ACTIVITY OF SOME ENZYMES IN THE BLOOD PLASMA OF HYLLA RABBITS FED VARIOUS PROPORTIONS OF SUBSTRATE FROM THE PRODUCTION OF THE *Pleurotus pulmonarius* MUSHROOM

B. LIKER[1], R. BOŽAC[1], I. HUSDŽINOVIĆ[2], S. MUŽIC[1], B. BIOĆINA[2], A. TUMPIĆ[3], V. RUPIĆ

[1]Faculty of Agriculture, University of Zagreb, 10000 Zagreb,
[2]Clinical Hospital Dubrava, 10000 Zagreb, [3]Medical Centre, 52000 Pazin, Croatia

Received April 28, 1997
Accepted March 3, 1998

Abstract

Liker B., R. Božac, I. Husdžinović, S. Mužic, B. Biočina, A. Tumpić, V. Rupić: *Activity of Some Enzymes in the Blood Plasma of Hylla Rabbits Fed Various Proportions of Substrate from the Production of the Pleurotus pulmonarius Mushroom.* Acta vet. Brno 1998, 67: 27-36.

The nutritive effect of different amounts of substrate from the production of *Pleurotus pulmonarius* on the activity of AST, ALT, LD, CK, GGT and AP in the blood plasma of rabbits, after a nine weeks experimental fattening, was investigated. Sixty male Hyla rabbits, divided in four groups equal in number, one control (C) and three experimental groups (E_1, E_2, E_3), were included in experiment. The control group (C) was fed the standard fodder mix without substrate. Added to the standard mix, the groups E_1, E_2 and E_3 received various proportions, 10%, 20%, and 30%, respectively, of the substrate left from the production of *Pleurotus pulmonarius*.

Different proportions of added substrate did not affect the activity of AST and GGT, respectively, whose values (x±s.d.) in the control group (C) amounted to 32.49 ± 12.8 $U.l^{-1}$ and 6.14 ± 1.35 $U.l^{-1}$, respectively. At the same time the activity of ALT was altered by experimental diets. The activity of ALT in the control group (C) amounted to 28.0±8.81 $U.l^{-1}$, whereas in the E_3 group it was significantly greater ($P<0.05$) than in the group C and E_2, i.e. 38.86±11.95 $U.l^{-1}$. Under the conditions of experimental feeding the activity of AP was greater too: in the control group (C) it amounted to 107 ± 42.5 $U.l^{-1}$, but in the group E_3 it was significantly greater ($P<0.05$) and amounted to 158.7 ± 27.7 $U.l^{-1}$. On the contrary, the activity of CK is decreased during the experimental feeding ($P<0.07$), and in the control group (C) it amounted to 2044 ± 647 $U.l^{-1}$, in the E_3 group to 1479.6 ± 927 $U.l^{-1}$. The activity of lactate dehydrogenase (LD) generally reflected the composition of fattening diets and is inversely proportionate to the share of substrate added. The highest values of LD activity was found in group C (94.57±24.78 $U.l^{-1}$), and the lowest was in group E_3 (63.49±14.12 $U.l^{-1}$). The difference was significant compared to the C and E_1, groups ($P<0.01$) and to the E_2 ($P<0.05$) group.

The results indicate B_1 hypovitaminosis and the development of compensatory metabolic acidosis.

Nutrition, substrate of mushrooms, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), creatine kinase (CK), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), hypovitaminosis, metabolic acidosis

The production of corn, barley, wheat, and oats is followed not only by grain but also by large quantities of by-products, e.g. corn stalks, straw, etc. Those products can be used in the nutrition of cattle, particularly of ruminants and other herbivores, whereas in the digestive system of other species of animals they are poorly utilised. The basic factors limiting their use are large quantities of lignin, cellulose, hemicellulose, aromatic matters, etc. (Gioffre et al. 1988). In order to increase the digestibility of potential fodder for rabbits (Lebas et al. 1978; McDonald et al. 1978; De Blas et al. 1979; Auxilia 1981; Jensen et al. 1986; Radwan et al. 1987; Gioffre et al. 1988; Mužic et al. 1990) and

Address for correspondence:
Doc. dr. sc. Branko Liker,
Faculty of Agriculture, University of Zagreb
Svetošimunska c. 25, 10000 Zagreb, Croatia

Phone: +385 01 239 3929
Fax: +385 01 239 3947
E-mail: bliker@agr.hr

for ruminants (Hartley 1987; Zadražil 1987) researchers treated it with NaOH, NH_3, CO_2, SO_2, etc., but without any positive results. As a biological approach to increasing the digestibility of straw and other secondary agricultural products the decomposition of cell walls by means of the mycelium of *Pleurotus* sp. or of other mushrooms can be considered. Henics (1987) fed steers with meals in which hay has been replaced by the rest of the production of *Pleurotus* in the proportion of 0, 3.0, 4.5 and 6.0 kg a day, respectively. In experimental steers he established the pH value of rumen contents higher and the total content of volatile fatty acids lower. As for final body weight, daily weight gain, food consumption, and carcass characteristics, there were no significant differences between the experimental steers and the controls. Research has been carried out (Rai and Gupta 1989; Mužic et al. 1990; Božac et al. 1991; Rupić et al. 1991) in which the exhausted substrate derived from the production of mushrooms was used in diet for rabbits. It was established that wheat straw the *Pleurotus* mushroom had grown on became more digestible (Zadražil 1987; Mužic et al. 1990) than the untreated one. After earlier investigations (Mužic et al. 1990; Božac et al. 1991) no negative influence of various proportions of that substrate in fodder could be found on the growth, the feed consumption and the feed conversion of Chinchilla and Hylla rabbits following 63 days of experimental fattening, although alterations of some hematologic parameters were found (Rupić et al. 1996).

