EFFECT OF AGE ON THE LEVEL OF SERUM ALKALINE PHOSPHATASE ACTIVITY OF JAPANESE BLACK STEERS

J. Sekine[1], K. Udagawa[2], Z. Morita and R. Oura

Department of Veterinary Science, Faculty of Agriculture,
Tottori University, Tottori 680, Japan

Summary

The level of serum alkaline phosphatase activity was determined in 7 Japanese Black steers at different ages. The isoenzyme activity of non-bone origin was estimated using a heat-inactivation technique. The activity of serum alkaline phosphatase (SALP, K-A unit) decreased as age (AGE, mo.) increased: SALP = 14.15 − 0.17 (±0.03) AGE, $r = -0.81$, $P < 0.01$, S.E. ± 0.28. The variation of the activity was greater in younger age than the older. The temperature of 58°C for the treatment of heat inactivation of bovine serum appeared to be suitable. The percentage of heat inactivated enzyme activity negatively correlated with age and positively with the level of serum alkaline phosphatase activity. The activity of SALP of non-bone origin was inferred to stay at about constant level irrespective of age and that of bone origin decreased with age.

(Key Words: Serum Alkaline Phosphatase, Heat Inactivation, Age in Month, Bone-origin Enzyme, Non-bone-origin Enzyme, Beef Steer)

Introduction

The alkaline phosphatase in serum has been well recognized to relate to the development of the bone structure because of its high activity in osteoblasts of young animals. Walawski et al. (1980) have revealed that young Polish Black-and-White Lowland bulls had the alkaline phosphatase generated almost exclusively from the bone, but the proportion of the isoenzymes originated from the liver or the mucosa of the small intestine increased in the blood serum of mature cattle. Serum isoenzymes of alkaline phosphatase have been analyzed in such diverse techniques as electrophoresis. The isolation of the isoenzyme generated from the bone using an electrophoretic technique encountered some difficulties to identify from the isoenzyme originated in the liver (Moss, 1982). An alternative approach has been presented for the identification of alkaline phosphatase isoenzymes by the difference in their heat stability (Posen et al., 1965; Cadeau and Malkin, 1973; Moss, 1982). There was, however, no report on the heat treatment of the bovine serum.

The present study is purposed to determine the level of serum alkaline phosphatase (SALP) activity in Japanese Black steers in varying age and to evaluate the proportion of the isoenzymes originated from the tissues other than the bone.

Materials and Methods

Animals used were 7 Japanese Black steers kept in the Experimental Farm of Tottori University. They were given a commercial formula feed, rolled barley, cubed alfalfa hay and rice straw under the conventional feeding regime of the farm (table 1). The blood samples were taken twice through the left jugular vein at an interval of one month. About 20 ml of the blood were taken into the heparinized vacuum tube. Blood samples were incubated for 60 min. at 37°C and then refrigerated for 60 min. at 5°C. After the refrigeration, samples were centrifuged at 700 g for 10 min. and then at 1000 g for 5 min. The serum obtained was analyzed for the activity of SALP by the method described by Morimoto et al. (1987). Healy (1975) has applied a heat-inactivation technique to the blood serum of sheep and obtained successful results in the identification of the bone alkaline phosphatase to the liver origin. The samples, therefore, were treated at 2 different temperatures that is 56°C reported to be suitable for

[1] Address reprint requests to Dr. J. Sekine, Dept. of Vet. Sci., Tottori Univ., Tottori 680, Japan
[2] Present address: Chichibu Highland Farm of Saitama Prefecture, Chichibu
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TABLE 1. FEEDS AND NUTRIENTS OFFERED (KG/HEAD/DAY) TO STEERS IN EACH AGE GROUP AND THEIR RANGE OF LIVE WEIGHT (KG)

Age (month)	6-10	14-20	24-26
Formula feed	3.3	6.3	6.0
Rolled barley	0.7	1.3	2.0
Cubed alfalfa	4.0	1.0	–
Rice straw		2.0	2.0
TDN	4.7	6.1	5.9
DCP	0.87	0.86	0.77
Live weight	157-281	415-470	550-608

human enzyme (Posen et al., 1965; Cadeau and Malkin, 1973; Moss, 1982) and 58°C for sheep (Healy, 1975).

Results

Figure 1 shows the change with age in SALP activity of Japanese Black steers. The SALP activity negatively correlated with age in month ($p < 0.01$). The regression analysis of SALP activity (SALP, King-Armstrong unit, K-A unit) (King and Armstrong, 1934) on age (AGE, mo.) resulted in a following equation: SALP = 14.15 – 0.17 (± 0.03) AGE, r = –0.81, P < 0.01, S.E. ± 0.28. The activity of SALP appeared to have a greater variation in younger age than the older one.

