calving)

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Milk fat percentage

As shown in [Fig. 3], milk fat percentages in treatments 1 and 2 were 3.19 and 3.20 percent, respectively. ANOVA of the data and comparison of he means using Tukey's test indicated there were no significant differences between the two treatments in milk fat percentage ($p>0.05$), but the effects of blocks on milk fat percentage were significant ($p<0.05$). The mutual effects of treatment and block and treatment and time were not significant either ($p>0.05$).

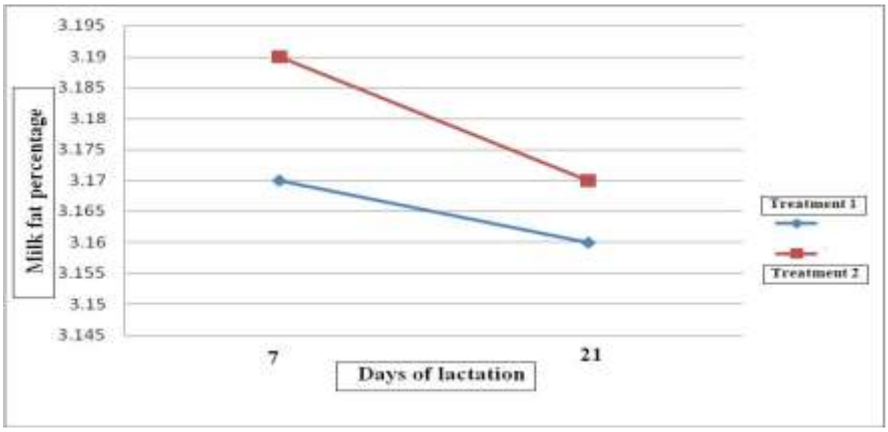

Fig. 3: Changes in milk fat percentage on days of lactation in treatments 1 (not injected) and 2 (injected immediately after calving)

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Blood parameters
Serum calcium

Treatments 1 and 2 had serum calcium concentrations of 8.39 and 9.41 mg/dL, respectively [Fig. 4]; i.e., the effects of treatment on calcium concentration were significant ($p<0.05$). So were those of block ($p<0.05$), days of taking blood samples ($p<0.05$), and the mutual effects of treatment and time ($p<0.05$). However, the mutual effects of treatment and block were not significant ($p>0.05$). The normal blood calcium level in cows is about 10 milligrams/dL. Use of commercial calcium chloride (54 grams of calcium) when there are signs of impending calving lowers milk fever and abomasal displacement 14-24 hours after parturition.

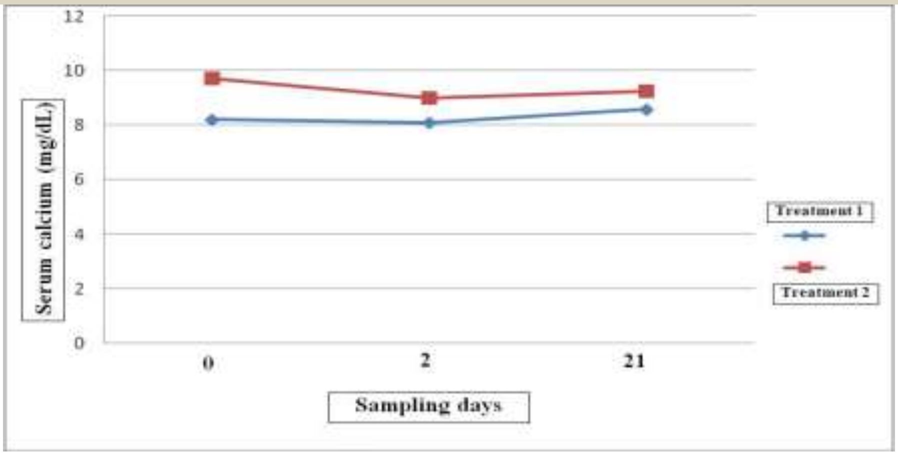

Fig. 4: Changes in blood serum calcium on days of lactation in treatments 1 (not injected) and treatment 2 (injected immediately after calving)

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Serum phosphorous

[Fig. 5] shows serum phosphorous concentrations were 4.6 and 4.75 mg/dL in treatments 1 and 2, respectively. The treatments had significant effects on phosphorous concentration ($p<0.05$), so did block ($p<0.05$) and time ($p<0.05$). However, the mutual effects of treatment and time and treatment and block were not significant ($p>0.05$). Mineral phosphorous in blood is almost completely distributed in blood plasma and in the fluid within the external tissues of blood vessels. Low phosphorous rations may increase renal activity of 1-alpha-hydroxylase, while high phosphorus rations can promote hypophosphite formation.

