Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.18, 2014

Effect of storage methods on viability of some hepatic enzymes in farm animals

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Abstract

In the course of a study of serum hepatic enzymes in the ruminants with storage effect was discovered, whose serum contained an Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) which was indistinguishable from sheep, cattle, and goats. However, the fundamental storage study of these enzymes is poorly understood in farm animals. Therefore this paper was aimed to determine the relations of two storage methods. The same enzyme was demonstrated in high concentration in the cattle and its linear decreases during storage over 8 weeks in all three animals tested. This single case demonstrates the possibility that elevated serum alkaline phosphatase in animals with -20 ^{o}C can be of neoplastic rather than of hepatic storage at 5 ^{o}C.

Introduction

Liver enzyme activity induction may be associated with changes in hepatic weight, histological evidence of abnormal hepatocytes, alteration of blood serum clinical biochemistry analyses and pleotrophic gene expression in the veterinary medicine (Buchet, Millán, & Magne, 2013; Filipowicz et al., 2013; 2013). The liver is accountable for maintenance of normal homeostasis and physiological purposes. It functions as a conditional system accomplished of relatively rapid responses to a different of incentives. Hepatic size is governed both by genetic factors and by the rate of biochemical activity to keep optimal purposeful mass. Following incentives such as toxic insult, infection, or partial hepatectomy, the liver rapidly restores its ideal mass to maintain normal purpose (K. Askar, A. C. Kudi, & A. J. Moody, 2011; Askar & Kudi, 2012; K. A. Askar, A. C. Kudi, & A. J. Moody, 2011a, 2011b; Lens, Leoz, Nazal, Bruguera, & Parés, 2014). The liver also eagerly responds to some stimuli by experiencing additive growth and function. Growing and functional demands that promote a hepatic response time a range of incentives, including hormonal fluctuations; pregnancy and lactation; bacterial and viral and infections that induce acute-phase proteins; dietary constituents such as carbohydrates, proteins, fat; and enzyme inductive responses to a variety of is a foreign chemical substance found within an organism that is not normally naturally produced by or expected to be present within that organism (Ramzy et al., 2013). The liver in the ruminants' response to these various stimuli may involve an increase in liver size and functional capacity attributable to an increase in size and/or number of hepatocytes (Daniel, 2013; Ramzy et al., 2013; Zanger & Schwab, 2013). Finally, the background information relative to liver enzyme induction as enclosed in the introductory outline is typically compartmentalized into Phase I and Phase II induction of liver enzymes functions (Daniel, 2013; Grimsley et al., 2013). Phase I oxidative metabolism is catalysed by several isoforms of the P450 super-family and follows in the micro-vesicles of the hepatocyte smooth endoplasmic reticulum. The arrangement and functions of the large family of P450 (CYP) enzymes have been reviewed with identification of species-specific isoforms (Grimsley et al., 2013; Sim & Ingelman-Sundberg, 2013; Zanger & Schwab, 2013).

Materials and Methods

The study carried out on 142 adult healthy animals, included three types of ruminants (cattle, sheep, and goat) in Kirkuk governorate. The number of mixed animals was 34 cattle, 74 sheep, and 34 goats. A blood sample was collected by sterile disposable syringe from jugular vein in the sterile serum test tubes (5 ml). The blood left to clot and centrifuged for 20 min at 300 rpm. Separated serum used for measurement of some hepatic enzyme activities. Serum enzyme activities of Alanine transaminase (ALT) and Aspartate transaminase (AST) were estimated according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) was determined according to Belfield and Goldberg (1971). The results read by spectrophotometrically at wavelength 500 nm. Statistically analysis was performed by using conventional statistical method was used to calculate the means, standard deviation (SD). All statistics was carried out using MiniTab statistical software version 15 (MiniTab Ltd., Coventry, UK).

