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Relationship Among The Igy Concentrations In Layers Blood Serum, Egg Yolk And Chicks Blood Serum In Two Improved Local Strains Of Chickens.

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***Abstract**: At 18 weeks of age,96 pullets from two local strains, Silver Montazah (SM) and Sinai chickens were taken at random to be used in the present experiment. The present study was carried out at the Department of Poultry and Fish Production, Faculty of Agriculture at Shibin El-Kom, Menoufia University. The aim of the present study was to investigate the simple correlations amongthe IgY concentrations in layers blood serum, egg yolk andchicks blood serum.*

***Key words:** IgY concentrations, hen blood serum, egg yolk , chicks blood serum, chickens.*

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The results were summarized as follows:

1. There were strong positive highly statistical significant simple correlation coefficients between IgY concentration in the layers (X1) and egg yolk (X2), between the layers (X1) and chicks (X3), and between the egg yolk (X2) and chicks (X3) in both Sinai and Silver MontazahStrains of chickens.
2. Sinai strain had higher strong positive significant correlations than Silver Montazah Strain of chickens. The values of r_{x1x2}=0.994 vs 0.934, r_{x1x3} = 0.937 vs 0.879, and r_{x2x3} = 0.930 vs 0.908 among IgY concentrations in layers blood serum (X1), egg yolk (X2) and newly hatched chicks (X3) of Sinai and Silver Montazah Strain of chickens, respectively.
3. Layers with higher IgY level may in turn lay eggs with higher IgY levels, which suggests that hens of good immune state were able to produce more immune response which were able to allocate more immune defense ability to eggs and their offspring.
4. The obtained highly significant positive correlations among these three traits, may emphasize the possibility of selection the parents of the next generation either from the IgY concentration in layers, chicks or egg yolk.

I. Introduction

In birds, maternal factors are mainly transferred via the egg to the chick **(Grindstaff*et. al*., 2003),**hence the time period for the uptake of maternal factors is restricted to a period before, and shortly after hatch. While, in mammals, maternal factors can be transferred via the placenta, in colostrums (the first milk transferred from the mother to her offspring just after birth) and in normal milk during lactation (**Glezen 2003 and Lemke *et. al., 2004).***

Among the maternal factors which can be transferred by the mother to her offspring are hormones, antibodies and nutrients **(Rubolini*et. al.*, 2006 and Biard*et. al*., 2007)**. In birds, maternal transfer of antibody via egg yolk to offspring has mainly been studied in poultry as means of improving chick survival early in life **(Blount *et. al.*, 2002 and Biard*et. al*., 2007).**

Early reports about the concentration of antibody classes in birds indicated that IgY makes up about 75% of the total immunoglobulin pool**(Rose *et.al*.,1974**) . Recently, **Ritu, *et al*., (2016),** compared the immunoglobulin Y (IgY) level in laying hens of four different breeds of local chickens. They reported that non-significant differences in IgY concentration were recorded among different laying breeds of chickens. The IgY concentration ranged from 3.35 to 5.83, 2.30 to 2.60 and 1.30 to 1.70 mg/ml in hens, egg yolk and chicks, respectively.In addition, there were significant differences among genetic lines or breeds. For example, the IgY concentrations reported were 2.2mg/ml in Single Comb White Leghorn, 2.0 mg/ml in line SLU-1329, and 1.7 mg/ml in Rhode Island Red **(Carlander, 2002).**

Also, **Hamal *et. al.*, (2006)** studied the concentration of IgY in the dam's plasma, and egg yolk in two meat lines of chickens. They found that the IgY concentration in dams plasma was 3.26 mg/ml, while it was 1.15 mg/ml in egg yolk with IgY in egg yolk total 22.5 (mg) in the first line. The corresponding concentrations

in the second meat line were 6.02 mg/mlin dams and 2.26 mg/ml in egg yolk with total egg yolk of IgY 43.9 (mg).

However, the total IgY concentration is estimated to be 100-200 mg per egg **(Woolley and London, 1995)**. Also, **Larrson*et. al.*, (1993)** found that IgY is more highly concentrated in egg yolk than it is in serum. It was reported that the average volume of egg yolk (15 ml) contains 50 to 100 mg of IgY (Michael et al., 2010).

