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BLOOD METABOLITES AND HAEMATOLOGICAL INDICES OF BEEF CATTLE FED RUMEN-PROTECTED METHIONINE

LIKER B*, VRANEŠIĆ N**, GRBEŠA D*, BAČAR-HUSKIĆ LINA**, MATIĆ IVANA*, KNEŽEVIĆ M*, ŠPERANDA MARCELA***, LETO J* and MAĆEŠIĆ D*

**Faculty of Agriculture, Zagreb, Croatia, **PLIVA, Research Institute, Zagreb, Croatia, ***Faculty of Agriculture, Osijek, Croatia*

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In the course of the trial which lasted 94 days, and involved growing beef cattle, a close examination of the nutritive effects of rumen-protected methionine on biochemical and haematological values in the blood (total protein, albumin, triacylglycerols, total cholesterol, glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), haemoglobin concentration (Hb), haematocrit value (Hct), white blood cell count (WBC) and differential blood count) was carried out. Twenty-six beef cattle were divided into two groups of equal number: control (C) and experimental group (E). Cattle were fed with meadow hay, maize grain silage and a 500 g of protein rich concentrate (35% CP). In addition to the fodder mix, animals in group E received 10 g DL-rumen-protected methionine per animal daily. Blood samples were collected on the 1st, 34th, 68th and 94th day of the trial. Plasma glucose concentration and the total cholesterol concentration in cattle in group E showed a tendency to increase at the end of the trial (P=0.065 and P=0.064, respectively). Plasma urea concentration had a tendency to decrease in group E (P=0.082) by the end of the trial. The activity of ALT in animals in group E increased on the 68th and 94th day (P=0.127 and P=0.104, respectively). No significant differences were found between the groups in total protein, albumin, triacylglycerols, creatinine plasma concentrations, AST and GGT activities.

The results indicate that excessive supplementation of rumen-protected methionine might increase the level of plasma glucagon.

Key words: beef cattle, rumen-protected methionine, biochemical values, haematological values, glucagon.

INTRODUCTION

Absorbed amino acids, and not protein *per se*, are one of the required nutrients for animals. Ruminants need the same amino acids and have at tissue level similar protein metabolism as non-ruminants (ARC, 1980). However, in ruminants the amount and profile of amino acids available at the site of absorption do not correspond to amino acids in the feed ration and depend on the complex protein degradation in the rumen. In the rumen crude protein from the feed is degraded to a mixture of amino acids, ammonia and peptides for the synthesis of microbial protein, which supplies most amino acids in the small intestine (Iburg and Lebzien, 2000). The status of amino acids in the small intestine of ruminants can be modified by the use of feeds of different degradability (Liker, 1992) thus creating conditions which enable an increased rate of passage through the rumen (Faichney, 1986), or by adding protected or by-pass protein supplements into the feed (Bačar-Huskić, 1996).

In ruminants digestible protein is provided by microbial protein as the first (50-90%), and ruminally undegraded feedstuff protein as the second significant source of amino acids (NRC, 2001).

Microbial proteins are not synthesized in an amount that covers requirements during intensive growth and diet supplementation with sources of undegradable protein is therefore necessary. However, the two protein sources differ markedly in the composition of amino acids (Mrchen and Titgemeyer, 1992).

Microbial and most feedstuff proteins are a poor source of methionine and lysine relative to animal requirements (Williams and Smith, 1974; Storm and Ørskov, 1984). Methionine is considered to be an essential amino acid which often limits ruminant growth (Richardson and Hatfield, 1978). The supply of methionine from rumen microbial proteins is generally limited (Bequette *et al*., 2003).

