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Age-Related Differences in Levels of Blood Chemistry Parameters and Cardiac Marker Proteins in Commercial Broilers

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ABSTRACT

The effect of age on levels of blood biochemical parameters in broiler chickens is largely unknown. The purpose of the present study was to investigate physiological alterations of blood chemistry variables in renal, liver, lipid and cardiac profile in commercial broilers at the age of 2(n=20) and 7 weeks (n=40). The results showed that compared with broiler chicks at the age of 7 weeks, those at the age of 2 weeks had significantly higher levels of blood urea nitrogen, creatinine, uric acid, triglycerides and the enzymes alanine aminotransferase and alkaline phosphatase. Older chicks, on the other hand, displayed significantly higher levels of total protein, albumin, total cholesterol and the enzyme gammaglutamyl transferase. Although a significantly higher activity of cardiac enzyme aspartate aminotransferase and a significantly lower activity of lactate dehydrogenase were observed in older chickens, younger broilers exhibited a significantly higher concentration of the cardiac-specific marker troponin T. There was no significant age-related difference either in the levels of cardiac enzymes creatine kinase, creatine kinase MB or in glucose level. Cardiac troponin T concentration correlated significantly with that of CK-MB in both groups of animals studied. These results underline the importance of age as a significant contributor of variation in levels of several blood chemistry parameters and cardiac marker proteins, especially cardiac troponin T in broiler chicks.

Key words: age-related difference, biochemical parameters, cardiac markers, broiler chicks

INTRODUCTION

Normal biochemical data are essential for evaluating animal health. In healthy animals, physiological variation in levels of several biochemical parameters exists that need to be considered in the interpretation of laboratory test results. In various bird species, for instance, seasonal variation in levels of total protein and the enzymes aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) has been reported (Schmid and Forstner, 1986). It is also known that in laboratory animals, physical stress induced by prolonged exercise or restraint can result in a significant elevation of the muscle enzyme creatine kinase (CK)(Holt, 1998). Changes in levels of blood chemistry parameters as a function of age represent another physiological alteration which can be observed for variables such as alkaline phosphatase (ALP) and total cholesterol (Samour, 2000).

To test the hypothesis that age-related changes in levels of biochemical variables exist in chickens, measurements of blood chemistry

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parameters in renal, liver, lipid and cardiac profile were performed on commercial broilers at the age of 2 and 7 weeks. Special reference was made to alterations in cardiac marker protein CK and its isoenzyme creatine kinase MB (CK-MB) as well as cardiac troponin T (cTnT), a novel specific marker of myocardial cell injury. Sophisticated immunoassay system for determining cTnT has recently been developed for human use (Collinson *et al*., 2001). Due to species conservation in the structure of cTnT molecules, this immunoassay was shown to be applicable across a wide range of animal species including birds (Fredericks *et al*., 2001). The results obtained from the present study could be served as baseline information for the interpretation of blood chemistry results in commercial broilers.

MATERIALS AND METHODS

The study consisted of 60 commercial broilers reared in pens to 2 weeks (group 1 ; n = 20) or to 7 weeks of age (group 2 ; n = 40) . Food and water were given *ad libitum.*

Laboratory analysis

Venous blood samples for clinical chemistry measurements were collected from the wing veins. The samples were immediately centrifuged and the plasma obtained were kept frozen at - 20°C until analysis of biochemical parameters. Glucose concentration was measured with hexokinase method. Levels of blood urea nitrogen (BUN), creatinine as well as activities of the liver enzymes alanine aminotransferase (ALT), ALP, gammaglutamyl transferase (GGT) and cardiac enzymes CK, AST and LDH were measured with standard enzymatic methods. Concentrations of uric acid, total cholesterol and triglycerides were determined by enzymatic colorimetric tests. Total protein and albumin were measured with Biuret method and bromocresol green method, respectively. Measurements of all these biochemical variables were performed using the fully-automated chemistry analyzer Cobas Integra 700 (Roche Diagnostics).