On the other hand, **Sun *et. al*., (2013)** studied the correlation between the levels of total serum IgY and egg yolk IgY in three different breeds (White Leghorn, Silkie and Dongxiang blue-shell) using indirect ELISA method. They found that there was a significant positive correlation between the levels of total serum IgY and total yolk IgY in all three breeds (White Leghorn, r = 0.404, $p \leq 0.001$, Silkie breed, r = 0.561, $p \leq 0.001$, and Dingxiang blue-shell, r = 0.619, $p \leq 0.001$). They also found significantly positive correlation between the total serum IgY levels in the 3-day old offspring hatched from the silkie hens with total hen IgY serum (r = 0.535, $p \leq 0.001$, and the yolk IgY levels (r=0.481, $p \leq 0.001$). They indicate that hens with higher IgY levels may in turn lay egg with higher IgY levels which suggests that hens of good immune state were able to allocate more immune defense ability to egg and their offspring.

Recently, **Gebriel*et.al.* (2018)** studied the concentrations of maternal antibody (IgY) in blood serum of layers and hatched chicks in addition to egg yolk of control, high and low IgYthreeantibody lines of Sinai and Silver Montazah strains of chickens as a genetic marker to improve fertility ,hatchability and livability percentages . They found that the high Igy antibody was significantly associated with these traits,which improved the percentages of them. So that, the Igy antibody can be used as a genetic marker to improve some productive traits in chickens.

II. Materials And Methods

The present study was carried out at the Department of Poultry and Fish Production, Faculty of Agriculture, Shibin El-Kom, Menoufia University andPoultry Research Station at Gimmizah, Institute of Animal Production and Agricultural Research Center, Ministry of Agriculture,in order to determine the relationships among the IgY concentration in layers blood serum, egg yolk and chicks blood serum in two improved local strains of chickens.

1. Chicken stock :

Two local strains of chickens were used in the present study in the Poultry Research Station at Gimmizah, Institute of Animal Production and Agricultural Research Center, Ministry of Agriculture.The strains of chickens used in the present study were:

1.1. Silver Montazah strain:

Silver Montazah strain is a synthetic local strain of chickens, which developed at the Ministry of Agriculture, Montazah Poultry Research Station. The formation of Silver Montozah strain started in early 1970 **(Mahmoud *et al.*, 1974).** The scheme of formation of Silver Montazah strain including two way cross between RIR × Dokki4, random mating and selection programs to select and develop the SilverMontazah strain as egg production strain. The plumage color is colonal white. The ear lobes color is red.

1.2. Sinai (Bedwin fowl):

Sinai chickens were originally obtained from the desert areas of North and West Sinai Governorates. The Sinai breed probably is originated from the natural cross between some foreign breeds with the local chickens reared in Sinai Governorate since War 1945.

2. Experimental Design:

At 18 weeks of age, 96 pullets from two local strains, Silver Montazah (SM) and Sinai chickens were taken at random to be used in the present experiment. The pullets were housed individually in wire individual cages in the Poultry Research Station at Gimmizah Pullets were hatched on the same day. They were reared under the same management practices, and they were immunized using the same vaccination protocols. Eggs were collected and recoded individually for each hen.

At sexual maturity, 4 hens were assigned at random for each sire which formed 12 families of each strain of chickens for reproducing the next generation. Each family contained one sire and 4 hens in both strains.

At 33 weeks of age, 42 eggs were taken at random as one egg from each hen from 21 hens of each strain, which represented 7 families of each strain. Fresh collected eggs were used to determine the concentration of yolk IgY, which transferred from the dam to egg yolk.At the same time, chick blood samples were taken from 80 chicks representing 80 dams (40 dams of each strain) at 4^{th} day of hatch for determination of IgY concentration in chicks blood serum. At the same time, 80 blood samples were collected at 34 weeks of age from 40 hens of each strain of chickens which used for determination of IgY concentration in hens blood serum.

To study the effect of IgY concentration on some economic traits, dams and chickens were divided into 3 lines on the bases of the IgY concentration blood serum (mg/ml) of each hen as the following:

2.1. High antibody (IgY) line (HL):

Hens of each strain reached IgY concentration more than $\bar{X} + S.Ex$ were selected and considered as the parents of HL to produce the offspring of the next generation.

2.2. Low antibody (IgY) line (LL):

Hens of each strain had IgY concentration lower than $\bar{X} - S.E$were selected and considered as the parents of LL to produce the offspring of the next generation.

