

## PHENOTYPIC AND GENETIC VARIATION IN VITAMIN B12 CONTENT OF BOVINE MILK

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### Abstract

*Vitamin B-12 (cobalamin) is essential for human health and current intake level of this vitamin is too low. Bovine milk is an important dietary source of vitamin B-12 and natural enrichment of the milk vitamin B-12 content may help to increase the intake levels. The aim of this study was to quantify the genetic variation in levels of vitamin B-12 in the milk of dairy cows. In this study milk (n= 194) samples were collected from first lactation Holstein Friesian cows, and analyzed for vitamin B-12 content. Vitamin B-12 content varied from 1.08 to 9.66 mg/l with a mean of  $3.93 \pm 1.58$  mg/l. The amount of genetic variation between cows in vitamin B-12 content in milk was reflected by an estimated heritability of 0.36. This heritability of 0.36, combined with a coefficient of variation of 40% for vitamin B-12 content in milk indicates that average milk vitamin B-12 content of the cow Holstein Friesian population can be increased by genetic selection.*

**Key words:** dairy cows, milk, vitamin B-12, genetic variation.

### INTRODUCTION

Only bacteria and archaeobacteria are able to synthesize vitamin B12 if cobalt supply is sufficient (Martens et al., 2002).

The natural source of vitamin B12 in human diets comes from animal-product, especially those from ruminants because of the close link between the animal and bacteria dwelling in its rumen.

The cow milk is a significant contributor to vitamin B12 intake.

In countries with a high dairy consumption approximately 40% of dairy vitamin B12 intake is from dairy products, and contributes to the low prevalence of vitamin B12 deficiency.

Low vitamin B12 status in people may be associated with megaloblastic anemia and neurologic disorders (Vogiatzoglou et al., 2009; Kim et al., 2008).

Also, vitamin B12 plays a role in prevention of osteoporosis, neurocognitive decline, cardiovascular diseases and neural tube defects in newborns.

Vitamin B12 acts as coenzyme in only two metabolic reactions.

One of these vitamin B12 is dependent enzyme, methylmalonyl - coenzyme.

A mutase, plays a major role for the entry of propionate in the Krebs cycle and gluconeogenesis (McDowell, 2000).

Beside this role, the vitamin is a coenzyme for the methionine synthase, the critical interface between folic acid and vitamin B12 metabolism.

Vitamin B12 is the largest of the B complex vitamins, with a molecular weight of over 1000.

It consist of corrin ring made up of four pyrroles with cobalt at the center of the ring (Weir and Scott, 1999).

In nature there are two other forms of vitamin B12: hydroxocobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt.

The synthetic form of vitamin B12 found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt.

These three forms of B12 are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

Most microorganism, including bacteria and algal, synthesizes vitamin B12 and they constitute the only source of the vitamin B12 (Chanarin, 1979).

The vitamin B12 synthesized in microorganism enters the human food chain through incorporation into food of animal origin.

Products from herbivores animals, such as milk, meat etc., constitutes important dietary source of the vitamin B12.

Some observational studies suggest that vitamin B12 from dairy products is better bioavailable than vitamin B12 from other sources (Tucker et al., 2000; Vogiatzoglou et al., 2009).

The absorption of vitamin B12 in humans is complex (Weir and Scott, 1999).

Vitamin B12 in food is bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid present in the stomach.

This process results in the free form of the vitamin B12, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands.

These glycoproteins, called R - binders, protect vitamin B12 from chemical denaturation in the stomach.

The stomach parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor.

Intrinsic factor binds vitamin B12 and ultimately enables its active absorption.

Russell et al. (2001) reported mean absorption of 55%, when radioactive vitamin B12 dissolved in milk was administered to human subjects.

Bioavailability of the synthetic form of vitamin B12 was reported to be poor (<4%) in humans and animals (Zittoun, 1996).

Ruminants require dietary cobalt (Co) for synthesis of vitamin B12 in the rumen. The dietary requirement of dairy cows for Co is 0.11 mg/kg (NRC, 2001).

