Theor. Appl. Genet. 62, 281–287 (1982)

Correlated Responses of Growth Traits to Selection for High and Low Plasma Alkaline Phosphatase in Mice

K. Yamaki and Y. Mizuma
Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai (Japan)

Summary. The effectiveness of two way selection for plasma alkaline phosphatase (ALP) was investigated in order to determine its influences on growth traits through thirteen generations. The responses of the two lines selected for high (HP) and low (LP) ALP at 45 days of age were compared to that of the mice selected for large (L) and small (SM) body size. The selection responses of plasma ALP were very effective for both HP and LP lines, with average responses per generation calculated from linear regressions of 0.227±0.037 and –0.088±0.022 respectively. The final levels of ALP in HP and LP were 5.54±0.71 and 1.27±0.20 in the thirtheenth generation, while the SM, L and base population had levels of 3.49±0.08, 0.86±0.55 and 2.77±0.56 respectively. The body weight at 45 days of age in LP (31.4±1.4 g) as a correlated response was significantly higher than HP (23.4±1.8 g) at generation 10. The correlated response of milk yield, measured by weight gain up to 12 days of age, was significantly greater in the LP line than in HP, but the correlated response of gains after weaning was not so different as the response of milk yield. The response of litter size and weight in LP showed significant higher levels than that of HP, but pups' birth weight did not differ between LP and HP. It is suggested that the correlated response of milk yield contributed more to the divergence of body size between HP and LP than the gain after weaning.

Realized heritabilities of ALP were 0.335±0.059 (HP) and 0.279±0.051 (LP). Realized genetic correlations between ALP and 45 days' body weight were –0.27±0.13 (HP with SM) and –0.52±0.19 (LP with L). Realized genetic correlations between ALP and milk yield were –0.95±0.03 (HP) and –0.37±0.29 (LP). Correlations between ALP and postweaning gains were fairly low.

Key words: Growth – Alkaline phosphatase – Selection – Correlated responses – Mice

Introduction

We have previously reported that mice selected for small body size have a significantly higher level of alkaline phosphatase (ALP) in plasma than large ones (Yamaki et al. 1970; Yamaki and Mizuma 1977). This finding promoted a study to obtain further information on this relationship and to determine whether these correlated responses are a general phenomenon or not.

The role of plasma ALP may be related to functions of growth, such as the dynamic absorption of nutrients through the intestinal microvilli (Glickman et al. 1970), calcium transport across cell membranes (Inesi 1971) and the regulation of fat or protein metabolism in the liver (Langman and Leuthold 1966). If the plasma ALP level is closely related to physiological functions of growth, we might be able to predict the growth potential of animals by the plasma ALP.

In poultry, the relationships between serum ALP and productive traits have been extensively investigated. The view has been presented that ALP activity is related to egg production in hens (Wilcox 1966), while others have presented contradictory results (Choudhary et al. 1971). Body weight during the later stage of growth, sexual maturity and egg weight showed negative correlations with serum ALP.

Both weight at birth and serum ALP correlated with weaning weight at 56 days in swine (Combs et al. 1959). The average serum ALP levels of immature Brahman cattle is approximately twice of European breeds of the same age (Kunkel et al. 1953). These reports suggest that plasma ALP levels are relevant to the growth of animals.

In mice, however, these relationships have not yet been investigated in a selection experiment. The purpose of this communication is to report on the response of certain growth traits to the selection for high and low plasma ALP, and to ascertain whether or not the difference of plasma ALP levels in mice selected for body size is genetically related to their growth.

Materials and Methods

Animals

The mice selected for large (L) and small (SM) body size at 45 days of age were derived from four way crosses of the inbred strains. The base population of the two lines selected for high (HP) and low (LP) plasma ALP was derived from reciprocal crossings between L and SM lines of generation 59. All these four lines were reared on the same diet in a conventional thermo-environment above 20 °C.

0040-5752/82/0062/0281/$ 1.40

Selection Procedure

One hundred thirty-two mice (male: 70, female: 62) were used as a base population, with five males and ten females selected truncationally for either high or low plasma ALP at 45 days of age. Two way selections were then carried out on the levels of plasma ALP in both sexes.

The traits of truncated populations in the base population are shown in Table 1. No significant differences were observed between body weights of low and high truncated populations for either males or females. Selection intensities, which were standardized selection differentials, were similar, ranging from 1.45 to 1.86.

