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Application of vitamins B_1 and B_{12} injection on milk yield and composition and some blood biochemical traits in Holstein dairy cows

Mohammad Ansari Ardali [1]and Farshad Andarzgoo [2]

Abstract: The current study was aimed to determine the effect of B_1 and B_{12} vitamins injection on milk production, milk composition and some blood biochemical in Holstein dairy cows. For this reason, 20 Holstein dairy cows with average milk production 30±2 kg and initial weigh 620±30 kg that they were in 60 days after calving as randomized experimental plan for 50 days were used. The treatments were control diet, injection of 10 Ml of Thiamine (vitamin B_{1}), 10 Ml vitamin Cobalamin (vitamin B_{12}) and 10 Ml vitamin B_1, 10 Ml vitamin B_{12} together on their neck muscle weekly. Milk production and its composition were measured after 5, 7, 9 and 15 weeks. Also, for determine some blood biochemical such as lactose, total protein, cholesterol and triglyceride content, blood samples were taken from tail vein of cows that they which under study. Data from this study showed that although milk production wasn't influenced by treatments but also lactose and protein percentage were higher on B_{12} treated cows in sixth week. There were no significant differences between treatment about fat, protein and lactose in 9, 15 weeks. As result relevant from this study B_1 and B_{12} injection had significant effect on milk other composition such as total protein, cholesterol and triglyceride at sixth week. Data from this study showed that B_1 and B_{12} vitamins injection had shown different values for triglyceride, cholesterol and total protein. In conclusion the study recommends adding B_1 and B_{12} improved the physiological status such as lactation performance and could be useful on milk fat, milk protein and some blood biochemical in Holstein dairy cows.

Key words: B_1 and B_{12} vitamins, Milk yield, Blood biochemical, Holstein dairy cows.

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*Corresponding author:
[1]Department of Animal Sciences, Shahre-Kord University, Shahre-Kord, Iran
[2] Department of Animal Sciences, Lorestan University, Khoram-Abad, Iran

Introduction

Vitamin B_1, also called thiamin. All B vitamins help the body to convert carbohydrates into glucose which is used to produce energy. These B vitamins also help the body to metabolize fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly, and are needed for good brain function. All B vitamins are water-soluble, meaning that the body does not store them. Like other B complex vitamins, thiamine is sometimes called an "anti-stress" vitamin because it may strengthen the immune system and improve the body's ability to withstand stressful conditions. Thiamine is found in both plants and animals and plays a critical role in certain metabolic reactions. The body needs it to form adenosine tri phosphate which every cell of the body uses it for energy. It is generally accepted that B-vitamin requirements can be met through synthesis by ruminal bacteria and dietary sources that escape from the rumen (National Research Council 2001). Consequently, very little research effort has been directed at defining dairy cow requirements for B complex vitamins. Over the past decades, 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. Moreover, vitamin B1 is necessary for healthy mucous membranes, helps in the digestion of food, provides strength to muscles and it is very useful for the proper functioning of heart (Mc DOWELL 2000).

This hypothesis is supported by studies that have reported beneficial effects from supplementation with thiamin (Shaver and Bal 2000). Vitamin B_{12}, also called Cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. Vitamin B_{12} consists of a class of chemically related compounds, all of which have vitamin activity. 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 (OVERTON, 1998). A cow producing 40 kg of milk per day requires approximately 3 kg of glucose per day (GIRARD, 1995). A lack of vitamin B_{12} in early lactation could reduce utilization of supplementary folic acid by the cow’s tissues, given that folic acid becomes “trapped” in the serum under its methylated form, 5-methyl-tetrahydrofolate. In fact, a lack of vitamin B_{12} inhibits methionine and S-adenosylmethionine synthesis. All available one-carbon units are diverted to the synthesis of 5-methyl-tetrahydrofolate. This reaction is irreversible, and de-methylation through the regeneration of mthionine is blocked by the lack of vitamin B_{12}. The three vitamin B_{12} dependent enzymes are involved in two reactions that they are trans methylation and isomerization reactions.

These enzymes are methionine synthase, methylmalonyl-CoA mutase and leucinemutase (Schneider and Stroinski, 1987). The present review highlights the fact that B vitamins, especially B_1 and vitamin B_{12} can influence metabolic efficiency through their role as coenzyme or cofactor of enzymatic reactions. However, research on these micronutrients is still in its infancy, so this study was aimed to determine the effect of B_1 and B_{12} vitamins injection on milk production, composition and some blood biochemical in Holstein dairy cows.

Material and methods

This study was carried out by cooperating Zagros Agricultural and Livestock Cooperative Company, in Shahrekord, Iran.

