

## Blood metabolites and haematological indices of pregnant beef cows fed rumen-protected methionine

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### ABSTRACT

The effect of rumen-protected DL-methionine on some blood biochemical and haematological values of cows in approximately the last 102 days of pregnancy was examined. Twenty-six cows in the third stage of gestation were divided into two groups of equal number, a control (C) and an experimental group (E). Cows were fed meadow hay, maize grain silage and a 500 g fodder mixture (35% CP). In addition to the fodder mixture, cows in group E received 15 g rumen-protected DL-methionine per animal daily (Mepron<sup>®</sup> M85). Blood samples were collected on approximately days 102, 68, 34 and 1 prior to parturition.

Plasma glucose concentrations of cows in group E were significantly decreased over a long part of the trial period (68 and 34 days prior to parturition) ( $P=0.046$  and  $P=0.0175$ , respectively). Plasma urea concentration in group C was decreased close to statistical significance ( $P=0.053$ ) on the 68<sup>th</sup> day prior to parturition. No significant differences were found in plasma concentrations of total protein, albumin, triacylglycerols, total cholesterol and creatinine between the two groups during the trial.

The activity of alanine aminotransferase in animals of group E was lower, close to statistical significance ( $P=0.052$ ) 34 days prior to parturition. No significant differences were found in plasma of aspartate aminotransferase, gamma-glutamyltransferase and alkaline phosphatase levels between groups during experimental feeding. Cows in group E had a lower red blood cell count on the

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68<sup>th</sup> and 34<sup>th</sup> days prior to parturition ( $P=0.316$  and  $P=0.153$ , respectively). On the 68<sup>th</sup> day before calving the lower number of leukocytes was close to statistical significance ( $P=0.0785$ ), with highly significant neutropenia in absolute ( $P<0.001$ ) and relative ( $P=0.039$ ) terms, and a decreased neutrophils/lymphocytes ratio ( $P<0.001$ ).

The results indicate that rumen-protected methionine could have an anti-stress effect on cows during late gestation in winter stable feeding and holding conditions.

**KEY WORDS:** cows, gestation, rumen-protected methionine, biochemical values, haematological values

## INTRODUCTION

Methionine is considered to be an essential amino acid that often limits ruminant growth (Richardson and Hatfield, 1978) and the first-limiting amino acid for beef cattle (Greenwood and Titgemeyer, 2000). Archibeque et al. (2002) identified methionine as a limiting amino acid for growing cattle. The supply of methionine from rumen microbial proteins is generally small (Bequette, 2003).

The current model estimating energy requirements for pregnancy appears adequate in contrast to the protein requirement model (Van Saun and Sniffen, 1996). Specific effects of protein deprivation on partitioning of amino acids between maternal tissues and the conceptus have not been examined in pregnant ruminants. In ewes that were fed the predicted requirement for dietary protein ( $>90$  g/day<sup>-1</sup> digestible crude protein), the available pool of circulating amino acids was augmented by the net mobilization of protein from maternal carcass tissues (mostly skeletal muscle) amounting to almost 10% of the digestible crude protein intake (Bell and Ehrhardt, 2000). Feeding protein at levels above predicted requirements (160 g/kg<sup>-1</sup> vs 120 g/kg<sup>-1</sup> DM) allowed significant net accretion of maternal carcass tissue protein during late pregnancy in ewes (McNeill et al., 1997).

Methionine was the limiting amino acid in preparturient dairy cows receiving a high crude protein and high rumen undegradable protein diet during the last two weeks of pregnancy (Bach et al., 2000). Cellular hypertrophy and the increased deposition of extracellular material in the foetus during the last third of gestation require large increases in nutrient supplies (Hay, 1999).

With reference to this, the effect of rumen-protected methionine as one of the potentially deficient amino acids in nutrition of cows in an advanced state of gestation was researched as a contribution to the composition optimization of the protein component in the feed given to pregnant ruminants. Some biochemical and haematological values as indices of metabolic status were used for this purpose, taking into account functional roles of amino acids in growth, general metabolism and health.

