

Specific Activities of Some Diagnostic Enzymes in Selected Tissues of Turkey (*Meleagris gallopavo*)

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Abstract

This study was undertaken to estimate tissue specific activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and creatine kinase (CK) in turkey. Tissue samples of liver, kidney, heart, brain, lung, proventriculus, duodenum, and breast muscle were collected from freshly killed adult male turkeys. The present study results reveal the presence of all five enzymes activities in studied tissues, although in different quantities. The highest specific activity of the AST and ALT was observed in the liver followed by the kidney, with statistically significant difference between them. The lowest specific activity of both AST and ALT was observed in the lungs. The specific activities of ALP were significantly higher in renal and duodenal tissues of turkey. The lowest ALP levels were seen in brain, lung, proventriculus, and muscle, with no statistically significant difference among them. Highest LDH and CK activities were observed in heart and muscle and their lowest specific activities were found in duodenum and proventriculus, with no statistically significant difference between them. Since each tissue has its characteristic complement of enzymes, such information might provide valuable data for clinical and diagnostic purposes in avian veterinary medicine. However, there is a need for further documentation of the clinical enzymology findings and also sensitivities and specificities of such tests in turkeys and other avian species.

Keywords

Enzyme, Tissue Distribution, Turkey

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1. Introduction

Enzymes are well known as essential catalysts in metabolism and any investigation of the cell metabolism requires a thorough understanding of enzyme action. Alterations in their activities are considered as sensitive clinical laboratory markers reflecting the metabolic disturbances and cellular damage in specific organs. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and creatine kinase (CK) are diagnostically valuable tools of clinical importance serving as indicators both for health and manifestation of some diseases. Estimation of them in circulation has some applications in the diagnosis and early detection of some

tissue injuries, differential diagnosis, and assessing therapy and prognosis of diseases.

Most tissues have characteristic enzyme patterns that are related to the function of the tissue. Advances in clinical enzymology was expanded rapidly by the recognition in diseases states of correlations between tissue specific enzyme activity patterns of the involved organs and the patterns of enzyme activities in the circulation. The organ specificity and intracellular location of enzymes are among important variables affecting the sensitivity and specificity of an enzyme in a particular species (Kramer *et al.*, 1997). Therefore interpretation of altered circulating enzyme activities for recognizing injury to various tissues requires precise knowledge concerning the distribution pattern of

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diagnostic enzymes in organs of the animal species under investigation. Tissue enzyme profiles of some mammalian and avian species have been established (Zimmerman *et al.*, 1968; Franson *et al.*, 1985; Lumeij, 1988; Bailey *et al.*, 1999, Kramer *et al.*, 1997; Fauquier, 2008). However, current understanding of avian clinical enzymology is less compared with knowledge of such data in mammals and normal physiological values for enzymes in many avian species had to be obtained.

Turkeys (*Meleagris gallopavo*) are reared all over the world for their tasty and high quality meat (Prabakaran, 2003) and their mass production is increasing in many countries as an important source of animal protein. Therefore, it is necessary to strengthen the physiological data base of turkeys in order to refine diagnostic aids for veterinarians caring for this species. To our knowledge there is relatively little information about enzymatic activities in various tissues of turkeys. The purpose of the present work was to determine and compare tissue enzyme activities of AST, ALT, ALP, LDH, and CK in various organs of the turkeys.

2. Materials and Methods

Tissue samples from freshly killed adult male turkeys were obtained from a local slaughterhouse. Samples of each tissue were cleaned free of extraneous material, washed a few times with physiological saline, and then stored at -70°C until analysis. Tissue samples were collected from liver, kidney, heart, brain, lung, proventriculus, duodenum, and breast muscle.

Tissue samples were rapidly thawed and homogenized in 10 volumes of ice cold 0.05 M sodium phosphate, pH 7.4. The suspensions were centrifuged at 4°C for 15 min at $4,000\times g$, and supernatants were used for enzyme assays.

The activities of aspartate aminotransferase (EC 2.6.1.1) and

alanine aminotransferase (EC 2.6.1.2) were determined by the colorimetric method of Reitman and Frankel, lactate dehydrogenase (EC 1.1.1.28) by the sigma colorimetric (Caboud Wroblewski) method, creatine kinase (2.7.3.2) by the sigma colorimetric (Modified Hughes) method, and alkaline phosphatase (EC 3.1.3.1) by the modified method of Bowers and McCOMB (Thrall *et al.*, 2004). Protein concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Enzyme activities in different tissues are expressed as specific activity (units per milligram protein).