Methionine was recognized as the first-limiting amino acid in diets which are based on small amounts of corn silage and grain added to high forage diets, or when soybean meal is the major source of rumen undegradable protein (Greenwood and Titgemeyer, 2000; NRC, 2001; Löest *et al*., 2002). Methionine, lysine and histidine might be the limiting amino acids in growing beef steers (Lapierre *et al*., 2000). Archibeque *et al*., (2002) identified methionine as a limiting amino acid for growing cattle. Supplement of rumen-protected methionine improves the average daily gain of growing cattle (Polan *et al*., 1991; Ainslie *et al*., 1993; Klemesrud *et al*., 1997; Klemesrud *et al*., 2000). However, even a modest excess of DL-methionine has toxic effects (Abe *et al.*, 2000). Blood metabolites and haematological indices of beef cows can be affected by feeding rumen-protected methionine during gestation (Liker *et al*., 2005).

Due to contradictory referential data, the effect of rumen-protected methionine, as one of the potentially deficient amino acids in nutrition of growing beef cattle, has been studied as a contribution to the optimalization of the composition of proteins feed to growing cattle.

MATERIAL AND METHODS

Animals and diets

Twenty-six Charolais growing beef cattle were randomly divided into two groups of 13 animals each; a control group (C) and an experimental group (E). Initial average body weight of beef cattle in the control group (C) and in the experimental group (E) was 251±30.7 kg and 249.6±43.8 kg, respectively. Cattle were fed a restricted amount of the basic diet consisting (as feed) of 3.5 kg/day natural grassland hay, 3.5 kg/day corn grain silage. In addition, cattle received 0.5 kg/day protein-rich concentrate to meet or exceed net energy, proteins digestible in the small intestine, and mineral requirements for growing beef cattle. Cattle in both groups were fed the same basic diet, but cattle in Group E were fed a mixture to which 10 g rumen-protected methionine per animal per day was added.

The rumen-protected methionine (Mepron M85®; Degussa Hülls AG, Hanau, Germany) used in this study is a methionine analogue that is physically protected by an ethylcellulose and stearic acid film. This enables it to resist the fermentation action of microorganisms, but also to quickly disintegrate in the abomasum due to the acid pH condition. This commercial form comes in small, 1.8 mm diameter and 2.5-4 mm long capsules containing 85% DL-methionine synthesized from DL-2-hydroxy-4-calcium methylithiobutanoic acid.

Feeding was performed individually at regular intervals. Cattle of both groups were housed in a common stall, i.e. in similar micro-climatic conditions. Water was provided *ad libitum* from automatic dispensers.

Cattle were weighed at the beginning and at the end of the trial period. The health status was checked daily.

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture.

Sampling and analyses

Chemical analysis was performed according to AOAC (1990) at the Department of Animal Nutrition, Faculty of Agriculture (Zagreb, Croatia). The net energy (MJ/kg DM) for growing (NE for growing) and the estimated contents (g/kg DM) for both the protein digested in the small intestine originated from the degraded dietary protein (PDIE) in the rumen and the protein digested in the small intestine originated from the organic matter fermented rumen (PDIN) were calculated (INRA, 1989). The concentration of methionine in the feed protein and PDIN was taken from NRC (2001) and Rulquin *et al*. (2001). Mepron 85M was considered to have a ruminal escape value of 85% and an intestinal digestible coefficient of 90% (Schwab, 1995).

Blood samples were taken for biochemical analysis on days 1, 34, 68 and 94 of the trial. Blood samples were drawn by venipuncture from the *v. jugularis*. The samples (10 ml) were stored in Greiner test tubes with EDTA, or without added anticoagulant. Immediately after drawing the sample the blood was centrifuged

(3500 r.p.m.) for 20 min; the plasma or serum separated and used for determination of total protein, albumin, triacylglycerols, total cholesterol, glucose, urea, creatinine, ALT, AST and GGT.

All biochemical values were determined with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland, manufactured for Olympus Diagnostic GmbH, Hamburg, using OLYMPUS AU 600 apparatus. Catalogue numbers: total protein, OSR6232; albumin, OSR6202; triacylglycerols, OSR6214; total cholesterol, OSR6216; glucose, OSR6222; urea, OSR 6234 and creatinine, OSR6118. Enzyme activities were determined in sera using Thermo Trace Ltd (Australia) tests. Catalogue numbers: ALT (IFCC), TR-1078; AST (IFCC), TR-1068; GGT, TR-1215 and ALP (IFCC), TR-1105 using OLYMPUS AU 600 apparatus.