2.3. Control line (CL):

Hens of CL were taken at random to form the control line of each strain to produce the offspring of the CL in the next generation. The unselected individuals were culled.

3. Mating system and reproduction :

The artificial insemination was used as a mating system for reproducing the next generation. Each family contains one sire and 4 dams. Dams were assigned at random to each sire for reproducing the next generation.Fertile eggs were collected two times a day and numbered according to their dams. Cracked, dirty, and misshapen eggs were removed. Then, fertile eggs were stored in egg storage room at 15 – 17 °C for 7 days, with 70% of relative humidity.

For hatching, all eggs were moved to the incubation room and left for at lest 12 hours at room temperature. Then, all eggs were set with wide end up in the setting trays according to their dams and incubated in a forced draft incubator at 99.5 °F (37.5 °C) with a relative humidity of 65%. Eggs were turned every two hours from the 2nd to 18th day of incubation. All fertile eggs were transferred to a separate hatcher in pedigree baskets according to their dams at 98.5 °F (36.9 °C) and 75% relative humidity.

4. Experimental stock management:

At hatching day, all chicks were removed from the hatcher, wing banded according to their dams for identification. All chicks were brooded in floor brooder with wood shaving litter. The starting brooder temperature was 34 °C during the first week, then the brooder temperature was decreased gradually from 2- 3 °C every week to reach 20- 22 °C at almost 42 days of age. The chicks were moved to rearing house at eight weeks of age.

All chicks were exposed to continuous artificial light for 24 hours during the first week of age, then; the artificial light was decreased gradually to reach the natural day light by about 8 weeks of age. All chickens were received only natural day light from 9 to 17 weeks of age. At 18 weeks of age, pullets were moved to individual cages in laying house, where they were kept until 42 weeks of age under 16 house light a day.

All chicks were fed *ad libitum* during brooding , rearing and growing periods on a diet containing 19.0%, 17.0% and 15.0% crude protein, and 2860, 2850 and 2850 Kcal ME/Kg diet, respectively. At 17 weeks of age (before sexual maturity), pullets were fed on a diet containing 17.0% crude protein and 2850 Kcal ME/Kg died. At 5.0% egg production, hens were fed on diet containing 16% crude protein and 2750 Kcal ME/Kg diet until 42 weeks of age. Then, hens were fed on diet containing 15% crude protein and 2750 Kcal ME/Kg a diet to the end of productive year. All chicks were vaccinated against diseases and were treated similarly throughout the experimental period.

5. Collection of hen blood samples:

At 34 weeks of age, 2 ml blood sample was collected in dry tube via the wing vein and one fresh laid egg from each hen were collected on week before bleeding for IgY determinations. The blood samples were centrifuged at 3.000 rpm for 10 min at 4 °C. The liquid that remained after blood had clotted was collected, placed in disposable tubes and frozen for subsequent laboratory analysis **(Siegel and Gross, 1980).**

5.1. Collection of chick blood samples:

Chicks were bled via the jugular vein at 4th day of hatch using a 0.5 ml heparinized insulin syringe with a 28-gauge needle.Plasma samples were collected and stored at –20°C until analysis **(Carlander, 2002**).

6. Extraction of egg yolk IgY:

In recent years, Polsons PEG (Polyethylene glycodal) precipitation method has become the most commonly used and most effective procedure **(Polson, 1990**). This method is used for extraction of egg yolk IgY. the volume of egg yolk (ml) was recorded during the extraction method to be used for calculating the IgY concentration in each egg yolk using the following formula:

IgY concentration in whole egg yolk (mg/egg)= egg yolk volume x IgY concentration in yolk.

The levels of the total IgY in the dams blood serum, egg yolk and chicks blood serum were determined in Lab Top in Zagazig City, Sharkia Governorate, using AMS Sat 450 system which imported from England using appropriate kits.

7. Studied traits:

7.1. The IgY concentrations in blood samples and egg yolk :

The concentrations of IgY were determined in both blood samples of hens at 34 weeks of age and chicks at 4^{th} day of hatch as mg/ml. Also, the IgY was determined in fresh egg yolk as mg/ml.

