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The effect of fasting on the concentrations of total proteins and of uric acid as well as on aminotransferase activity in duckling blood plasma

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ABSTRACT

The effect of a six-day fasting period on the variations of total protein and uric acid concentrations as well as on the activities of aspartate aminotransferase [aspartate:2-oxoglutarate aminotransferase (AST); EC 2.6.1.1] and alanine aminotransferase [L-alanine:2-oxoglutarate aminotransferase (ALT); EC 2.6.1.2] was investigated in Peking Duck ducklings. At the age of 28 days, the ducklings were divided into two groups: a normally fed control group (n = 28), and a group that was submitted to a six-day long fast. Eight ducklings from the experimental group and seven ducklings belonging to the control group were sacrificed by decapitation after the 3rd, the 4th, the 5th, and the 6th day of fasting. The blood for analysis was sampled from the neck, simultaneously in the experimental and in the control group, heparin being used as the anticoagulant. In the blood plasma obtained, the concentrations of total proteins and of uric acid as well as the levels of AST and ALT activities were assessed by spectrophotometer. Over the entire trial period, the concentration of total proteins in the blood plasma of the fasting ducklings was significantly lower than the control values: on day 3, $P = 0.0239$, and on days 4, 5, and 6, $P = 0.0012$. After the period of five days of the trial, a significantly higher ($P = 0.018$) value of uric acid concentration was measured in the experimental ducklings than in the controls, but at the same time, the lowest uric acid concentration in the entire trial period was found in the control group. In relation to the control group, significantly lower AST activity was found after the 5th day of fasting ($P = 0.0276$), and lower ALT activity after the 4th day of fasting ($P = 0.0410$). The low concentrations of total proteins over the entire trial period as well as reduced activities of the afore mentioned enzymes were probably a consequence of a reduced protein regeneration in the body due to the deficiency of amino acids from the gastro-intestinal tract during fasting.

Key words: fasting, ducklings, total proteins, uric acid, aspartate aminotransferase, alanine aminotransferase

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Introduction

Apart from being involved in the gluconeogenesis process, aminotransferases are also involved in the formation of some amino acids that the body does not receive in food during fasting. There are two principal AST isoenzymes: mitochondrial and cytosolic isoenzymes, the latter prevailing in the total blood plasma concentration due to its longer half-life (KRAMER and HOFFMAN, 1997). The activity of ALT in the blood plasma is dependant on the animal species, age and on the muscular activity (WEIGERT et al., 1980). According to FORENBACHER (1993), the half-life of ALT is approximately 75 hours. The values of AST and ALT in the blood plasma of the Black Duck are 20.1 and 18.6 IU/L respectively (FRANSON, 1982). The activities of ALT and AST in the serum of 63 day-old ducks measure 19.0 and 15.0 IU/L (JENKINS et al., 1982). Alterations in the levels of enzymes in the cells lead to their increased escape into extracellular space and into the blood in accordance with the principle of "endogenous-exogenous divergention in secretion". According to some investigations, there are positive correlations between AST and ALT levels in the liver and their respective levels in the blood plasma (ZIMMERMAN et al., 1968). The variations in these enzyme levels can be the consequences of an altered permeability of the cellular membrane, which indirectly suggests a lesion of the cells of a specific organ. Therefore, while increased AST and ALT values suggest muscular damage, toxic indigestion, and/or various metabolic disorders, decreased levels of enzymes speak for fasting or under-nourishment.

