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Relationship Between Some Serum Enzyme Activities, Liver Functions and Body Weight in Growing Local Chickens

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Abstract: An experiment was conducted to detect the relationship between some serum enzyme activities, i.e. lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) enzymes and liver functions, as well as evaluating the difference in some physiological parameters in relation to body weight in six body weight lines of Golden Montazah (GM) chickens. A total of 288 GM chickens 4-wk old were selected from 500 unsexed GM chickens and divided into six lines (three lines within each sex) heavy (HL), light (LL) and control (CL) according to their body weights. Each line was represented by 3 replicates (16 chicks each) over two generations (G_0 and G_1). Live body weight (LBW), body weight gain (BWG), mortality rates and the levels of some serum parameters, i.e. total protein (TP), triiodothyronine (T_3), LDH, CPK, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined at 4 WOA in G_0 and at 12 WOA in G_0 and G_1. The results showed that the average LBW and BWG for heavy lines and males were significantly ($p<0.01$) higher than that of light lines and females, respectively. The heavy line chicks recorded the lowest mortality rate compared to the light ones during the period from 4-12 WOA over the two generations. The HL body weight was associated generally with an increase in the serum TP, T_3 level and LDH activity and a decrease in AST, ALT and CPK activities as compared with the LL body weight. Converse trend was observed for these traits with the LL chicks. These results concluded that, it is possible to use these parameters as physiological indices to attain the goal of improving meat production and to produce a safety and healthy poultry production.

Key words: Chicken, physiological, liver, LDH, CPK and body weight

INTRODUCTION

In the production of meat, this goal is realized by breeding large bodies strains with a high proportion of desirable meat and little fat (Ricklefs, 1985). Body weight perse, is an important indicator of general health and lighter birds on the average produce the lowest meat and egg mass, because this class would include mainly more unhealthy birds than the heavier birds (Singh and Nordskog, 1982). Blood biochemical and hematological characteristics could be very important as indicator traits in breeding for the highest productive performance birds (Peterson *et al*., 1982; Hassan *et al*., 2006 and Hassaan *et al*., 2008).

Hematological parameters and blood enzyme activities were studied in most species of poultry in lines selected at 4 WOA for heavy and light body weights. Serum AST and ALT activities (liver enzymes) were used to evaluate liver functions, the increase in their activities are related to the degenerations of hepatocytes or liver damage "irrespective of its origin (Blackshow, 1978). The dystrophic hybrids and LL body weight chickens exhibited symptoms of high serum CPK and low LDH levels compared with normal and HL body weight birds (Wilson *et al*., 1988). Moreover, the overt muscle damage in birds is associated with an increase in the plasma activity of the intracellular muscle enzyme (CPK) (Siller *et al*., 1978 and Lumeij *et al*., 1988). In broiler chickens the CPK is released into the circulation in response to various pathologies and exposure to environmental stressors (Mitchell *et al*., 1992; Mitchell and Sandercock, 1995 and Bogin *et al*., 1997). However Hocking *et al*. (1998) compared plasma CPK activity in two lines of turkey, commercial large white and traditional turkey and reported no sex differences in the plasma CPK activity during rearing period. But, Hocking *et al*. (1999) reported that plasma LDH activity was higher from 12-24 WOA in *ad libitum* fed turkeys with the high body weight than the restricted ones whose body weight was the lowest. In Japanese quail selected at 4 WOA through three generations for heavy and light LBW, Shata (2001) reported that the activity of LDH increased significantly in the heavy line compared with the light body weight line and the activity of LDH in the G_2 increased by more than two folds over the G_1, as well the activity of CPK was decreased in the HL and increased in the LL body weight.

Hammouda *et al*. (2001) and Hassaan *et al*. (2008) demonstrated that the heavy line body weight chicks exhibited higher T_3 level than those of light line. On the other hand, Meluzzi *et al*. (1992) and Saleh (1997) reported that the values of plasma protein were higher in heavy chicks than light chicks. The present study was conducted to detect the relationship between some endogenous enzyme activities in the blood serum, i.e.

LDH and CPK enzymes and liver function and to evaluate the difference in some physiological parameters related to body weight among six lines of Golden Montazah chicks differing in their body weights. Golden Montazah (GM), is a developed strain from a cross between the Rohde Island Red and Dokki 4, using systems of breeding coupled with selection (Mahmoud *et al*., 1974 and El Hossari *et al*., 1992).