Concentrations of the MB-subfraction of CK (CK-MB mass) and cTnT were measured on an Elecsys® 2010 immunoanalyzer (Roche Diagnostics) with electrochemiluminescence technology. The lower limit of detection of CK-MB and the third generation immunoassay for cTnT used in the present study is 0.10 and 0.01 ng/ml, respectively. Levels of cTnT below 0.01 ng/ml are considered to be undetectable. As stated by the manufacturer, the monoclonal antibodies for cTnT immunoassay showed less than 0.01% cross-reactivity with other cardiac proteins (troponin I, myosin light chain) and skeletal muscle troponin T.

Statistical analysis

The results are presented as mean ± standard deviation. Differences in the means between the two animal groups were examined with Student's t test. Pearson or Spearman rank correlation coefficients were used to analyze the association among cardiac marker proteins as appropriate. A p value of < 0.05 was regarded to be statistically significant.

RESULTS AND DISCUSSION

The main findings in the present study were significant age-related differences in levels of most of the biochemical variables in renal, liver, lipid and cardiac profile. No statistical significant difference between the 2 animal groups was observed only for plasma glucose (Table 1) and the cardiac enzymes CK and CK-MB (Table 2). This result may be attributed to the small number of animals in the younger age group. Nevertheless, a trend for a higher glucose concentration ($p = 0.15$) and for a lower activity of CK ($p = 0.06$) and CK-MB ($p = 0.11$) was seen in the younger broiler chicks.

Table 1 Mean (±SD) levels of blood chemistry parameters in broiler chicks aged 2 weeks (group 1) and 7 weeks (group 2).

	Group 1 (n = 20)	Group 2 (n = 40)	p Value
Glucose (mg/dl)	253 ± 30	242 ± 22	0.15
BUN (mg/dl)	1.40 ± 0.60	0.53 ± 0.60	< 0.001
Creatinine (mg/dl)	0.53 ± 0.05	0.20 ± 0.04	< 0.001
Uric acid (mg/dl)	6.46 ± 1.75	3.77 ± 1.08	< 0.001
ALT (U/L)	3.50 ± 1.32	2.13 ± 1.30	< 0.001
ALP (U/L)	11601 ± 4407	2072 ± 938	< 0.001
GGT (U/L)	11.25 ± 2.59	26.08 ± 5.59	< 0.001
Albumin (g/dl)	0.89 ± 0.10	1.39 ± 0.11	< 0.001
Total protein (g/dl)	2.49 ± 0.25	3.72 ± 0.32	< 0.001
Total cholesterol (mg/dl)	97 ± 15	141 ± 22	< 0.001
Triglycerides (mg/dl)	66 ± 24	44 ± 36	< 0.05

Table 2 Mean (±SD) levels of cardiac marker proteins in broiler chicks aged 2 weeks (group 1) and 7 weeks (group 2).

	Group 1 (n = 20)	Group 2 (n = 40)	p Value
AST (U/L)	153 ± 26	210 ± 43	< 0.001
LDH (U/L)	958 ± 173	816 ± 172	< 0.01
CK (U/L)	2561 ± 1600	3448 ± 1752	0.06
CK-MB (ng/ml)	0.713 ± 0.421	0.892 ± 0.364	0.11
cTnT (ng/ml)	0.030 ± 0.020	0.009 ± 0.012	< 0.001

With regard to the parameters in renal profile, younger broilers showed a significantly higher level of BUN, creatinine and uric acid than the older chicks (Table 1). These age-related changes have also been observed in mallards reported by Fairbrother *et al*. (1990). However, BUN and creatinine are present in very low concentrations in both groups of broiler chicks in the present study. Since the main end product of nitrogen metabolism in birds is uric acid, measurements of uric acid levels represent the most sensitive indicators of renal function in chickens and other bird species (Fairbrother *et al*., 1990; Bogin *et al*., 1996).

Significant age-related differences have also been found in biochemical parameters in liver and lipid profile. Notably, a much higher activity of ALP was seen in younger animals. This may reflect, however, an accelerated bone growth in the younger age group. Younger broilers also exhibited a significantly higher activity of ALT and a higher triglyceride level, whereas a significantly higher activity of GGT and higher levels of albumin, total protein and total cholesterol

were observed in older chicks (Table 1). Similar findings for several of these parameters were reported in other bird species including the cockatoos and macaws (Samour, 2000).

