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Evaluation of Vit. B_{12}+Fe and Cu Administration on the Somatic Cell Count, Total Bacterial Count and Compounds Milk of Dairy Cattle

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Abstract: The aim of this study was studying the effect of vit. B_{12}+Fe and Cu on the somatic cell count, total bacterial count and compounds milk of Holstein cows. In this study, 4 groups contain 10 dairy Holstein cows were selected with same parity and milk production as control, vit. B_{12}+Fe injectable (10 mL day^{-1}), Cu injectable (2 mL day^{-1}) and vit. B_{12}+Fe along with Cu treatment groups. These were injected from 1 week precalving probably to 1 week after parturition. Milk samples were collected as twice weekly from the calving time to 1 week. Total Bacterial Count (TBC), Somatic Cell Count (SCC), fat, protein, Solid Non Fat (SNF) and lactose of milk were evaluated. Results showed the increasing rate of fat and lactose in vit. B_{12}+Fe along with Cu treatment group compared to other groups. All groups were lower than control group in protein level. On the other hand, vit. B_{12}+Fe treatment group was high in milk production, low in SNF and so, it had high effect on reducing bacterial total count compared to other groups. Furthermore, Cu treatment group was better than other groups in SCC. It was concluded that vit. B_{12}+Fe and Cu have beneficial effects on udder immune system and compounds milk of Holstein cows.

Key words: B_{12} + Fe, Cu, milk, udder, dairy cattle, Iran

INTRODUCTION

Bacteria present in the rumen of a cow produce high levels of B-vitamins even if the animal's diet provided very small amounts of those vitamins. Furthermore over the years, since the discovery of B-vitamins, it appears that true deficiency of these vitamins is rare in animals with a functional rumen.

It is generally accepted that B-vitamin requirements can be met through synthesis by ruminal bacteria and dietary sources that escape from the rumen. However, milk and milk component yields have increased dramatically. It is likely that the B-vitamin requirements of high-producing dairy cows have likewise increased and that ruminal synthesis alone is not sufficient to meet these new needs (Girard and Matte, 2006).

The primary functions of vitamin B_{12} involve metabolism of nucleic acids, proteins, fats and carbohydrates. Vitamin B_{12} is of special interest in ruminant nutrition because of its role in propionate metabolism as well as the practical incidence of vitamin B_{12} deficiency as a secondary result of cobalt deficiency (Lalman and McMurphy, 2005). The three vitamin B_{12}-dependent enzymes are involved in two reactions; transmethylation (transfer of a methyl group) and isomerization; methionine synthase, methylmalonyl-CoA mutase, leucine mutase (Girard and Matte, 2006). These enzymes are the link between metabolism of folic acid, biotin and vitamin B_{12} and also those has been identified in leukocytes. However, vit. B_{12} plays roles in increase milk yield and milk composition (Zimmerly and Weiss, 2001; Majee *et al*., 2003; Rosendo *et al*., 2004; Girard and Matte, 2005) improved neutrophil function and reduced SCC (Sato *et al*., 1999). Iron is an essential component in the structure of proteins involved in transportation and utilization of oxygen. Examples include hemoglobin, myoglobin, cytochromes and iron-sulfur proteins involved in the electron transport chain. Supplemental iron sources include ferrous (iron) sulfate, ferrous carbonate and ferric (iron) oxide.

Availability of ferrous sulfate is high while ferric oxide is very low in availability. Many commercial mineral mixes include ferric oxide to give it the traditional red appearance (Lalman and McMurphy, 2005). Iron deficiency lead to anemia (Lalman and McMurphy, 2005); reduce to binding to lactoferrin (Elliot *et al*., 1984). Anemia is associated with the development of insufficient milk. Anemia is the reduction in either the number of red blood cells or the amount of hemoglobin (iron containing portion) of the red blood cells. This results in a decrease in the amount of oxygen available to the cells of the body. As a result, they have less energy available to

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perform their normal functions. Important processes such as muscular activity, cell building and repair slow down and become less efficient. Iron deficiency can lead to impaired delivery of oxygen to the tissues to anemia, impaired immune function (inflammation of udder gland), decreased energy levels and to decreased physical performance (Scott, 2004). Lactoferrin is a Fe-binding protein with bacteriostatic properties, found in the whey fraction of milk in humans and domestic livestock species. It has been suggested that the bacteriostatic action of udder infectious such as mastitis in cattle (Elliot *et al*., 1984). The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction (redox) reactions and in scavenging free radicals.