Tiffany et al. (2006) recorded increased synthesis of vitamin B12 as the Co concentration of the diet increased from 0.1 to 1.0 mg/kg.

Thus, natural enrichment of the vitamin B12 in milk, by increasing cobalt levels of dairy feed may be a good way to increase dietary vitamin B12 intake of human consumer. This way seems limited, because it has been shown that milk vitamin B12 content level off between 4.2 mg/l, despite significantly higher cobalt levels of the cow's feed (0.93 vs. 0.57 mg/kg dry matter) (Kincaid and Socha, 2007).

Enhancing micronutrient (vitamin and mineral) concentration within milk and serum from dairy cows is important for both the health of the cow and the nutritive value of the milk for human consumption (Knaus, 2013).

Natural enrichment of the cow milk vitamin B12 content could be achieved through genetic selection.

The aim of this study was to quantify phenotypic and genetic variation in levels of vitamin B12 in the milk of Holstein Friesian dairy cows.

## MATERIALS AND METHODS

Animals involved in this study were from Agriculture Research and Development Station (ARDS) Simnic - Craiova, Romania.

All Holstein Friesian cows are results of a long-term selection experiment for genotype X environment interactions.

The selected cows have average to high genetic merit for kilograms of fat plus protein yield. The diet consists of high energy ration based on some by-products and one homegrown component (forage maize, grazed grass, alfalfa, forage beet, maize silage, grains and beans).

Additionally the ration is balanced with purchased minerals.

Average (minimum-maximum) nutrient and major ingredient composition was as follows: crude protein, 17% (15-19.5%); fat: 3.9% (3-5%); acid detergent fiber: 20% (16-26%); neutral detergent fiber 34% (23-41%); cobalt 0.52 mg/kg (0.19-1.27 mg/kg); net energy (lactation) 1.70 Mcal/kg (1.64-1.80 Mcal/kg); concentration: 50.2% (40-52%); corn silage 19% (0.00-47%) and hay 7% (0.00-24%).

Cows are milked two times daily (morning and evening) and for the present study milk samples were taken from first-lactation cows at morning milking. Milk samples (n = 194) were collected on 18 separate occasions between February 2014 to January 2019 at 60.4 ± 4.0 d in milk (DIM).

After collection, milk samples were poured into 15 ml conical tubes.

At the laboratory they were frozen at: -20°C and stored until analysis. Vitamin B12 in milk was analyzed in duplicate by enzyme immunoassay using a commercial kit

(RIDASCREEN FAST Vitamin B12, R-Bio pharm AG, and Germany).

Data analyses were performed using M.O. Excel. To estimate the genetic parameters and variance components the REML approach was used.

## RESULTS AND DISCUSSIONS

Vitamin B12 content in the milk of Holstein Friesian cows varied from 1.08 to 9.66 mg/l, with a mean of  $3.93 \pm 1.58$  mg/l.

Figure 1 illustrates the large variation in milk vitamin B12 content.

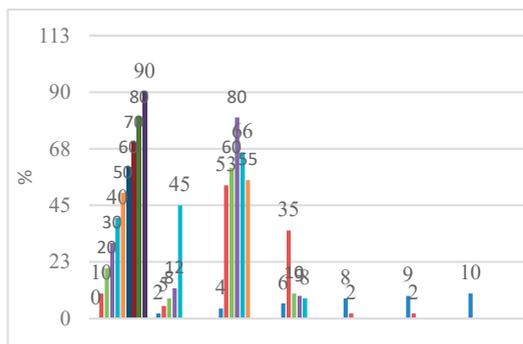


Figure 1. Vitamin B12 content in bovine milk samples (frequency distribution)

The lowest 25% of milk samples had an average vitamin B12 content of  $2.07 \pm 0.48$  mg/l and the highest 25% of milk samples had an average content of  $5.90 \pm 1.41$  mg/l, a difference of 3.83 mg/l.

Rutten et al. (2013) reported a variation of vitamin B12 from 1.0 to 12.9 mg/l, with a mean of 4.40 mg/l in raw sample of Dutch Holstein Friesian cows.

