



Original Contribution

EFFECTS OF FASTING ON BLOOD CELLS FROM LAMBS OF VARIOUS BREEDS

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ABSTRACT

Studies on the effects of fasting (48 h of food deprivation) were performed on lambs of different productive types. The animals were divided into four groups: I group – Stara Zagora breed (SZ) (n=14), II group – “Ile de France” (ILF) (n=14), III group – Trakia Merino breed (TM) (n=14), and IV group – Trakia Merino crosses with ¼ blood of Australian Merino (TM x 50 % AM) (n=14). The changes of specific blood cell parameters were observed (red blood cell counts, total and differential white blood cell counts, blood sugar, total protein, albumin and globulin). It was determined that 48 hours of fasting caused changes in the observed indicators, by increase in erythrocyte counts, white blood cell counts, basophils, eosinophils, metamyelocytes, blood sugar, total protein and albumin, while also causing a decrease in the values of band leucocytes and monocytes. These changes were most evident in the animals of the Trakia Merino breed, and the least affected being lambs of the Stara Zagora breed.

Key words: *fasting, blood laboratory indicators, lambs*

INTRODUCTION

The natural resistance and adaptive capabilities of animals, in the circumstances of the advanced technologies in sheep breeding, fall under the constant influence of various factors, some of which are stress-inducing (1 – 7). The most common among them are the regimens of feeding and husbandry, the environmental and climatic conditions, the number of production groups, the nutrition front, the microclimate, transportation, regrouping, the therapeutic and immunopreventive activities, etc. Strong and prolonged stress (4, 5, 8) causes higher energy expenditure and results in reduced adaptation mechanisms, increase in the sensitivity towards conditional pathogens, decreased quality of production, which leads to economical losses (4-6, 9). The introduction of new technological lines and complex mechanization in stockbreeding is accompanied by the impact of factors, various

in strength and type. Such factors are the concentration of a large number of animals in a limited space, the lack of movement and pasture breeding, the inclusion of growth promoters in the food together with other preparations, protecting the animals against infectious or parasitic agents, etc. These factors can lead to disruptions in the animals' metabolism and alterations in the homeostasis. The action of these factors and the adaptation capabilities determine the growth, development, productivity, and health status of the animals (4, 6, 10 - 12).

Under the influence of stressors, the body reacts by activating neurohormonal regulatory mechanisms, through which it maintains the homeostasis, the objective assessment of which are the blood indicators (3 - 5, 7, 9, 13, 14).

One of the most significant external factors is feeding, and its characteristics – type and quality, ratio of the various nutrients, diet balancing with regard to protein, carbohydrates, fats, macro- and trace elements, etc. Through all these elements, feeding exercises a considerable influence over the physiological condition and the homeostasis of the animal body (4, 5, 15 - 20).

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Research on the changes in the blood laboratory indicators under the conditions of feeding stress has been done on sheep (3 - 6), goats (16), cattle (7), rabbits (21), suncus (18) and rats (17, 19, 20, 22 - 25). Most of the reported data about the observed changes under starvation conditions are controversial, since the factors of interest are significantly influenced from the duration of the starvation, the different types of digestion in mono- and polygastric animals, breed- and age-related peculiarities as well as various other factors.

The aim of the current study was to determine the changes in some of the blood laboratory parameters in lambs of various breeds (Stara Zagora, Ile de France, Trakia Merino, and Trakia Merino crosses with ¼ blood of Australian Merino), with the purpose of establishing of the influence of feeding stress in animals from with different productive types (dairy, meat, and merino sheep).