In this paper the nutritive effect on the activity of some enzymes in the plasma of rabbits treated as in a previous study (Rupić et al. 1996) was investigated in order to complete the picture of the alterations found and to give an answer why they occurred. Besides, the wide range in the activity of some enzymes tested, as found in literature (McLaughlin and Fish 1994), was a motive for us to carry out additional investigations in order to determine their reference values.

Materials and Methods

Animals and diets

Sixty weaned male Hylla rabbits, aged 30 days, divided into 4 groups of 15 animals each, a control group (C) and three experimental groups (E_{1-3}), were included in the experiment. All the rabbits were housed in stainless steel cages in the same room. In each cage, 60 × 120 × 60 cm, there were five rabbits. The cages were lifted 120 cm above the bottom of the room. The room was air-conditioned, and thus the temperature was maintained between 18 and 20 °C, and the relative humidity between 60 and 70%. To decrease the risk of infection,

Table 1
Ingredients and chemical analysis of pelleted diets

Ingredients (% as fed)	C	E_1	E_2	E_3
Substratum	0	10.0	20.0	30.0
Corn	27.0	27.7	23.7	19.8
Soybean meal	0	0	9.3	13.9
Sunflower meal	16.0	14.0	5.0	1.5
Fish meal	1.0	4.0	5.0	5.0
Wheat middlings	6.0	2.0	2.0	0
Fat	4.2	4.2	5.2	6.5
Alfalfa meal	40.0	31.3	24.3	16.5
Barley meal	5.0	6.0	6.0	6.0
Iodised salt	0.3	0.3	0.3	0.3
Vitamin-mineral mix	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Analysis as fed				
Dry matter ($g.kg^{-1}$)	899.00	896.10	894.70	893.90
Crude protein ($g.kg^{-1}$)	172.50	172.40	170.90	173.50
Crude fat ($g.kg^{-1}$)	69.50	67.90	75.20	85.20
Crude fibre ($g.kg^{-1}$)	141.10	140.90	140.50	143.30
Ash ($g.kg^{-1}$)	59.80	57.40	53.70	51.10
Nitrogen free extract ($g.kg^{-1}$)	**456.10**	**457.50**	**454.40**	**440.80**
Ca ($g.kg^{-1}$)	6.39	7.20	7.00	7.10
P	5.40	5.40	4.50	4.10
Metabolizable energy, MJ/kg**	10.098	10.103	10.090	10.111

*Official methods were used throughout (A.O.A.C, 1984)
** Calculated data (Allen, 1993)

all the cages and equipment were treated with a chlorine disinfectant (Izosan G) before the experiment began.

The pelleted substrate was obtained from the commercial production of *Pleurotus pulmonarius*. By means of tissue culture, the Oyster mushroom culture spawn was developed, propagated on wheat grain and sown in the moistened and pasteurised wheat straw. The inoculated substrate (straw+spawn) was packed in polyethylene bags and incubated for 20 days at 22 °C. After picking the fungi (yield: 20%) the substrate residue (straw+spawn) was dried and ground in a hammer mill. The floury substrate was mixed with the other components into fodder mixes which were then pelleted with no binder added. Composition and metabolic energy of the fodder mixes are shown in Table 1.

The rabbits in the control group (C) were not fed the substrate. The proportion of substrate in the experimental groups was 10% (E_1), 20% (E_2), and 30% (E_3), respectively. The dried substrate obtained from the commercial production of *Pleurotus pulmonarius* contained in $g.kg^{-1}$: dry matter 886.2, crude fat 13.3, crude protein 67.9, crude fibre 255.2, nitrogen free extract 504.2 and ash 45.6.

Microbiological examination of one gram of fodder mix gave a germ count of 1,200,000 for the control group (C), 1,120,000 for the E_1, 900,000 for the E_2, and 1,300,000 for the E_3 experimental group. In the same quantity (1 g) of fodder mix no *Salmonellae* were found, while the anaerobic count was 1,000 and the total mould (*Ascomycetes, Phycomycetes*) count was also 1,000 in all groups.

Feed and drinking water were provided *ad libitum* for 63 days. At the end of the experiment blood samples were taken from all rabbits in the experiment, each sample in a separate test-tube. Blood samples were drawn between 08.00 and 11.00 h, by cardiac puncture. The blood was stored in Greiner test tubes with EDTA. The rabbits were put on back on special boards, with legs pulled out bound to board. Immediately after drawing the sample, the blood was centrifuged (3500 r.p.m.) for 20 min. and then the plasma separated from corpuscular elements. Within 2 hours the activity of above mentioned enzymes in the blood plasma was examined. In seven test tubes coagulation or haemolysis took place; these samples were excluded from the experiment. All enzyme activities were determined using commercial kits (Bayer Diagnostics Manufacturing S. A.), and the Technicon CHEM-1 apparatus.