MATERIALS AND METHODS

Experimental birds and management: This experiment was carried out at Poultry Farm, Faculty of Agriculture, Ain Shams University and Animal Production Research Institute Laboratories, Egypt. A total of 500 one-day old unsexed Golden Montazah (GM) chicks foundation flock were collectively brooded on littered floor pens till the 4^{th} wk of age (WOA). Chicks were fed for *ad libitum* on a diet contained 19.5% crude protein and 2878 Kcal ME/Kg during the growing period up to 8 WOA. During the finisher period, they were fed a diet contained 16.2% crude protein and 2928 Kca/ME/Kg. At 4 WOA a total number of 288 GM chicks were assigned and sexed. Chicks were wing banded and divided into six lines (three lines within each sex) heavy (HL), light (LL) and control (CL) according to their body weights, each line was represented by 3 replicates (16 chicks each). The male lines were designated to control "CM", heavy "HM" and light "LM" with the averages LBW of 233.13±1.36, 281.56±3.28 and 196.94±2.89 gm, respectively. The female lines were also designated to control "CF", heavy "HF" and light "LF" with the averages LBW of 183.42±2.26, 220.44±2.83 and 156.67±2.16 gm, respectively. At 30 WOA 27 cocks (9 for each line; 3/replicate) were transferred to their counterparts of hen lines. At 32 WOA and throughout 10 days thereafter a total of 360, 375 and 365 pedigreed eggs were collected from dams of CL, HL and LL, respectively. Eggs were set in an electric forced-draft incubator for 19 days. At the 19^{th} day incubation the eggs of each line were placed in a separate plastic net and were transferred to the hatcher. The hatched chicks; 278, 300 and 255 from CL, HL and LL, respectively were brooded and reared under the same managerial conditions as foundation flock (G_0) to form the first generation (G_1).

Measurements and observations: Individual live body weight (LBW) and body weight gain (BWG) for each line within each sex was biweekly recorded, to the nearest gram, from the age of 4 wk till the 12 WOA. Mortality rate was daily recorded and calculated as a percentage of total mortality due to the sex and line.

A total of 108 blood samples were withdrawn from wing vein (72 samples from foundation flock "G_0" at 4 and 12 WOA and 36 samples at 12 WOA from the first generation G_1) as six samples per line within each sex. Serum were harvested after centrifugation, decanted and stored at -20°C until biochemical analyses were done. Commercial kits were used for the determination of the following serum constituents according to the procedure outlined by the manufacturer. Total proteins (Sclavo INC. 5 Man. Sard Count, Wayne. N. J07470, USA). Serum triiodothyronine (T_3) was determined using a RIA kit produced by IMMUNO TECH (Beckman Couter Company -13276 Marseille Cedexg France-procedure No. IM 3287). Serum CPK and LDH (Stanbio-procedure No. 0910). Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) (Bio-Merieux, Marcyl, Etiols, France).

Data were subjected to analysis of variance using General Linear Model (GLM) procedure of SAS User's Guide (SAS, 2001). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Results of LBW and BWG at different ages showed significant ($p<0.01$) differences either between lines, sexes or generations (Tables 1 and 2). The heaviest LBW and BWG were recorded for birds of the G_1 and HL at all ages, as well males were significantly ($p<0.01$) heavier than females. These results are in agreement with the results of El-Soudany (2003) and Hassaan *et al*. (2008) who reported that sex and line showed highly significant differences in LBW and BWG of chickens at different ages. Also, Shata (2001) and El-Full *et al*. (2001) showed significant ($p<0.01$) differences between generations and lines, in which G_1 and HL had the highest LBW and BWG values compared with the other generations and lines of Japanese quail. In this connection, Abdel Latif and El-Hammady (1992) cited that sexual differences for LBW in chickens may be due to differences in the appetite between sexes during the growth period. However, Singh *et al*. (1990) concluded that sexual differences in LBW for chickens have polygenic basis of inheritance and selection could be effective in changing LBW relationship between male and female progenies.

As shown in Table 2, the different interaction figures had insignificant effects on LBW till the age of 8 wk except the G*L that was significant up to 12 WOA. Moreover, significant effects were found for G*S and L*S on LBW at 10 and 12 WOA. This result may be attributed to the highly significant effect of sex on LBW. Similar trend was approximately achieved for BWG, except the G*S interaction.