MATERIALS AND METHODS

The studies were performed in the premises of the Agricultural Institute in Stara Zagora. The animals were divided into four groups: I group – Stara Zagora breed (SZ) (n=14), II group – Ile de France (ILF) (n=14), III group – Trakia Merino breed (TM) (n=14), and IV group – Trakia Merino crosses with ¼ blood of Australian Merino (TM x 50 % AM) (n=14). All members of the groups were healthy, and were of uniform weight (35 kg) and gender. Before the experiment, they were freely fed with a starting mixture, containing 6.874 MJ net energy and 113.95 g digestible protein (in 1 kg), and alfalfa hay, containing 2.958 MJ net energy and 115 g digestible protein (in 1 kg). The experiment was performed in the month of June. For 48 hours, the animals were deprived of food, with *ad libitum* access to drinkable water.

Prior to fasting and on the 48th hour after that, blood samples were collected from *v. jugularis* for the determination of the following laboratory parameters – red blood cell counts (RBC; T/L), white blood cell counts (WBC; G/L) and differential white cell counts (DWC; %) on an automated analyzer (Cell dyn 4500, USA), blood glucose (Glu; mmol/l), total protein (TP; g/l), albumin (Alb; g/l) and globulin (Glob; g/l) concentrations on an automated biochemical analyser (Olympus AU 600, Japan).

All results were statistically processed using the ANOVA test (Statistica software). The significance of differences was evaluated vs the control group for each time interval.

The results were determined as significant at the level, $p < 0.05$.

RESULTS

The analysis of the results showed that fasting stress caused significant changes in the levels of the observed indicators in lambs. Together with that, variations between the blood cell indicators of the different breeds were also observed.

In the lambs of groups I, II, and III, increases in the red blood cell counts were observed on the 48th hour of the starvation (**Figure 1**). The greatest differences were found in the animals of the Ile de France breed (II group) – 9.74 ± 0.39 T/l, in comparison with the initial values – 7.79 ± 0.27 T/l ($p < 0.01$). In crossbred lambs (TMx50 % AM), no significant changes were detected.

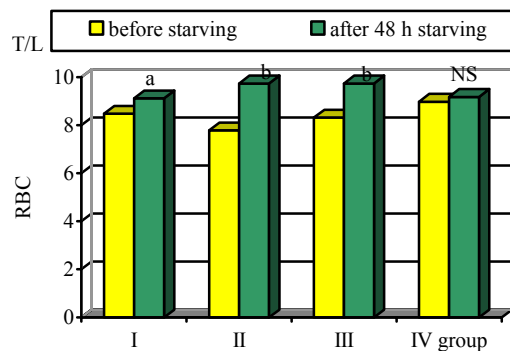


Figure 1. Changes in the red blood cell counts in lambs, subjected to 48 hours of fasting.

Under the effect of fasting, the white blood cell counts (**Figure 2**) increased for groups I and IV. The values were 8.63 ± 0.16 G/l and 8.75 ± 0.43 G/l, respectively, in comparison with the respective initial levels – 7.83 ± 0.23 G/l ($p < 0.01$) and 6.85 ± 0.49 G/l ($p < 0.01$). Significant changes in the parameters of this parameter were not found in the other two breeds (ILF and TM).

In the merino type groups (TM and TTx50 % AM) we observed an increase in basophils counts (**Figure 3**) – 1.52 ± 0.34 % and 1.75 ± 0.48 % respectively in comparison with the initial levels of 0.75 ± 0.23 % ($p < 0.01$) and 0.86 ± 0.14 % ($p < 0.01$). For the lambs of groups II and III, the variances were insignificant, compared to initial values.

Significant changes under the influence of fasting stress were observed in the counts of eosinophils (**Figure 4**). The studied parameter was higher for all groups on the 48th hour of starvation: 2.16 ± 0.44 % for group I, 3.67 ± 0.56 % for group II, 3.83 ± 0.54 % for group III, and 2.87 ± 0.58 % for group IV, in comparison with the respective initial levels – 1.78 ± 0.15 % ($p < 0.05$), 1.21 ± 0.21 % ($p < 0.01$),

1.13±0.13 % ($p<0.01$) and 1.42±0.20 % ($p<0.01$).

