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Original Research Article

The Effects of Intramuscular Injections of Vitamin B_{12} on Production Performance of Dairy Cows in Early Lactation Fed Dietary Supplements of Rumen-Protected Methionine

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ABSTRACT

To study the effects of rumen-protected methionine and vitamin B_{12} as well as their interactions on production performance of dairy cows in early lactation, 16 Holstein cows in early lactation in experiment with randomized complete block design with the 2×2 factorial arrangement used for 42 days. In this experiment, there were four treatments, which in each treatment is placed two cows primiparous and two cows multiparous. Treatments included: 1) The group receiving the basal diet, 2) The group receiving the basal diet with vitamin B_{12} injections, 3) The group receiving the basal diet with rumen-protected methionine, 4) The group receiving the basal diet with vitamin B_{12} injections and rumen-protected methionine. The results showed that in the use of vitamin B_{12} and rumen-protected methionine, there is no significant difference between the experimental groups in the dry matter intake, milk production, energy corrected milk, 4% fat corrected milk, fat, protein, lactose, solids, solids-non-fat and urea. Analysis of variance showed that the experimental unit in the second and sixth weeks of experiment there were statistically significant differences in dry matter intake. Use of vitamin B_{12} can increase the milk production in early lactation, but this increase was not significant and in the case of vitamin B_{12} associated with methionine this effect is more evident.

Keywords: Vitamin B_{12}, Rumen-protected methionine, Production performance, Dairy cattle

Abbreviations: **5-methyl-THF**= 5-methyl-tetrahydrofolate, **SAM**= S-adenosylmethionine, **THF**= tetrahydrofolate, **AA**= amino acid, **RPM**= rumen-protected methionine, **DMI**= Dry matter intake, **B-**= without of vitamin B_{12} injections, **B+=** Injection of vitamin B_{12}, **M-=** Non-methionine, **M+=** Receive rumen-protected methionine

INTRODUCTION

It is well known that ruminal bacteria can synthetize B vitamins, including vitamin B_{12} (NRC 2001; Duplessis *et al*., 2014). Vitamin B_{12} is a water-soluble vitamin produced by rumen microbes for their use and use by the host animal (Akins *et al*., 2013). According to NRC (2001), synthesis of B vitamins in the rumen is sufficient to meet requirements of dairy cows. Ruminal synthesis vitamin B_{12} was not sufficient to avoid fluctuations of serum concentrations of this vitamin around parturition in dairy cows (Girard and Matte 1999). Low serum concentrations of vitamin B_{12} have been observed in the some of dairy cows in early lactation (Girard and Matte 2005; Assan, 2014; Alameen *et al*., 2014). Duplessis *et al*. (2014) estimated apparent ruminal synthesis of vitamin B_{12} to be between 73.0 and 79.8 mg/d. Akins *et al*. (2013) reported serum vitamin B_{12} concentrations of 2.4, 2.0, and 1.2 ng/mL at −21, 7 and 120 d relative to parturition, respectively, and the decrease from 21 d prepartum to 7 days in milk (DIM) was greater for primiparous cows.

Vitamin B_{12} has the most complex structure of all the vitamins. The basic unit is a corrin nucleus, which consists of a ring structure comprising four five-membered rings containing nitrogen. In the active centre of the nucleus is a cobalt atom. A cyano group is usually attached to the cobalt as an artefact of isolation and, as this is the most stable form of the vitamin, it is the form in which the vitamin is commercially produced (Mcdonald *et al*., 2011).

The roles of vitamin B_{12} and methionine are closely interrelated in the methylation cycle (Figure 1) (Preynat *et al*., 2010). In mammals, two enzymes are vitamin B_{12}-dependent. The first enzyme is methionine synthase, which transfers a methyl group from 5-methyl-THF to homocysteine to regenerate methionine and THF, and second enzyme is methylmalonyl-CoA mutase, which transforms methylmalonyl-CoA into succinyl-CoA to enter the Krebs cycle and then gluconeogenesis (Mcdowell 2000; Girard and Matte 2005; Akins *et al*., 2013). Methylmalonyl-CoA mutase needs to adenosyl-cobalamin and methionine synthase to methyl-cobalamin