In birds, in contrast to mammals, ALT is not a liver-specific enzyme. Moreover, in the avian liver, more than 90 percent of ALT is found in the mitochondria, and only six percent in the cytosol. Similar activity is displayed by AST in duck liver and kidneys (FRANSON, 1982). In some birds, fasting is a side-effect during moulting and hatching, as well as during long flights, when the birds, saving their own proteins, satisfy their energy requirements primarily by mobilising glycogen, and then fat (BORDEL and HAASE, 1993; CHEREL et al., 1993; CHEREL et al., 1994). During fasting, the birds maintain high blood glucose levels (BRADY et al., 1978; FUJIWARA et al., 1994). The preservation of normoglycaemia during fasting involves the escape of glucose from the glycogen stored in the liver, the recycling of glucose metabolism by-products, and glucose synthesis from non-carbohydrate precursors. Considering the stability of glucose in the blood of birds during fasting as well as the rapid wasting of glycogen reserves (PEARCE, 1971; BELO et al., 1976; MILINKOVIĆ-TUR et al., 1996), it is to be expected that during fasting all metabolic pathways, also including protein metabolism, will have to rapidly and harmoniously change their functions. Amino acids that have not been used for the synthesis of tissue proteins, of enzymes and of other substances, are submitted to catabolic reactions in the liver, muscles and the kidneys. Apart from being used for recompensing the required energy as gluconeogenesis precursors, the released amino acids may serve in normoglycaemia maintenance. Since during fasting carbohydrates and fats are first used

to provide energy, they are called "protein preservers". The increased protein catabolism in the tissues and the inclusion of amino acids in the energy metabolism do not commence before approximately 80 percent of fat reserves have been spent (CHEREL et al., 1994). In birds, the final product of protein degradation and of non-protein nitrogen is uric acid. That is why its increased quantity as well as the decrease of free fatty acids in the blood plasma suggests the intensified expenditure of tissue proteins (HANDRICH et al., 1993).

Ducks are characterized by exceptionally rapid growth and increment, and play an ever greater role in meat production. They have recently become an increasingly important object of investigations in the field of veterinary biochemistry. As the estimate of metabolic events during fasting and of problems arising during refeeding of previously starved animals is of utmost importance, the purpose of the present paper was to investigate the impact of a six-day-long fast on protein metabolism during the period of intensive growth of ducklings.

Materials and methods

The experiments were conducted on Peking Duck ducklings (an English fattening breed). One-day old ducklings were placed in grated cages. Immediately after that, they were given feed and water. The ducklings were fed starter feeds for chicken fattening produced in "Sljeme" Animal Feed Factory in Zagreb, Croatia. Feed and fresh water, both available *ad libitum,* were offered in metal feeders and drinking troughs. At the arrival of the animals, the room temperature was 30 °C, and the relative air humidity 60 to 70 percent. Over the following days, the room temperature was gradually decreased, so that from the birds' 14th day of life it was decreased to 20 °C. Until the 14th day of life, the ducklings were kept in an all-day light regimen, and after the 24th day of life, the room was lighted by daylight. At the age of 28 days, the ducklings were divided into two groups: the control group, which continued to have access to feed and water *ad libitum* (28 ducklings), and the trial group, which was submitted to a six-day fast (32 ducklings). This was the point when the experiment period started. After the completion of days 3, 4, 5 and 6 of fasting, the ducklings were sacrificed by decapitation - eight ducklings from the trial group and seven ducklings from the control group at each sacrificing, always at the same time of day (8 a.m.), in order to avoid daily variations in the investigated parameters concentrations. The blood for analyses was sampled from the neck while the animal was bleeding, heparin being used as the anticoagulant agent. Blood plasma was separated by centrifuging the blood at 1,500 g for 10 minutes. In the plasma obtained, the concentrations of total proteins and of uric acid were assessed by spectrophotometry, using commercial kits manufactured by Herbos Dijagnostika Ltd., Sisak, Croatia. The activities of aspartate aminotransferase (AST; EC 2.6.1.1) and of alanine aminotransferase (ALT; 2.6.1.2) were measured using Boehringer commercial kits (Boehringer, Mannheim). The results of total

protein concentrations were expressed in g/L, the values of uric acid in μmol/L, and AST and ALT activities in U/L. The results obtained were statistically analysed and presented as median and as the upper and lower quartiles. The significance of the differences between the trial and control groups, as well as those occurring within the trial group associated with the trial period, were measured using the Kruskal-Wallis test.

Results

The results of total protein and uric acid concentrations as well as of the activities of AST and ALT enzymes in the blood plasma are presented in Tables 1 and 2.