All results were analyzed using one-way analysis of variance followed by Bonferroni's multiple comparisons test. The level of significance was set at $P < 0.05$. All calculations were performed using SPSS/PC software.

3. Results

The activities of the measured enzymes in different tissues of turkey are presented in Table 1 as mean \pm SEM. The present study results reveal the presence of all five enzymes activities in studied tissues, although in different quantities. The highest specific activity of the AST and ALT was observed in the liver followed by the kidney, with statistically significant difference between them. The lowest specific activity of both AST and ALT was observed in the lungs. Based on the present study results, the specific activities of ALP were significantly higher in renal and duodenal tissues of turkey than in other examined tissues. The lowest ALP levels were seen in brain, lung, proventriculus, and muscle, respectively, with no statistically significant difference among these tissues. Highest LDH and CK activities were observed in heart and muscle tissues as compared to other examined tissues. The lowest specific activities of LDH and CK were observed in duodenum and proventriculus, with no statistically significant difference between them.

Table 1. Mean (\pm SEM) specific activities (U/mg protein) of enzymes in different tissues of turkey (n=7).

Tissue	AST	ALT	ALP	LDH	CK
Liver	2.17 \pm 0.14 ^a	1.96 \pm 0.31 ^a	0.49 \pm 0.05 ^a	3.92 \pm 0.62 ^{a,d}	0.86 \pm 0.08 ^{a,c}
Kidney	1.65 \pm 0.07 ^c	0.89 \pm 0.08 ^b	0.87 \pm 0.12 ^b	3.56 \pm 0.62 ^{a,d}	1.05 \pm 0.20 ^{a,c}
Heart	0.81 \pm 0.11 ^d	0.49 \pm 0.08 ^{b,c}	0.13 \pm 0.01 ^c	8.48 \pm 1.10 ^c	3.81 \pm 0.56 ^b
muscle	1.44 \pm 0.08 ^c	0.27 \pm 0.04 ^{c,d}	0.28 \pm 0.04 ^{a,c}	6.12 \pm 0.76 ^{c,d}	4.22 \pm 0.55 ^b
Lung	0.33 \pm 0.02 ^b	0.07 \pm 0.02 ^c	0.08 \pm 0.01 ^c	0.83 \pm 0.07 ^b	0.48 \pm 0.04 ^c
Brain	0.39 \pm 0.06 ^b	0.131 \pm 0.01 ^{c,d}	0.05 \pm 0.01 ^c	1.49 \pm 0.26 ^{a,b}	2.14 \pm 0.21 ^a
Proventriculus	0.47 \pm 0.02 ^{b,d}	0.44 \pm 0.04 ^{b,c}	0.11 \pm 0.01 ^c	0.39 \pm 0.08 ^b	0.83 \pm 0.07 ^{a,c}
duodenum	0.43 \pm 0.06 ^b	0.67 \pm 0.12 ^{b,d}	0.83 \pm 0.07 ^b	0.32 \pm 0.06 ^b	0.75 \pm 0.08 ^{a,c}

Values in each column with no common superscript differ significantly ($P < 0.05$). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase.

4. Discussion

Enzymes are very sensitive markers for correct biological function and, consequently, also for metabolic disorders,

serving as indicators both for health and manifestation of some organ dysfunctions. The distribution of enzymes is markedly different between different organs and animal species (Bailey *et al.*, 1999). Investigations on the enzyme

activities and distribution in different organs of animal species might provide valuable data for clinical and diagnostic purposes in veterinary medicine. The present work describes some enzymatic characteristics in different tissues of turkey.

Transaminase enzymes of AST and ALT are distributed widely in animal tissues and play an important role in intermediary metabolism. Based on the present study results, AST is most active in the hepatic tissue of turkeys that is reminiscent of previously reported data in some mammalian and avian species (Zimmerman *et al.*, 1968; Franson *et al.*, 1985). Increment in plasma AST activity has been linked to hepatotoxicity in laying fowl (Ibrahim *et al.*, 1980). Indeed, as the present findings show, AST activity was appreciable in kidney and muscle of turkey in comparison to other examined tissues. On the other hand, in racing pigeons the highest activities of AST were found in the kidney and heart, followed by liver, pectoral muscle and brain and the lowest activities were found in duodenum (Lumeij *et al.*, 1988). Moreover, in houbarabustard highest AST activities were found in heart, pectoral muscle, and proventriculus, while lowest activities were detected in liver (Bailey *et al.*, 1999). It has been also documented that the concentration of AST was approximately the same in liver, myocardium, kidney and muscle of chicken and pigeon (Zimmerman *et al.*, 1968).