Data depicting mortality rate (MR) of GM chicks as influenced by generation (G), line (L) and sex (S) are shown in (Table 3). Regardless of sex and line it could be seen that the cumulative MR recorded for G_0 (6.25%) was higher than that recorded for G_1 (5.21%). Concerning line effect, the LL had the highest MR compared to their counterparts of HL and CL. This result

Table 1: Average of live body weight "grams" at different ages in Golden Montazah chicks as affected by generation (G), line (L) and sex (S)

Age (wk)	G: G_0	G: G_1	L: Control	L: Heavy	L: Light	S: Male	S: Female	SEM	Significance of difference: G	L	S	G*L	G*S	L*S	G*L*S
4	212.03[b]	242.79[a]	222.98[b]	270.01[a]	189.25[c]	255.48[a]	199.34[b]	2.96	**	**	**	**	NS	NS	NS
6	345.16[b]	390.01[a]	360.34[b]	442.76[a]	299.65[c]	400.85[a]	334.32[b]	4.16	**	**	**	**	NS	NS	NS
8	486.23[b]	549.68[a]	509.77[b]	612.39[a]	427.21[c]	559.61[a]	473.30[b]	5.70	**	**	**	**	NS	NS	NS
10	629.91[b]	697.71[a]	654.62[b]	799.42[a]	537.40[c]	722.41[a]	605.21[b]	6.77	**	**	**	**	*	*	NS
12	819.47[b]	910.37[a]	863.20[b]	1026.20[a]	705.30[c]	931.49[a]	798.35[b]	8.08	**	**	**	**	*	*	NS

[a-c]Means within the same classification with no common superscript differ significantly *($p<0.05$); **($p<0.01$), NS: not significant

Table 2: Average of body weight gain "grams" at different periods in Golden Montazah chicks as affected by generation (G), line (L) and sex (S)

Period (wk)	G: G_0	G: G_1	L: Control	L: Heavy	L: Light	S: Male	S: Female	SEM	Significance: G	L	S	G*L	G*S	L*S	G*L*S
4-6	133.13[b]	147.22[a]	137.36[b]	172.75[a]	110.40[c]	145.37[a]	134.98[b]	3.72	*	**	*	**	NS	*	NS
6-8	141.07[b]	159.67[a]	149.43[b]	169.63[a]	127.56[c]	198.76[a]	138.97[b]	5.55	*	**	**	**	NS	*	NS
8-10	143.68[b]	148.03[a]	144.85[b]	187.03[a]	110.19[c]	162.80[a]	131.91[b]	4.34	*	**	**	**	NS	*	NS
10-12	189.56[b]	212.66[a]	208.58[b]	226.78[a]	167.96[c]	209.08[a]	193.14[b]	7.20	**	**	*	**	NS	*	*

[a-c]Means within the same classification with no common superscript differ significantly *($p<0.05$); **($p<0.01$), NS: not significant

may be attributed to the LL would include mainly more unhealthy birds than the other lines particularly, the HL one (Singh and Nordskog, 1982).

The cumulative MR of females was slightly higher than that of males. However, Hussen (1997) found that the mortality rates of male and female GM chicken during the period from one to eight WOA were 8.0 and 7.14%, respectively.

From that the maximization of profit of meat type birds could be achieved from not only improving growth rate but also increasing livability of marketable chicks.

Serum total protein (STP) was significantly increased ($p<0.05$) in HL chicks compared with LL and insignificantly slight increase in females than males at four WOA (Table 4). Also, STP in G_1 was significantly ($p<0.05$) higher than G_0 and significantly ($p<0.01$) higher in HL than both CL and LL at 12 WOA (Table 5). These observed findings indicated that the increase in body weight was associated with the increase in STP level. These results are in full agreement with those reported by Meluzzi *et al.* (1992); Rizkalla (1996) and Hassaan *et al.* (2008) in chickens and Faisal *et al.* (2008) in Japanese quail chicks, they reported that chicks of heavy body weight line showed marked elevation in total blood protein than the chicks of low line. These results may be due to the differences in either the metabolic activities or the hormonal make up between lines (Patterson *et al.*, 1967).

