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Original Article

Investigation on Effects of Parenterally Given Vitamin B Complex on Ruminal Protozoa in Cattle

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B complex vitamins play an important role in the metabolism of carbohydrates, proteins and fats. The aim of this study was to determine the effects of B complex vitamins administered by exogenous route on rumen protozoa, hematological and blood biochemical parameters in clinically healthy cattles. Thirty cattles aged between 6 to 18 months, and breeding in Afyonkarahisar Province and surrounding regions were studied. Ten clinically healthy animals served as control group. Twenty study group animals were given 10-20 ml B complex vitamins, 3 times during 3 days, by intramuscular route. Clinical (body temperature, pulse and respiration rates, rumen contractions), hematological (total leukocyte count, erythrocyte count, hemoglobin and hematocrit measurements), and serum biochemical (aspartate aminotransferase, glucose, total protein, albumin) parameters along with rumen protozoa status were measured in all animals. Regarding hematological parameters, there was no significant difference between the study groups in term of time intervals ($P > 0.05$). The mean values of WBC, RBC, neutrophils, HGB, HTC, MCV, MCH and MCHC were different between the case and the control groups ($P < 0.05$), but the mean values of lymphocytes, monocytes, and basophils were not different ($P > 0.05$). AST, SDH, ALP enzyme levels averaged within the reference limits, but were significantly higher in the case group ($P < 0.05$). Consequently, the use of B complex vitamins have proved to be of great benefit as it did not caused adverse effects on the digestive system at certain intervals in cattle.

Keywords: Afyonkarahisar, B complex vitamins, cattle, rumen, protozoa

Rumen produced B complex vitamins play an important role in the metabolism of carbohydrates, proteins and fats (1-3). Ruminants remove their needs by digesting bacteria found in rumen or by taking B vitamins that are free in the body fluid (4-7). B complex vitamins include different vitamins such as thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3 or PP), choline (vitamin B4), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin, cyanocobalamine (vitamin B12). Vitamin B1 plays a key role in energy metabolism, and is destroyed by an enzyme called thiaminase, which is secreted by some bacteria in the rumen (8). When the thiamin level falls, neuro-transmitters do not form, and communication between the nerves may be interrupted (9-12). The lack of vitamin B1 is very common in ruminants as oral antibiotics, sulfa group medications, and antacids can cause thiamine deficiency (1, 13). Vitamin B2 is crucial for some enzymes for fatty acid and amino acid synthesis. In the absence of riboflavin, non-specific symptoms such as anorexia, chronic diarrhea, and growth regression are seen (13). Niacin plays a critical role in oxidation and

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glucose synthesis of rumen-forming volatile fatty acids (VFAs), and inhibits the formation of ketosis (14). Choline is the antioxidant of acetylcholine, which indirectly plays an effective role in fat metabolism by increasing carnitine synthesis (15). Vitamin B5 is an obligatory vitamin in coenzyme A production, and plays a vital role as a catalyst in the conversion of carbohydrates, fats and proteins into energy (4, 16-19). Pyridoxine is absorbed from the ileum by passive diffusion when it is produced in rumen. Absence of this vitamin causes axonal degeneration, demyelination in the peripheral nerves, cramps, inadequate growth, and anemia (20, 21). In cattle, dietary biotin (vitamin B8) supplement has been shown to improve nail wall damages and many nail diseases caused by wet soils, eliminate pregnancy and lameness problems, and improve liver fatigue (7, 21). In absence of folic acid (vitamin B9), DNA synthesis is disturbed, and conversion of glycine and serine to each other becomes more difficult (5, 7, 18). In ruminants, the absorption of vitamin B12 has many beneficial effects such as destruction of an important amount of dietary cobalt by the rumen microflora (7), food proteins digestion and vitamin B12 release for the production of internal factors by a functional abomasum, and trypsin secretion by a functional pancreas for protein digestion (13, 22).

Hematological and blood biochemical parameters as well as rumen fluid analyzes provide important information about the animal's health status (23-25). To the best of our knowledge, there is no literature report directly investigating the effect of B complex vitamins administered by the parenteral route on the content of rumen in cattle, although there are some studies examining the effects of orally administered B complex vitamins on the rumen protozoa (11, 12).

In this study, we aimed to determine the effects of B complex vitamins administered by intramuscular (IM) route on rumen protozoa, hematological and blood biochemical parameters, in clinically healthy cattles.

