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Research Article

EFFECTS OF RUMEN PROTECTED METHIONINE AND VITAMIN B_{12} ON RBC PARAMETERS OF DAIRY COWS IN EARLY LACTATION

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ABSTRACT: To study the effects of rumen-protected methionine and vitamin B_{12} as well as their interactions on the parameters of red blood cells 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 primi-parous and two cows multi-parous. 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 number of red blood cells, hemoglobin levels and blood hematocrit. Mean corpuscular (cell) volume and mean corpuscular (cell) hemoglobin did increase with vitamin B_{12} supplementation. In a general conclusion, it seems that increasing MCV and MCH may result in improvement in oxygenation and in turn lead to improvement on dry matter intake and milk production.

Key words: Vitamin B_{12}, Rumen-protected methionine, Red blood cell, Dairy cattle.

INTRODUCTION

The efficiency with which the dairy cow utilizes MP for protein synthesis is assumed to indicate how well the EAA profile in MP meets the profile of EAA required by the animal as well as by the total amount of EAA in MP (NRC 2001). Research has indicated that methionine is often a limiting AA for ruminants fed diets based on legume forages, corn silage, corn grain, and soybean meal (NRC 2001, Broderick *et al.* 2008; Ordway *et al.* 2009).

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

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much less (NRC 2001; Saulawa *et al.* 2012; Mahesh *et al.* 2013; Nabila *et al.* 2014).

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 some of dairy cows in early lactation (Akins *et al.* 2013; 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.

In the active centre of the nucleus of vitamin B_{12} is a cobalt atom. A cyano group is usually attached to the cobalt as an artifact 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). 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).

The roles of vitamin B_{12} and methionine are closely interrelated in the methylation cycle (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 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 dairy cows, this latter reaction is crucial for entry into the Krebs cycle of propionate produced in large amounts in rumen (Girard and Matte 2005; McDowell 2000). In ruminants, propionate is the major substrate for gluconeogenesis (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_a; Preynat *et al.* 2009_b). A 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 (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). 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 Schwab (1996), according to which, under intensive dairy systems, these AA may be limiting in certain diets. Under such circumstances, increasing methionine 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).

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 (Duplessis *et al.* 2014).

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). Because of the continuing need to replenish red blood cells, the erythropoietic cells of the bone marrow are among the most rapidly growing and reproducing cells in the entire body. Therefore, as would be expected, their maturation and rate of production are affected greatly by a person's nutritional status. Especially important for final maturation of the red blood cells are two vitamins, vitamin B_{12} and folic acid. Both of these are essential for the synthesis of DNA, because each in a different way is required for the formation of thymidine triphosphate, one of the essential building blocks of DNA. Therefore, 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). 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. The present study was undertaken to determine the effects of RPM and vitamin B_{12} supplementation and their potential interaction on red blood cells in lactating dairy cows at early lactation.

MATERIALS AND METHODS

Cows and treatments

Sixteen lactating (8 multiparous, and 8 primiparous) Holstein cows from the dairy herd at Malard were assigned to 2 blocks of 8 cows each according to number of parity. In each group four animals received the basal diet with the desired treatment. The cows in a randomized block design were individually maintained 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 began at approximately beginning lactation and continued until 6 wk of lactation. 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. Treatments, arranged as a 2 × 2 factorial, were infusion of no vitamin B_{12} (B-) or a dose of vitaminB_{12} (B+), and dietary supplementation of no (M-) or 15 g/d (M+) of rumen-protected Met. Based on product specifications, the 15 g/d of rumen protected Met (≥ 70% dl-Met, ≥ 90% protection in the rumen, and ≥ 90% release in the abomasum) supplied a minimum of 7.9 g/d of absorbable dl-Met. Rumen protected Met was divided into 2 equal portions and 7.5 g was thoroughly mixed with the diet before each feeding at 0700 and 1900 h. Within each block, the cows received a weekly intra- muscularly (I.M.) injection of 5 mg of vitamin B_{12}. In this study, an equal diet was formulated for all treatments. In all diets, forage to concentrate ratio was 35%:65%. Nutrient requirements were

determined using the table of feed standards in National Research Council (2001). A mixed ration was adjusted using the diet program software (NRC 2001). The ingredients and the nutrient composition of the basal diet were shown in Table 1.

Sampling procedure and measurements

Whole blood was collected by venipuncture of the caudal vein, using blood collection tube, at the beginning of the experiment (d 0) and every two wk thereafter (Girard and Matte 2005). Number of RBC, Hct, Hb values, MCH (Hb/RBC), MCV (Hct/RBC) and MCHC (MCH/MCV) were determined according to the methods described in Girard and Matte (2005).

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.

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

RESULTS AND DISCUSSION

The results of statistical analysis of red blood cell parameters listed in Table 2. RBC analysis showed that significant difference has not been established through the use vitamins B_{12} and supplemental methionine. There was a significant effect of time on the number of red blood cells ($P<0.05$) and was observed an increase in the number of cells at the end of the period (6670000 compared to 6471000 Number per microliter). Hemoglobin levels between experimental groups showed no significant difference. The effect of time on hemoglobin concentration was statistically significant ($P<0.05$). Hemoglobin at the end of the period was increased (11 vs. 9.7 g/dl). Hemoglobin concentration was increased due to the vitamins B_{12} involved in the synthesis of hemoglobin. Throughout the experimental period, supplementary vitamin B_{12} increased blood hemoglobin (Girard and Matte 2005). MCH showed a significant difference between treatments ($P<0.05$). Cows that were received vitamin B_{12} had a MCH higher than control cows (17.1 vs. 16.2 Pg) respectively. However, no significant difference was observed between M+ and M- animals in MCH. The effect of time on this trait was also not significant. Significant effect on measured MCV was observed between

Table 2. Effects of intramuscular injections of vitamin B_{12} on red blood cells parameters of dairy cows in early lactation fed dietary supplements of rumen-protected methionine.