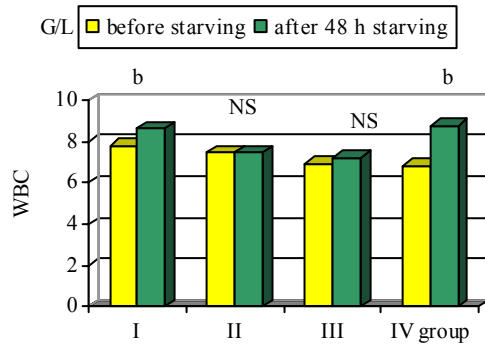


Figure 2. Changes in the white blood cell counts in lambs, subjected to 48 hours of fasting

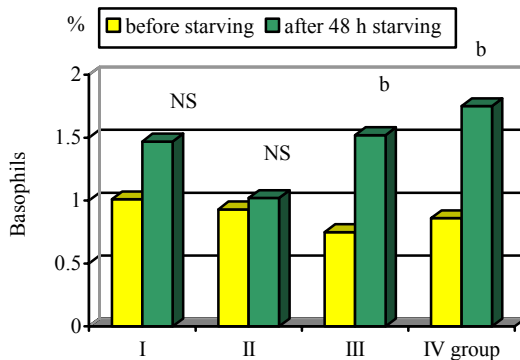


Figure 3. Changes in the basophils counts in lambs, subjected to 48 hours of fasting



Figure 4. Changes in the eosinophils counts in lambs, subjected to 48 hours of fasting

An analogical increase was observed in the counts of metamyelocytes as well (**Figure 5**) in fasted lambs of groups II, III and IV – 2.52±0.34 %, 1.83±0.30 % and 2.54±0.65 %, in comparison with the respective initial levels – 1.51±0.20 % ($p<0.01$), 1.75±0.16 % ($p<0.01$) and 1.75±0.25 % ($p<0.05$). No significant changes in the values of this parameter were observed for the first group.

The opposite tendency was observed for bands leukocyte counts (**Figure 6**). In the animals of second, third, and fourth group, a decrease was observed on the 48th hour of

food shortage. The values were 1.83±0.48 %, 2.25±0.48 % and 2.25±0.46 %, respectively vs the respective initial values – 3.29±0.42 % ($p<0.01$), 3.63±0.42 % ($p<0.01$) and 3.29±0.42 % ($p<0.01$). Again, no significant changes were detected in the first group.

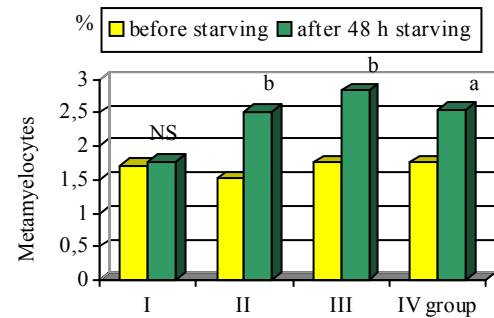


Figure 5. Changes in the counts of metamyelocytes in lambs, subjected to 48 hours of fasting

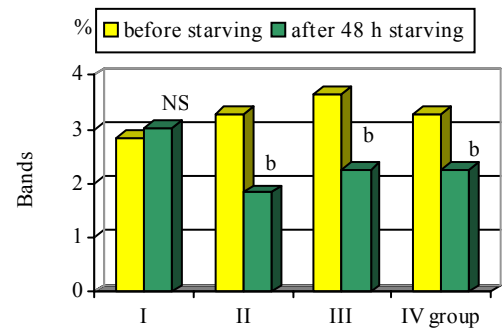


Figure 6. Changes in the bands leukocyte counts in lambs, subjected to 48 hours of fasting

In the lambs of the Trakia Merino breed, and its crosses with Australian Merino, we observed a decrease in the counts of monocytes under the conditions of feeding stress (**Figure 7**) – 2.53±0.56 % and 3.25±0.47 %, respectively in comparison with the respective figures before the starvation – 4.25±0.67 % ($p<0.01$) and 4.42±0.75 % ($p<0.05$). No significant changes were detected in the lambs of the first and second groups.