Materials and methods

Study design

The study was carried out in Afyonkarahisar Province on 30 cattles (6 to 18 months old) who were breeding public (study group). Ten clinically healthy animals having the same age range and nutritional requirements were selected as control group. 10-20 ml B complex vitamins (MUL-TİKOM-B, Çelikler Pharmaceuticals and Trade Co., Ankara / Turkey) were given by IM route for 3 days to twenty case group animals. The control group animals were not treated. Clinical status, some rumen contents, haematological and blood biochemical parameters were examined on days 1, 3 and 7. This study has been carried out in the framework of ethics rules of Afyon Kocatepe University Ethical Committee of Animal Experiments with the reference number of AKUHA-DYEK 503-15, and was supported by Afyon Kocatepe University Scientific Research Projects Coordination Unit (BAPK) the 16.SAĞBİL.13.

Clinical evaluation

Clinical examinations such as body temperature, respiration and heart rate, and number of rumen contractions were performed according to the method reported by Hungate (26).

Hematological evaluation

Blood samples were taken from jugular venous in all animals, and hematological indices such as erythrocyte (RBC) and total leukocyte count (WBC), hematocrit (HCT), hemoglobin (HGB) level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were measured using commercial kits and a blood cell counter (Mindray BC2800 Vet).

Serum biochemical parameters

Serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), ornithine carbamoyl transferase (OCT), sorbitol dehydrogenase (SDH) were measured spectrophotometrically (SHIMADZU UV). Total proteins (TP), creatinine (CREA), albumin (ALB),

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glucose (GLU), total bilirubin (TB), and direct bilirubin (DB) were measured by an autoanalyzer (Roche brand Cobas C111 Model) using commercial kits.

Rumen fluid analyzes

Methylene blue test, total infusoria count, and sedimentation test along with pH values (Mulristix 10 SG-Bayer®-Germany) were measured in fresh rumen contents taken by rumen tubes according to Boyne (27).

Statistical analyzes

Statistical calculations were made according to the ANOVA method. The Duncan test was used to determine the difference between groups. Statistical analyzes were performed using the Windows-compatible SPSS 18.0 (Inc., Chicago, II, USA) package program. Data were presented as mean ± standard error, and $P < 0.05$ was considered as statistically significant.

Results

Ten out of 20 animals assigned to the case group were female and the remaining were male. The mean age of the case group was 13.2 ± 3 months. In the control group, 3 out of 10 cattles were female. The mean age of control group animals was 13.3 ± 2 months, and there was no statistically significant difference between the two groups in terms of age ($P > 0.05$).

Clinical findings

No significant difference was found with regard to body temperature, respiration, and heart rates between the groups. However, with respect to the ruminal movements at 5 minutes, the mean values of the rumen movement in the case group were significantly higher than the control group ($P < 0.05$), although the values obtained were within normal limits (Table 1).

Ruminal fluid findings

The mean number of infusoria reached the highest level on the 7th day (320.30± 104.90 mm^3), and these higher levels were significantly higher than the infusoria counts of the control and the other working groups ($P < 0.05$). On the contrary, in both case and control groups the methylene blue average times on the 7th day were lower than the first day of the case group (Table 2).

Hematological findings

Mean WBC and RBC values of the case group were found to be significantly higher ($P < 0.05$) when compared to those of the control group. The mean HGB levels measured on days 3 and 7 in the case group were found to be significantly higher ($P < 0.05$) when compared to those of the first day, and the control group (12.38± 1.10 g/dL and 8.16± 1.10 g/dL, respectively). The mean MCV value on 7th day in the case group was significantly higher than those of the control group, and the 1st and 3rd days. Similarly, the MCHC levels of the study group were significantly higher ($P < 0.05$) than the control group. Furthermore, the average number of neutrophils on the control group was significantly lower ($P < 0.05$) than the average of the case group (Table 3).

Metabolic profile findings

Table 1. Comparison of clinical features of the animals

Groups/Parameters	Control	1st Day	3rd Day	7th Day	Reference values*	P
Temperature (°C)	38,81± 1.42	38.74± 1.66	38.60± 1.67	38.56± 1.44	37.5-38.6	> 0.05
Pulse (beats/min)	94± 6	95± 5	96± 6	96± 5	70-120	> 0.05
Respiration (breaths/min)	26± 3	27± 2	27± 3	26± 3	15-30	> 0.05
Rumen movement (contractions/5 min)	7± 2^c	10± 2^b	10± 3^b	12± 2^a	6-12	**< 0.05**

a,b,c Different letters within the same column indicate statistically significant differences between the mean values of the groups ($P < 0.05$).