Results

Each of the 8 freezing times and thawing rates combination were investigated the serum for sheep, cattle, and goats with freezing at -20 ^{o}C and 5 ^{o}C. One way analysis of variance performed and seen gradual decreases in enzyme activity in all weeks of freezing. One way ANOVA for the comparison of changes in the mean of AST in serum, during 8 weeks of freezing were significant (P<0.0.5) difference for ALT between week 3 and week 4 for sheep, and showed all different between weeks were significant differences (P<0.05) except insignificance between week 6 and week 8 for cattle, as well as AST observed all significant differences (P<0.05) in all weeks except insignificance (P>0.05) between week 2 and week 3 and between week 6 and week 8 for sheep, between week 5 and week 6, and between week 7 and week 8 for cattle (Table 2). Significant differences (P<0.05) in serum, AST activity between week 3 and week 4 and between week 6 and week 7 for sheep and between week 2 and week 3, between week 4 and week 5, and between week 6 and week 7 for cattle, as well as ALT activity significant differences (P<0.05) in all weeks except insignificance differences (P>0.05) between week 3 and week 4 and between week 7 and week 8 for sheep, between week 1 and week 2 and between week 4 and week 5, between week 7 and week 8 for cattle (Table 3). Serum activities for ALT, AST, and ALP sheep, goat and cattle are represented in Table 1. The mean value of serum ALT activity in healthy sheep, goat, and cattle were found to be 18.4-1.23, 116.3-2, and 21.66-6.31U/L, and ranged from 6-12,111-333 and15-35 respectively. The mean value of serum ATS activity was found to be 47.11-4.3, 211.1-13.3, and 14.3-3.31U/L, ranged from 40-111,157-261, and 11-89 respectively. Likewise, the mean value of serum ALP found to be 111.3-8.33, 431.21-11.8, and 120.11-4.11 U/L, and ranged from 75-213,310-550, and 215-330 respectively (Table 1). In all animals tested, the rank order of serum ALT was increased according to goat>cattle>sheep. While in serum AST was increased according to goat>sheep>cattle (Figure1). The rank order of serum ALP was increased according to goat>cattle>sheep (Table 1). The relative enzyme results indicated for ALT enzyme was recorded in goat six times greater than sheep and five times greater than cattle (Figure 1), but the AST enzyme of sheep recorded relatively fourteen times greater than cattle, whereas ALP enzyme in goat recorded four times greater than sheep and cattle (Figure 2). ALP enzyme in goat recorded four times greater than sheep and cattle (Figure 3). The two storage methods (-20 ^{o}C and 5 ^{o}C) for two hepatic enzymes was decreased in 8 weeks (Tables 1 and 2)

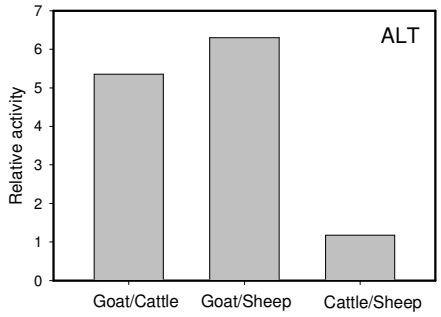

Figure 1: Relative activity of ALT enzyme activity in serum for farm animals.

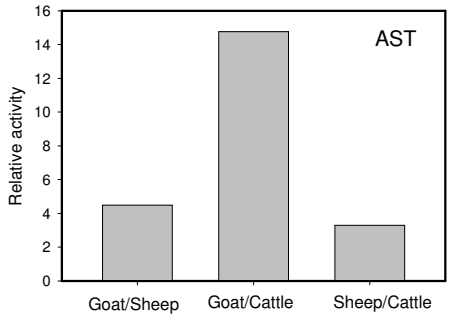

Figure 2: Relative activity of AST enzyme activity in serum for farm animals.