7.2. Simple correlation coefficients:

Simple correlation coefficients among IgY concentration in hen blood serum, egg yolk and chick blood serum were determined **(Snedecor and Cochran,1986)..**

8. Statistical analysis:

Least square means and their standard errors ($\bar{\times} \pm S.E$) for each studied trait were calculated for each line (HL,LL and CL) within each its strain. Data obtained were statistically analyzed using **SPSS (2004).** Probability values $\leq$ 5% were considered for significance.Duncans multiple range test was used for the multiple comparisons of means **(Duncan, 1955).**

The statistical model used in the present study was as follows:

$Yijk = U + Si + Lj + (S \times L)ij + eijk$

Where :

Yijk = The Ith observation of the individual over all means.

U = The common mean .

Si = The fixed effect of ith strains.

Lj = The fixed effect of jth lines.

(S × L)ij = The fixed effect of interaction between strains and lines.

eijk = experimental error .

III. Results And Discussion

1. Transfer analysis of the IgY levels from hens to egg yolk and then to chicks:

The transfer of IgY from hens to their newly hatched chicks is important to provide specific pathogens protection during the early post-hatched period. The effects of chicken strains and chicken lines on transfer percentages of IgY levels from laying hens to egg yolk and then to newly hatched chicks were studied. In addition, the correlation coefficients among IgY transferred from laying hens to their egg yolk and chicks were calculated.

1.1. Effect of chicken strains on IgY antibody transferred:

The effects of chicken strains on IgY transferred (%) form dams to egg yolk and chicks in two strains of chickens are given in Table (1) and Fig. (1). The average percentages of IgY transferred in both Silver Montazah and Sinai Strains were 81.79 vs 87.82% from laying hens to egg yolk, 76.88 vs 75.43(%) from egg yolk to chicks, and 62.88 vs 66.24 (%) from laying hens to chicks in both Silver Montazah and Sinai strains of chickens, respectively.

It is clear that Sinai strain had significantly higher IgY transferred (%) from laying hens to egg yolk and to newly hatched chicks than Silver Montazah strain of chickens. Where, the transferred IgY (%) from egg yolk to chicks was almost similar in both strans (Table 1) .

The present results were in good agreement with the finding of **Hamal *et. al.,* (2006)**. They reported that the transfer percentage of IgY from breeding layers to egg yolk and then to hatched chicks is depending on the amount of IgY concentrations in breeding layers. High IgY concentration in layers blood serum lead to high percentage of IgY transferred to egg yolk and then to chicks.

Also, similar results were recently reperted by **Ritu*et al.,* (2016)**, who studied the transfer of IgY from the parent layers to egg yolk and then to chicks. They found statistical significant differences among four local breeds of chickens. The transfer percentages were in range of 66.39 $\pm$ 1.42 to 74.92 $\pm$ 5.30 (%) from parent layers to egg yolk, 54.99 $\pm$ 1.93 to 66.52 $\pm$ 1.99 (%) from egg yolk to chicks and 25.62 $\pm$ 1.42 to 36.06 $\pm$ 4.34 (%) from parent layers to chicks.

On the other hand, the transferred of IgY from parent layers to egg yolk and then to hatched chicks are significantly affected by some factors. **Heller *et0 al.,* (1990) and Lemk*et. al.,* (2004)** reported that the diversity and amount of IgY transferred from layers to offspring are a reflection of the local disease environment. Also, **Hasselquist and Nilsson (2008)** reported that, parent layers tend to increase their immune response when food availability increases, which will also deposit higher levels of IgY in their eggs.

1.2. Effect of chicken lines on IgY antibody transfer:

The effects of chicken lines on IgY transferred from dams to egg yolk and chicks of control (CL) and selected lines for high (HL) and low (LL) IgY concentrations in two local strains of chickens are given in Table (2) and Fig. (2). The percentages of transfer of total IgY from the dams to their chicks were 61.89 vs 67.21 (%) in control lines, from 71.67 vs 76.47 (%) in high lines, and from 52.06 vs 58.17 (%) in low lines of both local chickens, Sinai and Silver Montazah strains, respectively.

In addition, the high lines of both chicken strains had the highest percentages of IgY transferred from the dams to their egg yolk and chicks, where the low lines had the lowest percentages of IgY transferred. The control lines occupied intermediate transferred IgY percentages.

Also, the data explained that statistical significant differences among lines were observed and the lines of Sinai strains had higher IgY transferred from the dams to egg yolk and then to their chicks than Silver Montazah strain.