In each of the samples the RBC, MCV, MCH, RDW, Hb, Hct, WBC and differential WBC count were determined using a Coulter Counter JT apparatus. For the differential WBC count the smears were coloured in accordance with the Papenheim method.

Statistical analysis

Differences between the control and trial groups were statistically tested using the repeated measurement model with PROC MIXED (SAS Institute, release 8.02.). Heterogeneity of variance was also tested, based on the previously mentioned method. Treatment differences were considered significant for $P<0.05$.

RESULTS

The chemical composition and nutritive values of the dietary ingredients of the basic diet are shown in Table 1.

The influence of the diet containing protected methionine on biochemical values in blood plasma compared with the control group is shown in Table 2.

Measurements performed at the end of the trial showed that the concentration of total cholesterol tended ($P=0.0640$) to increased in the experimental group.

On day 94 plasma glucose concentration tended to be higher and urea lower, due to methionine supplementation on the 94th day ($P=0.0650$ and $P=0.0818$, respectively).

Plasma concentrations of total protein, albumin, and triacylglycerols were not significantly affected by adding rumen-protected methionine, even though their levels in the experimental group were lower throughout the trial.

The activity of alanine aminotransferase in group E was higher throughout the experiment; its value on the 68th day and at the end of the trial tended to be higher ($P=0.1272$ and $P=0.1043$, respectively).

The influence of the diet containing protected methionine on haematological values is shown in Table 3.

Table 1. Chemical composition, nutritive value of dietary ingredients and daily nutrients intake

Item	Meadow hay	Corn grain silage	Mepron® M85	Protein-rich concentrate[21]
*Chemical composition, g.kg^{-1} of DM**				
dry matter	845.00	750.00	990.00	916.00
crude protein	82.00	78.00	500.00	377.00
ether extract	16.00	40.00	20.00	13.80
crude fibre	305.00	17.00	30.00	101.00
N-free extractives	534.00	852.00	445.00	355.00
ash	63.00	13.00	20.00	189.00
Ca	3.30	0.70		19.30
P	2.80	2.80		17.30
Calculated nutritive value				
NE for growing, MJ·kg^{-1} DM[3]	4.39	2.96	5.02	6.10
methionine, g·kg^{-1} DM	1,10	1,65	850,00	6,67
methionine, g·kg^{-1} CP[4]	13,0	21,10	1700,00	17,70
PDIN, g·kg^{-1} DM[5]	51.65	74.00	656.82	247,70
PDIE, g·kg^{-1} DM[6]	62.67	121.00	0,00	113.50
methionine DI[7] g·kg^{-1} of PDI	19,1	20,1	650,25[8]	17,40
Daily nutrients intake	*Group*			
	Control (C)		Experimental(E)	
total dry matter, kg·d^{-1}	6.05		6.05	
NE for growing, MJ·d^{-1}	30,1		30.1	
crude fiber, g·d^{-1}	883.3		883.3	
ether extract, g·d^{-1}	79.2		79.2	
Ca, g d^{-1}	37.7		37.7	
P, g·d^{-1}	20.5		20.5	
crude protein, g·d^{-1}	517.0		567.0	
PDIN, g·d^{-1}	379.7		386.2	
PDIE, g·d^{-1}	442.5		442.5	
methionine, g·d^{-1}	10.4		18.9	
methionine, g·kg^{-1} DM	1.7		3.2	
methionine, g·kg^{-1} CP	20.1		33.3	
methionine DI in PDI, g·kg^{-1}	5.95		10.13	

