

Vitamin B12 and homocysteine levels in blood of dairy cows during subacute ruminal acidosis

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Abstract

The aim of this study was to investigate the variations of vitamin B12 and homocysteine in blood of dairy cows during subacute ruminal acidosis (SARA). On 228 subjects ruminal liquid was collected through rumenocentesis technique and rumen pH was immediately measured by a portable pH-meter. On the basis of pH values all cows were classified (bovine class) in Group A (animals with rumen pH>5.7), Group B (animals with rumen pH between 5.6 and 5.7) and Group C (animals with rumen pH<5.6). In relation to the acidosis risk depending on the rumen pH (herd class), the herds were classified in Group 1 (normal herds: less than 33 % cows with rumen pH<5.8), Group 2 (critical herds: more than 33 % cows with rumen pH between 5.5 and 5.8) and Group 3 (acidosis herds: more than 33 % cows with rumen pH<5.5). On blood samples, collected by jugular venipuncture, vitamin B12 and homocysteine were measured by chemiluminescent immunological tests. One-way analysis of variance (ANOVA), followed by Bonferroni test, showed significant differences ($P<0.05$) for vitamin B12 in bovine class and significant differences ($P<0.05$) for homocysteine in herd class. The influence of rumen pH values resulted in adequate vitamin B12 and homocysteine levels to meet microbial and cow requirements and fatty acids modifications in dairy cows affected by SARA. Moreover, the increase of vitamin B12 could be due to the presence of analogues which interfere with the transport of the vitamin. These findings provide more information on blood modifications during SARA.

Keywords: dairy cows, dairy herds, homocysteine, rumen pH, subacute ruminal acidosis, vitamin B12

Introduction

Subacute ruminal acidosis (SARA) is thought to be a common condition in early lactating dairy cows. It is a common and serious health and production problem in dairy cows and herds (Nordlund 1996, Oetzel 2003). The determination of rumen pH is a key factor for the diagnosis of SARA (Morgante *et al.* 2007, Giancesella *et al.* 2010). Rumen pH values lower than 5.6 are considered abnormal and suggestive of either severe SARA, or possibly in combination with clinical signs, whereas rumen pH values of 5.6 to 5.8 are considered marginal and values of

rumen pH higher than 5.8 are normal (Nordlund & Garrett 1994). Generally spoken, SARA therefore has to be defined as an intermittent fall of rumen pH to non-physiological levels after uptake of a certain concentrate based diet because of a non-adaption of the ruminal environment in terms of flora and ruminal mucosa (Kleen *et al.* 2003). In fact, among its numerous manifestations, SARA impairs the activity of ruminal cellulolytic bacteria (Grant & Mertens 1992) and various reports have demonstrated a decreased fibre digestion both *in vitro* (Calsamiglia *et al.* 2002) and *in vivo* (Plaizier *et al.* 2001, Krajcarski-Hunt *et al.* 2002) under SARA conditions in dairy cows. In addition, a lower ratio of forage to concentrate in the diet is known to reduce ruminal synthesis of vitamin B12 (Kincaid & Socha 2007, Tiffany *et al.* 2006). This vitamin, also called cobalamin, is synthesised by the intestinal microflora in nonruminant species and by rumen microbes in ruminants. Its synthesis is influenced by many factors such as the cobalt availability and the rumen pH since an acidic environment may cause lysis of B12 synthesising bacteria. Several studies have demonstrated that vitamin B12 is an important growth factor for some ruminal microorganisms (Tanner & Wolfe 1988, Strobel 1992) and is utilised by others in pathways that produce propionate (Chen & Wolin 1981). After several transformations in the body, cobalamin as a cofactor is used in several reactions important to the citric acid cycle and gluconeogenesis. It is involved in metabolism of odd numbered fatty acid like propionate and in biosynthesis of methionine from homocysteine. This, a sulphur intermediate amino acid in the metabolism of methionine-cysteine, may undergo remethylation to methionine in a pathway dependent on vitamins B12 (via methionine synthase) and folate (via contribution of a substrate to the remethylation cycle) (Welch & Loscalzo 1998, Finkelstein & Martin 2000). Several studies have shown that plasma homocysteine levels are affected by diet factors such as protein and vitamin deficiencies (Kalantar-Zadeh *et al.* 2003, Yeh & Yeh 2006), by genetic background and by several pathological conditions (Ridker 1997, den Heijer 1996, Ray 1998, Langman 2000). Moreover, the hyperhomocysteinemia was also associated with chronic inflammatory bowel disease (Roblin 2005) and with vitamin B12 low levels (Romagnuolo *et al.* 2001).