Based on truncational selection, ALP levels the progenies (20 males and 40 females) from each line were determined, and 5 males and 10 females were used to establish the HP and LP lines after the second generation. One male mouse was mated to two female mice in each line, and pups were restricted to six (two male and four female) for suckling.

At the begining of the selection, the heritabilities of plasma ALP were calculated using the random bred line which was based on four way crosses of the inbred strains in the same way as L and SM lines (Yamaki et al. 1982a). Using a calculation of the components of variance in sib matings, the heritability of plasma ALP was estimated to be fairly high. Individual selection for plasma ALP was therefore adopted.

These selection procedures for HP and LP were performed throughout, from the first to the thirteenth generation. Full or half sib matings were avoided as much as possible. The effective population size of these lines was 13.3, with inbreeding coefficients per generation of approximately 3.75%.

Measurements of ALP

Approximately 0.05 ml of blood was withdrawn by orbital bleeding (Yamaki 1971) from mice of every generation at 45 days of age. Samples were centrifuged immediately and the plasma were stored at −20 °C. The plasma ALP were estimated within a week by the method of Bessy et al. (1946). The levels of ALP in the tables and figures are expressed in units of milli mole *p*-nitrophenol per hour per liter of plasma.

Statistical Analysis

The statistical analysis of these selection data generally adopted the Applied Program Package (STATPAC-6) of the Computer Center, Tohoku University.

The realized genetic correlations between ALP and body weight were calculated from the equation proposed by Falconer (1960):

$$rg^2 = (crx/rx)\,(cry/ry) \qquad (1)$$

where rg is the realized genetic correlation; crx and cry are correlated responses of ALP and the growth traits; rx and ry are direct responses of the two. Hence,

$$crx = i \cdot hy \cdot rg \cdot \sigma gx = i \cdot hy \cdot rg \cdot hy \cdot \sigma px \qquad (2)$$

$$ry = i \cdot hy \cdot \sigma gy = i \cdot h^2 y \cdot \sigma py \qquad (3)$$

where, i is the selection intensity; h^2x and h^2y are heritabilities of the character x and y; $\sigma^2 gx$, $\sigma^2 gy$, $\sigma^2 px$ and $\sigma^2 py$ are genetic and phenotypic variances of the two. Substituting (2) and (3) for (1). We obtain

$$rg = (cry/rx)\sqrt{h^2 x \cdot \sigma^2 px / h^2 y \cdot \sigma^2 py}. \qquad (4)$$

The realized genetic correlations between ALP and growth traits were calculated from formula (4). The heritabilities and phenotypic variances estimated by Yamaki et al. (1982a) were used. The standard errors of these realized genetic correlations were calculated by the formula proposed by Hill (1971).

Results

Response of Plasma ALP

Figure 1 shows the response of plasma ALP to the selection for high and low ALP. It is clear that the selection has been very effective. The SM line shows a significantly higher level (3.49±0.56) than L (1.86±0.55). The base population, developed from reciprocal matings of the two lines had an intermediate ALP level (2.77±0.56). The significant difference between HP and LP was observed from the first generation. After the sixth generation, the ALP level of the HP line was significantly higher than that of the SM. In the LP line, ALP became significantly lower than L after the ninth generation.

The final levels of ALP in HP and LP lines were 5.54±0.76 and 1.27±0.20, respectively. The HP level of

Table 1. Traits of truncated populations in the base population

Truncated populations	Female		Male	
	Low	High	Low	High
ALP level	1.86±0.23	3.49±0.27	1.82±0.19	3.74±0.27
Body weight at 45 days of age	22.0±1.6	22.0±2.8	25.7±1.4	25.6±1.6
Selection pressure	16.1%	16.1%	7.1%	7.1%
Selection differentials (i)	0.80	0.83	1.06	0.86
Selection intensity (i/σp)	1.45	1.51	1.86	1.51