The copper-dependent enzyme, cytochrome c oxidase, plays a critical role in cellular energy production. By catalyzing the reduction of molecular oxygen (O_2) to water (H_2O), cytochrome c oxidase generates an electrical gradient used by the mitochondria to create the vital energy-storing molecule ATP (Miroslaw *et al*., 2008). Copper is also a constructional part of blood cells and plays an important role in hematopoiesis. Thus, copper deficiency alters the activity of several enzymes which mediate antioxidant defenses and ATP formation. These effects may impair the cell immune functionality, affecting the bactericidal capacity and making the animals more susceptible to infection and anemia (Sol Morales *et al*., 2000). The recent studies have demonstrated the role of Cu on both incidence of Intramammary Infections (IMI) and response to infections during experimentally induced *E. coli* endotoxin mastitis in dairy cows (Scaletti *et al*., 2003). On the other hand, clinical mastitis is resulted in decreased milk production, increased numbers of leukocytes in milk, altered milk composition and appearance, increased body temperature and red, warm and swollen mammary quarters (Gunay and Gunay, 2008). Thus, the objective of this study is to determine an evaluation the impact of vit. B_{12}+Fe and Cu injection on udder immune system and milk compositions in Holstein dairy cows.

MATERIALS AND METHODS

Animals and management: This study was performed in one of the industrial dairy cattle farms located in the outskirt of Isfahan (city of Iran). Cows were housed in free stall and were offered fresh feed thrice daily (TMR) had free access to water. It was used anionic salts in diet during of preparturition for prevention of hypocalcaemia. Calves were separated from their mothers and were kept in a special compartment. After parturition, cows were checked in the light of extraction of placenta and uterine involution as daily. Also, cows were milked 3 times a day and were feed after milking.

Experimental design: A total of 80 Holstein dairy cows with a same milk production and parity were randomly divided into four groups of twenty cows as follow; control group (saline), vit. B_{12}+Fe group (10 mL/day/cow, Nasre Fariman, Iran), Cu group (2 mL/day/cow, Parnell, Australia) and vit. B_{12}+Fe (10 mL/day/cow) +Cu (2 mL/day/cow) groups. In this study, dosage of drugs was based on the manufacturer's recommendation. The vitamins and minerals applied as treatments were intramuscularly injected to the cows, from 1 week prior to the predicted parturition date to 1 week after parturition. Milk samples were taken 3 times in the beginning, end of 1st week and end of 2nd week of the trial in order to evaluate the effect of experimental treatments on udder immune system and milk composition. All the samples were sent to the microbiological laboratory for detection of Somatic Cell Counts (SCC) and Total Bacterial Count (TBC).

The samples were also analyzed to determine the concentration of fat, protein, lactose and Solid Non-fat (SNF) of milk with the use of Milkoscan 4000 (Combifoss, Denmark). Moreover, milk samples were taken 4 times at 3, 7, 11 and 14 days of the trial for the detection of Somatic Cell Counts (SCC) by Fossomatic 5000 (Combifoss, Denmark).

Statistical analysis: Data were subjected to the analysis of variance appropriate for a completely randomized design (SPSS Ver. 1.2). Duncan's Multiple Range Test (DMRT) was applied to separate means. Statements of statistical significance are based on a probability of ($p<0.05$).

RESULTS AND DISCUSSION

In this study, impact of vit. B_{12}+Fe and Cu injection on milk production, fat, protein, lactose and SNF as well as somatic cell count and bacterial count of milk was evaluated. Table 1 shows that vit. B_{12}+Fe injection resulted in a favorable increase in milk production compared to other treatments but it had only significant difference to control group in first sampling. On the other hand, B_{12}+Fe, Cu treatment groups had the least impact on milk production in total sampling ($p>0.05$).

Table1: Effect of treatment groups on milk production

	Milk production (kg)[1]		
Treatments	S_1	S_2	S_3
Control	26.88^{b}	30.02^{a}	28.92^{ab}
B_{12}+Fe	31.80^{a}	30.19^{ab}	30.18^{a}
Cu	28.80^{ab}	30.66^{ab}	28.71^{ab}
B_{12}+Fe, Cu	28.20^{ab}	29.62^{ab}	27.72^{abc}

[1]: S_1: First sampling; S_2: Second sampling; S_3: Third sampling. *In each column mean which contained at least one common letter are not significantly different at the 5% level