Table 2 demonstrates activities of cardiac enzymes AST, LDH and CK as well as levels of CK-MB and cTnT in the 2 groups of animals studied. It can be seen that compared with younger broilers, older chicks displayed a significantly higher activity of AST and a significantly lower activity of LDH. On the other hand, there was no age-related difference in the activity of CK. Similar results were obtained from studies on other bird species (Bailey *et al*., 1998; Samour, 2000). Due to their presence in a significant amount in tissues other than the myocardium, however, these enzymes lack specificity for myocardial injury (Schmid and Forstner, 1986).

Introduction in the early 1990's of sophisticated immunoassay systems for determining concentrations of CK-MB (CK-MB mass) and cTnT has allowed for cardiac marker protein testings with a high diagnostic specificity and sensitivity (Sribhen, 2001; Penttila *et al*., 2000). In the present study, a significant difference between the 2 groups of animals studied was found for cTnT, but not for CK-MB (Table 2). In this context, it has been reported that there is poor species conservation of CK-MB molecules and the immunoassays developed for human application are too selective for epitopes on the human form of CK-MB and will not cross-react to an appreciable extent with CK-MB from animals (Holt, 1998). In contrast, there is species conservation in the structure of cTnT (Holt, 1998) and the same cTnT immunoassay using specific monoclonal antibodies as in the present study has been found to be applicable across several animal species including birds (Fredericks *et al*., 2001). Our results are in accordance with data from human studies showing that levels of cTnT in the neonates were significantly higher than those in children of older age (Clark *et al*., 2001; Simbre *et al*., 2002). Our data also confirm the results from 2 previous studies performed on broiler chicks at different ages using the non-specific first-generation cTnT immunoassay (Maxwell *et al*., 1995 a; Maxwell *et al*., 1995 b). In an experimental model, Cooper and Ordahl (1985) have demonstrated that chick cardiac muscle contains two cTnT isoforms which differ only by 10 amino acid residues. These embryonic and adult isoforms are present at high levels in late embryonic heart but there is down regulation of the embryonic isoform production in the heart of young chickens. This protein switching could explain the difference in cTnT levels between different age groups observed in our study.

The associations between cTnT and other biochemical markers of myocardial injury are shown in Table 3 and 4. As stated above, the low concentration of CK-MB found in both animal groups in the present study was primarily due to the insensitivity of CK-MB immunoassay when used in animal species. However, significant correlations between CK-MB and cTnT observed in both groups of animals studied indicate that most of the CK-MB originated from the heart. In contrast, a significant association between cTnT and CK, which is abundantly present in both skeletal and cardiac muscle, was found only in the older age group. The activity of CK also correlated strongly with that of other non-specific cardiac enzymes AST and LDH in both animal groups.

CONCLUSION

Age-related changes in levels of several biochemical parameters in renal, liver, lipid and cardiac profile exist in commercial broilers. In particular, younger broilers exhibited a significantly higher cTnT concentration than the older chicks, thereby supporting the fetal origin hypothesis of this marker protein in the younger age group. There was a significant correlation between cTnT and CK-MB concentration in both

Table 3 Correlation coefficients of cardiac marker proteins in broiler chicks aged 2 weeks.

	cTnT	CK	CK-MB	AST	LDH
cTnT	1.000				
CK	-0.096	1.000			
CK-MB	0.517*	0.021	1.000		
AST	0.224	0.663**	0.057	1.000	
LDH	0.030	0.617**	-0.450*	0.619**	1.000

* $p < 0.05$; ** $p < 0.01$

Table 4 Correlation coefficients of cardiac marker proteins in broiler chicks aged 7 weeks.

	cTnT	CK	CK-MB	AST	LDH
cTnT	1.000				
CK	0.473**	1.000			
CK-MB	0.421**	0.367*	1.000		
AST	0.097	0.422**	0.018	1.000	
LDH	0.282	0.440**	0.219	0.275	1.000

* $p < 0.05$; ** $p < 0.01$

groups of animals studied. These results imply that age represents a significant contributor of variation in levels of several blood chemistry parameters, especially cTnT, that should be considered in the interpretation of laboratory test results in broiler chicks.

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