With regard to the serum concentration of T_3 hormone at G_0, the current results showed that it was significantly ($p<0.05$) elevated at 4 WOA in the HL compared to the LL and in males comparable to females (Table 4). Similar trend was observed at 12 WOA for the effect of both sex and lines and for the G_1 over the G_0 (Table 5). These results are in full agreement with those of Nyoman *et al.* (1989) and Hammouda *et al.* (2001) in broiler chicks; Hassaan *et al.* (2008) in local chicks and Shata (2001) in Japanese quail chicks. They reported that chicks of the HL whose LBW was the highest exhibited the highest values of T_3 compared with those had the lowest LBW (LL) and the males had higher T_3 level than the females.

These data confirmed the important role of T_3 hormone in the regulation of protein metabolism and protein turnover, especially in skeletal muscles (Hayashi *et al.*, 1992). In this respect, El-Husseiny *et al.* (2000) concluded that T_3 was 2 to 3 times as biologically active as T_4 in the chicks and oxygen consumption was significantly increased by T_3 or T_4 thereby, increased growth rate. Furthermore, the results of Nyoman *et al.* (1989) supported the hypothesis that the response of broiler chickens to thyroid hormones varied by sex and dose. Since, muscle growth and protein synthesis and muscle protein breakdown were accelerated to a greater degree in the male chicken, whereas abdominal fat deposition was reduced further in the female chicken. These differences might be explained by the interaction of thyroid hormones and steroid hormones as cited by Akiba *et al.* (1983). This result may point out the potential role of T_3 as a physiological marker for detecting the difference between sexes and among lines according to their body weights.

Significant effect for the G*S, L*S and G*L*S interactions was achieved on the serum T_3 level (Table 5).

Results of LDH activity recorded at 4 and 12 WOA are presented in Tables 4 and 5, respectively. It is of interest to note that, they followed similar trend observed for T_3 hormone.

As shown in Table 5, significant effect ($p<0.05$) was found for the interaction of G with each of L and S (G*L and G*S) on the LDH activity, while both of L*S and G*L*S had no significant effect.

These current findings signify a linear relationship between the LDH activity and the increase in LBW whether for the HL body weight over the LL or for males over females. These results coincided with the previous reports of Matsuzawa (1981) in chickens and Hocking *et*

Table 3: Average values of cumulative mortality rate (%) of Golden Montazah chicks from 4 till 12 WOA as affected by Generation (G), Line (L) and Sex (S)

		L			
Generation	Sex	Control	Heavy	Light	Overall mean
G_0	M	*6.25	2.08	8.33	5.55
	F	6.25	4.17	10.41	6.93
	Overall	6.25	3.13	9.37	6.25
G_1	M	2.08	4.17	6.25	4.17
	F	6.25	4.17	8.33	6.25
	Overall	4.17	4.17	7.29	5.21

M = Male F = Female, Mortality rate (%) = number of dead hens/total number of live hens x 100

Table 4: Average of some serum metabolites in Golden Montazah chicks at 4 WOA in Generation zero (G_0) as affected by Line (L) and Sex (S)

	L			S			Significance		
Parameters	Control	Heavy	Light	Male	Female	SEM	L	S	L*S
Total protein (g/dl)	2.98^{ab}	3.29^{a}	2.71^{b}	2.88	3.10	0.03	*	NS	NS
T_3 (ng/ml)	1.90^{ab}	2.16^{a}	1.62^{b}	2.07^{a}	1.71^{b}	0.02	*	*	NS
LDH (U/L)	195.59	228.05^{a}	169.61^{c}	206.38^{a}	189.11^{b}	10.52	**	*	NS
CPK (U/L)	119.35^{ab}	112.43^{b}	142.48^{a}	123.42	126.09	9.82	*	NS	NS
AST (U/L)	74.80^{ab}	64.58^{b}	84.76^{a}	68.99	80.44	5.19	*	NS	NS
ALT (U/L)	14.98^{ab}	11.19^{b}	19.06^{a}	16.86	13.28	1.62	*	NS	NS

$^{a-c}$Means within the same classification with no common superscript differ significantly *(p<0.05); **(p<0.01), NS: not significant

Table 5: Average of some serum metabolites in Golden Montazah chicks at 12 WOA as affected by Generation (G), Line (L) and Sex (S)