## MATERIAL AND METHODS

### *Animals and diets*

Twenty-six approximately five-year-old, pregnant (final 102 days of gestation) Charolais cows in the third stage of gestation were randomly divided into two groups of 13 animals, a control (C) and an experimental group (E). The initial average body weight of cows in the groups C and group E was  $558.3 \pm 77.5$  kg and  $558.6 \pm 76.5$  kg, respectively. The cows were fed restricted amount of a basal diet consisting (as feed) of 5 kg d<sup>-1</sup> natural grassland hay, 5 kg d<sup>-1</sup> wheat straw and 3 kg d<sup>-1</sup> maize grain silage. In addition, cows received 0.5 kg d<sup>-1</sup> protein-rich concentrate to meet or exceed net energy, protein digestible in small intestine, and mineral requirements of late pregnancy Charolais cows in average condition (BCS 2.5-3.0) that were calving from late winter to early spring (INRA, 1989). Cows of both groups were fed equally, but cows in group E were fed a mixture to which 15 g rumen-protected methionine was added daily, per cow.

The rumen protected methionine ((Mepron M85®; Degussa Hüls 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 the rumen 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 methylthio-butanoic acid.

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

Cows were weighed at the beginning and the end of the trial period. The cows' stage of health was checked daily.

The 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). The net energy for lactation (NEL) and both protein digested in the small intestine from rumen-degraded dietary protein (PDIE) and protein digested in the small intestine from rumen-fermented organic matter (PDIN) were calculated (INRA, 1989). The concentration of lysine and methionine in the feed's 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 and haematological analysis on four occasions, i.e. on approximately days 102, 68, 34 and 1 prior to parturition. Blood samples were drawn by venipuncture from *v. jugularis*. Blood samples (10 ml) were stored in Greiner test tubes with EDTA (2x) or without anticoagulant, and were taken from all cows involved in the experiment. Immediately after drawing, the blood sample was centrifuged (3500 r.p.m.) for 20 min, the plasma or serum was separated and was then used for determination of biochemical parameters, including total protein, albumin, triacylglycerols, total cholesterol, glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP). 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; creatinin, OSR6118. Activity of enzymes activities was determined in serum with Thermo Trace Ltd. (Australia) tests. Catalogue numbers: ALT (IFCC), TR-1078; AST (IFCC), TR-1068; GGT, TR-1215; ALP (IFCC), TR-1105 using OLYMPUS AU 600 apparatus.

In each of the samples, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), Hb, haematocrit value (Hct), thrombocyte count, mean platelet volume (MPV), white blood cell count (WBC) and differential WBC count were determined using a coulter counter JT apparatus. For the differential WBC count the smears were stained in accordance with the Papanheim method.

### *Statistical analysis*

Differences between the control and trial groups were statistically tested using repeated measurement model with PROC MIXED (SAS Institute, release 8.02.). Heterogeneity of variances was also tested based on the above method.

## RESULTS

The chemical composition and nutritive value of both dietary ingredients and the basal diet are shown in Table 1.