A. Experimental animals

Forty lactating Holstein dairy cow were assigned randomly into four experimental treatments. The experimental period lasted 50 days. Dairy cows weighed an average of 620±30 kg, with average of 30±2 kg milking received daily the basal diet plus, 10 Ml vitamin B_1, 10 Ml vitamin B_{12} and10 Ml vitamin B_1, 10 Ml vitamin B_{12} together on their neck muscle respectively. Feed and fresh water were providing *ad libitum* during the experiment period. The animals received their requirements according to (NRC, 2001).

B. Milk production

Milk yield of cows during the 15 weeks of suckling period was estimated by the cows suckling weight differential technique. Cows were milking at clock 7.00, 14.00 and 21.00. This procedure was repeated weekly during the whole suckling periods. Milk samples were taken for chemical analysis on 60 ml package and stored at 4$^{\circ c}$ for determine fat, protein and lactose content by Milkoscan device.

C. Sampling of blood

Blood samples from each animal in different treatments were taken after morning feeding from tail vein and collected at the beginning of experiment up to 15 weeks. Blood samples were allowed to clot

4$^{°c}$ temperature and serum was then separated by centrifugation at 1850 rpm for 20 minutes. Blood serum samples were divided into two parts and then transferred into dry glass vials and stored at -20 $^{℃}$ until subsequent analysis. After plan completion they were analyzed at Aryan Lab Company by using Pars Azmoon® kits and Auto Analyzer (TECHNICO, R. A 1000) device.

D. Statistical Analysis

Data were analyzed by using the general, linear model procedure of (SAS) 2001 and different means Duncan's multiple ranges test was used to detect the differences at level ($p<0.05$). The used statistical analysis in this trial was as below:

Yij=μ+Ti+eij

Whereas: Yij = Dependent variable, μ = Total average, Ti= Treatments, eij= Remained effects.

Result and discussion

1. Milk Yield

The presented results indicate that the milk yield (kg.d) was decreased by using B_1 and B_{12} vitamins. Data showed that there was no significant effect between treatments about milk yield (Table 1).

Table 1. Effect of vitamin B_1 and B_{12} injection on milk yield (Kg.d)

Experimental period (week)	Treatments				
	Control	Vit B1	Vit B12	VitB1+B12	SEM
7	41.2	39.2	39.4	37.8	1.82
9	41.5	38.2	39.5	38.7	2.16
15	39.8	37.2	39.3	38.5	1.74
Total	40.1	38.2	39.1	37.9	1.61

*Means within row with no common on letter are significantly different ($p≤0.05$).

2. Milk protein

The presented results indicate that the milk protein percentage was increased none significantly by using B_1 and B_{12} vitamins injection.

The milk protein improved as a result of B_1 and B_{12} vitamins effect and milk production (Table 2).

Table 2. Effect of vitamin B_1 and B_{12} injection on milk Protein (%)

Experimental period	Treatments				
	Control	Vit B1	Vit B12	VitB1+B12	SEM
7	2.74^{ab*}	2.75^{ab}	2.83^{a}	2.72^{a}	0.041
9	2.95	2.96	2.97	2.99	0.050
15	2.94	2.96	2.98	3.06	0.071
Total	2.82	2.88	2.90	2.91	0.049

*Means within row with no common on letter are significantly different ($p \leq 0.05$).

3. Milk Fat

Data showed that the milk fat was better for B_1, B_{12} and B_{1+} B_{12} respectively compared with control.

Milk fat percentage improved as a result of B_1 and B_{12} Injection (Table 3).

Table 3. Effect of vitamin B_1 and B_{12} injection on milk Fat (%)

Experimental period	Treatments				
	Control	Vit B1	Vit B12	VitB1+B12	SEM
7	2.74	2.92	2.81	2.94	0.18
9	2.28	2.34	2.33	2.75	0.21
15	2.38	2.68	2.64	2.31	0.19
Total	2.49	2.69	2.49	2.69	0.15

*Means within row with no common on letter are significantly different ($p \leq 0.05$).

4. Milk Lactose

Data reveled that thet significant difference for milk lactose at sixth week of experimental plan. Although adding B_1 and B_{12} decreased the milk lactose percentage in treatments but also there were no significant effect between treatment except first sixth week (Table 4).

Table 4. Effect of vitamin B_1 and B_{12} injection on milk lactose (%)

Experimental period	Treatments				
	Control	Vit B1	Vit B12	VitB1+B12	SEM
7	4.79^{a*}	4.62^{b}	4.77^{ab}	4.69^{ab}	0.09
9	4.72	4.77	4.79	4.75	0.057
15	4.78	4.68	4.78	4.68	0.065
Total	4.79	4.68	4.79	4.70	0.045

*Means within row with no common on letter are significantly different (p≤0.05).