The results about segmented leukocytes and lymphocytes counts did not show any significant changes after the 48 hours of food deprivation.

The glycaemic level (**Figure 8**) was raised on the 48th hour of hunger in all groups of lambs – 3.13±0.10 mmol/l for group I, 2.97±0.10 mmol/l for group II, 3.05±0.10 mmol/l for group III, and 3.12±0.17 mmol/l for group IV, compared to the respective initial values – 2.56±0.05 mmol/l ($p<0.01$), 2.73±0.10 mmol/l ($p<0.01$), 2.75±0.08 mmol/l ($p<0.01$) and 2.69±0.07 mmol/l ($p<0.01$).

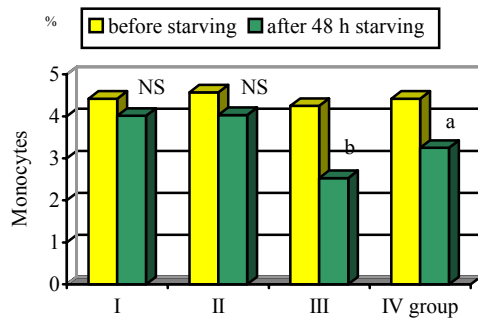


Figure 7. Changes in the counts of monocytes in lambs, subjected to 48 hours of fasting



Figure 8. Changes in the counts of blood glucose in lambs, subjected to 48 hours of fasting

A similar tendency was found in the total protein (Figure 9) and albumin concentrations (Figure 10). In the lambs of group II, both indicators were raised on the 48th hour of fasting – 69.93±1.65 g/l and 43.52±1.41 g/l, respectively as opposed to the respective initial values – 63.29±2.05 g/l ($p<0.01$) and 39.71±1.92 g/l ($P<0.01$). Identical changes in these parameters were detected in the animals of group III – 70.42±2.42 g/l and 41.77±1.31 g/l, respectively vs the respective values before the experiment – 58.13±2.36 g/l ($p<0.01$) and 39.13±1.27 g/l ($p<0.05$). No significant changes in the levels of blood total protein and albumin were detected in the lambs of groups I and IV.

Globulin levels in all groups had not changed after 48 hours of starvation.

DISCUSSION

The results obtained showed significant changes in the blood laboratory indicators in lambs of various breeds, subjected to feeding stress. Also, information on the differences between breeds, in regard to the extent of observed changes within haematological and blood biochemical parameters were obtained. The analysis of the changes in the erythrocyte counts showed an increase of this indicator in the blood of lambs after 48 hours of starvation. This was most probably related to the proved dehydration, caused by the

lowered intake of water during the fasting period, correlating to the reduced amounts of excreted urine (2, 4, 5, 9, 13, 19, 27). On the other hand, in polygastric animals, in periods of food deprivation, increases in the concentration of ammonia in rumen occurs, which results in impaired osmotic balance and blood vessels' permeability (6, 10, 16). Thus, blood and tissue fluids are transferred into the rumen, which is an additional reason for the occurrence of dehydration in animals subjected to feeding stress (1, 4 - 6, 16).

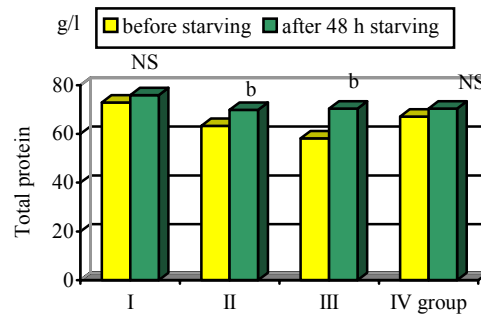


Figure 9. Changes in the counts of total protein in lambs, subjected to 48 hours of fasting