* AOAC, 1990; [1]Manufacture's standard (Degussa-Hülls AG, Hanau, Germany); [2]Protein-rich concentrates contain (50 g corn/kg, 100 g wheat bran/kg, 150 g soybean meal (44% CP)/kg, 320 g sunflower (34% CP)/kg, 200 g rape seed meal (32% CP)/kg, 50 g Benural S (42% urea)/kg, 40 g; limestone/kg, 50 g mono ammonium phosphate/kg, 20 g salt/kg, 20 g minerals/vitamins supplement/kg; [3]NE for growing =net energy for growing was calculated according to Ruminant Nutrition (INRA, 1989); [4]Data from NRC (2001); [5]PDIN Protein digested in the small intestine from rumen degraded dietary protein estimated from Ruminant Nutrition (INRA, 1989); [6]PDIE Protein digested in the small intestine from rumen fermented organic matter estimated from Ruminant Nutrition (INRA, 1989); [7]DI - concentration of the digestible methionine in the small intestine per kg PDI (protein digestible in the small intestine) according to Rulquin et al. (2001); [8]Mepron M85 was considered to have 85% DL -methionine, 85% escape protein and intestinal digestibility of 90%, so (850 x 0,85 x 0,90) 650 g/kg is in small intestine digestible DL-methionine.

Table 2. Biochemical values in blood plasma of beef cattle fed rumen-protected DL-methionine (n=13)

Bichemical parameters	Days of trial	Group		SEM	P
		control (C)	experimental (E)		
Total protein, g· L^{-1}	1	69.47	68.06	0.7542	0.8855
	34	73.22	70.95		0.4114
	68	73.31	70.71		0.2380
	94	71.52	69.03		0.2899
Albumin, g· L^{-1}	1	32.81	32.73	0.5378	1
	34	35.06	34.28		0.9686
	68	34.84	34.04		0.9648
	94	34.04	32.11		0.1984
Triacylglycerols, mmol· L^{-1}	1	0.2177	0.207	0.0137	0.9995
	34	0.1923	0.1546		0.5215
	68	0.2264	0.1823		0.3180
	94	0.2131	0.1992		0.9962
Total cholesterol, mmol· L^{-1}	1	3.06	3.05	0.1039	1
	34	2.24	2.31		0.9998
	68	3.05	3.34		0.5097
	94	3.20	3.65		0.0640
Glucose, mmol · L^{-1}	1	5.33	5.42	0.1093	0.9982
	34	5.7	5.86		0.9665
	68	5.39	5.68		0.5882
	94	4.58	5.05		0.0650
Urea, mmol· L^{-1}	1	1.42	1.45	0.1508	1
	34	2.79	2.6		0.9882
	68	3.08	2.84		0.9456
	94	2.69	2.06		0.0818
Creatinine, μmol· L^{-1}	1	127.26	131.81	4.2218	0.9945
	34	115.50	127.41		0.4920
	68	127.69	131.22		0.9989
	94	130.22	132.29		1
ALT, U·L^{-1}	1	31.02	30.62	1.0715	1
	34	31.32	34.01		0.6375
	68	31.35	35.51		0.1272
	94	29.94	34.22		0.1043
AST, U·L^{-1}	1	91.09	90.53	4.21	1
	34	90.70	94.39		0.9985
	68	94.81	99.21		0.9954
	94	116.08	115.22		1
GGT, U·L^{-1}	1	12.53	12.55	1.2023	1
	34	7.92	9.18		0.9955
	68	7.07	5.96		0.9980
	94	17.38	17.09		1

Table 3. Haematologic values of growing beef cattle fed rumen-protected methionine (n=13)