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The AST, ALP and SDH enzyme levels in the case group were found to be higher than the control group ($P< 0.05$), but the LDH and OCT levels did not differ significantly ($P> 0.05$). The case group urea level was found to be at the highest level (46.32± 6.04 mg/dL) on day 3, though within the reference limits. TP, ALB, CREA, TB, and DB mean concentrations were found to be significantly higher ($P < 0.05$) than the control group. The GLU concentration was interestingly at the lowest level (4.76 ± 1.32 mg/dL) on day 3 (Table 4).

Table 2. Lunar fluid analyzes of the icebergs in the control and study groups

Groups	pH	Infusoria (mm^3)	Methylen Blue Test (min)	Sedimentation Test (min)
Control	7.00± 0.00	162.80± 60.40^{b}	3.50±0.50^{a}	3.15±0.50^{b}
1st Day	7.00± 0.00	250.30± 90.20^{bc}	3.50±0.40^{a}	4.10±0.50^{a}
3rd Day	7.10± 0.00	230.60± 88.80^{b}	2.50±0.40^{b}	4.20±0.50^{a}
7th Day	7.10± 0.00	320.30± 104.90^{a}	2.50±0.40^{b}	4.30±0.50^{a}
P	> 0.05	**< 0.05**	p<0.05	p<0.05

a,b,c Different letters within the same column indicate statistically significant differences between the mean values of the groups ($P< 0.05$).

Table 3. Hematological findings and statistical analysis results

Groups	WBC (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fl)	MCHC (g/dL)	PLT (10^3/µL)	MCH (pg)	Lymphocytes (10^3/µL)	Monocytes (10^3/µL)	Neutrophils (10^3/µL)	Eosinophils (10^3/µL)	Basophils (10^3/µL)
Control	8.20± 2.20^{b}	5.10± 0.60^{b}	8.16± 1.10^{b}	26.80± 5.00^{b}	52.67± 8.20^{c}	30.40± 2.60^{c}	226.80± 19.48^{c}	16.10± 1.76^{b}	4.40± 0.40	0.72± 0.04	5.20± 1.80^{b}	1.48± 0.40	-
1st Day	9.80± 2.40^{a}	6.10± 0.40^{a}	12.38± 1.10^{a}	38.40± 4.60^{a}	62.94± 9.60^{ab}	32.28± 2.42^{b}	240.30± 34.30^{b}	20.22± 2.64^{a}	4.30± 0.60	0.70± 0.03	6.33± 2.18^{a}	1.65± 0.80	-
3rd Day	9.70± 2.60^{a}	6.30± 0.60^{a}	13.30± 2.10^{a}	39.10± 5.40^{a}	62.06± 9.88^{ab}	34.20± 3.68^{a}	242.12 ± [illegible]	21.10± 3.06^{a}	4.10± 0.80	0.70± 0.04	6.24± 2.30^{a}	1.63± 0.46	-
7th Day	9.90± 2.30^{a}	6.20± 0.60^{a}	13.60± 1.10^{a}	39.09± 4.80^{a}	64.40± 8.90^{a}	34.68± 2.38^{a}	284.28± 43.26^{a}	21.78± 2.20^{a}	4.30± 0.30	0.72± 0.02	6.32± 2.18^{a}	1.62± 0.66	-
Reference Values*	6-12	5-8	8-14	24-46	37-54	26-36	175-620	11-17	3-7.5	0.1-1.5	1.5-5	0.1–1.5	R
P	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05

a,b,c Different letters within the same column indicate statistically significant differences between the mean values of the groups ($P< 0.05$) R: rarely; * Filder (25).

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Table 4. Comparison of the metabolic profile parameters

Groups	AST (IU/L)	SDH (IU/L)	ALP (IU/L)	LDH (IU/L)	OCT (IU/L)	UREA (mg/dL)	CREA (mg/dL)	TP (g/dL)	ALB (g/dL)	GLU (mg/dL)	TB (mg/dL)	DB (mg/dL)
Control	102.28± 20.10^{d}	9.12±2.04^{b}	121.32±23.18^{b}	1246.78± 90.52	178.20± 12.28	40.12±50.22^{b}	1.22± 0.14^{b}	6.15± 0.10^{b}	2.94± 0.42^{b}	6.72± 1.28^{a}	0.32± 0.03^{d}	0.08± 0.001^{d}
1st Day	116.13± 24.40^{c}	10.44±2.20^{ab}	132.42±36.26^{a}	1240.20± 88.30	176.68± 14.24	45.24± 5.10^{a}	1.86± 0.20^{a}	6.96±1.12^{ab}	3.40±0.42^{ab}	5.45± 1.30^{b}	0.56± 0.02^{c}	0.10± 0.001^{c}
3rd Day	123.43± 32.22^{a}	11.24±1.60^{a}	134.32±40.40^{a}	1254.16± 72.12	177.30± 10.10	46.32± 6.04^{a}	2.02± 0.48^{a}	7.84± 1.40^{a}	4.24± 0.38^{a}	4.76± 1.32^{c}	0.70± 0.02^{a}	0.13± 0.001^{a}
7th Day	121.68± 36.20^{b}	11.32±1.88^{a}	136.42±38.30^{a}	1260.44± 80.00	183.22± 14.20	45.24± 6.12^{a}	1.96± 0.32^{a}	7.56± 1.28^{a}	4.36± 0.26^{a}	5.12± 1.44^{b}	0.62± 0.01^{b}	0.12± 0.001^{b}
Reference values	78-132	4.3-15.3	90-170	<1500	205± 135	42.8-64.2	1-2	6.7-7.5	3.0-3.6	4.5-7.5	0.1-0.5	0.04-0.14
P	**< 0.05**	**< 0.05**	**< 0.05**	> 0.05	> 0.05	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**