The present results concluded that the percentages of IgY transferred from the dam to egg yolk then to newly hatched chicks were directly related to the concentration of the IgY in the dam. Also, the high lines had the highest percentages of IgY transferred from the dams to egg yolk and then to newly hatched chicks, where the low lines had the lowest percentages. In addition, the Sinai strain had significantly higher transferred percentages of IgY than Silver Montazah strain of chickens.

The present results were in agreement with the results of **Hamal *et. al*., (2006)**. They reported that the transfer percentage of IgY from the dams to their egg yolk and then to newly hatched chicks is depending on the amount of IgY concentration in layers. High IgY concentration in layers blood serum lead to high percentage of IgY transfer to egg yolk and then to newly hatched chicks.

However, similar results were recently reported by **Ritu*et al*., (2016)**, who studied the transferred of IgY from the layers to egg yolk, and to then to newly chicks. They found statistical differences among four local breeds of chickens.

2. Simple correlation coefficients among the IgY concentrations in layers blood serum, egg yolk and chicks:

The correlation coefficients of IgY transferred from the layers to egg yolk and then to newly hatched chicks were calculated. A total of 42 layers with their eggs (42 eggs) and their newly hatched chicks (42 chicks) of both local strains of chickens were taken at random to calculate the simple correlation coefficients among the IgY concentration in layers blood serum, egg yolk and newly hatched chicks (Table 3) and Fig. (3 and 4).

The present results explained that there were strong positive highly statistical significant simple correlation coefficients between IgY concentration in the layers (X_1) and egg yolk (X_2), between the layers (X_1) and chicks (X_3), and between the egg yolk (X_2) and chicks (X_3) (Table 3) and Fig. (3 and 4), in both Sinai and Silver Montazah strains of chickens.

The results explained also that Sinai strain had higher strong positive significant correlations than Silver Montazah Strains of chickens. The values of $r\ x_1\ x_2 = 0.994$ vs 0.934, $r\ x_1\ x_3 = 0.937$ vs 0.879,and $r\ x_2\ x_3 = 0.930$ vs 0.908 among IgY concentration in layers blood serum (X_1), egg yolk (X_2) and newly hatched chicks (X_3) of Sinai and Silver Montazah strains of chickens, respectively.

The present results are in good agreement with the findings of **Hamal *et al*., (2006)**. They measured the IgY levels in dams blood serum, egg yolk and newly hatched chicks in two meat lines of chickens. They also concluded that the IgY levels in dams blood serum, egg yolk and newly hatched chickens had direct indicator of maternal antibody (IgY) transferred to the circulation of the chicks, with an expected percentages transfer of approximately 30%.

Similar results were reported by **Sun *et al*., (2013)** , who studied the correlation between the levels of total serum IgY and egg yolk in three different breeds. They found that there were significantly positive correlations between the levels of total serum IgY and total yolk IgY in all three breeds (White Leghorn, $r = 0.404$, $P \leq 0.001$, Silki breed, $r = 0.561$, $P \leq 0.001$ and Dongxiang blue-shell, $r = 0.619$, $P \leq 0.001$). They also found significantly positive correlation between the serum IgY levels in the 3-day hatched chicks with the IgY levels of hen serum.

The present results showed that the layers with higher IgY levels may in turn lay eggs with higher IgY levels which suggests that hens of good immune state were able to allocate more immune defense ability to eggs and their offspring.

Table (1): Effect of chicken strains on IgY transferred (%) from dams to egg yolk, and chicks in two local strains of chickens.

Traits	No	IgY level (mg/ml)	Transferred (%)
Silver Montazah strain			
Dams to egg yolk	**21**	**4.23 ± 0.632**	**81.79**

Egg yolk to chicks	**21**	**3.46 ± 0.501**	**76.88**
Dams to chicks	**21**	**2.66 ± 0.311**	**62.88**
Sinai strain			
Dams to egg yolk	**21**	**4.68 ± 0.739**	**87.82**
Egg yolk to chicks	**21**	**4.11 ± 0.671**	**75.43**
Dams to chicks	**21**	**3.10 ± 0.338**	**66.24**

Table (2): Effect of control and selected lines for high (HL) and low (LL) IgY levels on IgY transferred (%) from dams to egg yolk and chicks in two local strains of chickens.