Haematologicl parameters	Days of trial.	Group		SEM	P
		control (C)	experimental (E)		
RBC, $10^{12}L^{-1}$	1	9.12	9.02	0.1669	0.9999
	34	8.73	8.67		1
	68	8.70	8.54		0.9973
	94	8.75	8.42		0.8552
MCV, f L^{-1}	1	41.31	41.21	0.2155	1
	34	41.22	41.3		1
	68	40.78	41.05		0.9867
	94	40.4	40.4		1
RDW, %	1	33.41	34.32	0.3817	06899
	34	34.54	34.93		0.9959
	68	35.14	35.54		0.9954
	94	34.95	35.06		1
Haemoglobin, g L^{-1}	1	117.2	118.5	2.5168	1
	34	110.6	113.6		0.9975
	68	112.0	114.3		0.9980
	94	114.7	113.6		1
Haematocrit, L L^{-1}	1	0.3801	0.3731	0.0072	0.9971
	34	0.3608	0.3579		1
	68	0.3558	0.3518		0.9999
	94	0.3532	0.3415		0.9430
Thrombocytes, 10^{9} L^{-1}	1	440	410	30.97	0.9971
	34	550	487		0.8421
	68	497	455		0.9766
	94	483	396		0.4992
WBC, 10^9/L	1	9.51	9.51	0.3083	1
	34	9.54	10.22		1
	68	10.30	9.75		0.9664
	94	9.35	9.37		1
Basophils, %	1	3.38	3.38	0.4042	0.9922
	34	1.38	1.00		0.9975
	68	1.92	1.08		0.8152
	94	1.46	1.15		0.9994
Eosinophils, %	1	4.92	4.92	1.0474	1.0
	34	5.61	6.31		0.9998
	68	5.0	6.69		0.9449
	94	5.61	7.85		0.8018
Neutrophils, %	1	25.23	24.77	1.9746	1
	34	29.85	26.85		0.9601
	68	28.38	26.85		0.9993
	94	24.77	28.69		0.8521

Limfocytes, %	1	64.85	65.61	2.1026	1
	34	62.23	63.92		0.9991
	68	64.31	62.54		0.9988
	94	64.85	61.0		0.3957
Monocytes, %	1	1.6154	1.77	0.2502	0.9999
	34	1.77	1.54		0.9979
	68	1.23	2.0		0.3798
	94	1.08	1.0		1.0

DISCUSSION

Chemical analysis and calculated nutritive values of ingredients were consistent with reported values (NRC, 2001, Rulquin *et al*., 2001). The addition of Mepron 85 in the diet of the experimental group significantly increased the predicted methionine concentration in DM and CP by 188% and 165%, respectively (Table 1).

On average, the diet met or exceeded the net energy intake, digestible protein in the small intestine and mineral requirements for moderate (850 $g \cdot d^{-1}$) growing bulls from 250 to 350 kg body weight in the winter season (INRA, 1989). All animals consumed similar amounts of non-protein nutrients, but bulls in the experimental group ate more protein nutrients due to the added 10 g/day of Mepron 85. Supplementation of protected methionine increased the predicted concentration of intestinal digestible methionine from 1.57% to 2.9% of total digestible amino acid supply (PDI). The optimal use of PDI for combined maintenance and milk protein production requires a concentration of the digestible intestinal methionine of 2.4% (NRC 2001), or very close to a value of 2.5% (Rulquin *et al*., 1993). It is thought that beef cattle require less methionine DI/PDI for maintenance and growth, than do lactating cows for maximal milk protein production.

Since the diet containing protected methionine did not affect body weight gain it can be assumed that methionine was not the limiting amino acid. No evident changes were found in metabolic and haematological indicators which would support the findings of a deficiency of methionine in group C, which was found to be possible in late pregnancy (Liker *et al*., 2005). Potentially, the protein source of protein-rich concentrates may have provided an adequate concentration of methionine in the duodenum without the need for amino acid supplementation. A low level of urea in blood indicates a borderline energy supply of ruminant microorganisms.

This is in agreement with the findings by Hussein and Berger (1995); Tripp *et al*. (1998). In contrast, several groups were found to have increased N balance or improved average daily gain (ADG) in cattle supplemented with rumen-protected methionine (Titgemeyer and Merchen, 1990; Campbell *et al*., 1996; Campbell *et al*., 1997; Froidmont *et al*., 2000; Klemesrud *et al*., 2000; Greenwood and Titgemeyer, 2000; Lambert *et al*., 2002). Lysine was also found to be the limiting amino acid (Hill *et al*., 1980). The requirement of growing beef cattle for methionine is in the range from 207 to 324 $mg/kg^{0.75}$ (Williams, 1994; Froidmont *et*

al., 2000) and depends on cattle type and yield and composition of daily gain (NRC, 1996).