Duplessis et al. (2016) reported concentrations of vitamin B12 in milk from 2.309 to 3.878 mg/l in 15 commercial herds (386 Holstein and 13 Jersey cows in Canada). Vitamin B12 concentration in milk ranging from 1.575 to 4.781 mg/l has been reported by Preynat et al. (2009), and Akins et al. (2013).

Substantial amount of genetic variance in vitamin B12 content in milk was detected among the 194 milk sample.

Variance components and heritability of vitamin B12 content in milk of first lactation Holstein Friesian cows from ARDS Simnic - Craiova, Romania are presented in Table 1.

Table 1. Variance components and heritability of vitamin B12 content in milk of Holstein Friesian cows from ARD Simnic - Craiova, Romania

Source of variation	Vitamin B12
Genetic ( $6^2$ animal)	$0.92 \pm 0.22$
Residual ( $6^2$ e)	$1.59 \pm 0.33$
Phenotypic ( $6p = 6^2(\text{animal}) + 6^2e$ )	$2.51 \pm 0.18$
Heritability ( $h^2 = 6^2\text{animal}/6^2p$ )	$0.36 \pm 0.21$

In this study the phenotypic variance was 2.51 mg/l, and the genetic variance was 0.92 mg/l (Table 1).

Genetic variation between cows indicates that there is ample opportunity to influence the vitamin B12 content in milk. In the present study the heritability (the proportion of variation due to genetic variation) was 0.36, suggesting that genetic selection could modify milk vitamin B12 concentration (Table 1).

The genotype of the cow affects the amount of vitamin B12 that ends up in its milk.

Genotype of cows influences the microbial process in its rumen and after processes such as vitamin B12 absorbance from the digestive tract or secretion by the mammary gland.

Vitamin B12 concentration in milk varied among sampling months.

In this study, it was greater during spring and fall months than during winter and summer months (Figure 2).

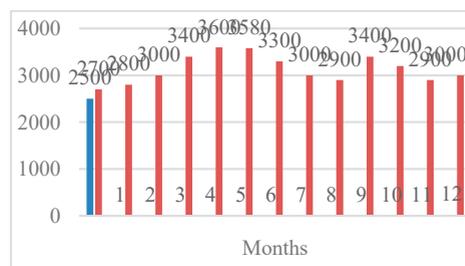


Figure 2. Vitamin B12 concentration in milk of cows among sampling months

Different species of microorganism in the rumen are involved in the degradation of forage and concentrate.

Petri et al. (2012) showed that some strains of ruminal bacteria could synthesize a greater amount of vitamin B12 than others.

As vitamin B12 is synthesized by ruminal microbes and a proportion of the synthesized vitamin is secreted into milk, we could reasonably hypothesize that what affects apparent vitamin B12 ruminal synthesis would also affect milk vitamin B12 concentration.

Apparent ruminal synthesis of vitamin B12 was positively correlated with dietary Neutral Detergent Fiber (NDF) and sugar contents and negatively correlated with Non Fiber Carbohydrate (NFC) contents in the trial of Schwab et al. (2006).

Santschi et al. (2005) observed that apparent ruminal synthesis of biologically active vitamin B12 was greater with a 60:40 forage - to - concentrate ratio diet.

Similarly, apparent ruminal synthesis was about 3 - fold greater for cows receiving a high - fiber diet compared with cows receiving a high - starch diet (Beaudet et al., 2016).

Duplessis et al. (2016) showed that negative relationship was observed between vitamin B12 concentration in milk and ration crude protein (CP).

Beaudet et al. (2016) concluded that apparent ruminal synthesis of vitamin B12 was not affected by 2 level of CP in the ration: 11.1% and 14.3% CP on a dry matter (DM) basis.

Present day genetic selection by dairy breeding organizations involves estimation of breeding values which requires phenotypes of many thousands of animals.

Phenotypes for vitamin B12 content in milk as determined in the current study, are too expensive for such large - sale collection.

Less expensive alternative phenotypes that correlate with milk vitamin B12 content are not currently available.

However, phenotype - based selection can also be substituted by genotype - based selection.