Line	**Transfer (%)***		
	1	**2**	**3**
Silver Montazah Strain			
Control line	**82.11 b**	**76.68 b**	**61.89 b**
High IgY line	**90.54 a**	**85.91 a**	**71.67 a**
Low IgY line	**71.14 c**	**66.51 c**	**52.06 c**
Sinai strain			
Control line	**88.14 b**	**81.96 b**	**67.21 b**
High IgY line	**97.19 a**	**89.67 a**	**76.47 a**
Low IgY line	**79.23 c**	**72.48 c**	**58.17 c**

* Transfer (1) = IgY transfer from dams to egg yolk.
* Transfer (2) = IgY transfer from egg yolk to chicks..
* Transfer (3) = IgY transfer from dams to chicks.

Table (3): Correlation coefficients of IgY levels among hen blood serum (X_1), egg yolk (X_2) and chicks blood serum (X_3) in two local strains of chickens.

Source of Variation	**No**	**Silver Montazah**	**Sinai**
X_1X_2	**21**	**r = 0.934****	**r = 0.994****
X_1X_3	**21**	**r = 0.879****	**r = 0.937****
X_2X_3	**21**	**r = 0.908****	**r = 0.930****

$P < 0.001$

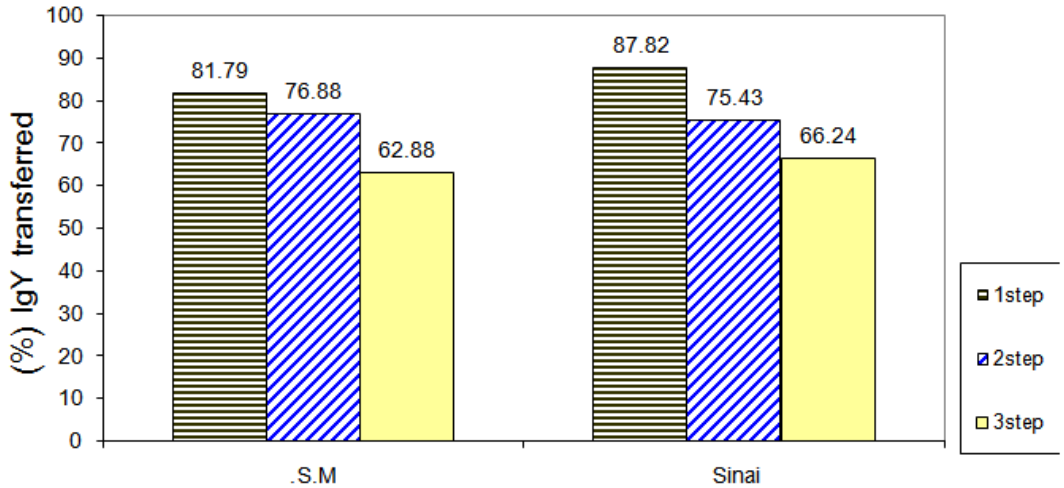

Fig. (1): Effect of chicken strains, Silver Montazah (S.M.) and Sinai on IgY transferred (%) from dams to egg yolk (step 1), egg yolk to chicks (step 2) and dams to chicks (step 3) of two local strains of chickens.

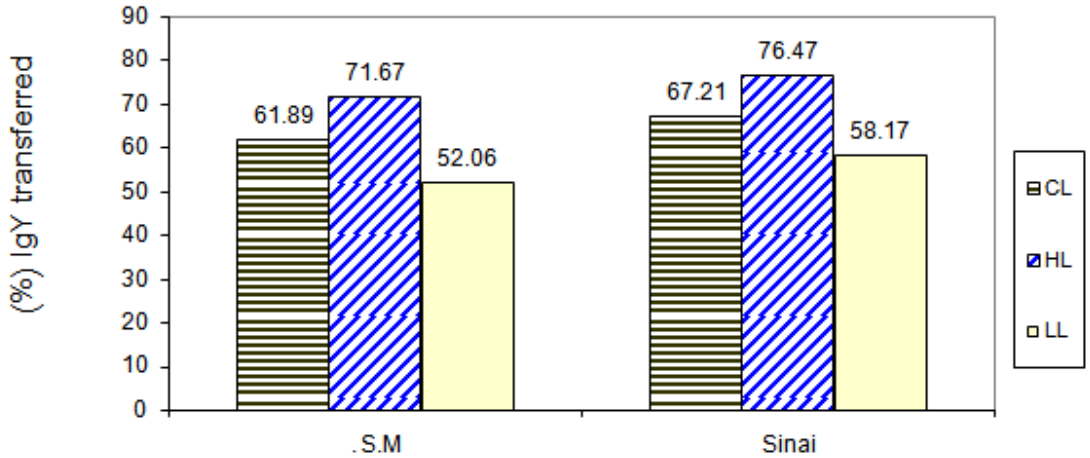

Fig. (2): Effect of control (CL) and selected lines for high (HL) and low (LL) IgY levels on IgY transferred (%) from dams to their newly hatched chicks in two local strains of chickens.