The highest ALT activity was found in the liver of turkeys that is different, to some extent, from the results of Franson *et al.* (1985) who reported higher ALT values in kidney and muscle of some birds. Moreover, it has been reported that in mallards, chickens and black ducks kidney had greater ALT activity relative to liver (Cornelius, 1963; Franson, 1982). However, high values of ALT activity has been found in liver of chickens and pigeons (Zimmerman *et al.*, 1968) and determination of its activity in plasma proposed as a specific marker of hepatic injury in waterfowl (Szaro *et al.*, 1978). Highest ALT activities in racing pigeons were observed in liver and kidney (Lumeij *et al.*, 1988). In houbara bustard the highest ALT levels were measured in the pectoral muscle and heart, followed by the proventriculus, liver, and duodenum and the lowest activities were found in pancreas and kidney (Bailey *et al.*, 1999).

ALP is a membrane-bound enzyme and catalyses the hydrolytic removal of phosphate group from a broad class of phosphomonoester substrates at the alkaline pH. In the present work, highest ALP activity was seen in kidney and duodenum followed by liver and muscle. The high renal and duodenal activity of ALP concurs with previously published work on racing pigeons (Lumeij *et al.*, 1988). However, Lumeij *et al.* (1988) detected only very low activities of ALP in brain and liver of racing pigeons and no activity in heart and pectoral muscle. Moreover, the high renal activity of

ALP has been reported in some other avian species (Franson *et al.*, 1985, Bailey *et al.*, 1999). However, as shown in the present work, ALP activities in renal and duodenal tissues are similar, while Bailey *et al.* (1999) showed that renal ALP activity in bustard is approximately fourfold greater than duodenum. Moreover, only low activities of ALP were detected in liver, muscle, proventriculus, and heart of bustard (Bailey *et al.*, 1999).

Creatine kinase is an enzyme expressed by various tissues and cell types and catalyses the reversible reaction of phosphorylation of creatine by adenosine triphosphate to form phosphocreatine. Based on the present findings, CK was distributed most abundantly in muscle, heart, and then brain of turkey. Relatively low quantities of CK were found in other examined tissues including kidney, liver, lung, proventriculus, and duodenum, with no significant difference between them. Relatively high activity of CK in three mentioned tissues suggests that circulating activities of this enzyme may have diagnostic importance in detecting muscle, heart, or brain injuries in this bird. In support of this suggestion, plasma CK activity has been used for identification of degenerative myopathy in turkeys (Holland *et al.*, 1980). In accordance to present work, Bailey *et al.* (1999) reported high activities of CK in muscle and heart of bustard (Bailey *et al.*, 1999); However, their findings also showed considerable difference in CK activity among other studied organs of bustard in the following order: proventriculus>duodenum>kidney>liver and pancreas (Bailey *et al.*, 1999).

LDH, which is an enzyme of anaerobic glycolysis, is also widely distributed among tissues and might provide some important diagnostic clues. In the present work LDH activity was predominantly found in heart and muscle. Intermediate activities were present in kidney, liver, and brain and the lowest activities were detected in proventriculus and duodenum. Similarly, the highest values of LDH activity have been found in the myocardium of pigeon and chicken, although its levels also were high in muscle, liver and kidney of these species (Dujovne *et al.*, 1969). Highest activities of LDH in racing pigeons were found in the kidney and heart, followed by liver, pectoral muscle and brain and the lowest activities were found in duodenum (Lumeij *et al.*, 1988). While the highest LDH activities were found in heart and proventriculus of bustard, its lowest activities were found in the pancreas and liver (Bailey *et al.*, 1999). Controversial pattern of enzyme activities in organs of various avian species, as mentioned above, may be due to technical factors, physiological and biochemical specificities, dietary differences, differences in the mean age and weight of sampled animals, and other unknown factors.

5. Conclusion

In summary, the present work described tissue distribution of some diagnostically important enzymes in turkey. Among studied organs of turkey, ALT and AST activities were highest in liver, ALP was highest in renal and duodenal tissues, and LDH and CK were distributed most abundantly in heart and muscle. Since each tissue has its characteristic complement of enzymes, such information can point towards establishment of biochemical diagnostic markers for identification of injured organs in turkeys. However, there is a need for further documentation of the clinical enzymology findings and also sensitivities and specificities of such tests in turkeys and other avian species.

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