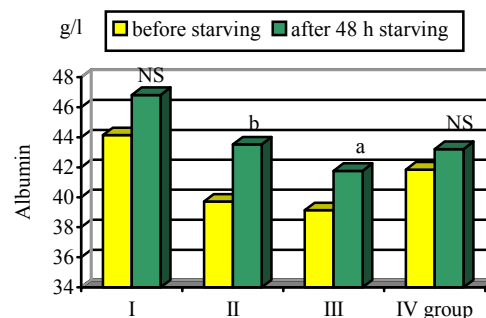


Figure 10. Changes in the counts of albumin in lambs, subjected to 48 hours of fasting

The changes in the white blood cell picture exhibited increases in total leukocytes counts, eosinophils, and metamyelocytes, as well as decreases in the counts of basophils and monocytes. The interpretation of the changes in basophil leukocytes is not yet provided (13). Our results supported the suggestion of Kaneko (13), that fasting causes decrease in the percentage of basophils. It could be assumed that the established leukocytosis with neutrophilia is a result of an inflammatory reaction, caused by the direct action of ammonia on the rumen wall (1, 16). Newer theories point out that the observed changes (leukocytosis, neutrophilia, eosinophilia, and monocytopenia) are typical for the so-called "stress leukogramme", which is due to the increased endogenous production of cortisol from the adrenal glands, following the action of stress factors, one of which is fasting (4, 5,

16 - 20). The established monocytopenia could correlate with the decreased adaptation and defence mechanisms, leading to higher sensitivity to pathogen influences (2, 9).

According to Ziegler (8), Sandner et al. (16), Peng and Coon (21), Yamazoe et al. (22), and Son et al. (25), stress-inducing factors cause an increase in the production of catecholamines in blood, as a result of adrenal gland hyperfunction and a simultaneous inhibition of the production of insulin in the pancreas. These data correlate with the established hyperglycaemia from our studies on the changes in the levels of blood sugar in lambs subjected to feeding stress. Some authors observed an opposite relationship of blood glucose lowering, that was explained by the fast depletion of glycogen in the liver (18). For rats, Popov et al. (24) did not find any changes in glucose levels after starvation. According to many researchers (8, 17, 21, 22, 25, 26), the process of glycogenolysis is only observed in the first 24 hours of fasting. Afterwards, the delivery of glucose is provided through the processes of gluconeogenesis from amino acid precursors and lipolysis from glycerol, as well as from lactate through the Cori cycle. Lactate is quickly transformed into pyruvate, and included in the gluconeogenesis together with the amino acids, which, after deamination, are included at various levels as carbohydrate residues. According to us, the detected hyperglycaemia is an objective criterion, which allows us to claim that for lambs, the 48-hour starvation serves as a stress factor. Our position correlates with the mechanism of inducing hyperglycaemia, namely the increase in the levels of catecholamines and glucagon in the first 48–72 hours of hunger (8, 21, 23), resulting in enhanced glycogenolysis and lipolysis (8, 16, 17, 25, 26). This process is an additional cause for the observed haematological changes because the increased production of catecholamines (epinephrine and dopamine) results in peripheral vasoconstriction and redistribution in blood, expressed with presence of erythrocytosis, leukocytosis, and neutrophilia (22).

The results from the changes in the levels of total protein, albumins, and globulins, showed the presence of hyperproteinaemia, which correlates with the increased levels of albumin in the blood. Most authors (19, 25) report the opposite tendency of hypoproteinaemia in starving animals. It could be assumed that the increase in the total protein is a result of an inflammatory process, correlating with the fact that

hyperproteinaemia appears together with hyperalbuminaemia, while globulins do not change. On the other hand, the presence of dehydration leads to a relative increase of total protein and protein fractions (27).