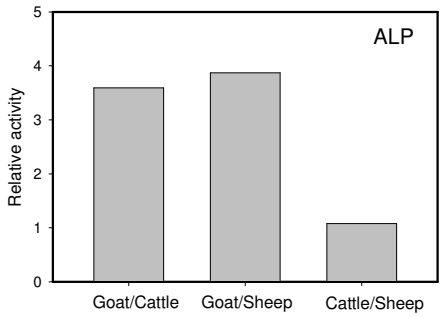

Figure 3: Relative activity of ALP enzyme activity in serum for farm animals.

Table1: Serum ALT, AST, and ALP enzyme activities in adult healthy sheep, cattle, and goat; range and mean ± SD.

Animal	No	ALT(U/L)		AST(U/L)		ALP(U/L)	
		Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD
Sheep	74	6-12	18.4±1.23	40-111	47.11±4.36	75-213	111.3±8.33
Cattle	34	15-35	21.66±6.31	11-89	14.34±3.31	215-330	120.11±4.11
Goat	34	111-333	116.3±16.2	157-261	211.1±13.3	310-550	431.21±11.8

Table 2: Hepatic enzyme activity (mean ± SE) in serum for sheep, cattle, and goats, stored at -20 °C.

	Sheep		Cattle		Goat	
Weeks	ALT*	AST*	ALT*	AST*	ALT*	AST*
2	48.6±2.28^{a}	28.6±2.64^{a}	44.0±2.04^{a}	24.2±2.68^{a}	66.04± 6.06^{a}	28.0±2.06^{a}
2	26.2±2.44^{b}	24.8±4.24^{b}	28.4±4.62^{b}	24±2.44^{b}	60±8.46^{ab}	24.6±4.28^{b}
4	22.8±2.84^{c}	28.2±2.66^{c}	24.8±0.064^{c}	22.6±0.060^{b}	46.04±4.64^{b}	22.6± 2.04^{b}
4	26.2±0.284^{d}	22.8±0.226^{d}	22.0±2.22^{cd}	4.4±0.466^{c}	24.0±2.64^{c}	0.22±0.842^{c}
6	26.02±2.28^{d}	20.4±0.880^{de}	22.6±0.828^{cd}	2.6±0.280^{c}	24.08±2.04^{c}	8.068±0.688^{cd}
6	24.0±2.20^{d}	8.4±2.26^{e}	20.2±2.62^{d}	2.6±0.442^{c}	22.2±2.26^{c}	6.0±2.06^{cd}
8	24.8±0.606^{d}	8.0±0.600^{e}	0.0±0.088^{d}	2.4±0.400^{c}	22.6±2.48^{c}	6.482±0.644^{cd}
8	24.2±0.666^{d}	8.2±0.842^{e}	0.6±2.24^{d}	2.2±0.266^{c}	20.6±2.46^{c}	6.8±0.644^{d}

$^{a-e}$ significantly different between means bearing different letters in the same column (P<0.06). N= 20.

Table 3: Hepatic enzyme activity (mean ± SE) in serum for sheep, cattle, and goats, stored at 5 °C.

	Sheep		Cattle		Goat	
Weeks	ALT*	AST*	ALT*	AST*	ALT*	AST*
2	6.28±0.446^{a}	2.8±0.226^{a}	8.60±0.668^{a}	2.82±0.260^{a}	20.6±0.806^{a}	2.42±0.224^{a}
2	4.4±0.626^{b}	2.28±0.0600^{ab}	6.06±0.846^{a}	2.62±0.224^{a}	8.68±0.486^{b}	2.86±0.200^{b}
4	4.4±0.824^{c}	2.2±0.266^{ab}	6.80±0.884^{b}	2.44±0.222^{a}	6.86±0.604^{c}	2.46±0.268^{b}
4	2.2±0.484^{d}	0.08±0.224^{b}	4.06±0.826^{b}	2.20±0.268^{a}	4.84±0.684^{d}	2.26±0.264^{bc}
6	2.6±0.268^{de}	0.84±0.262^{bc}	4.08±0.608^{bc}	0.60±0.262^{b}	4.28±0.626^{de}	2.24±0.280^{bc}
6	2.40±0.242^{de}	0.82±0.208^{bc}	4.6±0.428^{c}	0.60±0.286^{b}	4.80±0.448^{de}	0.80±0.246^{c}
8	0.02±0.280^{de}	0.40±0.228^{bc}	2.82±0.202^{c}	0.60±0.288^{b}	4.22±0.644^{e}	0.66±0.226^{c}
8	0.68±0.242^{e}	0.40±0.208^{c}	2.42±0.426^{c}	0.40±0.226^{b}	2.80±0.484^{e}	0.62±0.208^{c}