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

All statistical analyses were performed using the GLM (general linear model) procedure of SAS (1989).

Results

Average diet variables during the trial are given in Table 2.

The initial average body weight of rabbits in the control group (C) was 616 g, whereas in three experimental groups it was 626 g (E_1), 617 g (E_2), and 632 g (E_3), respectively. The analysis of variance showed that differences in the initial body weight of rabbits assigned to different groups were not significant ($P>0.05$) .

Table 2
Average feeding variables during the trial

Feeding variables	Group of animals*							
	C		E_1		E_2		E_3	
Body mass in g	x	s.d.	x	s.d.	x	s.d.	x	s.d.
starting	616	22.8	626	39.4	617	38.3	632	30.9
finishing	2044	316.8	2038	262.9	2123	335.4	2120	262.8
Daily weight gain (g/day)	23.3	5.8	23.0	4.7	24.6	6.3	24.0	4.7
Feed conversion (kg/kg)	3.88		4.19		3.82		3.89	

*Number of rabbits in each group, n = 15.
In feeding varieties no significant differences ($P>0.05$) between experimental groups during the trial were found out.

After 63 days of feeding the following mean body weights were attained: 2044 g (C), 2038 g (E_1), 2133 (E_2), and 2120 g (E_3). No significant differences were found in the body weight among the four groups at the end of the trial. In the course of experiment the feed conversion in the control group (C) amounted to 3.88 kg/kg, and in the experimental groups (E_{1-3}) amounted to 4.19, 3.82, and 3.89 kg/kg, respectively.

The influence of experimental diets containing different proportions of substrate from the production of the *Pleurotus pulmonarius* mushroom on the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), creatine kinase (CK), gamma-glutamyltransferase (GGT), and alkaline phosphatase (AP) in the blood plasma of rabbits in the control group (C) and in three experimental groups (E_1, E_2, E_3) at the end of the experimental period is shown on the Table 3.

Table 3

Activity of enzymes AST, ALT, LD, CK, GGT and AP in blood plasma of Hylla rabbits at the end of the experiment ($U.I^{-1}$)

Enzyme activity ($U.I^{-1}$)	Groups of rabbits							
	C		E_1		E_2		E_3	
	n = 14		n = 12		n = 13		n = 14	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
AST	32.79	12.80	29.92	8.76	31.31	16.57	31.14	9.15
ALT	A 28.00 I	8.81	31.67	11.45	B 24.23	4.38	A, B 38.86	11.95
LD	A 94.57	24.78	B 79.37	11.04	a 78.20	16.72	A, B, a 63.49	14.12
CK	2044.00	647.10	1708.20	338.30	1668.90	487.70	1479.60	927.30
GGT	6.14	1.35	5.50	1.17	5.23	1.48	6.00	2.63
AP	A 107.86	42.50	139.92	46.80	a 130.40	36.20	A, a 158.70	27.70

Legend: Average values marked by the same letter are significantly different at the levels
A:A and B:B = $P<0.01$,
a:a and b:b = $P<0.05$,

The activity of AST did not differ significantly among the groups. However, it was numerically the highest one in the control group (C).

Diets containing the experimental fodder mix caused an increase in ALT activity, which was commensurate with the proportions of substrate in the groups E_1 and E_3 and was the highest in the E_3 group, with a highly significant difference from the values in the C and E_2 groups ($P<0.01$).

The activity of lactate dehydrogenase (LD) generally reflected the composition of fattening diets and is inversely proportionate to the share of substrate added. The highest values of LD activity were found in the C group. They decreased gradually with changes in the diet composition. Diets containing 10% substrate caused already a decrease of the LD activity statistically significant at the 7% level ($P<0.07$). The lowest mean value was found in the E_3 group, it was highly significantly lower than those ones in the C and E_1 groups ($P<0.01$), and significantly lower than the decrease value in the E_2 group ($P<0.05$).

The activity of creatine kinase (CK) in the fattened rabbits plasma was similar to the LD activity. It was the highest in the C group and gradually decreased to the E_3 group; the results in those two groups were close to statistical significance ($P>0.05$; $P<0.07$).

The concentration of plasma gamma-glutamyltransferase (GGT) was not effected by the composition of diets.

On the other hand, the values of alkaline phosphatase (AP) were different among the groups. The lowest value was found in the C and the highest one in the E_3 group; the difference between them was highly significant ($P<0.01$). A significant difference was

found also between the high activity in the E_3 group and somewhat lower one in the E_2 group ($P<0.05$). The influence on the difference in the level of AP activity between the C group and the experimental groups E_1 - E_3 is supported by that one between the C and E_1 groups, where the proportion of only 10% substrate added caused a difference close to statistical significance ($P>0.05$; $P<0.08$).