	G		L			S			Significance						
Parameters	G_0	G_1	Control	Heavy	Light	Male	Female	SEM	G	L	S	G*L	G*S	L*S	G*L*S
Total protein (g/dl)	4.38^{b}	4.71^{a}	4.53^{b}	5.04^{a}	4.05^{c}	4.51	4.58	0.17	*	**	NS	NS	NS	NS	NS
T_3 (ng/ml)	2.34^{b}	2.92^{a}	2.66^{b}	3.01^{a}	2.22^{c}	2.88^{a}	2.38^{b}	0.21	**	**	**	NS	*	*	*
LDH (U/L)	228.75^{b}	282.64^{a}	254.25^{b}	272.15^{a}	240.69^{c}	267.49^{a}	243.90^{b}	13.42	**	**	**	*	*	NS	NS
CPK (U/L)	137.76	141.47	136.21^{b}	127.16^{c}	155.48^{a}	137.47	141.76	10.69	NS	**	NS	NS	NS	NS	NS
AST (U/L)	84.64	86.48	85.59^{ab}	75.56^{b}	95.54^{a}	79.63	91.49	4.64	NS	**	NS	NS	NS	NS	NS
ALT (U/L)	21.65	24.44	22.07^{ab}	19.1^{b}	26.84^{a}	21.59	24.50	1.57	NS	**	NS	NS	NS	NS	NS

$^{a-c}$Means within the same classification with no common superscript differ significantly *(p<0.05); **(p<0.01), NS: not significant

al. (1999) in turkey. They illustrated that males showed higher level of LDH activity than females at the same age. Also, Wilson *et al*. (1988) and Hassaan (2008) noticed that the HL body weight chickens had higher levels of LDH activity than LL ones and the dystrophic body weights birds. However, Krasnodebska *et al*. (2000) reported no difference between female and male chickens in the activity of LDH enzyme.

The muscular metabolic activity can be assessed by serum LDH enzyme activity (Bazea *et al*., 2000). Besides, this enzyme could be used as marker of physiological state of growth and the significant differences in serum LDH activity among lines and between sexes. This may be attributed to the genetic make-up of each line and sex (Shata, 2001).

From Tables 4 and 5, it could be noted that the activity of CPK was significantly elevated in the LL body weight compared with HL at both 4 and 12 WOA over the two generations (G_0 and G_1). These results revealed that the increase in the CPK activity is negatively correlated with the increase in the LBW.

These results are in agreement with those found in different poultry species i.e., in chicken (Bogin *et al*., 1997 and Krasnodebska *et al*., 2000); in turkey (Hocking *et al*., 1998) and in Japanese quail (Shata, 2001). All of them reported collectively that no sex differences in serum CPK activity during rearing period while increased significantly in dystrophic and light line body weights (LL) compared with normal and HL birds. The significant differences in serum CPK activity estimated in this study may be attributed to the genetic make up of each line or the variations in synthesis or clearance of this enzyme, this explanation was supported by Kaneko (1989).

The elevation of both AST and ALT enzymes is considered as an index of hepatic dysfunction. In which, the AST is not specific for hepatocellular damage but is highly sensitive in detecting liver damage. The ALT is found in hepatocyte cytosol as well as in muscle and other tissues of birds. ALT has poor specificity for liver disease and the clinical relevance of an increased ALT value is decreased (Harr, 2002).

Results presented in Tables 4 and 5 showed insignificant differences between generations (G_0 and G_1) or sexes in the AST and ALT activities at both 4 and 12 WOA. Further, their activities were significantly

($p<0.05$) and highly significantly ($p<0.01$) elevated in the LL body weight comparable to the HL at similar ages. This results could be due to an attempt of the LL body weight birds to face their increasing demand for non-essential amino acids. Also, Guyton (1981) reported that changes in the serum transaminases level may depend on the rate of protein metabolism.

These results indicated that the HL body weight birds had better liver functions compared to the LL ones. In harmony with the present findings, Singh and Nordskog (1982) reported that lighter birds on the average produce the lowest meat and egg mass, because this class would include mainly more unhealthy birds than the heavier birds, which confirm the results of the present study.

On the other hand, Faisal *et al.* (2008) found that the activities of both AST and ALT were not significantly different between the high and low lines of body weight in Japanese quail chicks.

Conclusion: In conclusion, the results obtained herein pointed out the presence of a positive relationship between CPK and serum transaminases activities in the LL body weight and a negative relationship between LDH and serum transaminases in the HL one. These results signifying hepatic dysfunction and better hepatic expression in the LL and the HL body weight, respectively.

Therefore, these results could be used as indices of the highest productive performance birds in attaining the goal of improved meat production and to produce a safety and healthy poultry production.

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