Effect	MCV[5]	Hct[4]	MCHC[1]	MCH[3]	RBC[2]	Hb[1]	Treatment
Effect of vitamin B_{12}	51.8 [a]	33.4	33.1	17.1 [a]	6481000	11.08	B+
	48.6 [b]	32.3	33.2	16.2 [b]	6660000	10.70	B-
	0.9	0.9	0.28	0.27	241000	0.3	SEM
	0.03	0.41	0.74	0.03	0.63	0.48	P-value
Effect of methionine	50.5	32.9	32.8	16.5	6565000	10.80	M+
	49.9	32.7	32.5	16.8	6577000	11.00	M-
	0.9	0.9	0.27	0.26	248000	0.3	SEM
	0.67	0.87	0.08	0.5	0.97	0.69	P-value
Interactions of vitamin B_{12} and methionine	49.7 [ab]	32.7	33.4	16.6	6591000	10.90	B-M-
	50.1 [ab]	32.7	33.7	16.9	6563000	11.06	B+M-
	47.4 [b]	31.8	33.1	15.7	6730000	10.50	B-M+
	53.5 [a]	34	32.4	17.3	6400000	11.10	B+M+
	1.3	1.2	0.39	0.3	343000	0.42	SEM
	0.04	0.39	0.21	0.1	0.63	0.91	P-value

[1,] Expressed in grams per deciliter, [2,] Number per microliter, [3,] Expressed in picograms, [4,] Percent, 5- Expressed in femtoliters.

Values with different superscript letter within a column differs significantly ($P \leq 0.05$).

the experimental groups ($P<0.05$). Cows receiving vitamin B_{12} had a higher MCV compared to control cows (51.8 vs. 48.6 Fl) respectively. In MCV was not observed significant differences between the M+ and M- animals. Mean MCV in cows fed experimental diets B-M-, B+M-, B-M+, B+M+, was 49.7, 50.1, 47.4 and 53.5 Fl, respectively. Cows that were received both complementary had the highest MCV ($P<0.05$). An increase in the MCV is due to increased blood Hb. Hb that has increased by vitamin B_{12} increases the MCV. Due to increased MCV increases the power of transfer of oxygen to the tissues. Data analysis did not show significant effect on blood Hct in the experimental groups. The effect of time on blood Hct was significant ($P<0.05$) and the cows had a higher Hct at the end of the period (33.3 vs. 29.2 %), respectively.

The factor that can affect dry matter intake is the role of vitamin B_{12} as a cofactor in methylmalonyl-CoA mutase. methylmalonyl-CoA mutase transforms methylmalonyl-CoA into succinyl- CoA for entry into the Krebs cycle. Vitamin B_{12} deficiency can be induced by adding levels of propionic acid of diet. Propionate metabolism in ruminants is important. Propionate production is increased very much because of fermentation of carbohydrates. Propionate normal production continues, but is reduced levels usual of propionate in blood and accumulates

methylmalonyl-CoA as a result of the lack of cobalt and vitamin B_{12}. These circumstances increase the urinary excretion of methyl-malonic acid and reduce appetite, because 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 in this experiment, increase blood propionate in control cows can be a limiting factor feed intake. One of cause of increased dry matter intake of the use of vitamin B_{12} could be related to efficiency of oxygen, and its effect on feed intake control. DMI can also be affected by oxygen consumption. Animals consume net energy at a rate that optimizes the use of oxygen and minimizes production of free radicals that lead to aging (NRC 2001). The effects of vitamin B_{12} on the volume of red blood cells were clearly visible. This increase, improves oxygen transport by red blood to cells. On the other hand, vitamin B_{12} has also increased the amount of hemoglobin. This is also a factor in increasing oxygen transport.

CONCLUSION

In the present experiment, mean corpuscular (cell) volume and mean corpuscular (cell) hemoglobin significantly increased by vitamin B_{12}. In a general conclusion, it seems that increasing MCV and MCH may result in improvement in oxygenation, which in turn leads to improvement dry matter intake and milk production.

[Abbreviations used: Hct= hematocrit, MCV= mean corpuscular (cell) volume, MCH= mean corpuscular (cell) hemoglobin, MCHC= mean corpuscular (cell) hemoglobin concentration, RBC= red blood cell, Hb= hemoglobin, 5-methyl-THF= 5-methyl-tetrahydrofolate, SAM= sadenosylmethionine, THF= tetrahydrofolate, EAA= essential amino acid, AA= amino acid, MP= metabolizable protein, RPM= rumen-protected methionine, DMI= dry matter intake, B-= lack of vitamin B_{12} injections, B+= injection of vitamin B_{12}, M-= non-methionine, M+= receive rumen-protected methionine].

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