Table 4

Enzyme activity in serum (plasma) of rabbits obtained in the investigations of various authors ($U.I^{-1}$)

	AST	ALT	LD	CK	GGT	AP
Weils 1969			50–70			
Friedel and Mattenheimer 1970	12.7±3.2	18.3±2.6	36.9±7.2			
Schievelbein *et al.* 1970	10.9±2.8	11.7±3.5	73.2±33.6			18.0±7.3
Grötsch and Hajdu 1971	2–15	6–20	100–450			30–106
Kozma *et al.* 1974	42±6	35±3				
Dabew *et al.* 1976	12.1±1.9	18.9±2.4	86.4±10.2			11.8±2.6
Yu *et al.* 1979	19.1±1.9	33.3±13.4	77.5±32.7	310.5±169.9	2.46±1.66	39.8±25.8
Lindena and Trautschold 1986	17.2±7.2	37.8±10.4	36.3±8.5	91.4±23.8	3.80±1.20	71.0±24.2
Hewit *et al.* 1989			107±44	610±352	3.00±2.4	71.0±34.0
Lepitzki and Woolf 1991	70.2±11.3	64.1±6.2				10.2±2.7
our results	32.8±12.8	28.0±8.8	94.57±24.78	2044.6±647.1	6.1±1.35	107.9±42.5
normal values in humans (TECHNICON) x ± s.d.	5–35	5–35	130–300	19–105	8–38	28–76

Discussion

In accordance with the obtained feeding results the different proportions (10%, 20% and 30%, respectively) of substrate added to diets did not influence on the body weight, the weight gain, and the feed conversion of rabbits control and both experimental groups. Different proportions of substrate added to diets did not affect to nutritional parameters of rabbits within the feeding period of 63 days (Table 3).

A survey of the activity of some enzymes in the rabbits plasma after 63 days of experimental fattening (Table 3) indicates that fodder mixes containing a 10%, 20%, and 30%, respectively, level of substrate from the production of *Pleurotus pulmonarius* affected the metabolism of rabbits in whose cells the examined enzymes had originated and from which they had entered into the extracellular fluid.

That influence on the activity of enzymes in the plasma of experimental rabbits had a various

effect. The activity of LD, of CK and to a certain extent of AST has decreased most noticeably, that of LD, the E_3 group showed markedly the lowest value. The difference was significant, compared both to the C and E_1 groups ($P<0.01$) and to the E_2 ($P<0.05$) group.

The variability of CK activity among the groups was the same as that one of LD, i.e. a successive and regular decrease from the C towards the E_3 group, only that the difference between the mean values of the extreme groups was somewhat less significant ($P<0.07$). The ratio between the LD and CK activities was 0.046 in the C and E_1, 0.047 in the E_2, and 0.043 in the E_3 group.

The AST activity in the experimental groups E_1 - E_3 was insignificant and can therefore be compared with GGT, i. e. it ranged in the group on which the experimental diets had no effect.

The third entity comprises ALT and AP. Theeir activity increase was in accordance with the increase of the proportion of substrate, i.e. the activity of enzymes grew from the control to the E_3 group. The exception was the E_2 group in which the ALT value was the lowest among the values of other groups, and the AP value lower than in the E_1 group.

Determining the plasma enzyme activity enables to evaluate the state of health of particular organs because nearly all diagnostically important cell enzymes are in the plasma of so-called normally healthy units. Depending on their activity within the cell and on the extent of cell damage, the cellular enzymes leak from the cells into the plasma, where the level of their activity indicates the place and extent of cellular damage.

Various way of the cellular enzyme activity in the plasma of our experimental animals - in some it remained the same (AST, GGT), in some it grew (AP, ALT), in others it fell (LD, CK) - gives evidence that the experimental fodder mix did not impair seriously the function of organs and tissues nor damage their cells. However, it clearly brought about metabolic disorder affecting the activity of the examined enzymes in the cells. A consequence was increased synthesis of some, and the inhibition of synthesis of other enzymes. The described variety of the activity of examined plasma enzymes could be explained by the state of organism during the development of metabolic acidosis, in fact during the compensation of it when the level of bicarbonates in the blood is decreasing.

Fungi, bacteria, and certain plants contain the thermolabile enzyme thiaminase which destroys vitamin B_1. Signs of vitamin B_1 deficiency developed in animals fed such a diet (Smith 1977; Scheunert and Trautmann 1987). Pyruvic acid and its reduced form lactic acid are accumulated in the blood and tissues of such animals (McDonald et al. 1978; Church and Pond 1988). In addition, the vitamin B1 deficiency leads to a pathological increase of pyruvic acid. The congenital defect of insufficient forming the pyruvate dehydrogenase, i. e. its catalytic unit pyruvate decarboxylase during the neonatal period brings about lactic acidosis, later turning to chronic metabolic acidosis (Leonard 1982).

Feeding of rabbits with the mycelium of *Pleurotus pulmonarius* could act in the same way but because of B_1 hypovitaminosis cause the same state of health as the aforementioned genetic defect. The results of haematological investigations in rabbits fed in the same way point at the risk of the acid-base balance (Rupić et al. 1996) also. In our experiment such acidosis was probably successfully compensated for because no significant alterations of the Cl ions concentration in the blood of our experimental rabbits had been ascertained (unpublished data).