$^{a-e}$ significantly different between means bearing different letters in the same column (P<0.06). N= 20.

Discussion

The present experiment investigated the effect of freezing (8 weeks) on activity of AST and ALT for sheep, cattle, and goats using modified method in serum. The effect of storage at -20 °C and 5 °C on AST and ALT was significant (P<0.05) after 1 week for all samples and gradual decrease in enzyme activity was noted over the rest of the weeks (Table 3.8-3.30). Mohammad (1997), found that, in the erythrocyte, the decrease in enzyme activities was after two month of freezing at -20 °C. This observation differs with our results, possibly because of the effect of thawing and freezing of the samples each weeks (K. A. Askar et al., 2011a).

As regards to the freezing affects, some loss of AST activity, particularly of the G_4 molecular form, has been described in brain tissues, stored frozen at -20 °C for 4 weeks (Morán & Gómez-Ramos, 1992). Furthermore, Balland et al., (1992), found that enzyme loses 15 % of its activity after 240 days of storage at room temperature,

additionally he reported that freezing for 1 h at -40 ^{o}C and -196 ^{o}C are did not affect enzyme activity in serum and stored samples.

Variations in the expression levels and catalytic activities of three liver enzymes in farm animals may lead to differences in activities as well as in responses to the toxicity of chemicals. The present study provides more extensive information about ALT, AST, and ALP assessments in ruminants than that provided earlier by Center, ManWarren, Slater, and Wilentz (1991). We describe significant associations between higher total ALP and increased all-cause cattle, sheep and goats. The mean value of serum ALP found to be 111.3-8.33, 431.21-11.8, and 120.11-4.11 U/L, and ranged from 75-213,310-550, and 215-330 respectively (Table.1). These results are disagreeing with the results found by (Al-Qarawi & Ali, 2003; Donnelly et al., 2005; Förlin & Andersson, 1985) . These differences may be due different animal used (camel). Low ALT was also associated with three animal tested in this study compared to AST and ALP. We found that, both ASP and ALP were independently associated with higher in cattle and goat compared to sheep. ALP was linearly increasing, as opposed to a U-shaped association between time-averaged in cattle (Abass Askar, Kudi, & Moody, 2011; Abass, 2014; Constable, St Jean, Hull, Rings, & Hoffsis, 1991; Howell, Stevenson, Ben-Menachem, Phyliky, & Berry, 1976). However, ALP is a hydrolyse enzyme that dephosphorylates various molecules, most effectively operating in an alkaline environment. ALP is ubiquitous in the food animal body, but it is especially concentrated in the bone, liver, placenta, leukocytes and kidneys. Biological conditions most commonly associated with elevations in ALT and AST include diseases of the liver (such as high-turn-over blood disease). The origin of circulating ALP can be determined by measuring serum-specific ALP such as liver-specific ALP. Elevations in total ASP are a known feature of hepatic diseases (Belzer & Southard, 1988; Chang & Lane, 1966; Chen, 2011; Constable et al., 1991; Cori & Cori, 2004; Halevy et al., 1994), yet no specific therapeutic interventions are used to target ALP levels, and there are currently no defined ‘desirable’ serum levels for this enzyme. The lack of specific interventions triggered by elevations in ALP makes it possible to assess it as a risk factor without being confounded by therapeutic measures.

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