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Storage stability of certain serum enzymes of chicken (*Gallus domesticus*)

V RAMNATH [1], C C LINI [2], P S REKHA [3] and K S SUJATHA [4]

Kerala Agricultural University, Mannuthy, Thrissur, Kerala 680 651 India

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Enzymes comprise an extremely heterogeneous class of biological macromolecules. Except few, most enzymes are proteinous in nature. Proteins are often unstable when they are not in the native environment. Enzymes can lose activity as a result of proteolysis by proteinase, aggregation and suboptimal buffer condition (Herrmann *et al.* 2001).The study was taken up to evaluate the *in-vitro* stability of certain important serum enzymes of diagnostic interest of normal healthy chicken and to study the time dependent effect of different storage temperatures on their activities.

Clinically healthy male egg type cockerels (8), 6 months old and of 1 kg body weight was procured from University Poultry Farm, Kerala Agricultural University, Thrissur and used in this study. Blood (5 ml), was collected through wing vein puncture into sterile tube and serum was harvested 30 min following clot formation by centrifugation at 500 g for 20 min at room temperature. Aliquots of serum samples were either assayed within 1 h of serum separation (day '0'), or stored at room temperature (RT; 28° C), 4° C, –20° C or incubation temperature (IT) of 37° C. Aliquoted samples stored at different temperature were then assayed on 1, 2,3,4,5 and 10 days post collection.

On the day of estimation, samples were thawed to room temperature and by using semi-automated clinical analyser; assays on enzyme activities were carried out, following standard methods (Keiding 1974, Stromme *et al.* 1976). Activities of serum enzymes, viz. creatine kinase (CK, EC 2.7.3.2), alkaline phosphatase (ALP, EC 3.1.3.1), lactate dehydrogenase (LDH, EC 1.1.1.27), choline esterase (ChE, EC 3.1.1.8), alanine amino transferase (ALT, EC 2.6.1.2) and aspartate amino transferase (AST, EC 2.6.1.1) were determined using commercial kits.

Paired 't' test was used to compare variations within the temperature group at different days, with respect to day '0' sample. One way ANOVA using Duncan's multiple range tests (Snedecor and Cochran 1994) was used to compare variation between different groups of temperature of storage. The values thus obtained were referred to the day'0' value to find out the% activity with respect to initial activity on day '0'which was considered 100%.

Within a period of 10 days of storage, there was highly significant ($P < 0.01$) reduction in the activity of CK at all temperatures tested. On 10th day of incubation at 37° C, serum CK activity was reduced markedly to 6% of the original value at day '0', while the corresponding sample stored at –20° C showed 28.5% of its initial activity (Table 1). Activity of ALP was significantly ($P < 0.01$) affected by storage, on comparing with its initial sample at day '0'. Activity of ALP was conserved to a maximum extent, even on day 10 post collection, especially at refrigerated (4° C) and freezing (–20° C) storage temperatures. (Table 1).

LDH activity was reduced just below 50% on day 10 at –20° C and at 4° C storage temperature, while storage at RT/ IT adversely affected the activity of LDH. (Table 1).The activity of AST did not change significantly on day 10 upon storage at refrigerated and freezing temperature (Table 1). Serum ALT activity showed a gradual but significant ($P < 0.01$) fall in its activity especially in those samples stored at or below refrigerated temperature. (Table 1). As far as ChE activity was concerned, –20° C storage alone assured comparatively highly significant ($P < 0.05$) stability than other storage temperature conditions (Table 1).

On day '10' post collection, the activity of enzymes like ALP, LDH and AST in the serum, stored at 4° C was found to be significantly higher ($P < 0.05$) than other temperature opted for storage, while the activity of CK, ALT and ChE in the serum at storage temperature of –20° C was found to be significantly higher ($P < 0.05$) than the other temperature considered. Sera maintained in sterile condition either at RT of 28° C, or at IT of 37° C showed better activity for ALP when compared to other enzymes tested on day'10' post collection. There was considerable instability in serum enzyme activity on storage.

A class of group-specific proteinase has been described that attack enzymes. Such proteinases, if are functional at –20° C could account for loss of activity of some enzymes

Present address: [1]Associate Professor, [2]Research Assistant, [3]Senior Research Fellow, *Adhoc* ICAR Scheme, Department of Veterinary Physiology, College of Veterinary and Animal Sciences; [4] Associate Professor, Department of Statistics.