σp = phenotypic variance

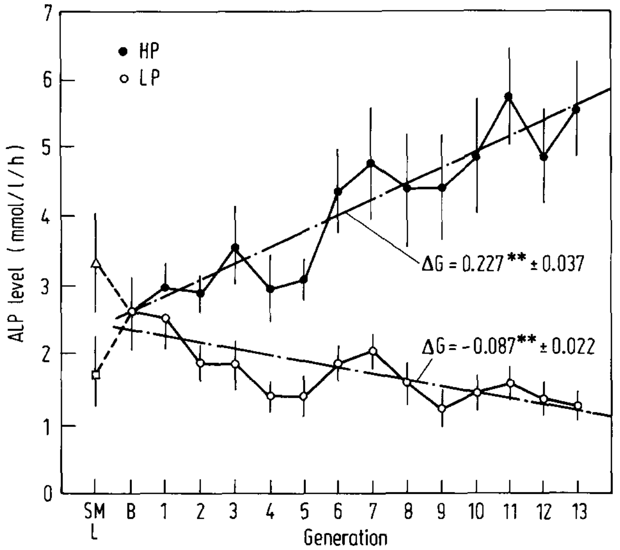

Fig. 1. Response of plasma ALP to selection for high and low ALP. ΔG = gentic gain per generation; SM = the value of SM line (original line); L = the value of L line (original line); B = base population; vertical line = S.D.

ALP became 4.36 times higher than LP by the thirteenth generation. The genetic gains per generation, calculated from linear regressions of the responses on generation, are 0.227±0.037 (HP) and -0.087±0.022 (LP), both highly significant. These results showed asymmetrical responses, with greater responses in the HP line.

Correlated Response of Body Weight

The correlated responses of body weight in male mice at 45 days of age to the selection for high and low plasma ALP are shown in Fig. 2.

The body weight of the LP line became significantly greater than that of the base population (25.8±1.7 g) after the second generation, yet both HP and LP lines remained between the L (38.5±3.7 g) and SM (17.6±1.4 g) lines. The LP line responded effectively in body weight until the third generation, after which it tended to reach a plateau, while the HP line gradually decreased in body weight throughout. The LP line had a significantly greater body weight than that of HP throughout all generations except the first. The correlated responses per generation, calculated from simple regressions, were 0.273±0.090 (HP), -0.093±0.153 (LP, up to generation 13) and -0.334±0.187 (LP, up to generation 10).

In generation eight, we detected that the mice were infected by Sendai Virus (Hemagglutinating Virus of Japan: HVJ) and, moreover, that suckling pups were suffering from Epidemic Diarrhea of Infant Mice (EDIM). It is not clear whether the increased body weight after the eleventh generation was caused by recovery from these infections or not. We therefore adopted -0.334 as the correlated response of the HP line and calculated the genetic parameters described later using this value.

In females, the correlated response of body weight followed a very similar pattern to that found in the males.

The correlated response of body weight at weaning in male mice is shown in Fig. 3. In the male LP line, body weight reached the level of the L line in the second generation, and was significantly higher than HP after that.

In the HP line, body weight at weaning remained close to that of the base population. Average body weight decreased sharply in generations seven and nine

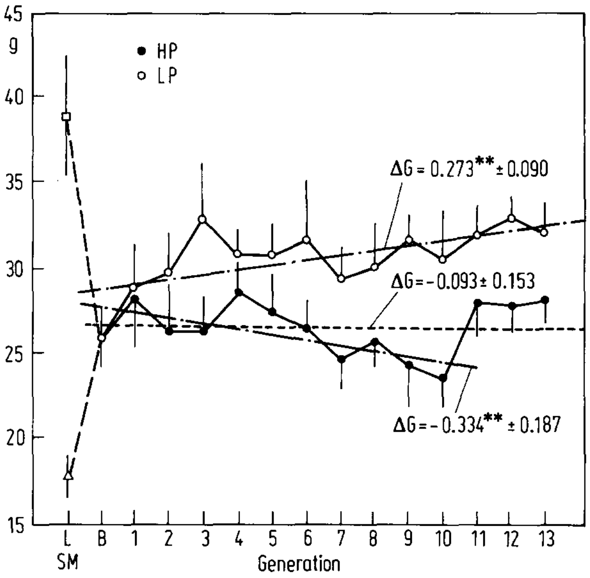

Fig. 2. Correlated response of body weight in male mice at 45 days of age to the selection for ALP

