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Observations on Immunogenetic Constitutions of Serum and Egg Proteins in Local Egyptian Hens

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Abstract: The present study was carried out to investigate immunogenetic constitutions of blood serum and egg proteins of local Egyptian breed hens. Five blood serum proteins (albumin-transferrin- pos-transferrin-esterase and cholinesterase) and 6 egg proteins (ova albumin- ova transferrin- globulin2- globulin3-ova macroglobulin and conalbumin)were investigated. The most predominant blood protein loci were Alb^A 0.693, Tf^B 0.707, Ptf^F 0.757 and $Es\text{-}5^B$ 0.721. All of these genetic loci were polymorphic. Concerning egg protein loci, the high frequency of $OV\text{-}Al^A$ (0.830), $OV\text{-}Tf^A$ (0.750), $OV\text{-}Al^A$ (0.840) and Pr^D (0.700) were recorded. The most important results of the present study is the closely association between $OV\text{-}Al^A$ and $G2^B$ alleles and body weight, egg weight. Therefore, more attention is urged to be paid by poultry breeding companies to enhancing general health or disease resistance and welfare of the laying hens, next to the production traits. It could be concluded that the internal genetic merit about maintaining health is highly required.

Key words: Immunogenetic constitutions · Serum Proteins · Egg Proteins · Hens

INTRODUCTION

Consumer demand for poultry meat and eggs is increasing, as poultry meat is a high- quality dietary protein source for human. Nowadays, the chicken meat production has shown a dramatic increase [1]. During the past decades, poultry breeders have successfully improved the production performance, either egg production in layers or meat production in broilers. However, considering the ever-increasing social concern, future animal husbandry is also required to pay more attention to enhancing animal welfare [2].

Local breeds of poultry are highly adapted to the prevailing environmental conditions. The performance of these breeds depends largely on the management system. Their true genetic potential can be expressed only under improved conditions [3].

In the past, genetic improvement programs for increasing chicken productivity in developing countries was mainly focused on the use of imported temperate breeds. Many exotic breeds of chicken (White and brown Leghorns, Rhode Island Red, New Hampshire, Cornish, Australorp, Light Sussex etc.) were introduced over the years. The other approach to improve productivity of the indigenous chicken production has been the use of crossbreeding to exploit heterosis. This approach involved crossing of unselected indigenous chicken to different levels of exotic breeds [4].

Most traits of economic importance in farm animals show continuous variation; however, their underlying genetic natures are very complex. Molecular marker assisted selection is efficient and leads to improvements in production performance. A candidate gene approach is a powerful method for understanding the direct genetic basis involved in the expression of quantitative differences between individuals [5, 6].

Reproduction is a comprehensive reflection of the development of various parts of a chicken body and its final expression is the result of interaction among genetic, nutritional and environmental factors [7].

The pattern of serum protein electrophoresis results depends on the fractions of two major types of protein: albumin and globulin. Albumin is the major protein component of serum is produced by the liver under physiological conditions. Globulins comprise a much smaller fraction of the total serum protein. The subsets

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of these proteins and their relative quantity are the primary focus of the interpretation of serum protein electrophoresis [8].

Several studies have been carried out to recognize and evaluate the immunogenetic constitution and genetic polymorphisms of poultry [9-16].

The objective of the present study was to identify polymorphisms of some blood and egg protein loci of local Egyptian indigenous breed. In particular, we searched for a genotypic interaction of these markers, with some economic values such as, egg production, reproduction and genetic resistance to some infectious diseases.

MATERIAL AND METHODS

The present study was carried out in a small holder Farm at El-Sharkia Governorate, Egypt.

Hen: Seventy purebred local breed hens during their first laying season (approximately 6 months old and 800-1000 g body weight) were nested at the natural prevailing conditions. Hens were fed and watered *ad libitum* on a commercial ration birds were subjected to vaccinal programe available by the General Egyptian Organization for Veterinary Services.

Design:

- Eggs were collected for analyzing of egg proteins (egg albumin and egg yolk).
- Blood samples were taken from the heart after starvation for 12 hours by syringes. Serum samples were obtained by centrifugation (x 1300/15 minutes /4°C) and kept at - 20°C till electrophoretic analysis.
- Data were computed and statistically analyzed using student and Chi square [17].
- Electrophoretic patterns of serum and egg proteins were performed using polyacrylamide gel electrophoresis (PAGE), [18].
- Gene frequencies of different protein loci were determined by Hardi-Waiberg formula:

$$P^2+2pq+q^2=1$$

whereas

P = Frequency of homozygotic genotype A A

q = Frequency of homozygotic genotype BB

Chi-square ($x2$) analysis was carried out to determine the gene drift.

The theoretical and expected numbers of genotypes were determined as follows:

$$N\,(PA)2 + N2PAqB + N\,(qB)\,2 = 1$$

whereas

PA = Frequency of allele A

qB = Frequency of allele B [19]

RESULTS AND DISCUSSION

Poultry products (meat and eggs) are a major source of animal protein on which the world is increasingly reliant to feed a rapidly growing population. Improved breeds and advances in farm management practices have had a large impact on the poultry industry [20].