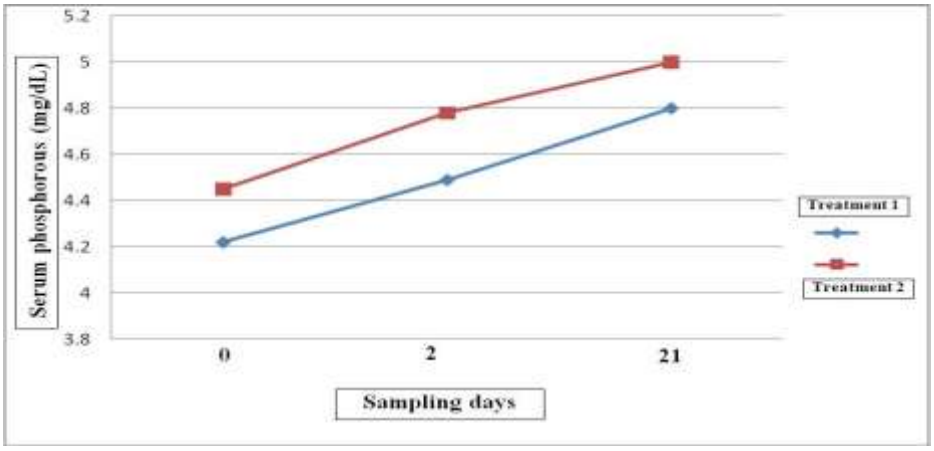

Fig. 5: Changes in blood serum phosphorous on days of lactation in treatments 1 (not injected) and 2 (injected immediately after calving)

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Serum magnesium

[Fig. 6] shows Serum magnesium concentration in treatments 1 and 2 were 2.2 and 2.39 mg/dL, respectively. ANOVA of the data and comparison of the means of the treatments showed that the treatments had significant effects on serum magnesium concentration ($p<0.05$), but block had no significant effect on magnesium concentration ($p>0.05$). Moreover, the effects of time on serum magnesium concentration were significant ($p<0.05$) and so were the mutual effects of treatment and time ($p<0.05$), while the mutual effects of treatment and block on serum magnesium concentration were not significant ($p>0.05$). Therefore, we can say that calcium absorption inversely affected magnesium absorption. Furthermore, if plasma magnesium concentration drops below 0.74 mM/L, stock becomes susceptible to hypomagnesaemia.

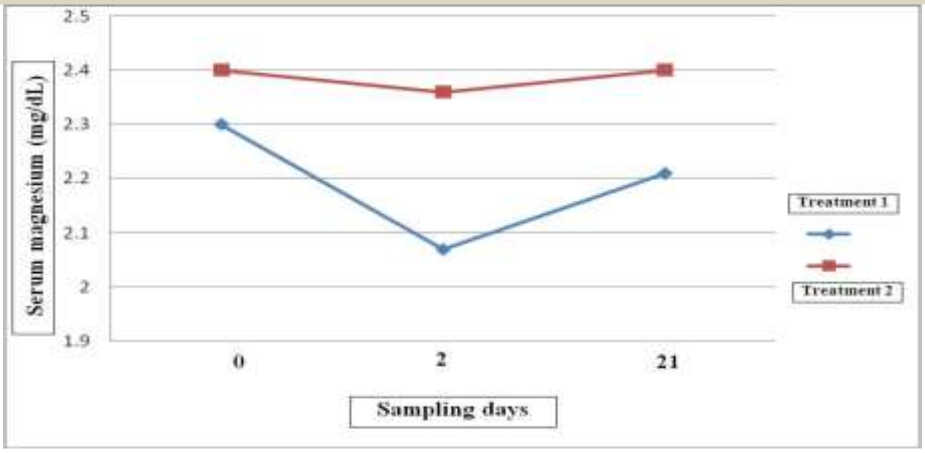

Fig. 6: Changes in serum magnesium on days of lactation in treatments 1 (not injected) and 2 (injected immediately after calving)