Acidosis inhibits glycolysis and the production of lactate (Macklear and Guest 1953). In acidotic rats (acidosis induced by NH_4Cl) a significant decrease of the level of lactate in the blood as well as of pyruvate, lactate, and other metabolic intermediate products of glycolyse in the skeletal muscle cells takes place, and a reduced level of pyruvate in the liver cells is found out also (Cuthbert 1980). The highest LD activity, in relation to other examined tissues, was determined in the liver and skeletal muscles cells (Schmidt and Schmidt 1960). In our experimental rabbits the inhibition of glycolytic processes and possible reduction of the cell

pyruvate level led to a decline of activity, i.e. of the concentration of lactate dehydrogenase in cells and consequently in plasma (the relation between the LD concentration in muscles and liver to that one in plasma is 1400:1; Schmidt and Schmidt 1962).

Thus the LD level in the blood of our experimental rabbits could be equalled to the reaction way of some other glycolytic enzymes - phosphorylase, phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase, which responded to acidosis by diminishing their activity (Alberti and Cuthbert 1982). Such decrease could be a part of the compensatory mechanism of acidosis.

Acidosis induces to a great extent the increase of the adenine nucleotide level in the skeletal muscle cell, so the infusion of ammonium chloride led to a significant increase of ATP, ADP and AMP within the muscles of experimental rats (Alberti and Cuthbert 1982), and the same occurred in the liver also. The increase of the ATP level should mean a higher creatine phosphate level too. A decrease of the CK plasma activity in our experimental rabbits, presuming high ATP and creatine phosphate levels in the muscle cell, which is almost exclusively the CK source, indicates a reduction in the dynamics of the creatine phosphate consumption and restitution in the same cell. That could be a part of the protective mechanism in compensating for the acidosis.

Thus the ATP dynamics is affected, too: on the one hand its hydrolysis is linked to the H ions release (Alberti and Cuthbert 1982), on the other hand a rapid restitution is disrupted by the inhibited glycolysis.

In addition a high ATP level inhibits glycolysis, and so the pyruvate production is co-ordinated with the acid-base capacity of the organism.

Alanine aminotransferase develops its most intensive activity in the liver cells. In our experiment the significantly highest ALT activity was found in the E_3 group. Since the plasma activity increase as a consequence of the damage of liver cells within the total profile of determined enzymes can be neglected, it can be assumed that the activity increase is due to the need of intensified glutamine synthesis from the glutamic acid in the liver, in order to increase the production of ammonia in the kidney, which is essential for the excretion of H ions appearing excessively in the state of metabolic acidosis. During the chronic acidosis the glutamine synthesis in the liver is increased and then a share of NH_4 is used for it, the share that normally is built into the urea (Ganong 1991). In rats the chronic metabolic acidosis led to the intensified ammonium production, gluconeogenesis and renal excretion of glutamine (Alleyene et al. 1982). The ammonium production by deaminating the glutamine is a process stimulated by the reduced pH value or decreased bicarbonate level. It takes place exclusively in the proximal renal tubules (Brewer and Cruise 1994). In rabbits the reduced blood level of bicarbonate has a pronounced role in this process (Simpson and Sharrard 1969).

Although the concentration of ALT by gram/tissue in the skeletal muscles is considerably lower than in the liver; they could by reason of their share in the total body mass contribute remarkably to the level of ALT activity in the blood. Since the main store of glutamine is in the muscles (Lemieux et al. 1980) and the glutamine production in the muscles increases considerably during acidosis (Oliver et al. 1977; Schrock et al. 1980), its increase must be accompanied by intensified transamination, also by intensified activity of ALT, which penetrates into the plasma from the muscle cell considerably easier than from the liver cell (in the relation 750:1 and 7600:1, respectively; Schmidt and Schmidt 1962). In addition, this would mean that the increased ALT concentration is a part of the system for compensating the metabolic acidosis, a process in which the alanine originating from pyruvate is further transformed by the cell into the glutamic acid and glutamine.

Significantly increased alanine concentration is recorded in the hepatic cells of acidemic rats (Alberti and Cuthbert 1982).

In this experiment the AST activity in the experimental groups did not differ statistically from

the one in the control group, although it was the highest in the control group; that would mean that the transaminase activity in liver and muscles is directed, as already mentioned, towards the glutamine synthesis, at the expense of aspartic acid. From the diagnostic point of view such a result, by unchanged GGT concentration, is the best evidence that the integrity of hepatic cells in the rabbits of experimental groups has been completely preserved.