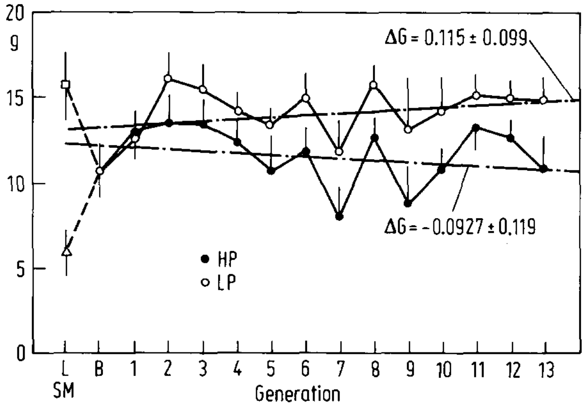

Fig. 3. Correlated response of body weight in male mice at weaning to the selection for ALP

for both the HP and LP lines. Infection by EDMI and HVJ may have caused these decreases. Almost the same results were obtained in the females.

Correlated Responses of Milk Yield and Gain

Figure 4 shows the gains from birth to 12 days of age per six pups in the litter as an index of total milk yield during the suckling period. The peak of the lactation curve in mice occurs between 12 and 13 days postpartum, so that weight gain up to 12 days provides a good prediction of actual total milk yield.

The LP line showed an effective response to selection, increasing rapidly to the same level as that of the L line by the second generation. The LP line showed a significantly higher level of milk yield than HP line after the second generation. The correlated responses of milk yield per generation calculated from linear regressions were –0.527±0.272 g (HP) and 0.443±0.292 g (LP).

These results suggest that one of the causes for increased body weight at 45 days of age can be attributed to the change of milk yield. On the other hand, the gains of the LP line after weaning (from 21 to 45 days of age) were not significantly different from those of HP except in a few generations (Fig. 5).

For both HP and LP lines, the gains after weaning in male mice were between the values for L and SM, yet the gains at pre-weaning were near the level of the L line. In females, the gains after the weaning of the HP and LP lines were near the SM ones and only differed significantly in a few generations.

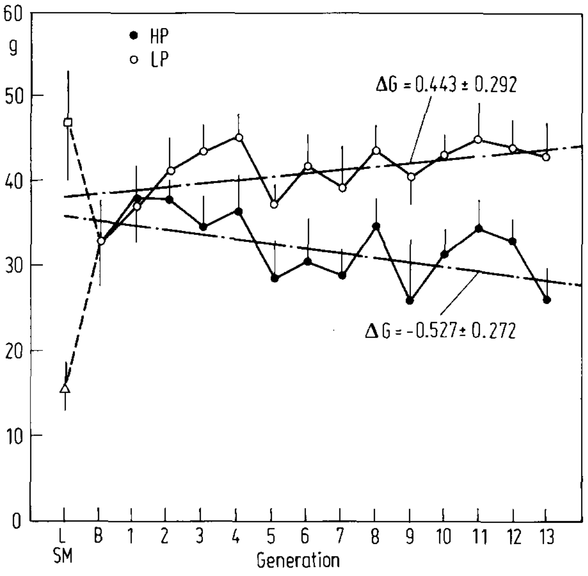

Fig. 4. Correlated response of milk yield measured by weight gain from birth to12 days of age, per six pups