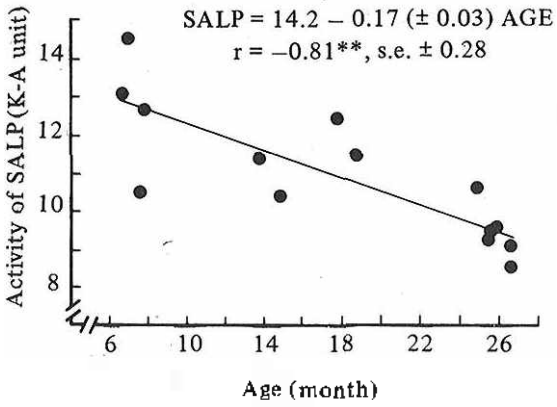

Figure 1. Change with age in activity of serum alkaline phosphatase of Japanese Black steers.

Results of a heat-inactivation of SALP treated at 56 and 58°C were shown in figures 2 and 3. Heat treatment at 56°C which is recommended in the analysis of human isoenzymes showed greater variation in heat inactivation of bovine enzymes than the treatment at 58°C at which bovine serum has been treated. Although both treatments at 56 and 58°C inactivated SALP activity in the present study, the coefficient of determination of the regression between SALP inactivation percentage and age was lower in the treatment at 56°C than that at 58°C. The mean percentage of isoenzyme inactivated at 58°C was

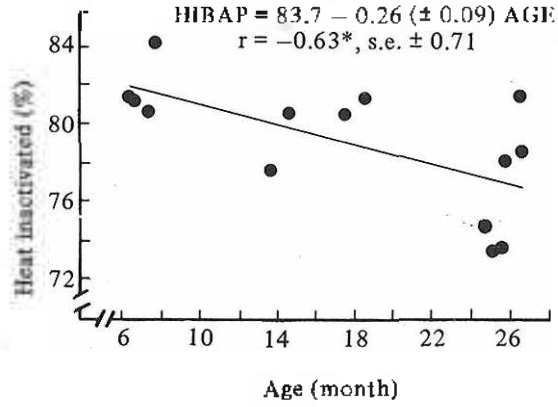

Figure 2. Effect of age on heat inactivation of bovine alkaline phosphatase(HIBAP) treated at 56°C for 10 minutes.

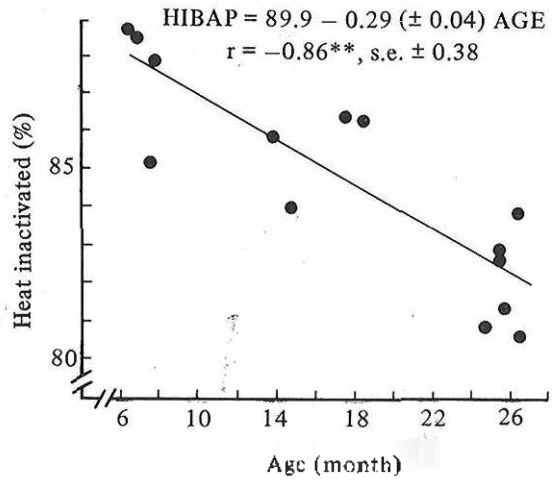

Figure 3. Effect of age on heat inactivation of bovine alkaline phosphatase treated at 58°C for 10 minutes.

significantly greater than that at 56°C ($P < 0.01$). The percentage of heat inactivated SALP (Y, %) decreased as age (X, mo.) increased in both treatments at 56 and 58°C. The following equations were obtained for both treatments:

at 56°C; $Y = 83.7 - 0.26\ (\pm 0.09)X$, $r = -0.63$, $P < 0.05$, S.E. ± 0.71, at 58°C; $Y = 89.9 - 0.29\ (\pm 0.04)X$, $r = -0.86$, $P < 0.01$, S.E. ± 0.38.

Assuming that heat-inactivated isoenzyme was the bone origin, SALP activity generated by other isoenzymes than the bone origin was calculated and correlated with age and SALP level (table 2). There was no statistically significant correlation between the non-bone-origin isoenzyme and the age nor the level of SALP activity. The non-bone-origin isoenzyme, however, negatively correlated with the percentage of heat inactivation at 58°C ($P < 0.05$). The regression coefficient was calculated to be −0.034 (S.D. ± 0.013). The level of SALP activity significantly and positively correlated with the percentage of SALP inactivated at 58°C ($r = 0.84$, $P < 0.01$).