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as a cofactor (Lesson and Summers 2001). Methylmalonyl-CoA is formed as an intermediate in the catabolism of valine and by the carboxylation of propionyl-CoA arising in the catabolism of isoleucine, cholesterol, and, rarely, fatty acids with an odd number of carbon atoms—or directly from propionate, a major product of microbial fermentation in ruminants (Mcdowell 2000; Murray *et al*., 2003; Akins *et al*., 2013). In ruminants, propionate is the major substrate for gluconeogenesis (Danfar *et al*., 1995; Duplessis *et al*., 2014). Methionine is the precursor of SAM, the major donor of methyl groups in mammals (Murray et al., 2003; Preynat *et al*., 2010). The major function of the methylation cycle is to provide SAM (Preynat *et al*., 2009). The vitamin B_{12} deficiency blocks the transfer of a methyl group from 5-methyl-THF leading to a secondary folate deficiency by interfering with folate utilization in cells (Scott 1999; Murray *et al*., 2003). Supply of methionine, because of its role as donor of preformed labile methyl groups, affects the needs for methylneogenesis (Preynat *et al*., 2010). In monogastric animals, methionine, choline and creatine in the diet are major sources of methyl groups (Snoswell and Xue 1987). Methionine has an important role in protein synthesis (Brosnan *et al*., 2007). In the latest edition of nutrient requirements of dairy cattle (NRC 2001), recommendations were made for 2 AA, lysine and methionine, based on works of Rulquin *et al*. (1993) and Schwab (1996), according to which, under intensive dairy systems, these AA may be limiting in certain diets. Under such circumstances, increasing Met supply through feeding RPM has the potential to augment milk protein and fat concentrations and yields in high-producing dairy cows, probably through increased protein synthesis (NRC 2001).

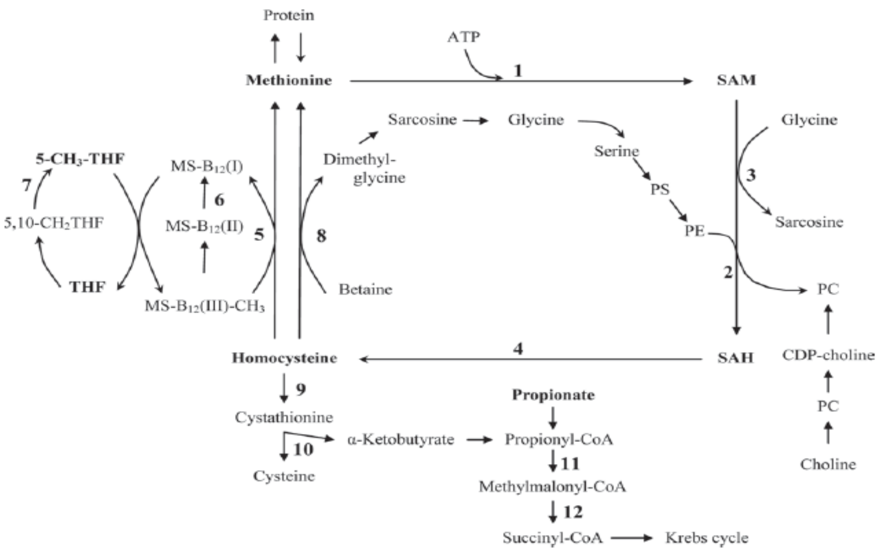

Figure 1. Methylation cycle and simplified vitamin B_{12}-dependent pathways. Key enzymes: 1 = methionine adenosyltransferase; 2 = phosphatidylethanolamine methyltransferase; 3 = glycine N-methyltransferase; 4 = S-adenosylhomocysteine hydrolase; 5 = methionine synthase (MS); 6 = methionine synthase reductase; 7 = 5,10-methylenetetrahydrofolate reductase; 8 = betaine homocysteine methyltransferase; 9 = cystathionine β-synthase; 10 = cystathionine β-lyase; 11 = propionyl-CoA carboxylase; 12 = methylmalonyl-CoA mutase. 5-CH3-THF = 5-methyl-tetrahydrofolate; 5,10-CH2-THF = 5,10-methylene-tetrahydrofolate; THF = tetrahydrofolate; PS = phosphatidylserine; PE = phosphatidylethanolamine; PC = phosphatidylcholine; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine. Adapted from Preynat *et al*. (2010).