The performed studies on the effect of a 48-hour fasting period for lambs from different productive types, showed several differences between breeds in the blood laboratory changes. In the animals of the Stara Zagora breed, the changes were the least exhibited, or the least number of indicators had changed. From that could be assumed that lambs of this breed are the most resistant to starvation. The opposite tendency was observed in the animals of the Trakia Merino breed. With them, the highest number of tested indicators changed, and the extent of changes was the greatest. From that it could be assumed that merino type lambs were the most sensitive to starvation stress. The changes of blood laboratory parameters after 48 hours of starvation, for IIF and TMx50 % AM took a middle position, compared to the data obtained from TM and SZ, with regard to the extent of detected changes. We assume that the heterosis effect in TMx50 % AM did not have any significant influence on the resistance of lambs to feeding stress.

CONCLUSIONS

1. The changes in blood laboratory parameters in lambs subjected to 48 hours of starvation are exhibited through increases in the counts of erythrocytes, leukocytes, basophils, eosinophils, metamyelocytes, blood sugar, total protein, and albumin, and decreases in the counts of band leukocytes and monocytes.
2. Merino-type animals (TM) were the most sensitive to fasting stress, while dairy lambs (SZ) were the most resistant.

REFERENCES:

1. Potter, E. L. and Dehority, B. A., 1973. Effects of changes in feed level, starvation, and level of feed after starvation upon the concentration of rumen protozoa in the ovine. *Appl. Microbiol.*, 26, 5, 692–698.
2. Burxer, G. V., 1974. Stress in farm animals. *Veterinaria*, 8, 92-94.
3. Blum, J. W., M. Gingius, P. Vitins and H. Bickel, 1980. Thyroid hormone levels related to energy and nitrogen balance during weight loss and regain in adult sheep. *Acta Endocrinologica*, 93, 440-447.