Table 2: Effect of treatment groups on milk composition

	Fat[1] (%)			Protein (%)			Lactose (%)			SNF (%)		
Treatments	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
Control	3.29^{a}	3.17^{a}	3.49^{a}	2.83^{d}	2.90^{cd}	2.83^{c}	4.98^{a}	4.98^{ab}	4.98^{a}	9.25^{a}	9.20^{a}	9.15^{a}
B_{12}+Fe	3.67^{a}	3.48^{a}	3.64^{a}	3.17^{ab}	3.09^{ab}	3.05^{ab}	4.92^{a}	4.98^{ab}	4.93^{a}	8.88^{b}	8.85^{b}	8.89^{b}
Cu	3.55^{a}	3.33^{a}	3.39^{a}	2.98^{abcd}	2.97^{abcd}	3.04^{ab}	4.97^{a}	4.84^{b}	5.01^{a}	9.08^{ab}	9.02^{ab}	9.18^{a}
B_{12}+Fe, Cu	3.61^{a}	3.28^{a}	3.38^{a}	2.94^{bcd}	2.93^{bcd}	2.95^{bc}	5.00^{a}	5.08^{a}	5.01^{a}	9.06^{ab}	9.02^{ab}	9.09^{ab}

[1]: S_1: First sampling; S_2: Second sampling; S_3: Third sampling. *In each column mean which contained at least one common letter are not significantly different at the 5% level

In the relation can be mentioned that the high-producing dairy cow requires a large supply of energy and glucose. Vitamin B_{12} deficiency causes an accumulation of methylmalonic acid which can disrupt glucose and glutamic acid metabolism. Therefore, it is likely that this metabolic pathway plays an important role in the energy metabolism of dairy cows (Girard and Matte, 2006). However, it can be suggested that the mechanism leading to the positive overall production response with B-vitamin supplementation was due to improvements in metabolic efficiency of intermediary metabolism rather than increased metabolic activity. On the other hand, administration of Fe could be affected on reduction of anemia and blood flux sufficient was resulted to improve milk production (Scott, 2004). However if Fe administration had increased milk production, the most likely mode of action would be increased oxygen carrying capacity of the blood or improved health (e.g., less mastitis) via reduced oxidative stress because of enhanced antioxidant status (Weiss *et al*., 2010).

Table 2 shows that vit. B_{12}+Fe group increased fat percentage of milk compared to control and other groups but it was not statistically significant. The vit. B_{12}+Fe treatment group was greater than control group in milk protein but it was not significant to other treatment groups. Furthermore, vit. B_{12}+Fe, Cu treatment group increased milk lactose compared to control and other treatment groups but no significant difference was found. Percentage of milk SNF in control group was significantly higher than vit. B_{12}+Fe treatment group but it was not significant in comparison to other treatment groups. The vit. B_{12}+Fe treatment group was higher than other groups in fat percentage of milk but it was not statistically significant. The primary functions of vitamin B_{12} involve metabolism of nucleic acids, proteins, fats and carbohydrates.

Vitamin B_{12} is of special interest in ruminant nutrition because of its role in propionate metabolism. In young ruminant animals, vitamin B_{12} deficiency can occur when rumen microbial flora have not reached adequate populations or are depleted due to stress. Vit. B_{12} administration has effect on improvement of rumen microbial flora, propionate metabolism and finally improving of fat milk (Lalman and McMurphy, 2005). The vit. B_{12}+Fe treatment group was higher than other groups in milk protein and it was significant compared with control group. Vitamin B_{12} is the coenzyme of methionine synthase, the enzyme essential for the transfer of a methyl group from the 5-methyl-tetrahydrofolate to Homocysteine (Hcy) for regeneration of Met. This amino acid is the precursor of S-Adenosylmethionine (SAM), the major donor of methyl groups. However, vitamin B_{12} plays a major role in the entry of propionate in the Krebs cycle and gluconeogenesis through the vitamin B_{12}-dependent enzyme methylmalonyl-CoA mutase and Met has an important role in protein synthesis (Preynat *et al*., 2010).

The vit. B_{12}+Fe, Cu treatment group increased milk lactose compared to control and other treatment groups but not significantly. The high-producing dairy cow requires a large supply of energy and glucose. Huge amounts of glucose are required by the lactating mammary gland to synthesize lactose, the primary osmotic controller of milk volume. The nature of the ruminant digestive system imposes a huge dependence on gluconeogenesis as very little glucose is absorbed. Unlike the situation in non-ruminants in ruminants, the rate of gluconeogenesis increases with feed intake and the major substrates for gluconeogenesis are propionate, glucogenic amino acids and lactate. Lactate metabolism is closely related to propionate metabolism because in addition to lactate produced in the rumen, lactate is formed during the catabolism of glucose by peripheral tissues or the degradation of propionate by the ruminal epithelium. During early lactation, the demand for methyl groups is high and there is a concomitant increase in pressure to synthesize glucose in order to support lactose output and provide energy (Girard and Matte, 2006).