In ruminants, choline by rumen microflora is destroyed, as well as plant material in their diet contains adequate amounts of creatine or creatinine were not, as a result, these products should be provided through endogenous synthesis, in addition, the supply of methionine in ruminants, especially during lactation is much less (NRC 2001; Saulawa *et al*., 2012; Mahesh *et al*., 2013; Nabila *et al*., 2014).

Especially important for final maturation of the red blood cells are two vitamins, vitamin B_{12} and folic acid. Lack of either vitamin B_{12} or folic acid causes abnormal and diminished DNA and consequently, failure of nuclear maturation and cell division (Guyton and Hall 2006).

During lactation in dairy cattle, large amounts of glucose are required for lactose synthesis and as an energy source. As very limited amounts of glucose are absorbed from intestinal digestion of starch, the dairy cow relies heavily on gluconeogenesis for glucose supply (Reynolds 2006). In high-yielding dairy cows, propionate is the major glucose precursor, followed by glycerol, lactate, and glycogenic amino acid (Danfar *et al*., 1995).

Deficiency of folic acid itself or deficiency of vitamin B_{12}, which leads to functional folic acid deficiency, affects cells that are dividing rapidly because they have a large requirement for thymidine for DNA synthesis. Clinically, this affects the bone marrow, leading to megaloblastic anemia (Murray *et al*., 2003; Aboamer *et al*., 2015).

Milk secretion requires substantial supplies of both glucose and amino acid. Given the roles of vitamin B_{12} in protein and energy metabolism, this vitamin should play an important role in the regulation of these metabolic pathways in lactating dairy cows. In addition, because of their direct link with the regeneration of methionine from homo-cysteine, their metabolic effect and subsequent response on animal performance might depend on Met supply. Thus, it was hypothesized that supplements of vitamin B_{12} would affect lactation performance 1) through the methylation cycle, in which case, this effect would be more important when methionine supply is limited, and 2) by improving gluconeogenesis because of the role of vitamin B_{12} in this metabolic pathway. The present study was therefore undertaken to determine the effects of RPM and vitamin B_{12} supplementation and their potential interaction on dry matter intake and production performance in lactating dairy cows at early lactation.

MATERIALS AND METHODS

Cows and treatments

Sixteen Holstein cows from the dairy herd in a dairy farm in the city of Malard were placed in two blocks. Animals intended, using a computer information system of dairy cattle breeding, cows and heifers with reviews based on the time calving and parity were selected. Block 1 (Primiparous cows), which includes eight head of cattle (body weight: 564 ±30) and block two (second and third calving) of eight animals (body weight: 603 ±30). Four animals in each group the basal diet with the desired treatment received. Cows were randomly assigned to each of the treatments. The cows were kept in a tie-stall barn under 16 h/d of light (0530 to 2130 h) and were milked thrice daily at 8-h intervals. The experiment lasted for 42 days. Animals were grouped on the basis of parity. So that in each experimental group there were two cows calving the first and two cows second calving. Treatments included: 1) The group receiving the basal diet, 2) The group receiving the basal diet with vitamin B_{12} injections, 3) The group receiving the basal diet with rumen-protected methionine, 4) The group receiving the basal diet with vitamin B_{12} injections and rumen-protected methionine. Vitamin B_{12} injection, the amount of five milligrams, the distance took place once every seven days. Vitamin used (Gedra, France) was a four-ml ampoule which was contains 1000 mg of cyanocobalamin. The group receiving the methionine, 15 g rumen-protected methionine (Mepron, product of the Degussa Company Germany, 80% rumen by-pass) on a daily basis, receiving in three meals. At each meal, methionine for each head of cattle, fully manual with concentrates were mixed. In this study, a diet was formulated for all treatments. In all diets, forage to concentrate ratio was 35 to 65. Forage resources in the diet of alfalfa hay were selected. Cows nutrients needs was determined using the table of feed standards national research council (2001). Feed rations were adjusted using the diet program software (NRC 2001). The ingredients and the nutrient composition of the basal diet were shown in Table 1.

Table 1. Ingredients and nutrient composition of the basal diet.