The aim of this study was to investigate the variations of vitamin B12 and homocysteine in blood of multiparous Holstein dairy cows during SARA.

Materials and methods

The study, conducted from June to October 2010 in 21 intensive Italian dairy herds, located in North Italy, was carried out on 228 multiparous Holstein dairy cows. As far as surveyings on the single animals, for every herd we chose 10 or 11 bovine, a statistically significance number inside of the herd (Nordlund & Garrett 1994, Nordlund 2001, Kleen *et al.* 2003). All housing and care conditions were conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals, Directive 2010/63/EU and Directive 1998/58/EU.

All cows were between 7 and 90 days in milk (DIM) and all dairy herds had a high average farm milk production (about 10 000 kg per year). Cows were housed in free stalls, fed a total mixed ration, and had received a steam-up diet in the final part of the dry period. Feed were analysed by near infrared spectroscopy (NIRS) using NIRS 5000 (Foss, Hillerød, Denmark) and an in-house calibration. Table 1 shows the chemical composition of diets consumed by dairy cows during steaming-up and subsequent early lactation. The value of neutral detergent

fibre, acid detergent fibre, non fibre carbohydrates, starch and crude protein, are the same in all herds, with values within the normal range for each period and this chemical composition represent the normal condition in Italian dairy herds.

Table 1

Mean chemical composition of total mixed rations fed to dairy cows during steaming-up and early lactation

Chemical composition of diet in dry matter, %	Steaming-up	Early lactation
Crude protein	13.37	16.59
Ethreal extract	4.30	6.01
Ash	7.38	7.42
Neutral detergent fibre	40.77	30.17
Non fibre carbohydrates	34.17	38.81
Dry matter degradable	59.02	68.48
Acid detergent fibre	24.82	20.37
Starch	14.74	28.46
Dietary cation-anion balance	35.49	49.39

On all subjects rumen fluid was collected by rumenocentesis as previously described by Morgante *et al.* (2007), without sedation, using a 13 gauge 105 mm needle. The sampling time was between 4 and 6 h post total mixed ration distribution as recommended previously. Rumen pH was immediately determined after sampling using a portable pH meter (Piccolo, Hanna Instruments, Leighton Buzzard, UK). On the basis of pH values obtained all subjects were classified (bovine class) into three different Groups: Group A (animals with rumen pH>5.7), Group B (animals with rumen pH between 5.6 and 5.7) and Group C (animals with rumen pH<5.6). In addition, using the classification scheme proposed by Nordlund & Garrett (1994), the herds were classified, in relation to the acidosis risk depending on the rumen pH (herd class in Group 1 (normal herds: less than 33% cows with rumen pH<5.8), Group 2 (critical herds: more than 33% cows with rumen pH between 5.5 and 5.8) and Group 3 (acidosis herds: more than 33% cows with rumen pH<5.5).