Increased AP activity in blood most frequently indicates disorders in liver or bony tissue. Considering the AP activity in relation to the total enzymatic situation and the above observations, it can be hypothesised that bony tissue is responsible for increased plasma AP activity in experimental rabbits. Increased plasma AP activity in the experimental groups (E_1, E_2, E_3) in relation to the control group (C), with different statistical significance ($C:E_3=P<0.01$, $C:E_2=P<0.05$, $C:E_1=P<0.08$), would mean that osteoblasts have intensified their activity in the AP synthesis, resp. that their number has increased due to the intensified absorption of bony tissue. Such intensified absorption is performed by osteoclasts which act under the influence of parathyroid hormone (PTH); the plasma level of PTH is raised in part by acidosis, and acidosis itself induces an intensified activity of osteoclasts (Ciba Collection of Medical Illustrations 1991). Intensified AP activity indicates a disorder in metabolism of bony tissue, resp. an impaired mineralization, but it could be also a consequence of disorders in production of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$ D) from D vitamin caused by metabolic acidosis (Ganong 1991). It can be presumed that both mechanisms were involved, i.e. the increased PTH concentration, which in normal conditions induces the 1,25-$(OH)_2$ D synthesis, and the 1,25-$(OH)_2$ D deficiency due to its impaired synthesis as a consequence of metabolic acidosis.

The results of enzyme activity in the rabbits serum or plasma, obtained in investigations by different authors, are shown in Table 4. The results of the control group obtained in this investigations are shown in the Table also. It can be seen that the data on enzyme activity differ significantly from author to author. The differences in the activity among some enzymes are so great that, in spite of the fact that all values are expressed in the same units, it is almost impossible to compare them; therefore it can be looked upon as different quantification of the mentioned activities is in question, resulting before all from the differences in analytic methods, equipment and reagents used.

The physiological values of humans, obtained on a Chem-1 (TECHNICON) apparatus, which was also used for analysing the blood samples in this experiment, are given in Table 4, too.

The values of AST and ALT in rabbit and humans would be of the same order of magnitude, those of GGT and LD slightly lower in rabbits, and the AP value slightly higher in rabbits than in humans. The rabbits in this experiment were young animals, in which the AP value is 2-4 times higher than in adult ones (Kramer 1989). The CK activity in rabbits is considerably higher than in humans. Increased levels of CK, but also of LD and AST could be a consequence of the blood taking procedure, due to damaged muscle (Mitruka and Rawnstey 1981; Lindena and Trautschold 1986).

Aktivita enzymů v krevní plazmě králíků Hylla krmených různým množstvím substrátu houby *Pleurotus pulmonarius*

V této studii byl zkoumán výživný účinek různého množství substrátu houby *Pleurotus pulmonarius* na aktivitu enzymů AST, ALT, LD, CK, GGT a AP v krevní plazmě králíků po 9 týdnech experimentálního krmení. 60 králíků samčího pohlaví bylo rozděleno do 4 skupin po dvaceti, z nichž jedna sloužila jako kontrolní a ostatní experimentální skupiny bly označeny E_1, E_2, E_3.

Kontrolní skupina byla krmena standardní krmnou směsí bez substrátu. Experimentální skupiny obdržely krom standardní krmné směsi 10 %, 20 % a 30 % substrátu houby *Pleurotus pulmonarius*.

Různé množství přidaného substrátu neovlivnilo enzymů AST ani GGT, jejich hodnoty se u jednotlivých skupin nelišily 32,49±12,8 $U.I^{-1}$; 6,14±1,35 $U.I^{-1}$. Aktivita enzymu ALT naměřená ve stejnou dobu činila v kontrolní skupině 28,0±8,81 $U.I^{-1}$, u skupiny E_3 byla významně vyšší ($P< 0,05$) než u skupiny kontrolní a u skupiny E_2 byla naměřena hodnota 38,86±11,95 $U.I^{-1}$.

Hladina enzymu AP byla rovněž vyšší; u skupiny kontrolní byla naměřena hodnota 107±42,5 $U.I^{-1}$, zatímco u skupiny E byla významně vyšší ($P< 0,05$) 158,7±27,7 $U.I^{-1}$. Aktivity enzymu CK byla naopak během experimentálního krmení nižší ($P< 0,07$) než u skupiny kontrolní 2044±647 $U.I^{-1}$, jejíž hodnota u E_3 činila 1479,6±927 $U.I^{-1}$.

Získané výsledky indikují hypovitaminózu B_1 a vývoj kompenzační metabolické acidózy.

References

ALBERTI, K. G. M. M., CUTHBERT, C. 1982: The hydrogen ion in normal metabolism. In: Metabolic acidosis (Porter, R., Lawrenson, G., Eds). Ciba Foundation Symposium 87, Pitman Books, London, pp. 1-19

ALLEN, R. 1993: Ingredient analysis table. Feedstuffs Issue **65**, 24-37

A.O.A.C. 1984: Official Methods of Analysis (14th ed). Association of Official Analytical Chemists, Arlington, VA, USA

AUXILIA, M. T.1981: By-products in diets for rabbits (In Italian). Rivista di coniglicoltura **18**: 15-19

ALLEYENE, G., LUPIANEZ, J. A., McFARLANE-ANDERSON, N., BENJAMIN, J., BARNSWELL, J., SCOTT, B. 1982: Glutamine metabolism in metabolic acidosis. In: Metabolic acidosis (Porter, R., Lawrenson, G. Eds). Ciba Foundation Symposium 87, Pitman Books, London, pp. 101-119