TABLE 1

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

Item	Meadow hay	Wheat straw	Maize grain silage	Mepron® M85 <sup>1</sup>	Protein-rich concentrate <sup>2</sup>
<i>Chemical composition, g · kg<sup>-1</sup> of DM*</i>					
dry matter	845.00	867.00	750.00	990.00	925.00
crude protein	82.00	39.00	78.00	500.00	367.00
ether extract	16.00	36.00	17.00	10.00	18.00
crude fibre	305.00	405.00	40.00	30.00	80.00
N-free extractives	534.00	424.00	852.00	445.00	355.00
ash	63.00	96.00	13.00	15.00	180.00
Ca	3.30	1.70	0.70		16.00
P	2.80	0.60	2.80		16.00
<i>Calculated nutritive value</i>					
NEL, MJ · kg <sup>-1</sup> DM <sup>3</sup>	4.30	2.96	8.55	5.20	6.33
lysine, g · kg <sup>-1</sup> CP <sup>4</sup>	42.50	32.50	26.40		44.40
methionine, g · kg <sup>-1</sup> CP <sup>4</sup>	14.30	11.90	21.10	1700.00	18.80
PDIN, g · kg <sup>-1</sup> DM <sup>5</sup>	51.65	24.80	74.00	656.82	395.25
PDIE, g · kg <sup>-1</sup> DM <sup>6</sup>	62.67	48.40	121.00		303.05
methionine DI <sup>7</sup> % of PDI	19.1	19.8	20.1	650.25 <sup>8</sup>	17.40
<b>Group</b>					
<i>Daily nutrients intake</i>	control		experimental		
total dry mater, kg d <sup>-1</sup>	11.3		11.3		
NEL, MJ g · d <sup>-1</sup>	53.0		53.0		
crude fibre, g · d <sup>-1</sup>	3172.0		3172.0		
ether extract, g · d <sup>-1</sup>	238.0		238.0		
Ca, g · d <sup>-1</sup>	64.0		64.0		
P, g · d <sup>-1</sup>	29.0		29.0		
crude protein, g · d <sup>-1</sup>	1012.0		1019.5		
PDIN, g · d <sup>-1</sup>	607.4		617.3		
PDIE, g · d <sup>-1</sup>	799.2		799.2		
methionine, % in DM	1.30		2.50		
methionine, % in CP	1.55		1.83		
methionine DI in PDI, %	0.97		1.94		

\* AOAC, 1990; <sup>1</sup> Manufacture's standard (Degussa-Hülls AG, Hanau, Germany); <sup>2</sup> protein-rich concentrates contain, g/kg: maize 50, wheat bran 100, soyabean meal 150 (44% CP), sunflower 320 (34% CP), rape seed meal 200 (32% CP), Benural S 50 (42% urea), limestone 40, mono ammonium phosphate 50, salt 20, minerals/vitamins supplement 20; <sup>3</sup> NEL=net energy for lactation were calculated according to Ruminant Nutrition (INRA, 1989); <sup>4</sup> Data from NRC (2001); <sup>5</sup> PDIN Protein digested in the small intestine from rumen degraded dietary protein estimated from Ruminant Nutrition (INRA, 1989); <sup>6</sup> PDIE Protein digested in the small intestine from rumen fermented organic matter estimated from Ruminant Nutrition (INRA, 1989); <sup>7</sup> DI is concentration of the digestible methionine in the small intestine per kg PDI (protein digestible in the small intestine) according to Rulquin et al. (2001); <sup>8</sup> Mepron M85 was considered to have 85% DL methionine, 85% escape protein and intestinal digestibility of 90%, so (850 × 0.85 × 0.90) 650 g/kg is in small intestine digestible DL-methionine

Plasma concentrations of total protein, albumin, triacylglycerols, total cholesterol and creatinine were not affected by adding rumen-protected methionine (Table 2).

TABLE 2  
Biochemical values in blood plasma of pregnant cows fed rumen-protected DL- methionine (n=13)

Biochemical parameters	Days prior to parturition	Group		SEM	P
		control	experimental		
Total protein, g·L <sup>-1</sup>	102	76.07	75.36	1.20	1
	68	78.85	78.24		1
	34	81.95	79.90		0.93
Albumin, g·L <sup>-1</sup>	1	77.82	78.36	0.49	1
	102	32.18	31.00		0.98
	68	32.80	32.94		1
Triacylglycerols, mmol·L <sup>-1</sup>	34	33.59	32.27	0.01	0.58
	1	31.40	30.39		0.84
	102	0.19	0.20		1
Total cholesterol, mmol·L <sup>-1</sup>	68	0.19	0.21	0.10	0.99
	34	0.21	0.20		1
	1	0.21	0.19		0.97
Glucose, mmol·L <sup>-1</sup>	102	2.70	2.58	0.11	0.99
	68	2.29	2.38		1
	34	2.54	2.41		0.99
Urea, mmol·L <sup>-1</sup>	1	2.68	2.60	0.17	0.99
	102	3.73	3.76		1
	68	3.68	3.17		0.04
Creatinine, µmol·L <sup>-1</sup>	34	3.87	3.31	9.66	0.01
	1	3.21	3.35		0.99
	102	1.95	1.98		1
ALT, U·L <sup>-1</sup>	68	3.73	4.51	1.72	0.05
	34	4.08	4.05		1
	1	3.03	3.20		0.99
AST, U·L <sup>-1</sup>	102	176.96	179.45	4.1	1
	68	155.16	178.64		0.67
	34	143.16	157.63		0.96
ALT, U·L <sup>-1</sup>	1	147.94	158.85	1.72	0.99
	102	26.8 5	25.65		1
	68	27.42	25.34		0.99
AST, U·L <sup>-1</sup>	34	26.79	19.24	4.1	0.05
	1	22.76	20.99		0.99
	102	87.08	85.18		0.81
AST, U·L <sup>-1</sup>	68	93.72	87.37	4.1	0.96
	34	92.44	91.66		1
	1	91.84	86.16		0.97