As presented in Table 1, the concentration of total proteins in the blood plasma of the experimental ducklings was significantly lower than in the controls over the entire experiment period: after the 3rd day of fasting, this level of significance was P = 0.239, and after the 4th, 5th and 6th days of fasting, the level of significance was P = 0.0012. When the values of total protein concentrations in the blood plasma of the fasting ducklings were compared, a significant reduction in relation to prior trial periods was observed after the 4th day of fasting (P = 0.0045) and after the 5th day of fasting (P = 0.0008). After the 6th day of fasting, the concentration of total protein in the fasting ducklings significantly increased (P = 0.0011) in relation to the previous fasting period, these values, however, still being significantly lower than in controls (P = 0.0012).

Uric acid concentration in the blood plasma of the fasting ducklings did not significantly differ from the values obtained in the control group until the 5th day of fasting. After the 5th day of fasting, a significantly higher uric acid concentration (P = 0.018) in the fasting ducklings in comparison to the controls was observed. However, during the same trial period, the lowest uric acid concentration (155.794 μmol/L) was observed, while over the remaining period of the experiment, this concentration varied between 297.425 μmol/L and 339.914 μmol/L. In the fasting animals, uric acid concentration gradually decreased with fasting, but on comparison of these values, no significant differences were found between individual trial periods.

Significantly lower AST activity in the blood plasma of the fasting ducklings in comparison to the control group was detected after the 5th day of fasting (P = 0.0276), and significantly lower ALT activity (P = 0.0410) than in controls after the 4th day of fasting. Over the six-day experimental period, AST activity in the blood plasma of the fasting ducklings did not change significantly, while ALT activity in the blood plasma decreased with the duration of fasting, as follows: from 10.500 U/L after the 3rd day of fasting to 2.500 U/L after the 6th day of fasting. A significant reduction of ALT activity in the fasting ducklings was observed after the 4th day (P = 0.0104) and after the 6th day of fasting (P = 0.0080) in comparison to the previous trial period.

Table 1. Concentration of total proteins and uric acid in the blood plasma of the normally fed group and the fasting group of ducklings

Days of experiment	Variable	Concentrations of total proteins in the blood plasma (g/L)		Concentrations of uric acid in the blood plasma (μmol/L)	
		Control	Fasted	Control	Fasted
3	median	41.182	37.997*	297.425	346.996
	quartiles	40.727-42.092	35.722-39.589	184.120-311.588	296.009-410.730
4	median	42.774	33.332**^{a}	339.914	311.588
	quartiles	27.451-45.277	30.716-34.243	184.120-396.567	240.773-382.404
5	median	32.991	23.662**^{c}	155.794	269.099*
	quartiles	29.578-33.673	22.162-24.686	99.142-169.957	212.446-418.545
6	median	35.494	27.189**^{b}	311.588	245.022

The significance of differences in total protein concentration by days of fasting between duckling control group and trial group: *P = 0.0239, **P = 0.0012; the significance of differences within the duckling fasting group in relation to the earlier trial period: ^{a}P = 0.0045, ^{b}P = 0.011, ^{c}P = 0.0008; The significance of differences in uric acid concentration between the duckling control and trial groups by days of fasting: *P = 0.018.

Table 2. Activity of AST and ALT in the blood plasma of normally fed group and fasted group of ducklings

Days of experiment	Variable	Activity of AST in the blood plasma (U/L)		Activity of ALT in the blood plasma (U/L)	
		Control	Fasted	Control	Fasted
3	median	22.000	21.000	11.500	10.500
	quartiles	17.500-42.000	16.250-29.750	10.000-16.000	9.000-11.750
4	median	16.500	15.000	8.500	6.000*^{a}
	quartiles	13.000-20.500	13.000-19.250	7.000-9.000	6.000-7.250
5	median	24.000	18.500*	6.000	4.000
	quartiles	22.000-38.000	12.250-22.750	6.000-8.000	3.000-6.750
6	median	14.000	14.000	3.000	2.500^{b}
	quartiles	13.000-17.500	11.250-15.250	3.000-4.000	2.000-3.000

The significance of differences in activity of AST by days of fasting between duckling control group and trial group: *P = 0.0276; the significance of differences in activity of ALT by days of fasting between duckling control group and trial group: *P = 0.0410; the significance of differences within duckling fasting group between single experimental periods for the activity of ALT: ^{a}P = 0.0104, ^{b}P = 0.0080