It is experimentally proved that all traits of production, reproduction and genetic diseases are controlled by the biochemical activities in the body of the individuals and these are accomplished by the several types of proteins such as serum protein, enzyme, hormone etc. These different proteins show their effects in different ways. Anabolic, catabolic, activating the substrate etc. which controls the growth, production and reproduction of the Individual [15].

Plasma proteins have multiple physiological functions and reflect performance status and health in chickens, e.g. total protein is an indicator of growth and nutritional status [21]. Albumin, the main protein in plasma, has a good binding capacity for water, Ca2+, Na+, K+, fatty acids, hormones, bilirubin and drugs. Its main function is in the regulation of the colloidal osmotic pressure of blood [22]. Globulins are produced by the liver and the immune system. Albumin makes up more than half of the total protein within the blood and globulins make up the remainder [23]. Globulins serve as antibodies and transport substances, transport various substances in the blood and are involved in primary defense mechanisms [24].

In the present study, 5 blood serum proteins (albumin-transferrin- pos-transferrin- esterase and cholinesterase) and 6 egg proteins (ova albumin- ova transferrin- globulin2- globulin3-ova macroglobulin and conalbumin) were investigated. results revealed that the most predominant serum protein loci were Alb^A 0.693, Tf^B 0.707, Ptf^F 0.757 and $Es\text{-}5^B$ 0.721. Moreover, all of these genetic loci were polymorphic as the frequency of each allele ‹ 0.95 [25]. This finding agree with those reported by Mahfudz *et al*. [12], Johari *et al*. [14] and Sabra *et al*. [16], specially for Alb^A, Tf^B and $Es\text{-}5^B$ alleles.

Table 1: Genotyping and gene frequencies of some blood serum proteins of local Egyptian hens (No. 70)

Blood protein loci	Genotyping: Observed and expected no. of genotypes			$\times^2$	Gene frequency
Albumin					
Alb	AA	35	(33.6)		$Alb^A = 0.693$
	AC	27	(27.9)		$Alb^B = 0.307$
	CC	8	(6.6)	0.6	
Transferrin					
Tf	BB	42	(34.9)		$Tf^B = 0.707$
	BC	15	(29.0)		$Tf^C = 0.293$
	CC	13	(6.1)	16.2	
Post-transferrin					
Ptf	FF	48	(40.1)		$Ptf^F = 0.757$
	FS	10	(25.7)		$Ptf^S = 0.243$
	SS	12	(4.1)	26.5	
Esterase					
Es-2	$Es\text{-}2^{AA}$	28	(19.5)		$Es\text{-}2^A = 0.528$
	$Es\text{-}2^{Aa}$	18	(34.8)		$Es\text{-}2^a = 0.471$
	$Es\text{-}2^{aa}$	24	(15.5)	16.4	
Cholinesterase					
Es-5	AA	14	(5.4)		$Es\text{-}5^A = 0.278$
	AB	11	(28.1)		$Es\text{-}5^B = 0.721$
	BB	45	(36.4)	26.13	

Table 2: Genotyping and gene frequencies of some egg proteins of local Egyptian hens (no. 50)

	Genotyping: Observed and expected no. of genotypes				
Egg protein loci		Obs.	Expec.	$\times^2$	Gene frequencies
Ova- albumin					
OV	AA	39	(34.4)		$OV^A = 0.830$
	AB	5	(14.1)		$OV^B = 0.170$
	BB	6	(1.4)	21.5	
Ova-transferrin					
Tf^{BW}	BW/BW	10	(3.1)		$Tf^{BW} = 0.250$
	BW/A	5	(18.7)		$Tf^A = 0.750$
	A/A	35	(28.1)	27.03	
Globulin					
G3	LL	18	(15.68)		$G3^L = 0.560$
	LA	20	(24.64)		$G3^A = 0.440$
	AA	12	(9.68)	1.76	
Globulin					
G2	AA	3	(1.2)		$G2^A = 0.160$
	AB	10	(13.4)		$G2^B = 0.840$
	BB	37	(35.3)	3.6	
Ova-macroglobulin					
Omg	AA	8	(9.24)		$Omg^A = 0.430$
	AB	27	(24.51)		$Omg^B = 0.570$
	BB	15	(16.24)	1.5	
Con- albumin					
Pr	BB	10	(4.5)		$Pr^B = 0.300$
	BD	10	(21.0)		$Pr^D = 0.700$
	DD	30	(24.5)	13.6	

Concerning of homozygotic and heterozygotic genotypes of studied loci, the present results revealed a high frequent of homozygotic genotypes and this result is not agree with the finding recorded by Badawy *et al*. [26] who reported that the Dandarawi Egyptian native chicken characterized by heterozygote genotype Cr for crest gene which improve the abnormality and viability of spermatozoa, as well as, increase egg production. The condition may be due to genetic diversity among chicken populations.