continue on the next page

TABLE 2 continued

Biochemical parameters	Days prior to parturition	Group		SEM	P
		control	experimental		
GGT, U·L <sup>-1</sup>	102	13.55	13.08	0.99	1
	68	11.75	8.71		0.38
	34	6.54	5.15		0.97
	1	11.27	9.32		0.86
ALP, U·L <sup>-1</sup>	102			5.51	
	68	85.36	80.64		0.99
	34	71.27	61.82		0.83
	1	67.18	55.00		0.62

ALT - alanine aminotransferase, AST - aspartate aminotransferase  
 GGT - gamma-glutamyltransferase, ALP - alkaline phosphatase

TABLE 3

Haematologic values of pregnant cows fed rumen-protected DL-methionine (n=13)

Haematological parameters	Days prior to parturition	Group		SEM	P
		control	experimental		
RBC, 10 <sup>12</sup> ·L <sup>-1</sup>	102	6.65	6.56	0.18	1
	68	6.74	6.17		0.22
	34	6.66	5.99		0.80
	1	6.18	6.27		1
MCV, fL	102	43.55	43.95	0.62	1
	68	44.31	45.06		0.99
	34	44.59	45.27		0.99
	1	44.01	44.10		1
MCH, pg	102	15.54	15.67	0.39	1
	68	14.92	15.64		0.99
	34	15.36	15.72		1
	1	15.64	15.83		1
MCHC, g·L <sup>-1</sup>	102	356.70	356.00	3.50	1
	68	343.82	347.18		1
	34	344.18	347.00		1
	1	354.91	358.70		0.97
RDW, fL	102	30.67	30.51	1.99	1
	68	31.76	29.61		1
	34	31.95	32.00		1
	1	30.85	32.29		1
Haemoglobin, g·L <sup>-1</sup>	102	102.92	102.69	2.93	1
	68	98.92	96.15		1
	34	101.92	94.54		0.63
	1	96.62	99.00		1
Haematocrit, L·L <sup>-1</sup>	102	0.29	0.29	0.01	1
	68	0.30	0.28		0.68
	34	0.30	0.27		0.48
	1	0.27	0.28		1

TABLE 3 continued

Haematological parameters	Days prior to parturition	Group		SEM	P
		control	experimental		
Thrombocytes, $10^9 \cdot L^{-1}$	102	361.73	365.73	41.79	1
	68	488.64	399.64		0.80
	34	455.00	450.09		1
	1	374.64	345.60		1
MPV, fL	102	5.64	5.92	0.18	0.95
	68	5.87	5.97		1
	34	5.57	5.49		1
	1	5.53	5.78		0.97
WBC, $10^9 \cdot L^{-1}$	102	7.81	7.58	0.36	1
	68	8.99	7.48		0.08
	34	7.61	7.10		0.97
	1	7.38	6.99		0.99
Neutrophils, $10^9 \cdot L^{-1}$	102	2.13	2.06	0.18	1
	68	2.71	1.61		0.00
	34	2.11	1.61		0.49
	1	2	1.62		0.79
Eosinophils, $10^9 \cdot L^{-1}$	102	0.97	0.77	0.13	0.96
	68	1.35	1.29		1
	34	1.02	1.11		1
	1	0.93	0.98		1
Basophils, $10^9 \cdot L^{-1}$	102	0.09	0.07	0.01	1
	68	0.1	0.08		1
	34	0.09	0.05		0.77
	1	0.06	0.07		1
Lymphocytes, $10^9 \cdot L^{-1}$	102	4.18	4.13	0.23	1
	68	4.33	4.16		1
	34	3.95	3.92		1
	1	3.99	3.94		1
Monocytes, $10^9 \cdot L^{-1}$	102	0.435	0.325	0.07	0.95
	68	0.51	0.34		0.65
	34	0.44	0.41		1
	1	0.39	0.38		1