Discussion

During fasting as well as during an inadequate diet, all metabolic pathways must act rapidly and synchronously, with the aim to maintain normoglycaemia and procure much needed energy. This induces the rapid consumption of the supplies of glycogen stored in the liver, and then of that stored in the muscles (MILINKOVIĆ-TUR et al., 1996). After the glycogen supplies have been spent, fats and, to a smaller extent, body proteins are mobilised (LIEN et al., 1999). A more intensive breakdown of body proteins and amino acid entrance into the metabolic pathways starts when approximately 80 percent of stored fat is consumed (CHEREL et al., 1994). Up to that time, amino acids needed for the synthesis of vital proteins, for instance, of hormones and enzymes, are procured by tissue proteolysis. When the quantity of proteins in any cell is reduced, circulating proteins can serve as a source for rapid compensation. In this way, protein concentrations in the blood plasma are tightly connected with the synthesis and breakdown of proteins in the body. During fasting, due to the lack of amino acids from the gastro-intestinal tract, protein regeneration in the body is reduced.

According to the results of these investigations, when total protein concentration was compared to the same parameter in the normally fed duckling group, a reduction of this concentration was observed in the blood plasma of the fasting ducklings during the entire experimental period. This reduced concentration of total proteins in the blood plasma of the fasting ducklings could therefore be attributed not only to reduced plasma protein regeneration, but also to the consumption of plasma proteins, with the purpose of synthesising indispensable body proteins. The decrease of protein concentrations in the blood plasma could have been a consequence of their more intensive breakdown in order to maintain the required glucose levels in the blood as well as of direct energy production. If amino acids are used as a source of energy, or as a gluconeogenesis precursor, only the keto-acid part is used, while the amino group is excreted from the body as so-called non-proteinic nitrogen. As non-proteinic nitrogen in birds is eliminated in uric acid, the concentration of uric acid in the blood plasma is closely connected with protein breakdown.

In the present investigations, the concentration of uric acid in the blood plasma of the fasting ducklings was found to be higher in relation to controls after the 5^{th} day of fasting. However, as, at the same time, uric acid concentration in the controls' blood plasma was found to be the lowest, it could not be asserted that the levels of uric acid actually increased, i.e., whether tissue proteins were broken down at that point of fasting. Some authors have stated that, during fasting, more ample gluconeogenesis is going on, simultaneously with reduced protein biosynthesis (AYUSO et al., 1986). This reduced protein biosynthesis saves amino acids for their transformation into glucose. This suggests the conclusion that gluconeogenesis is vitally a more important pathway of biosynthesis during fasting.

As aminotransferases play a significant role in linking carbohydrate and protein metabolisms, it can be expected that, during fasting, alterations in their activity would occur. Because liver enzymes as well as blood plasma enzymes are proteins, their activity, generally speaking, is also dependent on the diet. In case of the absence of proteins in a meal or in total fasting, in principle, plasmatic enzymal activity is reduced in parallel with protein content in the liver, or even more rapidly (FORENBACHER, 1993).

According to the results of the present research, a significant reduction of AST activity occurred after the 5th day of fasting, whereas the activity of ALT significantly fell after the 4th day of fasting when these enzymal activities where compared to those in the control group. The activity of the enzyme ALT decreased with the duration of fasting, so that significant activity reduction was assessed after the 4th and 6th days of fasting in comparison to the prior days of fasting. The reduced activity of the examined enzymes in the blood plasma was probably a consequence of the reduced protein regeneration in the body induced by the deficiency of amino acids from the gastro-intestinal tract, as well as by the low level of gluconeogenesis from amino acids during the fasting period examined. The results obtained are in accordance with the results of the earlier investigations of normoglycaemia maintenance during fasting in ducklings. According to the results reported by POLJIČAK-MILAS et al. (2003), a six-day fast in ducklings results in an increase of activity of the enzymes involved in the gluconeogenetic pathway above the stair of triose phosphate in the liver and in the kidneys, as well as in the increase of phosphoenolpiruvate carboxykinase (PEPCK) in the kidneys. As in the kidneys, in contrast to the liver, PEPCK is a cytosol enzyme; the kidneys are considered as important organs where during fasting gluconeogenesis from amino acids, piruvates and glycerol takes place in chickens and pigeons (TINKER et al., 1983).