Genotype - based selection require knowledge on regions of the bovine genome associated with vitamin B12 content in milk.

Rutten et al. (2013) reported on a genome - wide association study to identify regions of the bovine genome associated with milk vitamin B12 content.

Identification of associated genomic regions also contributes to the understanding of the biological mechanism responsible for the observed genetic variation in vitamin B12 content in milk.

Significant association [ $-\log_{10}(P - \text{value}) > 3$ ] was found between 68 SNP and vitamin B12 content in raw milk of 487 first - lactation Dutch Holstein Friesian cows (Rutten et. al., 2013).

Significantly associated SNP were spread over 16 *Bos taurus* (BTA) chromosomes.

Among the 68 significantly associated SNP, cluster of at least 3 significantly associated SNP, could be discriminated on BTA 5, BTA 8, BTA 10, BTA 13, BTA 14 and BTA 26.

Table 2 shows that most of the candidate genes for vitamin B12 were actually genotyped for one or more SNP as part of the genome wide association study (Rutten et al., 2013), and none of these SNP were significantly associated with vitamin B12 in milk.

The lack of association between SNP and most known candidate genes for vitamin B12 indicates that variation in other genes causes most of the observed genetic variation in vitamin B12 content in milk (Rutten et al., 2013).

One glass (250 ml) of milk from cows in this study, would have provided between 25-45% of the recommended daily allowance of vitamin B12.

Among individual cows, however, this prevision varied between 20 and 62% of the recommendation.

Our results will need to be confirmed, but they open new research avenues on factors affecting the vitamin B12 concentration in milk of dairy cows.

## CONCLUSIONS

Genetic variation between Holstein Friesian cows in vitamin B12 content in milk was established.

The average heritability of 0.36 combined with a coefficient of variation of 40% for vitamin B12 content in milk indicates that the average milk vitamin B12 content of Holstein Friesian cow population can be increased by genetic selection.

Our results will need to be confirmed on a large Holstein Friesian cow population.

Table 2. Candidate genes for vitamin B12 content in bovine milk with their genetic position and significance of genotype SNP

Gene symbol	Gene name	Chromosome	Genomic position (Kbp) <sup>a</sup>	#SNP in gene <sup>b</sup>	Highest -log <sub>10</sub> (p-value) <sup>b</sup>
LRP2	low density lipoprotein receptor - related protein 2 (Megalin)	2	27, 603, 435.27, 857, 248	3	0.747
MMADNC	methylmalonic aciduria (cobalamin deficiency)	2	47, 991, 127.48, 006, 635	1	1.481
MMACHC	methylmalonic aciduria	3	105, 516, 664, 106, 521, 668.	0	-
CD320	CD320	7	15, 415, 307.15, 421, 023	0	-
LMBRD1	LMBR1 domain containing 1	9	8, 373, 879.8, 506, 806	4	0.614
CUBN	Cubilin	13	31, 053, 631.31, 312, 338	10	2699
GIF	gastric intrinsic factor	15	83, 763, 128.83, 782, 200	0	-
TCN1	transcobalamin I haptocorrin	15	83, 791, 407.83.806, 715	1	13.19
MMAA	methylmalonic aciduria	17	73, 590, 935.13, 603, 835	1	0.169
MMAB	methylmalonic aciduria	17	66, 719, 631.66, 730, 086	0	-
TCN2	transcobalamin II	17	72, 841, 020.72, 857,003	0	-
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	20	68, 917, 318.68, 946, 683	1	1051
AMN	Amnionless	21	67, 751, 621.67, 762, 225	0	-
MUT	Methylmalonyl CoA mutase	23	22, 488, 908.22, 530, 197	1	0.206
ABCC1	ATP - binding cassette, sub-family C	25	15, 453, 547.15, 606, 736	2	0.656
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	28	7, 953, 588.8, 080, 514	2	0.599

a - based on genome assembly BTAU 461

b - number of genotyped SNP in the gene and - log<sub>10</sub> (P - value) of the SNP that was most significantly associated with milk vitamin B12 content.

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