SE - standard error of least squares means, RBC - red blood cell count, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, RDW - red cell distribution width, MPV - mean platelet volume, WBC - white blood cell count

Cows from group E had a significantly lower concentration of glucose in plasma on the 68<sup>th</sup> and 34<sup>th</sup> days prior to parturition ( $P=0.046$  and  $P=0.017$ , respectively) than those in group C. Plasma urea concentration was lower in cows from group C than those in group E; on the 68<sup>th</sup> day prior to calving the difference was close to statistical significance ( $P=0.053$ ).



The effect of rumen-protected methionine on the activity of ALT, AST, GGT in blood plasma and ALP in blood serum of cows in groups C and E is shown in Table 2. Their activities throughout the experiment were higher in cows in group C. The activity of ALT in group C animals on day 34 prior to parturition was close to statistical significance ( $P=0.052$ ) compared with group E.

The haematological values of cows in the control and experimental groups during the trial are shown in Table 3. Cows in group E had a lower RBC on the 68<sup>th</sup> and 34<sup>th</sup> days prior to parturition ( $P=0.3157$  and  $P=0.1533$ , respectively) and a lower WBC. On the 68<sup>th</sup> day prior to calving the difference in the number of leukocytes was very close to statistical significance ( $P=0.0785$ ). The decreased number of leukocytes in group E was the result of neutropenia which, on day 68 prior to parturition, was highly significant in the absolute ( $P<0.001$ ) and significant in the relative ( $P=0.0389$ , not shown) terms. In group C, relative neutrophilia caused a relative drop in the lymphocyte count ( $P=0.148$ ) (not shown). Cows in control group had higher values for Hb, Hct and thrombocyte count on the 68<sup>th</sup> day before calving, although the difference was insignificant.

The average body weight of cows at the beginning and at the end of the trial period was 558.3 and 546.7 kg in group C and 558.6 and 550.8 kg in group E, respectively.

## DISCUSSION

The chemical analysis and calculated nutritive values of ingredients were consistent with reported values (NRC, 2001; Rulquin et al., 2001). The addition of the Mepron 85 to the diet of the experimental group significantly increased the predicted methionine concentration in DM, CP and PDI by 192, 118 and 200%, respectively (Table 1).

The amounts of dry matter offered to the beef cows were well below average intake capacity of 13.2 kg/d DM (INRA, 1989) which ensures complete and constant consumption of both diets (Table 1). All cows consumed similar amounts of non-protein nutrients, but cows in experimental group ate more protein due to the added 15 g/d Mepron 85. Despite the low dry matter intake, both diets met the INRA (1989) nutrient requirements for pregnant beef cows calving from late winter to early spring. Supplementation of protected methionine increased the predicted concentration of intestinal digestible methionine from 0.97 to 1.94% of total digestible amino acids supply (PDI). Optimal use of PDI for the combined of maintenance and milk protein production requires a concentration of intestinal digestible methionine of 2.4% (NRC 2001) or very close to the value of 2.5% (Rulquin et al., 1993). It is supposed that in late pregnancy beef cows require less

methionine DI/PDI for maintenance, growth of the conceptus, and of the dam than lactating cows for maximal milk protein production.

The experimental diet containing rumen-protected methionine influenced the concentrations of glucose and urea, the activity of ALT, as well as some haematological values.