The experimental diet containing rumen-protected methionine tended to influence the concentration of glucose, urea, and total cholesterol in the final phase of the trial and the activity of ALT in the second half of the experiment. In group E there were decreased concentrations of total protein, albumin and triacylglycerols throughout the experiment.

Such changes could be the result of supplemented methonine and the influence of the increased level of methionine on the endocrine system. The concentration of an amino acid in the blood should remain low and relatively constant when its supply is less than its requirement. The concentration of an amino acid in the blood increases when the supply is above the animal's need (Bergen, 1979). The splanchnic flux of essential amino acids increased with increasing feed intake, leading to elevated arterial blood concentration, with the exception of histidine, lysine and methionine, and there was a strong correlation between portal release of glucagon and hepatic removal of total amino acids (Lapierre *et al*., 2000).

Amino acids are a potent signal that regulates protein metabolism, either directly or indirectly (Jefferson and Kimball, 2001).

The control of hepatic removal of essential amino acids seems to be under tight control, and catabolism of histidine, methionine and phenilalanine is believed to occur mainly in the liver (Bequette *et al*., 2003).

Several amino acids are capable of stimulating hormone synthesis and secretion (Kuhara *et al*., 1991). A protein meal and an infusion of various amino acids increase glucagon secretion (Peret *et al.*, 1981). Patients with a glucagonoma have a diminished level of plasma amino acids (Barazzoni *et al*., 1999). It seems appropriate that the glucogenic amino acids are particularly potent in this regard, since these are the amino acids that are converted into glucose in the liver under the influence of glucagon. The glucagon response to oral administration of amino acids is greater than the response to intravenous infusion of amino acids, suggesting that a glucagon-stimulating factor is secreted from the gastrointestinal mucosa. Cholecystokinin (CCK) and gastrin increase glucagon secretion (Ganong, 2005).

The best predictor of plasma glucagon is plasma methionine (Calbert and Maclean, 2002). Methionine transport was elevated two-fold in hepatocytes isolated from glucagon-treated rats (Jacobs *et al*., 2001). Methionine supply to growing steers affects hepatic activities of methionine synthase and betaine-homocisteine methyltransferase (Lambert *et al.*, 2002). Methionine treated beef cattle (6 g/animal^{-1}/day) tended ($P=0.1$) to consume more feed than untreated animals throughout most of the treatment period, with similar daily gains ($P=0.82$) (Tripp *et al*., 1998).

Just as the surplus of methionine initiates the release of glucagon, so its deficit stimulates the activity of the adrenal cortex. Infusion of methionine, lysine and leucine to Angora goats decreased plasma cortisol concentrations ($P<0.05$) (Puchala *et al.*, 1995). A deficit of methionine in beef cows in late pregnancy can

cause the reaction manifested as increased secretion of glucocorticoids (Liker *et al*., 2005).

Increased levels of glucose, total cholesterol and ALT in group E can be explained by glucagon increase. Glucagon increases glucose output from the liver, lowers insulin-glucagon ratio, increases plasma glucose (Ganong, 2005) and tends to increase the concentration of plasma HDL_2-cholesteryl ester, plasma LDL-free cholesterol, plasma LDL-cholesteryl ester and plasma HDL_1-protein (Bobe *et al*., 2003). The increased gluconeogenesis in liver of lactating cows (Baldwin and Smith, 1983) and in the *in vitro* trial, was accompanied by an increase in ALT and AST activities (Leena *et al*., 1999).