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Metabolic abnormalities

Clinical hypocalcemia (milk fever)

During the experiment, no case of milk fever was observed in either of the treatments. This could indicate desirable management of the difference in the anion-cation of the ration prior to calving using anionic salts. Milk fever may be caused by incomplete absorption of calcium from the intestines or by excessive excretion of calcium via feces. About 85% of the cows that exhibited symptoms of milk fever, and were immediately injected with intravenous calcium, responded to calcium treatment. Milk fever is muscle weakness or hypocalcemia at parturition. Hypocalcemia is a metabolic disorder in dairy cows that happens after calving. Incidence of milk fever varies among different breeds, and has been estimated to be 12.4 to 30% in the Jersey breed but about 3.9% in other breeds. Milk fever may affect 50 to 80 percent of the herd, and it varies depending on the breed, age, and rations of cows. At calving and following parturition, the demand for calcium suddenly rises sharply. Stimulation of transport and absorption of calcium by the intestines must take place about 15 to 24 hours before the absorption process begins so that it can sufficiently prevent development of milk fever.

Subclinical hypocalcemia

Logistic regression analysis of the data related to calcium concentrations lower than 8 mg/dL after calving indicated that 29.16 and 12.5% of the cows in treatments 1 and 2, respectively, had calcium concentrations lower than 8 mg/dL, and that there were no significant differences between the two treatments ($p>0.05$). However, incidence of hypocalcemia in treatment 1 was twice that in treatment 2. Data revealed susceptibility to hypocalcemia was less in treatment 2 as compared to treatment 1. Hypocalcemia and milk fever occur when cows are unable to remove calcium from their bones or absorb it through the intestines. The normal calcium concentration in a mature cow is maintained at 8.5 to 10 mg/dl.

Abomasal displacement

Logistic regression analysis of the data related to abomasal displacement showed that this abnormality happened in 4.16 and 3.7% of the cows in treatments 1 and 2, respectively. This difference was not statistically significant ($p>0.05$). Abomasal displacement, also called twisted stomach, happens when the actual stomach loses its normal position, and is twisted to the left or to the right. About 90% of abomasal displacement is to the left and happens between 5 to 6 weeks after calving. If feed intake is low in a freshly calved cow, its rumen may not be filled and, hence, the abomasum will have a larger space for displacement. However, in this research, the rumen was rapidly filled because of the increase in the quantity of dry matter consumption, which prevented abomasal displacement.

Percentage of retained placenta

The mean percentages of retained placenta in treatments 1 and 2 were 20.83 and 8.33%, respectively, and the treatments were not significantly different in this respect ($p>0.05$). However, incidence of retained placenta tended to be lower in treatment 2. Normally, placenta expulsion should take place one to several hours after calving, and if it does not happen after 12 hours, the abnormality is called retained placenta

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CONCLUSION AND SUGGESTIONS

Decreased appetite is a problem frequently observed in freshly calved cows, and one of the reasons for this reduced feed intake is the drop in blood calcium levels and malfunction of masseter muscles and of the digestive system. The most rapid way of improving the negative calcium balance can be the use of injected calcium solutions before and immediately after calving. In this research, this injection led to increased dry matter consumption during the first 24 hours after parturition. Calcium injection led to significant increases in dry matter intake following which daily milk production increased and there were improvements in the immune system including a drop in somatic cell count and improved condition of the uterus after parturition (improved metritis and endometritis). Calcium injection led to significant reductions in metabolic abnormalities including hypocalcemia and ketosis.

Results of this research indicate that cows experience severe negative calcium balance on calving day despite the use of anionic salts, especially cows that have calved several times because they produce large quantity of colostrum. Considering the relationship between hypocalcemia and reduced feed intake (which is an important factor in the development of other abnormalities including ketosis and fatty liver), injection of calcium solutions can be recommended for all cows immediately after calving. This will help improve the negative calcium balance and will be beneficial as there is high incidence of hypocalcemia, which affects production and reproduction capacity in stock. Moreover, injection of calcium is an easy and low- cost management practice compared to supplying it via drinking water.

CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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