However, glucose is the primary precursor for mammary lactose synthesis. Therefore, the amount of glucose available may have an effect on milk production because lactose is the major osmoregulator for mammary uptake of water and a close relationship between whole-body glucose flux and milk volume has been proposed (Preynat *et al*., 2009). Figure 1 shows that vit. B_{12}+Fe group had the most significant effect on the reduction of Somatic Cell Count (SCC) of milk in 3, 7, 11 and 14 days of the experiment.

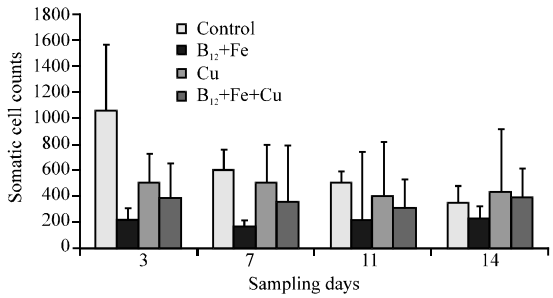

Fig. 1: Effect of vit. B_{12}+Fe and Cu administration on Somatic Cell Count (SCC)

Table 3: Effect of treatment groups on Total Bacterial Count (TBC)

	Total bacterial count (mL^{-1})		
Treatments	S_1	S_2	S_3
Control	12.53[a]	12.52[a]	12.76[a]
B_{12}+Fe	12.36[a]	12.01[a]	12.19[a]
Cu	12.45[a]	12.12[a]	12.68[a]
B_{12}+Fe, Cu	12.41[a]	12.02[a]	12.30[a]

[1]: S_1: First sampling; S_2: Second sampling; S_3: Third sampling. *In each column mean which contained at least one common letter are not significantly different at the 5% level

Vitamin B_{12}+Fe treatment group was different ($p<0.05$) compared to other treatments groups in the total sampling except to the sampling day 11. Table 3 shows that vit. B_{12}+Fe administration resulted in a favorable decrease in total bacterial count compared to control and other treatments but it was not significant.

Furthermore, copper is a co-factor of superoxide dismutase enzymes which prevents the detrimental peroxidative impact of free radicals on defensive and immune system of udder gland (Scaletti *et al*., 2003). However, copper was influenced to the udder immune system and clearing of free radicals that leading to improved composition and quality of milk.

The result of this study indicated that vit. B_{12}+Fe treatment group had the most significant effect on the reduction of somatic cell count and total bacterial count of milk. These findings might be due to an increase in the activity of Polymorphonuclear Neutrophils (PMN), immune potency and resistance of the animal against infectious diseases (Scaletti *et al*., 2003). Fe is binding to Lactoferrin (LF). LF is an iron-binding glycoprotein that is synthesized by specific granules in PMNL and glandular epithelial cells. LF is present in milk and on mucosal surfaces.

In milk, LF plays a key role in the defense mechanisms of the mammary gland of lactating animals (Cheng *et al*., 2008). *In vitro* studies indicate that bovine apo-LF (lactoferrin incompletely saturated with iron) at physiological concentration inhibits growth of organisms causing mastitis (Nonneck and Smith, 1984). Iron supplementation can affect immune function and oxidative balance in animals. Beef cattle fed diets with high concentrations of supplemental Fe (500 mg kg^{-1} from Fe sulfate) had reduced neutrophil function (phagocytosis and killing ability) which was ascribed to reduced Cu status.

In one study (Gengelbach *et al*., 1997), neutrophil kill was substantially higher (although not statistically higher) when cattle were fed 600 mg kg^{-1} of supplemental Fe (from Fe sulfate) compared with cattle not fed supplemental Fe.

Iron-deficient rats had evidence of increased oxidative damage compared with rats fed Fe-adequate diets (Rao and Jagadeesan, 1996). One presumed mode of action for that response is via catalase, an Fe-containing enzyme that catalyzes the reaction that converts hydrogen peroxide to water. However in the Rao and Jagadeesan (1996)'s study, hepatic catalase activity was not different between Fe-deficient and Fe-adequate rats although, several measures confirmed increased oxidative stress with Fe deficiency. Cows fed supplemental Fe had lower log10 SCC ($p<0.05$) than control cows and increased mastitis is related to oxidative stress and antioxidant status (Weiss and Spears, 2006). On the other hand, injections of vit. B improved neutrophil function and reduced SCC in cows with intramammary infections of *Staphylococcus aureus* but did not affect cure rates (Sato *et al*., 1999).

CONCLUSION

The results of this study shows that the injection of vit. B_{12}+Fe in precalving and postcalving period improved levels of yield, fat, protein, lactose, SNF of milk and reduced the SCC of milk at dairy cows.

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