Ingredients	**%**
Alfalfa	35
Barley	28
Corn	6
Cottonseed	6.7
Cottonseed meal	4.4
Soybean meal	8.4
Wheat bran	9.85
Fish meal	0.25
Salt	0.42
Calcium carbonate	0.49
Sodium bicarbonate	0.49
Dry matter (%)	87.5
NEL (Mcal/kg)	1.59
Crude protein (%)	17.6
Methionine (Percent of CP)	1.93
Cobalt (mg/kg)	6.87

Sampling procedure and measurements

Feed intake (forage and concentrates) which were individually available livestock daily measured and to determine the DMI from remaining amount was deducted. Cows were milked three times daily in a milking parlour at 06.00, 14.00, and 22.00, with no provision of water or concentrate while milking. Milk yield was recorded weekly for all cows. The milk samples from individual cows were sent to the laboratory weekly for the analysis of milk percentages of fat, protein and lactose, solids-non-fat, total solids and urea by Milk-O-Scan (Foss Electric, Denmark). 4 % fat corrected milk (FCM) of each cow was calculated as follows: FCM= ([0.4 × kg milk] + [0.15 × kg milk × fat %]) (NRC 2001). The yield of energy corrected milk (ECM) was calculated by the following formula: ECM= ([0.327 × kg milk] + [12.95 × kg fat] + [7.2 × kg protein]) (Anderson *et al*., 2007).

Statistical analysis

Design used in this experiment was a randomized complete block design with 2×2 factorial arrangement. In each group is placed two cows with first calving and two cows with more than once calving. After collecting data in Excel recorded and categorized. SAS (version 9.1, 2003) statistical software was used to analyze the data and analysis. Results are reported as least squares means and SE.

RESULTS AND DISCUSSION

Dry matter intake and milk production

Effect of intramuscular injections of vitamin B_{12} on DMI and milk production of dairy cows in early lactation fed dietary supplements of rumen-protected methionine are shown in Table 2.

Table 2. Effects of intramuscular injections of vitamin B_{12}, rumen-protected methionine and their interaction given to cows in early lactation on DMI, milk production, ECM and FCM (kg/d).

Effect	Treatment	DMI	Milk production	ECM	FCM
Effect of vitamin B_{12}	B+	23	32.9	23.7	28
	B-	22.6	30.8	21.8	25.8
	SEM	0.2	1.51	1.09	1.2
	P-value	0.17	0.37	0.25	0.26
Effect of methionine	M+	22.8	32.2	23.4	27.6
	M-	22.9	31.19	22.1	26.2
	SEM	0.19	1.51	1.06	1.1
	P-value	0.82	0.54	0.4	0.43
Interactions of vitamin B_{12} and methionine	B-M-	22.6	29.9	21.1	25.1
	B+M-	23.1	32.4	23.1	27.3
	B-M+	22.6	31.7	22.5	26.5
	B+M+	23	33.3	24.4	28.7
	SEM	0.28	2.18	1.5	1.7
	P-value	0.82	0.83	0.95	0.99

B-= without of vitamin B_{12} injections, **B+=** Injection of vitamin B_{12}, **M-=** without rumen-protected methionine, **M+=** Received rumen-protected methionine

DMI for cows fed with experimental diets on a daily basis measured and data were analyzed on a weekly basis. Statistically significant differences were not observed in dry matter intake between treatments, but cows receiving vitamins B_{12} had a higher average dry matter intake of cows that did not receive vitamin B_{12}. The highest dry matter intake was between experimental groups of animals that received only vitamin B_{12}. Analysis of variance showed that the experimental groups in the second and sixth weeks showed statistically significant differences in dry matter intake ($P<0.05$) (Figure 1).

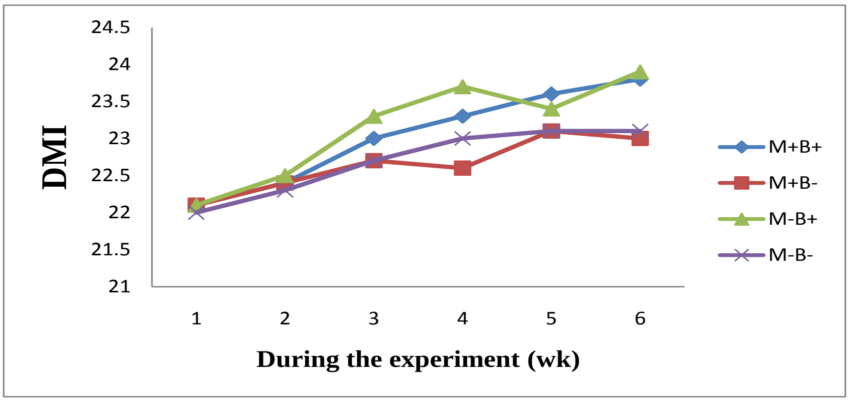

Figure 1. Dry matter intake during the six weeks experimental period in cows receiving weekly I.M. injections 5 mg of vitamin B_{12} and fed with a diet supplemented rumen-protected methionine.
B-= without of vitamin B_{12} injections, **B+=** Injection of vitamin B_{12}, **M-=** without rumen-protected methionine, **M+=** Received rumen-protected methionine.