BOŽAC, R., MUŽIC, S., RUPIĆ, V., JURIĆ, I., ROGINA, Ž. 1991: A contribution to the knowledge of the effect of feeding Chinchilla rabbits with substratum from the production of *Pleurotus pulmonarius* on performance and meat quality (In Italian). Rivista di coniglicoltura **28**: 29-33

BREWER, N. R., CRUISE, L. V. 1994: Physiology. In: The Biology of them Laboratory Rabbit (Manning, P.J., Ringler, D. H., Newcomen, C. E., Eds). 2nd ed. Academic Press, pp. 63-68

CHURCH, D. C., POND, W. G. 1988: Basic Animal Nutrition and Feeding. 3rd ed. J . Wiley and Sons, New York, pp. 245-247

CUTHBERT, C. R. 1980: Studies of insulin resistance in experimental diabetic ketoacidosis. PhD thesis, University of Southampton.

DABEW, D., MAHLBERG, F. A., STRAUCK, H. 1976: Enzymaktivitäten im Serum von Kaninchen und Ratten Referenzwerte und Tagesschwankungen. J. Clin. Chem. a. Clin. Biochem. **14**: 561-567

DE BLAS, J. C., MERINO, J., FRAGA, F. M., GALVEZ, J. F. 1979: A note on the use of sodium hydroxide treated straw pellets in diets for growing rabbits. Anim. Prod. **29**: 427-430

FRIEDEL, R., MATTENHEIMER, H. 1970: Release of metabolic enzymes from platelet dusting blood clotting of man, dog, rabbit and rat. Clin. Chim. Acta **30**: 37-46

GANONG, W. F. 1991: Review of Medical Physiology. 15th ed. Prentice-Hall International Inc., London, Sydney, Toronto, Mexico, New Delhi, Tokyo, Singapore, Rio de Janeiro, Englewood Cliffs, New Jersey, pp. 679-687

GIOFFRE, F., PASSARI, M., PROTO, V. 1988: Straw in feeding of rabbits. Rivista di coniglicoltura **25**: 43-46

GRTSCH, H., HAJD, P. 1971: Automatische Bestimmung von Enzymaktivitäten in tierischen Seren mit dem Reaction Rate Analyzer 8600. Z. Klin. Chem. Klin. Biochem. **9**: 123-125

GRTSCH, H., HAJD, P. 1972: Automatische Bestimmung von Enzymaktivitäten in tierischen Seren mit dem Reaction Rate Analyzer 8600. Z. Klin. Chem. Klin. Biochem. **10**: 334-337

HARTLEY, R. D. 1987: The chemistry of lignocellulosic materials from agricultural wastes in relation to processes for increasing their biodegradability. In: Degradation of Lignocellulosics in Ruminants and in Industrial Processes (Van Der Meer, J. M., Rijkens, B. A., Ferranti, M. P. Eds). Elsevier Applied Science, Elsevier, London and New York, pp. 3 -11

HENICS, Z. 1987: Effect of wheat straw upgraded by *Pleurotus ostreatus* on rumen fermentation and fattening performance of steers. World Rev. Anim. Prod. **23**: 55-60

HEWITT, C. D., INNES, D. J., SAVORY, J., WILLS, M. R. 1989: Normal biochemical and hematological values in New Zealand White rabbits. Clin. Chem. **35**: 1777-1779

JENSEN, N. E., CHWALIBOG, A., BOISEN, S., MARLOU, B., OG, B. S., KJAERGAARD, J. 1986: NaOH-treated straw in pelleted rations for rabbits in 6 generations. 602 Berretning fra Statens Husdyrbrugsfosog (*Report from the National Institute of Animal Science, Denmark*), pp. 1-72

KOZMA, C., MACKLIN, W., CUMMINS, L. M., MAUER, R. 1974: The anatomy, physiology and biochemistry of the rabbit. In: The Biology of the Laboratory Rabbit (Weisbroth, S. H., Flatt, R. E., Kraus, A. L. Eds). Academic Press, New York, pp. 50-72

KRAMER, J. W. 1989: Clinical enzymology. In: Clinical Biochemistry of Domestic Animals (J. J. Kaneko, ed.). 4th ed. Academic Press, San Diego, pp. 338-360

LEBAS, F., COLIN, M., MERCIER, P., TREMOLIERS, E. 1978: Use of straw treated with sodium hydroxide in rabbit feeding. (In French). 2čme Journes de la recherche cunicole, 4 et 5 Avril, Toulouse, pp. 11-16

LEMIEUX, G., WATFORD, M., VINAY, P., GOUGOUX, A. 1980: Metabolic changes in skeletal muscle during chronic metabolic acidosis. Int. J. Biochem. **12**: 75-80

LEONARD, J. V. 1982: Problems in the congenital lactic acidosis. In: Metabolic acidosis (Porter, R., Lawrenson, G. Eds). Ciba Foundation Symposium 87, Pitman Books, London, pp. 340-356

LEPITZKI, D., Woolf, A. 1991: Hematology and serum chemistry of cottontail rabbits of southern Illinois. J. Wildlife Diseases **27**: 643-649

LINDENA, J., TRAUTSCHOLD, B. 1986: Catalytic enzyme activity concentration in plasma of man, sheep, dog, cat, rabbit, guinea pig, rat and mouse. J. Clin. Chem. Clin. Biochem. **24**: 11-18