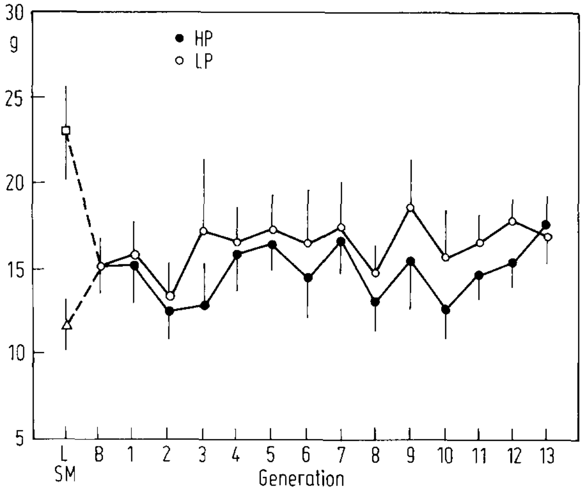

Fig. 5. Correlated response of gain after weaning to the selection for ALP

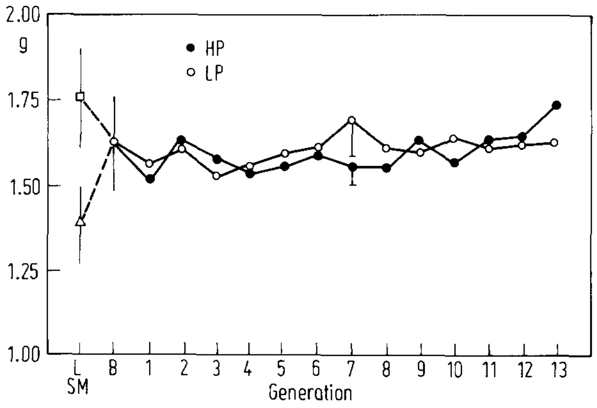

Fig. 6. Correlated response of the average pup's weight to the selection for ALP

Litter Size and Weight

Figure 6 shows the response of average pups' weights at birth to the selection for ALP. Litter size of the LP line was not significantly different from L throughout all generations, and HP showed an intermediate number between L and SM. Litter weight of these HP and LP lines followed the same tendency to litter size, so that average pups' weights at birth were almost the same and only differed significantly in the seventh generation.

Realized Heritabilities and Genetic Correlations

Table 2 shows the realized heritabilities of plasma ALP and realized genetic correlations between ALP and growth traits. Realized heritabilities of plasma ALP were calculated from simple regressions of the direct responses of the selection for ALP on cumulated selec-

Table 2. Realized heritabilities of plasma ALP and realized genetic correlations between ALP and growth traits

Line	Sex	Realized heritabilities of plasma ALP	Realized genetic correlations				
			21 days body weight	45 days body weight	45 days body weight[a]	Milk yield	Gain postweaning
HP	male	0.336[b] ± 0.046	-0.25 ± 0.15	-0.64 ± 0.22	-0.36 ± 0.10	-0.95 ± 0.03	0.19 ± 0.24
	female		-0.28 ± 0.13	-0.25 ± 0.23	-0.34 ± 0.11		0.34 ± 0.38
LP	male	0.279[b] ± 0.051	-0.28 ± 0.17	-0.34 ± 0.25	-0.40 ± 0.11	-0.37 ± 0.29	-0.14 ± 0.44
	female		-0.39 ± 0.04	-0.25 ± 0.20	-0.52 ± 0.09		-0.21 ± 0.27

The realized heritabilities and the correlation with milk yield were calculated from the pooled data of males and females

[a] Calculated from the equation (1) with the direct and indirect responses of HP with SM and LP with L. Other correlations were calculated from Eq. (4)

[b] High significance of regression coefficient ($P < 0.01$)

tion differentials (Falconer 1960) with the data pooled for males and females. The realized heritabilities were 0.336±0.046 (HP) and 0.279±0.051 (LP), with highly significant regression coefficients.

Realized genetic correlations between plasma ALP and growth traits are also shown in Table 2. Realized genetic correlations between ALP and the body weight at weaning ranged from −0.25 to −0.39 and all were negative. Similarly, those of weight at 45 days of age were all negative and in the same range as at 21 days of age, except for the males in the HP line.

The realized genetic correlations calculated from direct and indirect responses of the LP and L lines were higher (−0.52 to −0.40) than those of HP with SM lines (−0.36 to −0.34). Those of milk yield estimated from the gain up to 12 days of age per six pups in the litter showed fairly high and negative values, but those of the gains after weaning (21 to 45 days of age) were low or positive in the HP line.

Discussion

Selection for high and low plasma alkaline phosphatase has not been reported previously in mice. Wilcox (1966) and McClung et al. (1972) have carried out similar selections in laying hens. Wilcox showed that the level of ALP in the high line was significantly greater than the control, and that the difference was maintained to the end of the second laying year. McClung also showed that the plasma ALP of the high line was about twice that of the low line at the end of selection in generation 3.

Our selection was more effective: the HP line was about four times higher than the LP line at generation 13. These results show the effectiveness of selection for plasma or serum ALP, and suggest its usefulness as a selection criterion.