In an experiment the effect of vitamin B_{12} on DMI was not significant (Graulet *et al*., 2007), however, in their study well as group receiving vitamin B_{12}, 700 g dry matter intake was higher. Preynat *et al*. (2009) observed no significant effect of DMI between experimental groups. According to the results of Preynat and Lapierre (2009) there was no significant effect of the combination of folic acid and vitamin B_{12} and protected methionine on DMI. Girard and Matte (2005) reported a non-significant increase (1 kg) of DMI by injecting B_{12}. Factors that can affect DMI are role of vitamin B_{12} as a cofactor in methylmalonyl-CoA mutase. It undergoes vitamin B_{12}- dependent rearrangement to succinyl-CoA, catalyzed by methylmalonyl-CoA isomerase. Also succinyl-CoA turn to enter the citric acid cycle is converted to succinate. By adding levels of propionic acid diet,

B_{12} vitamin deficiency can be induced. Propionate metabolism in ruminants is important, because of fermentation of carbohydrates; propionate production is increased very much. Propionate normal production continues, but in cobalt and vitamin B_{12} deficiency is reduced levels usual of propionate in blood and accumulates methylmalonyl-CoA. These circumstances increases the urinary excretion of methylmalonic acid and reduces appetite, because of defects in the metabolism of propionate, leading to higher levels of blood propionate which there is an inverse correlation with feed intake. Due to the high concentrate diets were used in this experiment, increase blood propionate in cows that did not receive the vitamin B_{12}, can be a limiting factor feed intake.

Milk production in the experimental group was not affected by the use of vitamin B_{12} and RPM. Cows fed diets containing RPM and injection vitamin B_{12}, in numerical terms, they produce more milk, but statistically not significant.

Vitamin B_{12} and methionine had no significant effect on ECM and FCM. The highest ECM and FCM was achieved when the cows received the dietary supplement methionine and injections vitamin B_{12}. Increase ECM needs to be more glucose and amino acids. Glucose is needed to provide NADPH for the production of milk fat (Van Soest *et al*., 1994) and supply of ATP to produce the protein of amino acids (Lobley 1992). Increase ECM suggest that the rumen-protected methionine can have a positive effect on milk production. Increased FCM is obtained from increased milk production and milk fat. In experiments that addition of methionine increases milk production and milk fat is due to the increase in production of choline from methionine (Sharma *et al*., 1988; NRC 2001).

Milk components

Effect of intramuscular injections of vitamin B_{12} on milk components of dairy cows in early lactation fed dietary supplements of RPM are shown in Table 3. and Table 4.

Table 3. Effects of intramuscular injections of vitamin B_{12}, RPM and their interaction given to cows in early lactation on fat, protein and lactose of milk.

Effect	Treatment	Fat (%)	Protein (%)	Lactose (%)	Fat (kg/d)	Protein (kg/d)	Lactose (kg/d)
Effect of vitamin B_{12}	B+	3	3.02	4.8	1	0.99	1.6
	B-	2.99	3.09	4.8	0.91	0.94	1.5
	SEM	0.08	0.08	0.07	0.04	0.06	0.09
	P-value	0.8	0.5	0.38	0.23	0.48	0.41
Effect of methionine	M+	3	3.08	4.9	0.99	1	1.6
	M-	2.97	3.02	4.7	0.92	0.93	1.5
	SEM	0.08	0.07	0.07	0.04	0.04	0.09
	P-value	0.65	0.5	0.29	0.3	0.3	0.39
Interactions of vitamin B_{12} and methionine	B-M-	3	2.98	4.7	0.88	0.88	1.4
	B+M-	2.96	3.07	4.8	0.96	0.99	1.6
	B-M+	2.93	3.2	5	0.94	1.01	1.6
	B+M+	3.13	2.9	4.8	1.03	0.99	1.6
	SEM	0.11	0.11	0.09	0.06	0.06	0.13
	P-value	0.42	0.17	0.28	0.87	0.29	0.54

B-= without of vitamin B_{12} injections, **B+=** Injection of vitamin B_{12}, **M-=** without rumen-protected methionine, **M+=** Received rumen-protected methionine

Table 4. Effects of intramuscular injections of vitamin B_{12}, RPM and their interaction given to cows in early lactation on solids, solids-non-fat (SNF) and urea of milk.