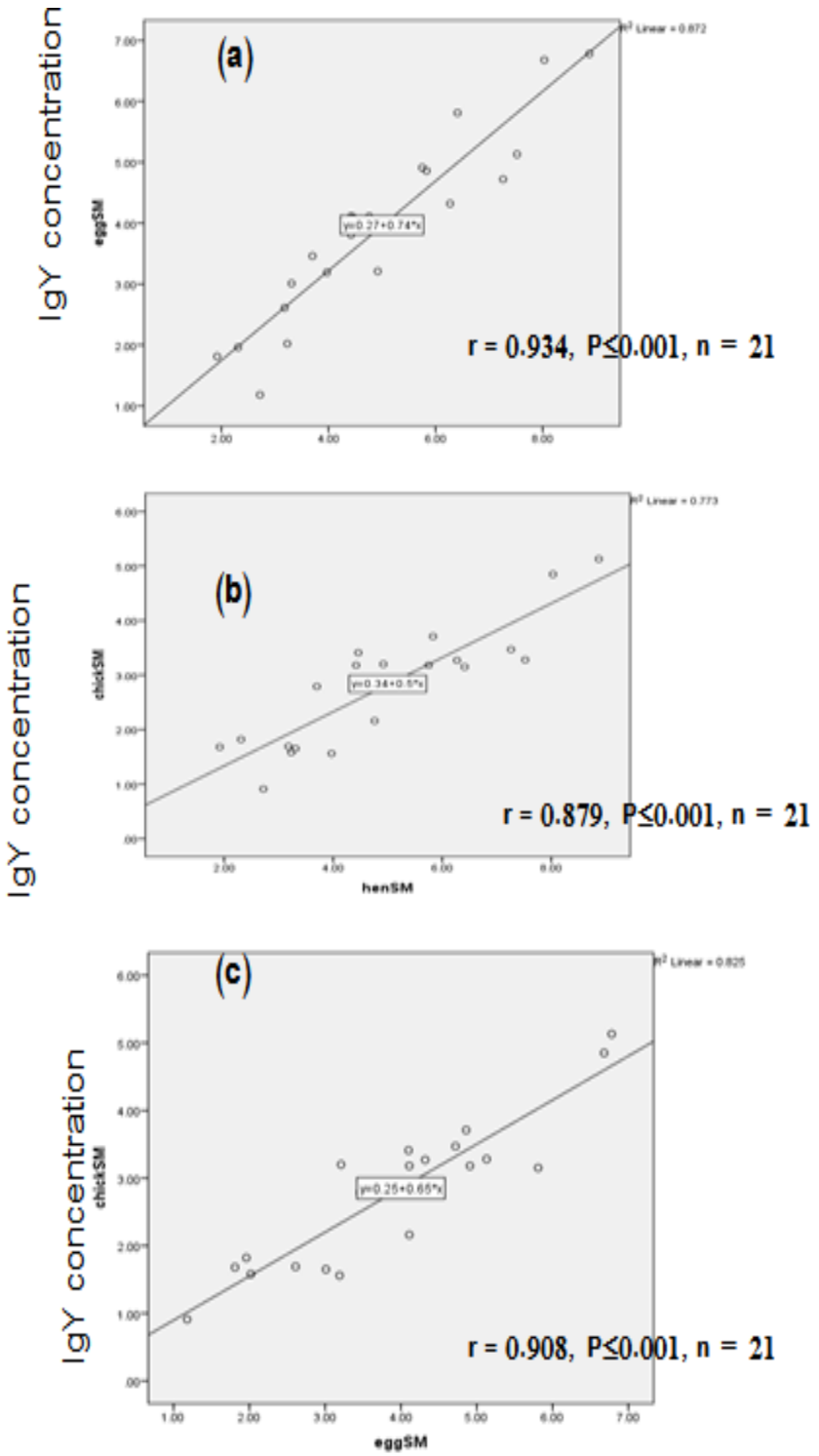

Fig. (3): Correlation coefficients of IgY levels among hen and egg yolk IgY levels (a), hen and chicks IgY concentration (b) and egg yolk and chicks IgY levels (c) in Silver Montazah chickens.