All Groups showed a similar body condition score and average values were 3.59 ± 0.23 , in a 1 to 5 scale, according the procedure of Edmonson *et al.* (1989). On all animals blood samples were collected by jugular venipuncture, under aseptic conditions, and included into tubes containing 1.2 mg of anhydrous salt of ethylenediaminetetraacetic acid (EDTA) per ml of blood. The determination of vitamin B12 was performed with a solid phase, competitive chemiluminescent enzyme immunoassay (Immulate 1000 Vitamin B12, Medical System s.p.a., Genoa, Italy) by means of immunoassay automated system (Immulate One, Medical System s.p.a., Genoa, Italy). Plasma total homocysteine was measured by a competitive chemiluminescent immunological test again (Immulate Homocysteine, Medical System s.p.a., Genoa, Italy) by means of the same analyser Immulate One. One-way analysis of variance (ANOVA) was applied to compare the Groups A, B and C in bovine class and one-way ANOVA was applied to compare the Groups in herd class (Groups 1, 2 and 3). Bonferroni's test was applied for post hoc comparison. A P -value<0.05 was considered statistically significant. All data were analysed using Statistica 7 (StatSoft Inc, Tulsa, OK, USA).

Results

The average values of rumen pH, vitamin B12 and homocysteine, together with their standard deviation of the means (SD) and statistical significances, in dairy cows of Groups A, B and C are presented in Table 2. Instead, the mean values (\pm SD) of the parameters studied, together with statistical significances, in dairy herds of Groups 1, 2 and 3 are presented in Table 3.

Table 2

Average values (\pm SD) of rumen pH, homocysteine and vitamin B12, together with the relative statistical significances in experimental groups

Parameters	Experimental groups (Bovine class)		
	Group A (n=155)	Group B (n=49)	Group C (n=24)
Rumen pH	6.17 \pm 0.29	5.69 \pm 0.06*	5.45 \pm 0.14*, **
Vitamin B12, pg/mL	208.50 \pm 43.55	210.20 \pm 41.86	241.90 \pm 32.27*, **
Homocysteine, μ mol/L	4.18 \pm 0.97	3.99 \pm 0.94	3.77 \pm 0.81

Group A: animals with rumen pH>5.7, Group B: animals with rumen pH between 5.6 and 5.7, Group C: animals with rumen pH<5.6, * P <0.01 vs. Group A, ** P <0.05 vs. Group B

Table 3

Average values (\pm SD) of rumen pH, homocysteine and vitamin B12, together with the relative statistical significances in experimental groups

Parameters	Experimental groups (Herd class)		
	Group 1 (n=65)	Group 2 (n=71)	Group 3 (n=92)
Rumen pH	6.16 \pm 0.37	6.01 \pm 0.31	5.85 \pm 0.34*, **
Vitamin B12, pg/mL	206.30 \pm 41.94	208.40 \pm 39.36	219.50 \pm 46.20
Homocysteine, μ mol/L	4.57 \pm 1.09	3.97 \pm 0.82*	3.85 \pm 0.85*

Group 1: normal herds – less than 33% cows with rumen pH<5.8), Group 2: critical herds – more than 33% cows with rumen pH between 5.5 and 5.8, Group 3: acidosis herds – more than 33% cows with rumen pH <5.5, * P <0.001 vs. Group 1, ** P <0.05 vs. Group 2

The application of one-way ANOVA in bovine class showed a statistical significance on rumen pH ($F_{(2,225)}=127.80$; P <0.0001) and vitamin B12 ($F_{(2,225)}=6.34$; P =0.0029). Data elaboration no pointed out statistically significant differences for homocysteine.

One-way ANOVA in herd class showed a statistical significance on rumen pH ($F_{(2,225)}=14.86$; P <0.0001) and homocysteine ($F_{(2,225)}=12.39$; P <0.0001). Data elaboration no pointed out statistically significant differences for vitamin B12.

Discussion

The analysis of the results obtained in the present study indicated an increase of vitamin B12 values in dairy cows with lower rumen pH and a decrease of homocysteine values in dairy herds with lower rumen pH.