Concerning serum esterase loci, results of present study showed an almost genetic equilibrium of the gene frequency of both alleles (Es-2^{A} and $Es2^{a}$) and the condition is attributed to predominated of the heterozygote genotype.

Concerning egg protein loci, the current results revealed high frequency of OV-Al^{A} (0.830), OV-Tf^{A} (0.750),$G2^{B}$(0.840) and Pr^{D}(0.700), these results compatible with those reported by Obeidah *et al.* [27] and Mahfudz *et al*. [12] who found an association between body weight, egg weight and ovalbumin genotype AA. They also, reported that the hens with G2 globulin in heterozygote form laid more egg than either homozygotes and this result was not similar to our finding, whereas the allele $G2^{B}$ in the present study showed the highest frequency among studied loci in this respect, Mahfudz *et al.* [12] studied the genetic variation through polymorphism of blood and egg proteins in three kinds of Kedu chickens at laying period and they reported that blood and egg have different potential of protein content, but both of them can be used to study the genetic variation.

The comparison between the protein fractions of serum and egg in present study showed priority of egg white (albumin = 0.830 and transferrin = 0.750) and this result confirm those reported by Barker, Ann *et al.* [28] who reported that the useful protein polymorphisms have been found in chicken egg white than in serum, egg yolk, or erythrocytes. Furthermore, the genetic polymorphism of transferrin in serum and egg yolk is paralleled by the polymorphism of conalbumin in egg white.

Mapping the quantitative trait loci (QTL) associated with individual metabolites has been combined with economic traits for investigating the genetic basis of variation and co-variation in metabolites. In this respect Shigang *et al*. [29] suggested that the SNP on *GGA9* were identified by *CMLM* is associated with Alb concentration. Pundir *et al*. [30] reported that the *PPP4R1* gene, identified as a regulator of protein phosphatase enzyme, plays a role in cell division and this gene (*PPP4R*1 gene) is located within a QTL that controls egg albumin height. Furthermore, they added that the gene *KATNAL1*, that is located on *GGA1*, encodes a protein called Katanin, which involved in the ATPase activity and is essential for spermatogenesis. In addition, *PLOD2* gene was located within two QTLs that control chicken albumen height [31]. On the other hand, Esmailnjada and Nikbaknt [32] reported that the *IGFI B/B* genotype is associated with higher egg weight in the Iranian native population, as well as associated with a higher expression in ovarian follicles in Korean native chickens. So, B/B genotype could be a regulatory factor of follicular development and egg production.

Mortality due to diseases is responsible for 10 to 20% of the gross production value in the poultry industry and likely higher in the developing countries [2].

The close links between the triad of animal disease, food security and public health is more and more realized. Animal diseases affect production and productivity of animals. Additionally, some animal diseases transmissible to man (zoonosis) also affect public and human health as evidenced by outbreak of emerging diseases such as different strains of avian influenza [33]. To overcome these challenges, the internal genetic merit about maintaining health is therefore highly required and more attention is urged to be paid by poultry breeding companies to enhancing general health or disease resistance and welfare of the laying hens, next to the production traits. In this respect, the involvement of immune components has been established in a variety of metabolic and behavioral disorders [34, 35]. The adaptive immune response to some specific pathogens or vaccines has been considered in selection for disease resistance and survival [36].

The impact of genetic resistance/ tolerance to pathogens is high when all levels of genetic resistance/ tolerance acts synergistically [37]. The *KATNAL1* and *STARD13* genes were located near two co-localized QTL for spleen and caecal bacterial burden after challenge with Salmonella enteritidis [38]. In this respect, Shabolina and Iotova [39] found an additional inheriting alyestrase fraction Al appears in blood serum of resistant chickens after contamination with Salmonella. Furthermore, Most chicken with resistant to salmonella, have heterozygote genotype of cholinesterase (Es-5).

In addition, *MAN2A1* was located within two QTL for Marek's disease in chickens, which showed these regions were important related to immune defense against

pathogens [40]**.** For instance, the fayoumi chicken is renowned to have resistance against infectious diseases. A recent study has identified differential gene expression patterns to AIV infection in two distinct genetic lines of chicken species; the fayoumi and Leghorn chickens. Further investigations on these differentially expressed genes and introduction of those salient genomic variations to the chicken genome using gene editing technology can provide new insights into the production of disease-resistant chickens [41]. In this regard, chicken hyperimmune egg yolk IgY antibodies offer a practical alternative to mammalian serum antibodies because of their feasibility for large-scale commercial production and the relative noninvasive methods used for their preparation [42].

In conclusion the internal genetic merit about maintaining health is therefore highly required and we must rely on modern genetic applications for genetic improvement and increase production as well as the strengthening of the disease- resistant genetic component in local poultry breeds.

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