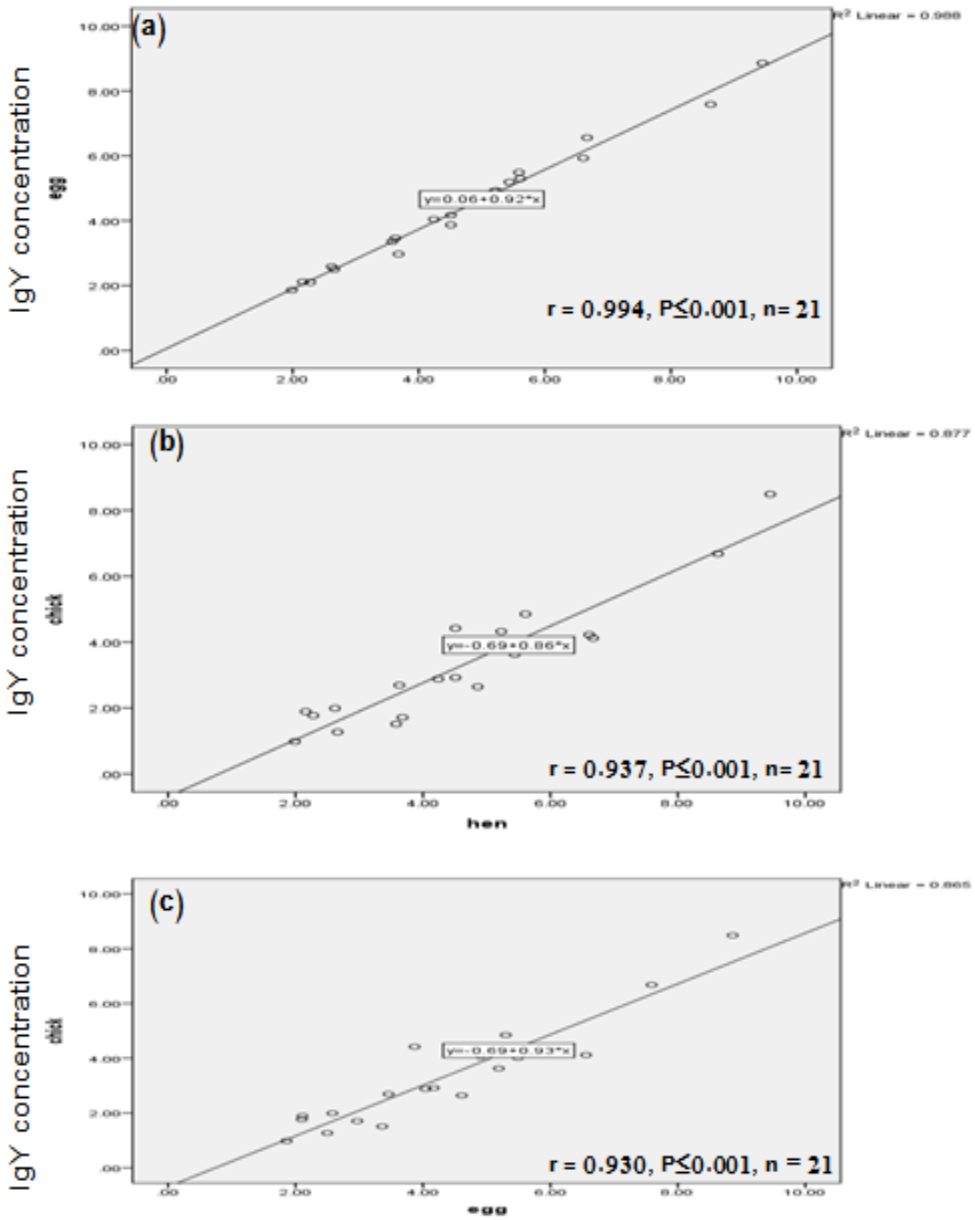

Fig. (4): Correlation coefficients among hen and egg yolk IgY concentration (a), hen and chicks IgY levels (b) and egg yolk and chicks IgY level (c) in Sinai chickens.

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