Vitamin B12 concentration increased in animals with lower rumen pH, with significant values only in dairy cows with rumen pH<5.6, in fact in critical and acidosis herds vitamin B12 increased slightly. This significant increase obtained in Groups B and C vs. Group A could be explained considering the different microorganisms present in the rumen, their nutrient requirements and metabolism, what roles they play and how a perturbation or

an imbalance in the microbial population may lead to several metabolic disorders which can have a direct impact on productivity and health. In fact, together with the decrease of rumen pH significant quantities of vitamin B12 are synthesised in the rumen since microbes, particularly sensible to variations of rumen fluid, produce vitamins required by the cow (NRC 2001). The rumen contains a complex and diverse array of anaerobic bacteria and fungi, and specialized protozoa that live only in the rumen and synthesise vitamin K and all B vitamins inside their cells. They in turn supply these vitamins to other rumen microbes and finally to the animal confirming that the vitamins are an important growth factor for ruminal microorganisms (NRC 2001). Rumen bacteria have their own requirements for some of the B vitamins such as vitamin B12 that as demonstrated increased in our study. Moreover, as previously demonstrated (Schwab *et al.* 2006) a restricted roughage, a high-concentrate ration results in significantly lower liver and milk B12 levels and a higher total vitamin B12 activity in serum (Walker & Elliot 1972). So, the increase of obtained vitamin B12 values is due to the presence of analogues whose presence in considerable quantities interfere with the transport of the vitamin or with metabolic reactions involving in it (Sutton & Elliot 1972). In agreement with previous researches the vitamin B12 level seems to be linked to pattern of fatty acids in the rumen (Strobel 1992). In dairy cows affected by SARA during early lactation, rumen papilla are not fully developed leading to lower absorption of short chain fatty acids, increased concentration of them within the rumen and decrease of pH below physiological limits (Kleen *et al.* 2003). The ruminal wall and its papillae herein play an important role and their development has been described (Dirksen *et al.* 1984). The ruminal papillae are then of crucial importance in the absorption of short chain fatty acids and their proliferation is promoted by fatty acids arising from the fermentation (Morgante *et al.* 2007). This suggests that the increase of vitamin B12 was also dependent on propionate and butyrate because when the pH was below 5.6 ratio between acetic, propionic and butyric acid is shifted towards propionic and butyric acid (Strobel 1992, Hibbard *et al.* 1995). The suspect that vitamin B12 concentration in blood could decrease in cows affected by SARA because of lysis of bacteria synthesising this vitamin was rejected by our results which confirm that the rumen pH decrease during SARA, characterised by a transient character, determines minor damages in comparison to acute acidosis. We must also remind that vitamin B12 is stored in the liver (60 %) and in muscles (30 %) then signs of deficiency are delayed due to relatively high depot reserves, and a possible lack of production by rumen bacteria related to higher demand should be hid by these depots.

Although homocysteine unchanged in dairy cows, its values are significantly decreased in critical and acidosis herds. These modifications are linked to vitamin B12 levels and consequently to the fall of rumen pH. In fact, since homocysteine may undergoes remethylation to methionine in a pathway dependent on vitamin B12 and folate, its decrease results inversely proportional to the increase of vitamin B12 (Welch & Loscalzo 1998, Finkelstein & Martin 2000). The decrease reflects also the influence of vitamin deficiencies shown previously on plasma homocysteine levels (Kalkantar-Zadeh *et al.* 2003, Yeh & Yeh 2006).

The present data resulted in adequate vitamin B12 and homocysteine levels to meet microbial and cow requirements and fatty acids modifications in dairy cows affected by SARA. These findings may be useful to provide more information on blood modifications during the

subacute ruminal acidosis in bovine and may be used with advantage in occupational health examinations and in special clinical work. Since we know that consequences from SARA would arise after a certain delay from the initial insult (which make difficult the diagnosis) it is important to prevent the development of disease in critical dairy cows. So, further investigations should be done to assess the relationship between the rumen pH values, vitamin B12 and homocysteine levels in order to investigate blood levels of these parameters within a fixed period from SARA diagnosis.

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