In the late pregnancy of ditocus ewes that were fed to predicted energy and protein requirements, a large portion of amino acids was directed towards the gravid uterus, and calculations showed that as much as 80% of apparently digested crude protein was partitioned to it, with the remainder being used to support increased metabolism and net deposition of amino acids in developing mammary glands and visceral organs (Bell and Ehrhardt, 1998).

In contrast to glucose, the foetus, rather than the placenta, is the principal site of amino acid utilization reflecting the importance of this substance for foetal growth.

The effects obtained in group E in relation to group C during certain periods of the experiment could have resulted from a relative methionine deficiency in group C cows and its influence on the endocrine system. Since food deprivation leads to increased corticosteroid secretion, and infusion of methionine, lysine and leucine to Angora goats were decreased plasma cortisol concentrations ( $P < 0.05$ ; Puchala et al., 1994), it appears that nutritive substances, in this case a deficit of methionine in cows in group C, could cause a reaction manifested as increased secretion of glucocorticoids. Glucocorticoids have an important influence on the intermediary metabolism in that they increase hepatic gluconeogenesis, contribute to the maintenance of the plasma glucose concentration, and in states of glucocorticoid excess there may cause hyperglycaemia.

In addition, glucocorticoids influence renal function through increasing the glomerular filtration rate. Increasing glomerular filtration decreases of blood urea and creatinine levels. In our study, the glucose and urea concentrations had equalized within groups immediately prior to parturition, indicating the level of glucocorticoids in group C cows has equalized with that in group E cows. The foetus has completed its growth by this time and therefore amino acid and glucose requirements had decreased.

The level of activity of all enzymes monitored was always somewhat higher in group C than in group E. A major difference was noted in the activity of ALT ( $P = 0.052$ ) in the middle of the trial. A significant increase of this enzyme ( $P < 0.01$ ), was stated in rabbits that were fed with various proportions of substrate from the production of the *Pleurotus pulmonarius* mushroom, considered to contain the enzyme thiaminase, causing symptoms of metabolic acidosis (Liker et al., 1998). Chronic acidosis increases gluconeogenesis (Alleyene et al., 1982). The increased gluconeogenesis in the 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 higher activity of ALT in cows in group C in our experiment could be a result of the increased gluconeogenesis.

In certain determinations of group C animals (68 or 34 days prior to parturition) an increase in the number of erythrocytes (34<sup>th</sup>, P=0.153), leukocytes (68<sup>th</sup>, P=0.078), neutrophils (68<sup>th</sup>, P=0.001) and neutrophil/lymphocyte (N/L) ratio (68<sup>th</sup>, P<0.001) and a decrease in the percentage of lymphocytes (68<sup>th</sup>, P=0.148, not shown) was found. These changes are considered to be typical of increased ACTH and glucocorticoids secretion. A physiologically high periparturient blood level of glucocorticoids has been suggested as an explanation for the increased neutrophil count around parturition (Kehrli et al., 1999). A significant positive correlation was found between cortisol and total white blood cells and neutrophils, respectively, in two groups of Norwegian cattle. Cows from a lower cortisol group also had lower N/L ratios (Kulberg et al., 2002). It has been shown that transport-induced stress in different species leads to elevated cortisol blood concentrations, increases in total white blood cell and neutrophil levels, and an elevated N/L ratio (Stull and Rodiek, 2000). The proliferative ability of peripheral blood T lymphocytes increased for cows consuming 30 g/d Mepron<sup>®</sup>85 (Soder and Holden, 1999).

Blood analysis reference values (Kaneko et al., 1997) and results obtained from investigations by various authors (Reece, 1997; Xu et al., 1998; Lischer et al., 2000) are shown in Table 4. The average results from both groups obtained in this investigation are also shown in the same Table.