Table 1. Storage stability of certain serum enzymes at different temperatures in chicken (n=8)

Serum enzymes	'0' day	10th day			
		Storage temperature			
		-20°C	4°C	28°C (RT)	37°C(IT)
Alkaline phosphatase (U/L)	1091±2 (100.00)	$_{b}$918**±2.2 (85.00)	$_{a}$927**±1.6 (47.00)	$_{c}$512**±1.2 (37.00)	$_{d}$403**±3.8 (84.00)
Creatine kinase (U/L)	1695±4.2 (100.00)	$_{a}$480**±3.3 (28.00)	$_{b}$425**±4.3 (25.00)	$_{c}$109**±1.6 (6.50)	$_{d}$101**±1.7 (6.00)
Aspartate amino transferase (U/L)	207±1.6 (100.00)	$_{b}$143**±0.9 (69.25)	$_{a}$158**±1.3 (76.50)	$_{c}$102**±2.0 (49.25)	$_{d}$51**±1.1 (24.50)
Lactate dehydrogenase (U/L)	1239±4.3 (100.00)	$_{b}$600**±5.1 (48.50)	$_{a}$617**±2.8 (49.75)	$_{c}$221**±2.0 (17.75)	$_{c}$226**±2.2 (18.25)
Choline esterase (U/L)	6937±13.5	$_{a}$5314**±3.3	$_{b}$4034**±3.6	$_{c}$761**±2.1	$_{d}$750**±2.2
Choline esterase (U/L)	(100.00)	(76.50)	(58.25)	(11.00)	(10.75)
Alanine amino transferase (U/L)	48.5±0.7 (100.00)	$_{a}$35.0**±0.7 (71.00)	$_{b}$30.0**±0.9 (62.25)	$_{d}$8.0**±0.5 (16.00)	$_{c}$15.0**±0.7 (31.00)

Paired't' test was used to compare within group variations from day '0' sample. Means±SE carrying ** are highly significant (P<0.01). One way ANOVA using Duncan's multiple range test was used to compare variations between groups. Means±SE carrying different subscripts (a-d) differ significantly (P<0.05). Values in parenthesis indicate percentage initial activity of enzyme. RT, room temperature; IT, incubation temperature.

(Gosling 1986) stored at freezing temperature. In the present study, stability of serum CK was found to be the least among 6 enzymes tested under all temperature considered for storage, while ALP showed the most satisfactory stability over other enzymes. Similar results were reported in sheep, dairy cows and in camels, as the CK the most labile enzyme (Jones 1985, Eshani *et al.* 2008, Saeed *et al.* 1995) and it is clearly advisable to cool the samples for CK determination as soon as possible. The present investigation indicated greater stability of enzymes like ALP, AST and ALT during the first 2 days at RT. Even at 37° C, the degradation was rather lesser for ALP. This is of much importance as it enables the transportation of samples under sufficiently stable conditions from the farm to a local diagnostic laboratory.

Cooling, while often unnecessary for short term storage, usually enhances sample stability, but the deleterious effects on ALT and á-hydroxybutyrate dehydroenase in sheep serum should be noted. For long term studies, deep freezing can usually be recommended although ALT and AST in serum (but not in plasma) from both sheep and cattle were highly unstable after freezing (Jones 1985).Another common reason which could be attributed to loss of activity being the intolerance of thawing from freezing to analytical temperature (Thoresen *et al.* 1995).

To summarise, temperatures of –20° C and 4° C were regarded as ideal for storage of biological samples. Storage temperature of –20° C was found to be ideal for serum ALT and ChE, whereas 4° C storage temperatures provided better stability for AST, ALP and LDH. The results of the enzyme stability in the trial may serve as a guide to those involved in clinical veterinary analysis.

SUMMARY

Many times loss of activity of some of the diagnostically important enzymes before estimation would lead to false interpretation of the result in veterinary practice. The present study was designed to assess the effect of time and various storage temperatures on the activity of chicken serum enzyme like creatine kinase (CK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), choline esterase (ChE), alanine amino transferase(ALT) and aspartate amino transferase (AST). It was observed that at the end of 10 days of post collection, the activity of ALT and ChE was significantly ($P < 0.05$) higher in the serum samples stored at freezing (–20° C) temperature when compared to sample stored at 4° C and 37° C. Sera stored at 4° C showed significantly ($P < 0.05$) higher values for the activity of ALP, LDH and AST, while comparing with other temperature considered for storage on day '10' post collection. It was also found that among these six enzymes tested CK was more thermo labile.

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