A significant increase in mitochondrial ALT activity in chicken livers on the 7th day of fasting was reported by FUJIWARA et al. (1994). DICKSON and LANGSLOW (1978) reported that lactate was a much more efficient gluconeogenetic precursor than piruvates in the hepatocytes of fasting chickens. Moreover, the authors reported that alanine and glycerol were very weak precursors of gluconeogenesis in the hepatocytes of fasting chickens. They attributed the low response to alanine as gluconeogenetic precursor in hepatocytes partly to the low ALT activity in the chicken liver, which fell to only one percent of that in the liver of rats (DICKSON and LANGSLOW, 1978). According to *in vivo* investigations conducted by these authors, alanine is probably metabolised to lactate in tissues, for example in muscles. Lactate is then in a range of reactions transformed to glucose. SOLING et al. (1973) also reported that the role of the liver in gluconeogenesis actually consists of recycling lactates. Normoglycaemia maintenance in birds during long-term fasting is actually attributed to more ample gluconeogenesis in the kidney from various precursors (TINKER et al., 1983; YAMANO et al., 1988), and to gluconeogenesis from the liver, predominantly from lactates (SARKAR, 1971).

The reduced protein and uric acid concentration as well as the reduced aminotransferase activity in the fasting ducklings suggest reduced plasma protein regeneration and indicate that no significant mobilization of tissue proteins started during the six-day fasting period, either to obtain energy or to initiate gluconeogenesis.

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SAŽETAK

Istražen je utjecaj šestodnevnog gladovanja na koncentraciju ukupnih bjelančevina i mokraćne kiseline, te aktivnosti aspartat-aminotransferaze [aspartate:2-oxoglutarate aminotransferase (AST); EC 2.6.1.1] i alanin-aminotransferaze [L-alanine:2-oxoglutarate aminotransferase (ALT); EC 2.6.1.2] u krvnoj plazmi pačića pekinške patke. U dobi od 28 dana pačići su bili podijeljeni u dvije skupine: kontrolnu skupinu (n

= 28) i pokusnu skupinu koja je podvrgnuta šestodnevnom gladovanju (n = 32). Nakon 3., 4., 5. i 6. dana gladovanja žrtvovano je po 8 pačića pokusne skupine i po 7 pačića kontrolne skupine. Krv za analizu uzimana je s heparinom kao antikoagulansom, istovremeno pokusnoj i kontrolnoj skupini. U dobivenoj krvnoj plazmi spektrofotometrijski su određene koncentracije ukupnih bjelančevina i mokraćne kiseline, te aktivnosti AST i ALT. Tijekom pokusnoga razdoblja koncentracija ukupnih bjelančevina u krvnoj plazmi izgladnjivanih pačića bila je značajno niža od kontrolnih vrijednosti i to 3. dana (P = 0,0239) te 4., 5. i 6. dana (P = 0,0012). Nakon petodnevnog pokusnog razdoblja u izgladnjivanih pačića utvrđena je značajno viša (P = 0,018) vrijednost koncentracije mokraćne kiseline nego u kontrolnoj skupini, no istodobno je u kontrolnoj skupini zabilježena najniža koncentracija mokraćne kiseline tijekom cijeloga pokusnoga razdoblja. U odnosu na kontrolnu skupinu značajno niža aktivnost AST zabilježena je nakon 5. dana gladovanja (P = 0,0276), a aktivnosti ALT nakon 4. dana gladovanja (P = 0,0410). Niska koncentracija ukupnih bjelančevina u pačića pokusne skupine i smanjena aktivnost navedenih enzima vjerojatno je posljedica smanjene obnove bjelančevina u organizmu zbog nedostatka aminokiselina iz probavnoga trakta u vrijeme gladovanja.

Ključne riječi: pačići, gladovanje, ukupne bjelančevine, mokraćna kiselina, aspartat-aminotransferaza, alanin-aminotransferaza