4. Kock, M. D., Clark, R. K., Franti, C. E., Jessup, D. A. and Wehausen, J. D., 1987. Effects of capture on biological parameters in free-ranging bighorn sheep (*Ovis canadensis*): evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. *J. Wildl. Dis.*, 23, 4, 652-662.
5. Kock, M. D., Jessup, D. A., Clark, R. K. and Franti, C. E., 1987a. Effects of capture on biological parameters in free-ranging bighorn sheep (*Ovis canadensis*): evaluation of drop-net, drive-net, chemical immobilization and the net-gun. *J. Wildl. Dis.*, 23, 4, 641-651.
6. Iliev, Y., N. Sedloev, B. Bivolarski, G. Kutsarov, 1995. Metabolic and functional changes in sheep submitted to feeding stress. *Veterinary Medicine, Suppl.* 1, 86-90.
7. Kutsarov, G., Y. Iliev, B. Bivolarski, 1995. Effect of rearing technology of pregnant cows upon the adaptative potential of their offspring. *Veterinary Medicine, Suppl.* 1, 64-66.
8. Ziegler, R., 1991. Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta*. *J. Comp. Physiol. (B)*, 161, 2, 125-131.
9. Golikov, A. N., 1985. Adaptation in farm animals, Agropromizdat, Sofia.
10. Petrov, Av., 1982. Processes of food digestion and feeding behaviour in lambs. Dissertation, VIZVM, Stara Zagora.
11. Alonso, A. J., de Teresa, R., Garcia, M., Gonzales, J. R. and Vallejo, M., 1997. The effects of age and reproductive status on serum and blood parameters in merino breed sheep. *Zentralbl. Veterinarmed. A*, 44, 223-231
12. Bickhardt, K., Dudziak, D., Ganter, M. and Henze, P., 1999. Investigations on the dependence of haematologic and blood chemical parameters on the age of health lambs - a contribution to the definition of reference values in sheep. *Dtsch. Tierarztl. Wochenschr.*, 106, 445-451
13. Kaneko, J. J., 1989. Clinical Biochemistry of Domestic Animals. 4th edn. San Diego, Academic Press. Inc. Chapters 6 and 18, and Appendix VII.
14. Desco, M., Cano, M. J., Duarte, J., Rodriguez, F., Fernandez-Caleya, D., Alvarez-Valdivielso, M., Antoranz, J. C., Rubio, M. A., Garcia-Barreno, P. and del Canizo, J. F., 1989. Blood biochemistry values of sheep (*Ovis aries ligeriensis*). *Comp. Biochem. Physiol. A*, 94, 4, 717-719.
15. Hill, C. H., 1982. Interaction of dietary amino acids with the immune response. *Fed. Proc.*, 41, 2818-2820.
16. Sandner, N., Strangassinger, M. and Giesecke, D., 1990. The effect of glucocorticoid on the glucose metabolism of pigmy goats. 1. Selected metabolites of energy metabolism. *Zentralbl. Veterinarmed A*, 37, 1, 35-44.
17. Duhault, J., Lacour, F., Espinal, J. and Roland, Y., 1993. Effect of activation of the serotonergic system during prolonged starvation on subsequent caloric intake and macronutrient selection in the Zucker rat. *Appetite*, 20, 2, 135-144.
18. Ohama, T., Matsuki, N., Saito, H., Tsukamoto, K., Kinoshita, M., Katsuragawa, K., Okazaki, S., Yamanaka, M. and Teramoto, T., 1994. Effect of starving and refeeding on lipid metabolism in suncus. *J. Biochem. (Tokyo)*, 115, 2, 190-193.
19. Munch, I. C., 1995. Influences of time intervals between meals and total food intake on resting metabolic rate in rats. *Acta Physiol. Scand.*, 153, 3, 243-247.
20. Chung, H. C., Sung, S. H., Kim, J. S., Kim, Y. C. and Kim, S. G., 2001. Lack of cytochrome P450 2E1 (CYP2E1) induction in the rat liver by starvation without coprophagy. *Drug. Metab. Dispos.*, 29, 3, 213-216.
21. Peng, H. M. and Coon, M. J., 1998. Regulation of rabbit cytochrome CYP2E1 expression in HepG2 cells by insulin and thyroid hormone. *Mol. Pharmacol.*, 54: 740-747.
22. Yamazoe, Y., Murayama, N., Shimada, M., Yamauchi, K. and Kato, R., 1989. Cytochrome P450 in livers of diabetic rats: Regulation by growth hormone and insulin. *Arch. Biochem. Biophys.*, 268, 567-575.
23. Chen, D., Andersson, K., Iovanna, J. L., Dagorn, J. C. and Hakanson, R., 1993. Effects of hypercholecystokinemia produced by pancreaticobiliary diversion on pancreatic growth and enzyme mRNA levels in starved rats. *Scand. J. Gastroenterol.*, 28, 4, 311-314.
24. Popov, V. N., Volvenkin, S. V., Eprintsev, A. T. and Igamberdiev, A. U., 2000. Induction of glyoxylate cycle enzymes in various tissues from starving rats. *Izv. Akad. Nauk. Ser. Biol.*, 6, 672-678.
25. Son, M. H., Kang, K. W., Kim, E. J., Ryu, J. H., Cho, H., Kim, S. H., Kim, W. B.

- and Kim, S. G., 2000. Role of glucose utilization in the restoration of hypophysectomy-induced hepatic cytochrome P450 2E1 by growth hormone in rats. *Chem-Biol. Interact.*, 127, 13-28.
26. Sinsch, U., Seine, R. and Sherif, N., 1992. Seasonal changes in the tolerance of osmotic stress in natterjack toads (*Bufo calamita*). *Comp. Biochem. Physiol. Comp. Physiol.*, 101, 2, 353-360.
27. Kaneko, J. J., 1989a. Serum proreins and dysproteinaemias. In Kaneko, J. J. (ed) *Clinical Biochemistry of Domestic Animals*. 4th edn. *Academic Press. San Diego*, 156-163, 365-375.