Effect	Treatment	Solids (%)	SNF (%)	Urea (mg/dl)
Effect of vitamin B_{12}	B+	11.6	8.7	14
	B-	11.6	8.8	14.1
	SEM	0.2	0.11	0.2
	P-value	0.92	0.47	0.78
Effect of methionine	M+	11.8	8.8	15.1
	M-	11.5	8.7	14.2
	SEM	0.24	0.11	0.2
	P-value	0.43	0.43	0.42
Interactions of vitamin B_{12} and methionine	B-M-	11.2	8.6	14.06
	B+M-	11.8	8.8	14.22
	B-M+	12	9	15.3
	B+M+	11.5	8.6	14.85
	SEM	0.37	0.16	0.22
	P-value	0.16	0.07	0.55

B-= without of vitamin B_{12} injections, **B+=** Injection of vitamin B_{12}, **M-=** without rumen-protected methionine, **M+=** Received rumen-protected methionine

Vitamin B_{12} and RPM had no significant effect on milk components. between vitamin B_{12} serum or liver with the amount and percentage of milk fat, there is no significant correlation (Elliot *et al*., 1975 and 1979). Weekly intramuscular injections of cobalamin hydroxy during weeks 9 to 11 or 13 to 15 after calving there was no effect on production and fat percentage of milk (Croom *et al*., 1981). Injection of vitamin B_{12} of the second week until the eighth week after calving there was no effect on milk fat percentage, but increased production of milk fat from 0.73 to 0.84 kg/d, which was accompanied by an increase in milk production from 26.2 to 30 kg/d. (Elliot 1981). Milk fat production, is highly correlated with the percentage of acetates in the rumen and blood concentrations of acetate. Diets containing high in cereal and low in fiber, increase propionate production in the rumen causing the vitamin B_{12} deficiency. Daily intramuscular injection of vitamin B_{12} (hydroxy cobalamin), in 50 mg, increased the production of milk fat in 200 grams per day, and it was expressed that increase propionate production in the rumen is associated with a reduction in the amount of its cofactor (vitamin B_{12}), for propionate metabolism (Frobish *et al*., 1976).

It is not clear why increased amounts of methionine in metabolizable protein may sometimes increase fat content of milk. One reason may involve a possible effect of methionine on de novo synthesis of short- and medium-chain fatty acids in the mammary gland (Pisulewski *et al*., 1996). Another reason may relate to the role of AA in the intestinal and hepatic synthesis of chylomicrons and very low density lipoproteins (VLDL) (Bauchart *et al*., 1996).

It has been reported that the use of RPM increases the milk protein content of dairy cattle (Overton *et al*., 1996; Armentano *et al*, 1997; Socha *et al*., 2005; Davidson *et al*., 2008; Broderick *et al*., 2009; Ordway *et al*., 2009). Graulet *et al*. (2007) reported no significant effect of vitamin B_{12} on the amount of milk protein. It seems that crude protein and crude fiber in diet affect on the potential effect of RPM on milk protein. Also may low NDF in the diet, rumen pH is reduced, and thereby reduce the efficiency of microbial protein synthesis (Van Soest *et al*., 1994).

Changes in the amount of lactose in the use of methionine and vitamin B_{12} may be due to the participation of methionine in the gluconeogenesis pathway, subsequently, increase in blood glucose increases the milk lactose. Well as increase in glucose can increase breast osmotic pressure, and to increase the milk production (Girard *et al*., 2005).

CONCLUSIONS

In the present experiment, supplementary vitamin B_{12} did not increase milk and milk component yields when given in early lactation to cows fed a diet supplemented with rumen-protected methionine. However, there was some increase in milk production. During the experiment, an increase in dry matter intake was observed, that this increase could have an effect on increasing milk production. Therefore, it is recommended that the effect of methionine and vitamin B_{12} in early lactation are examined in greater quantities.

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