TABLE 2. MEAN VALUES WITH STANDARD DEVIATION OF SALP ACTIVITY AND PERCENTAGE OF HEAT INACTIVATION, AND CORRELATIONS AMONG THEM

Age, mo.	6-10	14-20	24-26
SALP activity (K-A unit)			
Total	12.8±1.5	11.6±0.8	9.5±0.6
Non-bone-origin	1.6±0.07	1.6±0.01	1.7±0.2
Inactivated (%) (58°C, 10 min.)	87.7±1.5	85.7±1.1	82.1±1.2

Correlations

	SALP	Age	% inactivated
Non-bone-origin	-0.06^{ns}	0.35^{ns}	−0.58*
% inactivated	0.84**	−0.86**	
Age	0.81**		

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

Discussion

The activity of SALP has long been utilized as a diagnostic measure for the disorders of the liver, the bone and other related tissues in the medical and veterinary fields (Moss, 1982; Morimoto et al., 1987). The level of SALP activity, however, has been found to relate to the growth rate of chicken (Okada and Tsutsumi, 1963), mouse (Yamaki et al., 1970) and cattle (Roubicek and Ray, 1974; Walawski et al., 1980) with its involvement in the osteogenesis of animals. The activity of SALP decreased as age increased in Japanese Black steers, which agreed with the results found in mouse and cattle (Yamaki et al., 1970; Walawski et al., 1980). A greater variation was observed in those of younger age than the older ones. It may be related with a variation of growth rate in young steers since steers with higher level of SALP activity appeared to grow faster than those with lower level (Morimoto et al., 1987; Udagawa et al., 1989).

Posen et al. (1965) have found that alkaline phosphatase in bone homogenate was inactivated at a rapid rate to 27.6% of the untreated while that in bile which is considered to be the liver origin, at a slow rate to 75.0% of the untreated after heating samples at 56°C for 10 min. The coefficient of determination (r^2) revealed that the variation of age was able to explain only 0.40 of the variation of the percentage of heat inactivated SALP in the equation calculated from the results for heat treatment at 56°C, while 0.74 in that at 58°C. The accuracy of the second equation is much higher than the first one to predict the percentage of heat inactivation of bovine SALP with age. Thus, the temperature for heat inactivation of bovine SALP is recommended to apply 58°C as done in the sheep (Healy, 1975). The activity of SALP decreased to 12 to 18% of the untreated after heating samples at 58°C for 10 min. in the present study. Heat inactivation was more intense than that found by Posen et al. (1965). The enzyme inactivated by heating bovine serum at 58°C for 10 min. may be of bone origin, if not all but majority. Heat treatment at 58°C may have inactivated non-bone-origin SALP since the percentage of heat inactivation negatively correlated with non-bone SALP activity which was calculated by assuming that heat inactivated isoenzyme was the bone origin.

The level of isoenzymes generated in other tissues than the bone was not significantly correlated with age. The level of activity of SALP of non-bone origin may have stayed at about a constant level throughout the growing period of ani-

mals used in the present study. Furthermore, the age in month negatively correlated with the level of SALP activity and the percentage of heat inactivation of SALP. The level of SALP activity positively correlated with the percentage of heat inactivation. Multiple regression analysis of the percentage of heat inactivation (HINAC, %) on age (AGE, mo.) and level of SALP activity (LSALP, K-A unit) resulted in a following multiple regression equation:

$$HINAC = -0.17\ (\pm 0.07)\ AGE + 0.7\ (\pm 0.3)\ LSALP + 80.3,\ R^2 = 0.804,\ P < 0.01.$$

The absolute values of the standard partial regression coefficients were 0.51 and 0.43 for AGE and LSALP, respectively. The percentage of heat inactivation may be influenced by both parameter with about the same degree with a tendency to be higher in AGE. Results of multiple regression showed that age and the level of SALP activity influenced the percentage of heat inactivation at 58°C. Thus, steers with a low level of SALP activity may have a lower level of the isoenzyme of bone origin than the one with a higher level of SALP activity eventhough they are of the same age. Considering the results shown by Udagawa et al. (1989), those steers with a lower level of SALP activity is assumed to gain their weight at a slower rate than those with a higher SALP activity at the same age. Results of the present study, however, were not able to clarify the relationship between the variation of SALP activity and growth rate. Thus, more study is required to clarify the relationship between the SALP of bone origin and growth rate in cattle.

It is concluded from the above discussion that the SALP activity of non-bone origin stays at about a constant level irrespective of age and the level of activity of SALP generated from the bone decreases with age resulting an increase in relative composition of the iscenzyme of non-bone origin as observed by Walawski et al. (1980).

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