a,b,c Different letters within the same column indicate statistically significant differences between the mean values of the groups (P<0.05).

Discussion

Although ruminants are able to produce their own B complex vitamins by microbial synthesis in their rumen, some recent researchs have shown that ruminal synthesis of B complex vitamins may not be sufficient for an optimal production and health of today's cattle (especially dairy cattle) (12, 24, 25). Most B vitamins' microbial synthesis is increased by energy uptake, and decreased in the presence of B vitamins. In addition, there is a problem of complete destruction of the rumen which is added to the baits (4, 6). It has also been reported that the addition of vitamin B12 to food does not lead to a significant increase in blood B12 levels in the absence of methionine (25). A similar situation exists in cobalt (Co) deficiency (24, 28). This is why in the present study we used B complex vitamins by IM route. Moreover, studies have shown that injections of B complex vitamins are more effective than tablets, gels or liquids (22, 29-31).

In the present study, body temperature, respiration and heart rates were not significantly different from those of the control group, but the average of the case group was higher than the control group in terms of the number of rumen contractions at 5 minutes. These findings are consistent with those reported by other researchs showing that B complex vitamins stimulate the nervous system, and might lead to muscle contractions increase, and consequently higher rumen motility (32, 33).

Furthermore, in the present study, rumen total infusoria counts on the 7th day were higher than the mean values obtained in the control group or other

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time periods (days 1 and 3 after the beginning of the study), while sedimentation test averages were short. However, some researchs where B complex vitamins were administered intramuscularly led to significant positive changes on the ruminal microflora and fauna (12, 34).

The mean values of the WBC, RBC, neutrophils, HGB, HTC, MCV, MCH and MHCH in the case group were higher compared with those of the control group, but the mean values of lymphocytes, monocytes, and basophils were not different from those of the control group. Smilar findings were obtained by Uslu et al. (35) who reported that immunocapacitance increased after application of B complex vitamins.

We found that AST, SDH, and ALP enzyme levels in the case group were found to be significantly higher when compared with those of the control group, while being within the reference limits. A possible reason for this condition may be that B complex vitamins cause an increase in liver and kidney metabolism. In fact, B complex vitamins stimulate fat and protein metabolism (13). Similarly, higher urea and CREA levels can be attributed to increased ruminal fauna, and renal metabolic activity (3). It has also been reported that despite the ammonia flow in animals, nitrite conversion may decrease, resulting in an increase in blood urea levels (36). However, the increase in the concentration of CREA following the application of B complex vitamins has been adhered to increase the reabsorption activity of the kidneys (from the tubules), and possible irritation of the kidney tissue under this condition (37).

In the present study, we detected higher TP and ALB concentrations in cases compared to those of the control group. These findings were compatible with some other reports (38-40).

B vitamins, help particularly to convert foods taken by the body (vitamin B12) into glucose that can be further used by the body (24, 41, 42). In the present study, the lowest GLU concentration on day 3 (4.76± 1.32 mg/dL) might be due to the fact that B complex vitamins caused an increase in glucose utilization and catabolism. However, a quite large amount of B complex vitamins was needed for conversion of VFA's into energy (43).

Concentrations of TB and DB in our study were at the highest level on the 3rd day. This can be interpreted as a sign of an increase in liver metabolic activities as some researchers' reported previously (44-46). Correspondingly, some studies have shown that TB and DB concentrations can increase with the increase of liver and bile system activity (47-49).

Consequently, the use of B complex vitamins by İM route at certain intervals in cattle has proven to be of great benefit as it was not affected by the negative effects of their digestive system.

Conflict of interest

The authors declare that they have no competing interest.

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