The combined effect of glucagon and growth hormone can not be excluded. Glucagon stimulates the secretion of growth hormone. Concentrations of IGF-I were increased and the longissimus muscle area tended to increase in the two control points in methionine-supplemented animals (Tripp *et al*., 1998). Since high density lipoproteins (HDLs) are predominant lipoproteins in ruminants, and serve to deliver cholesterol to steroidogenic tissues (Drackley, 2000), and the application of growth hormone significantly increased HDL-cholesterol (Tanriverdi *et al*., 2005) the increase in total cholesterol in our trial could have been caused by growth hormone. All the more so, since it is difficult to explain the tendency of the urea reduction differently. It is possible that in the final period of the experiment occurred a mild methionine deficit in animals in group C, which caused increased ureagenesis. Gain weight in growing animals decreases the efficiency of utilization of supplemental methionine (Titgemeyer, 2003). If we were to take the values from 207 to 324mg/$kg^{0.75}$ (Williams, 1994; Froidmont *et al*., 2000) as being borderline with regard to the methionine requirement of high growing beef cattle, then the first signs of methionine deficiency in animals could have appeared at the very end of the experiment in the form of increased urea concentration in the blood. Supplementation with a higher concentration of methionine decreased urinary N excretion (Awawdeh *et al*., 2004). If the nutrients are inadequate, or i dietary intake of N exceeds the metabolic capacity to retain N, then urea production will increase (Archibeque *et al*., 2002).

CONCLUSION

In the present experiment, methionine did not appear to be a limiting amino acid in the diet of growing bulls in the winter season. Supplementation of rumen-protected methionine above nutritive requirements could have an influence on the endocrine system, causing a possible increase in glucagon and growth hormone.

Address for correspondence:
Branko Liker, Faculty of Agriculture,
University of Zagreb, Svetošimunska 25,
Zagreb, Croatia,
E-mail:bliker@agr.hr

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VREDNOSTI HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA U KRVI TOVNE JUNADI HRANJENIH ZAŠTIĆENIM METIONINOM

LIKER B, VRANEŠIĆ N, GRBEŠA D, BAČAR-HUSKIĆ LINA, MATIĆ IVANA, KNEŽEVIĆ M, ŠPERANDA MARCELA, LETO J i MAĆEŠIĆ D

SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja uticaja, u buragu nerazgradljivog, zaštićenog (by-pass) metionina na neke vrednosti hematoloških i biohemijskih parametara u krvi tovne junadi, uključujući ukupne proteine, albumine, triacilglicerole, ukupni holesterol, glukozu, ureu, kreatinin, aktivnost alanin-aminotransferaze (ALT), aktivnost aspartat-aminotransferaze (AST), aktivnost gama-glutamil-transferaze (GGT), broj eritrocita (RBC), srednji volumen eritrocita (MCV), srednju vrednost hemoglobina eritrocita (MCH), raspodelu eritrocita po volumenu (RDW), koncentraciju hemoglobina (Hb), hematokrit (Hct), broj leukocita (WBC) i diferencijalnu krvnu sliku.

Istraživanje je trajalo 94 dana i izvršeno je na 26 životinja podeljenih u dve jednake grupe, kontrolnu (C) i oglednu (E). Junad je hranjena livadskim senom i kukuruznom silažom uz dnevni dodatak 500 g krmne smese (35% CP) po grlu. Životinje iz grupe E su dnevno, po grlu, dobijale i 10 g DL-metionina zaštićenog od razgradnje u buragu. Uzorci krvi su uzimani 1., 34., 68. i 94. dana ogleda.

Nivo glukoze i ukupnog holesterola u plazmi grupe E je pokazivao tendenciju rasta prema kraju ogleda (P=0,065 odnosno P=0,064), dok je nivo uree imao tendenciju pada (P=0,082). Aktivnost ALT, kod životinja grupe E, je pokazivala 68. i 94. dana tendenciju porasta (P=0,127 odnosno P=0,104). Razlike u koncentraciji ukupnih proteina, albumina, triacilglicerola, kreatinina i aktivnosti AST i GGT između grupa nisu bile signifikanatne. Ovi rezultati ukazuju da bi suvišak metionina mogao da izazove porast nivoa glukagona u krvnoj plazmi.