5. Some blood biochemical

The presented results in (table 5) indicate that the blood serum total protein, cholesterol and triglycerides values in the different treatments are significantly different (P≤0.05). The result showed that total protein was increased by B_1 and B_{12} injection. The cholesterol and triglycerides blood levels were increased significantly by B_{12} injection (P≤0.05).

Table 5. Effect of vitamin B_1 and B_{12} injection on some blood biochemical (mg.dl)

Blood Biochemicals	Treatments				
	Control	Vit B1	Vit B12	VitB1+B12	SEM
Total Protein (mg.dl)	7.49^{b*}	8.29^{a}	7.57^{b}	7.58^{b}	0.19
Cholesterol (mg.dl)	298.8^{ab}	257^{b}	302.7^{a}	266.4^{ab}	15.4
Triglyceride (mg.dl)	14.7^{a}	12.2^{b}	15.6^{a}	13.7^{ab}	0.89

**Means within row with no common on letter are significantly different (p≤0.05).

Discussion

The presented results in currents study showed that the milk yield had decreased by B_1 and B_{12} injection, but milk fat and milk protein percentage had tended to increase.

Solouma *et al* (2014 showed that protein improved as a result of thiamin effect and milk production improved as a result of total protein improving in blood by 37.50% in thiamine groups in comparison with control.

Majee et al (2003) indicated that milk production was increased when cows were fed a mixture of B-vitamins (biotin, folic acid, niacin, pantothenic acid, B-6, riboflavin, thiamin, and B-12) compared with cows not fed supplemental B-vitamins but was not different from a treatment in which only biotin was supplemented.

The improvement in the milk yield performance as a result of increases total protein in blood may be due to the positive effect of these treatments on the digestibility coefficient of different nutrients and nutritive values as suggested by (FARAHAT et al., 2007).

These results of this study indicate that there is an increase in the metabolism and decrease in the catabolism as a result of improve total protein in blood. Increasing of metabolism and decreasing of catabolism may be led to improve the milk yield performance. These results are in agreement with those of (SAPIENZA, 1981).

Research conducted in recent times suggests that levels of water-soluble B vitamins may be insufficient to meet the needs of dairy cows during lactation (EVANS and MAIR, 213).

Girard et al (1995) found that the combined supplement of biotin and vitamin B_{12} increased milk production by 1.1 kg d–1 from 30.5 to 31.6 kg d–1 and milk protein yield from 1.04 to 1.07 kg d^{-1} without affecting dry matter intake ($P \leq 0.05$).

Graulet et al (2007) found higher milk yield in early lactation with high levels of dietary folic acid, and speculated that the folic acid supplementation improved synthesis of purine and pyrimidine compounds in the mammary gland.

Preynat et al (2010) observed that effects of the combined supplement of folic acid and vitamin B_{12} on lactation performance of dairy cows probably result from an improvement of energy metabolism during early lactation. Changes in plasma concentrations only reflect differences between the supply and tissue demands. In the experiment concerned, all cows received the same basal diet; hence, the nutrient supply should be similar among the treatments and differences in plasma concentrations are likely to reflect changes in tissue utilization.

Evans and Mair (2013) suggested that a response can be anticipated at all levels of milk production. As with milk protein percentage, the increase in milk protein yield was not influenced by milk yield. In cows fed supplementary folic acid, it seems that tissue utilization of biotin was increased by vitamin B_{12} supplementation and resulted in increased formation of glucose. It is possible that dietary supplements of vitamins may have modified ruminal fermentations and the cows' nutrient supply.

In this study milk fat tended to increase by $B_1$and B_{12} injection. Evans and Mair (2013) showed that milk fat % on average increased when cows were given the B vitamin blend. Nonetheless, the results seem to indicate that supplementary vitamin B_{12} acts on the two vitamin B_{12} dependent metabolic pathways. Neither the interrelationships among the three B vitamins nor the effect that a suboptimal supply has on the major metabolic pathways are well understood (GIRARD and MATTE, 2005). The mechanisms of action of dietary supplements of B vitamins should also be elucidated, in order to dissociate their effects on ruminal microflora and fermentation products from their direct effect on the cow post absorptive metabolism.

Conclusion

These results of this study indicated that dairy cows may require supplemental $B_1$and B_{12} vitamins. Cows respond to a $B_1$and B_{12} vitamin with increased milk components. These changes seem to be independent of level of production. Increases in components can be expected when the components are low, and there appears to be less opportunity for improvement when fat or proteins are currently high. Fat and protein appear to be more responsive to change on a% basis as lactation progresses with multiparous cows. There were also differences between blood parameters between treatments.

These results generated can be used to predict the likely outcome from the use of the $B_1$and B_{12} vitamin and can be used in the development of improved nutritional requirement models for lactating dairy cows.

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