On this point, we make the assumption that the effectiveness of selection for ALP activity can be attributed to the close relation between gene and enzyme, i.e. the relationship of the gene to enzyme activity is more direct than to such other traits as body size, which results from the overall regulation of many complex pathways of metabolism. This assumption, however, is contradicted since the realized heritabilities of plasma ALP were about 0.3, and those of body weight were of the same order (Yamaki et al. 1982a). However, as ALP is a nonspecific enzyme and participates in several metabolic pathways, the heritability of ALP might be decreased because of the confounding effects of the various pathways. Hence, it is necessary to search for a specific enzyme involved in protein or fat metabolism, and obtain a more accurate breeding criterion able to estimate the genetic potential of growth.

The selection was more effective in increasing than in lowering plasma ALP. McClung et al. (1972) reported the same results in that the selection for ALP had a tendency to be more effective in the high ALP line of laying hens. Falconer (1960) pointed out possible causes of the asymmetry of selection response, i.e. selection differential, genetic asymmetry, selection for heterozygotes, inbreeding depression and maternal effects.

In the case of selection for ALP, we are able to rule out these possible causes except for the maternal effects. The selection differentials and inbreeding coefficients of HP and LP lines were almost the same and the asymmetry did not appear in the first few generations, which according to Falconer implicates "directional dominance and gene frequencies". On this account, we suggest that high levels of ALP in plasma may be more meaningful in relation to physiological or pathological function than low ones.

There ae several reports (Matsumoto et al. 1960; Smith et al. 1970) concerned with the genetic correlation between ALP and body weight in the fowl. In this experiment, the correlated response of body weight was negative. Yamaki et al. (1982a) estimated the negative genetic correlations (–0.25 to –0.49) between plasma ALP and body weight at 45 days of age with 565 random bred mice population by two methods of analysis of variance and regression on the offspring on dams. These results showed that negative genetic correlations between plasma ALP and body size may be a general phenomenon in animals.

If plasma ALP was adopted as a breeding criterion, it is important to know why the genetic correlation was negative. On this account, it is necessary to undertake further detailed studies of how the phenotypic value of plasma ALP activity appears through physiological processes. Yamaki et al. (1979) suggested that the tissue sources of plasma ALP in mice were mainly liver ALP with a smaller contribution from bone. Priestley and Robertson (1973) reported measurements of rates of protein turnover and calculated rates of protein degradation, suggesting that protein might be degraded more rapidly in small mice.

From these results, small body size animals are assumed to have a faster metabolic rate of chemical body component turnover than those of large ones. Consequently, the negative genetic correlation between plasma ALP and body weight can be explained by the following hypothesis, i.e. several genes of small body size regulate high degradation rates of chemical body components in the dynamic state, resulting in small body size overall.

In fact, Yamaki (1982b) explained the negative genetic relation between ALP and body size by fitting the large (L) and small (SM) strains to the Gompertz equation, and computing the relative growth rates ($dw/dt \cdot 1/w$) which were consistent with the developmental patterns of plasma ALP on age, namely that SM showed a high relative growth rate. This assumption must be verified by detailed study of the relationship between ALP and turnover rate of assimilation and degradation of body components.

Milk yield showed a remarkable negative response to the selection for plasma ALP, but the postweaning gain showed positive or little response to it. The results of realized genetic correlations were consistent with these findings. Positive correlations between mammary gland ALP and lactation in the early period of suckling was reported in rats (Arole and Kate 1980). From a random bred population, Yamaki et al. (1982a) estimated the genetic correlations between postweaning gain, milk yield and body weight during suckling and at weaning. Milk yield and postweaning gain showed fairly strong negative genetic correlations (–0.49 to –0.86), but a positive one (0.51 to 0.52) was estimated between postweaning gain and body weight at 45 days of age. These results suggest that selection for ALP would mainly contribute a genetic improvement of milk yield or preweaning gain.

There is no report of realized genetic correlations between ALP and growth traits. The genetic correlations estimated using another random bred population agreed fairly well with these realized in the present study, suggesting that the genetic parameters reported here are fairly reliable. There may be some objections that the correlated responses of selection were specific phenomena in this population because these four lines originated from the same population and gene pool, or a mere random genetic drift could have occurred. However, random genetic drift is denied by the fact that the four lines and the random population indicated the same responses, especially in body size and ALP. Further studies which investigate the physiological function of ALP need to be carried out to generalize the genetic relation between the plasma ALP and growth observed in this study.

Acknowledgement

We wish to thank Dr. T. E. C. Weekes for a critical reading of this manuscript and Y. Ohtomo for technical assistance.