TABLE 4

Cow plasma parameters obtained in investigations performed by various authors

	Kaneko et al., 1997	Reece, 1997	Xu et al., 1998	Lischer et al., 2000	Our results <sup>1</sup>
	x ± s.d.				
Total protein, g·l <sup>-1</sup>	71 ± 1.8	70 - 85			78.38 ± 5.0
Albumin, g·l <sup>-1</sup>	32.9 ± 1.3				32.10 ± 1.9
Triacylglycerols, mmol·l <sup>-1</sup>	0 - 0.2		1.2		0.20 ± 0.05
Total cholesterol, mmol·l <sup>-1</sup>	2.1 - 3.1	2.1 - 4.7		5.6	2.55 ± 0.42
Glucose, mmol·l <sup>-1</sup>	3.19 ± 0.38	2.2 - 4.4	3.3	3.2 ± 0.4	3.51 ± 0.43
Urea, mmol·l <sup>-1</sup>	7.14 - 10.7	3.4 - 10.7	3.3 - 5.6	4.9 ± 1.25	3.31 ± 0.45
Creatinine, μmol·l <sup>-1</sup>	88.4 - 177	88 - 177			160 ± 38
ALT, U·L <sup>-1</sup>	27 ± 14		16		24 ± 7
AST, U·L <sup>-1</sup>	105 ± 27		61	87 ± 14.9	90 ± 15
GGT, U·L <sup>-1</sup>	15.7 ± 4.0			16 ± 4.7	10 ± 5
ALP, U·L <sup>-1</sup>	194 ± 126				72 ± 41

<sup>1</sup>average values in groups

## CONCLUSIONS

It can be concluded that the feeding of pregnant beef cows in advanced pregnancy in winter-keeping conditions with meals containing added rumen-protected methionine had an anti-stress effect, which means that methionine is not only as a deficient amino acid in the feed of highly productive dairy animals during lactation, but may also be deficient in the feed of beef cows in late pregnancy.

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## STRESZCZENIE

**Stężenie metabolitów i hematologiczne wskaźniki u cielnych krów ras mięsnych otrzymujących dodatek chronionej metioniny**

Badano wpływ dodatku metioniny chronionej przed rozkładem w żwacu na niektóre biochemiczne i hematologiczne wskaźniki krwi u 32 krów w ostatnich 102 dniach ciąży. Zwierzęta podzielone na dwie równoliczebne grupy: kontrolną (C) i doświadczalną (E) żywiono dawką o następującym składzie: siano łąkowe, kiszonki z ziarna kukurydzy i 500 g mieszanki treściwej (32% białka ogólnego). Do dawki grupy E dodawano po 15 g chronionej DL-metioniny (Mepron® M85)/zwierzę/dzień. Krew do oznaczeń pobierano w około 102, 68, 34 i 1 dniu przed wycieleniem.

Stężenie glukozy w osoczu krwi grupy E obniżyło się istotnie w ciągu okresu doświadczalnego (w 68 i 34 dniu przed wycieleniem;  $P=0,05$  i  $P=0,02$ , odpowiednio), a w grupie C obniżenie stężenia mocznika w 68 dniu przed wycieleniem było bliskie istotności ( $P=0,05$ ). Nie stwierdzono istotnych różnic w stężeniu białka całkowitego, albumin, trójgliceroli, cholesterolu całkowitego i kreatyniny pomiędzy grupami. Aktywność aminotransferazy alaninowej w osoczu krwi krów grupy E była niższa (różnica bliska istotności,  $P=0,05$ ) w 34 dniu przed wycieleniem. W ciągu doświadczenia nie stwierdzono natomiast istotnych różnic w stężeniu w osoczu krwi aminotransferazy asparaginianowej, gamaglutaminotransferazy oraz fosfatazy zasadowej między grupami. Liczba czerwonych ciałek krwi w 68 i 34 dniu przed wycieleniem krów grupy E była niższa ( $P=0,32$  i  $P=0,15$ , odpowiednio). W 68 dniu przed wycieleniem liczba białych ciałek krwi była mniejsza (różnica bliska istotności;  $P=0,08$ ), a neutropenia różniła się wysoce istotnie zarówno w wartościach absolutnych ( $P<0,001$ ) jak i względnych ( $P=0,04$ ), obniżył się istotnie stosunek neutrofilii do leukocytów ( $P<0,001$ ).

Otrzymane wyniki wskazują, że dodatek chronionej metioniny w warunkach zimowego żywienia i utrzymania krów, w końcowym okresie cielności, może mieć działanie antystresowe.