MACKLEAR, B., GUEST, G. M. 1953: Blood glycolysis: mechanisms inhibited by glycolysis. Am. J. Physiol. **174**: 269-272

McDONALD, P., EDWARDS, R. A., GREENHALGH, J. F. D. 1978: Animal Nutrition. 2nd ed. Longman, London, pp. 59-88

McLAUGHLIN, R. M., FISH, R. E. 1994: Clinical Biochemistry and Hematology. In: The biology of the laboratory rabbit (Manning, P. J., Ringler, D. H., Newcomber, C. E. Eds). Academic Press Inc., pp. 111-128

MITRUKA, B. M., RAWNSTEY, H. M. 1981: Clinical Biochemical and Hematological references values in normal experimental animals and normal humans. 2nd ed. Masson, New York

MUŽIC, S., BOŽAC, R., LIKER, B., RUPIĆ, V. 1990: Digestibility of substrate used for commercial production of *Pleurotus pulmonarius* fungi in rabbit (In Croatian). Krmiva **32**: 1-94

OLIVER, J., KOELZ, A. M., COSTELLO, Y., BOURRE, E. 1977: Acid-base induced alterations in glutamine metabolism and ureogenesis in perfused muscle and liver of rat. Eur. J. Clin. Inves. **7**: 445-449

RADWAN, M. A. H., PATRIDGE, C. C., ALLEN, S. J., FORDYCE, R. 1987: The use of treated straws in diets for growing rabbits. Agric. Res. Review **61**: 41-52

RAI, S. N., GUPTA, T. K. 1989: The chemical composition and nutritive value of rice straw after treatment with urea or *Coprinus fimentarius* in a solid state fermentation system. Animal Feed Sci. Techn. **26**: 81-91

RUPIĆ, V., BOŽAC, R., MUŽIC, S., ROGINA, Ž., VRANEŠIĆ, N., JERGOVIĆ, I. 1991: Effects of spent substratum from commercial production of *Pleurotus pulmonarius* fungi on some hematological and biochemical blood parameters of Chinchilla rabbits (In Croatian). Vet. glasnik **45**: 17-23

RUPIĆ, V., BOŽAC, R., MUŽIC, S., ROMIĆ, Ž., LIKER, B. 1996: Haematologic values of Hyla rabbits fed different levels of by-product arising from the propagation of *Pleurotus pulmonarius*. Acta Vet. Hung. **44**: 75-83

SAS Institute (1989): SAS Users Guide, Version 6, 4th ed. vol. 1 and 2, Cary, NC, SAS Institute Inc.

SCHEUNERT, A., TRAUTMANN, A. 1987: Lehrbuch der Veterinär-Physiologie. 7. Aufl. Paul Parey, Berlin und Hamburg, pp. 84-85, 160-207

SCHIEVELBEIN, H., LONDONG, W., LONDONG, H., GRUMBACH, H., REMPLIK, V. 1970: Nicotine and Arteriosclerosis. Z. Klin. Chem. Klin. Biochem. **8**: 190-196

SCHMIDT, E., SCHMIDT, F. W. 1960: Originalen Enzym - Muster menschlicher Gewebe. Klin. Wochenschr. **38**: 957-961

SCHMIDT, E., SCHMIDT, F. W. 1962: cit. on: Enzymes - Fundamentals of enzyme diagnostics. In: Geigy Scientific Tables, Vol.3, eight, revised and enlarged edition (Lentner, C. ed.). Ciba - Geigy, Basle, pp. 166-176

SCHROCK, H., CHA, C., GOLDSTEIN, L. 1980: Glutamine release from kind limb and uptake by kidney in the acutely acidotic rat. Bichem. J. **188**, 557-560

SIMPSON, D. P., SHARRARD, P. 1969: Regulation of glutamine metabolism *in vitro* by bicarbonate ion and pH. J. Clin. Inves. **48**: 1088-1096

SMITH, S. E. 1977: Vitamins. In: Dukes Physiology of Domestic Animals, Ninth Edition (Swenson, M. J. Ed .). Cornell University Press, Ithaca and London. pp. 378-394

The Ciba Collection of Medical Illustrations (2nd print., 1991. vol. 8, part I. Musculosceletal system. Anatomy, Physiology and Metabolic Disorders (prepared by Netter, F. H.). Ciba - Geigy Corp., New York, pp. 178-179

WEILS, I. G. 1969: Release of intracellular enzymes into serum. Can. J. Biochem. **47**: 347-351

ZADRAŽIL, F. 1987: White rot fungi and mushrooms grown on cereal straw: aim of the process final products, scope for the future. In: Degradation of Lignocellulosics in Ruminants and in Industrial Processes (Van Der Meer, J. M., Rijkens, B. A., Ferranti, M. P. Eds). Elsevier Applied Science, Elsevier, London and New York, pp. 55-62

YU, L., PRAGAY, D. A., CHANG, D., WICHER, K. 1979: Biochemical Parameters of normal Rabbit Serum. Clin. Biochem. **12**: 83-87