Literature

Arole, V.U.; Kate, B.R. (1980): Alkaline phosphatase activity in the myoepithelial cells of albino rat mammary glands during pregnancy and lactation. J. Anat. Soc. India **29**, 94–99

Bessy, O.A.; Lowry, O.H.; Brock, M.J. (1946): A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. **164**, 321–329

Choudhary, R.P.; Krishna, S.T.; Verina, S.K.; Shivaprasad, H.L. (1971): Serum alkaline phosphatase polymorphism and its association with economic traits in White Leghorn. Indian J. Poult. Sci. **6**, 22–26

Combs, G.E.; Wallace, H.D.; Alsmeyer, W.H.; Koger, M. (1959): Growth potential of swine as measured by serum alkaline phosphatase. J. Anim. Sci. **18**, 361–364

Falconer, D.S. (1960): Introduction to Quantitative Genetics. London: Longman

Glickman, R.M.; Alpers, D.H.; Drummey, G.D.; Isselbacher, K.J. (1970): Increased lymph alkaline phosphatase after fat feeding effect of medium chain triglycerides and inhibition of protein synthesis. Biochim. Biophys. Acta **201**, 226–235

Hill, W.G. (1971): Design and efficiency of selection experiments for estimating genetic parameters. Biometrics **27**, 293–311

Inesi, G. (1971): p-Nitrophenyl phosphate hydrolysis and calcium ion transport in fragmented sarcoplasmic reticulum. Science **181,** 901–903

Kunkel, H.O.; Stokes, D.K., Jr.; Anthony, W.B.; Futrell, M.F. (1953): Serum alkaline phosphatase activity in European and Brahman breeds of cattle and their crossbred types. J. Anim. Sci. **12,** 765–770

Langman, M.J.S.; Leuthold, E. (1966): Influence of diet on the "intestinal" component of serum alkaline phosphatase in people of different AB0 blood groups and secreter status. Nature **212,** 41–43

Matsumoto, K.; Tonoue, T.; Okada, I. (1960): Heritability of Physiological characters of chickens III. Serum alkaline phosphatase activity and its relation to growth. J. Facul. Agr. Hokkaido Univ. **51,** 315–312

McClung, M.R.; Hyre, H.M.; Martin, W.G. (1972): Two-way selection for serum alkaline phosphatase in laying hens. Poult. Sci. **51,** 1428–1437

Priestley, G.C.; Robertson, M.S.M. (1973): Protein and nucleic acid metabolism in organs from mice selected for larger and smaller body size. Genet. Res. **22,** 255–278

Smith, J.L.; Goodman, B.L. (1970): Heritability and correlation estimates for serum alkaline phosphatase and growth in the chick. Poult. Sci. **49,** 1728–1729

Wilcox, F.H. (1966): Effect on performance of selection for high level of alkaline phosphatase in serum. Poult. Sci. **45,** 776–784

Yamaki, K.; Yamagishi, T.; Nishida, S. (1970): Growth and alkaline phosphatase relation in mice I. Growth and plasma alkaline phosphatase of the mice selected for large and small body size. Jpn. J. Zootech. Sci. **41,** 314–318

Yamaki, K. (1971): Investigation into blooding of experimental animals and enzyme activity. Sci. Through Exp. **22,** 1082–1085

Yamaki, K.; Mizuma, Y. (1977): Growth and alkaline phosphatase II. Selection response of mice selected for body size. Tohoku J. Agric. Res. **28,** 111–117

Yamaki, K.; Ohtomo, Y.; Mizuma, Y. (1979): The tissue sources of plasma alkaline phosphatase in mice. Tohoku J. Agric. Res. **30,** 24–31

Yamaki, K.; Ohtomo, Y.; Mizuma, Y. (1982a): Genetic parameters of alkaline phosphatase and growth traits in mice. Jpn. J. Zootech. Sci. (in press)

Yamaki, K. (1982b): Breeding studies of growth and alkaline phosphatase in mice. Doctor thesis, Tohoku Univ.

Received December 28, 1981
Accepted February 18, 1982
Communicated by H. Abplanalp

Dr. K. Yamaki
Dr. Y. Mizuma
Department of Animal Science
Faculty of Agriculture
Tohoku University
Sendai 980 (Japan)