



**Effect of Breed and Management System on the Production
and anti-bacterial Effect of IgY**

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Abstract

The high concentration of immunoglobulin yolk (IgY) found in the egg yolk is the major antibody produced by chickens. IgY have been applied successfully for scientific, diagnostic purposes and for alternative of mammalian antibodies. The effect of poultry breed (Hisex, Sudanese Baladi) and management system (Semi-closed and Traditional) on the production and antibacterial effect of IgY was investigated. The IgY concentration was determined by modification of polyethylene glycol method (PEG). The result reveal that, the egg weight (g) and IgY (mg/ml- yolk) were significantly higher in Hisex than Sudanese Baladi. While the yolk volume (ml) and IgY (mg/g-egg) were significantly lower in Hisex compared to Baladi. The result also revealed that the bird kept under Semi close produces significantly heavier egg compared to bird kept under Traditional system but significantly lower on IgY (mg/g-egg). IgY have antibacterial effect against bacteria (*E. coli*, *Staphylococcus* and *Pseudomonas*). Irrespective of the breed and management system IgY showed antibacterial (*E. coli*, *Staphylococcus* and *Pseudomonas*) effect.

Keywords: Semi close system, Traditional system, *E. coli*, *Staphylococcus*, *Pseudomonas*

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Introduction

Avian eggs are the largest source of major nutrient which consist of various type of proteins, fats, vitamins, growth factors and minerals that are required for the developing embryo and also significant number of defense factors for protecting against many viral and bacterial infection. Chicken egg yolk contain antibodies called immunoglobulin Y. IgY which is the major antibody present in birds, playing similar role like mammalian antibody (IgG) (Kowalczyk, *et al.*,1985). There is increasing

interest in the use of chicken egg yolk for polyclonal antibody production due to economical reasons and have been applied successfully for scientific, diagnostic, prophylactic, therapeutic purposes and veterinarian therapy against bacteria. (Gassmann *et al.* 1990., Bellon *et al.*1996., Schade *et al.* 1997., Sarker *et al.* 2001., Tini *et al.* 2002 and Amaral *et al.* 2002). Chicken have potential to be use to complete the spectrum of animal used for antibody production (Kumaran, 2016). To obtain non specific IgY antibodies against an antigen of

interest IgY purification, fast and low cost isolation process that produces a high quantity of non specific antibody. The production and amount of IgY produced from chicken egg yolk can be affected by certain different factors, such as the breed and climatic condition and methods of preparing IgY yolk extract, (Groos and Siegeel., 1990; Carlander and Larsson. ; 2001). Several methods were used for purifying IgY based on the strategy of separation of proteins from lipoproteins and the rest of the yolk lipids (Schade *et al.*, 1996; Kitaguchik *et al.*, 2008). In the present study polyethylene glycol PEG precipitation technique was performed with slight modification. First objective was to study the effect of breed (Hisex strain and Sudanese Baladi breed) and management system (Semi-closed and Traditional), on IgY concentration in egg yolk. The second objective to study the effect of immunoglobulin Y on bacteria.

Materials and method

Collection of eggs and separation of yolk:

A total of 24 eggs were collected from three groups of hens (30 Wks of age). first group is Hisex bird kept under Traditional management system (n= 8), second group is Sudanese Baladi bird kept under Traditional management system (n= 8) group three is Hisex bird kept under Semi close system (n=8). Each eggs was weighted, the eggs were carefully cracked and The yolk was transferred to a modified yolk spoon to remove the remaining albumen as possible. Then the yolk was rolled over a filter paper to remove albumen adhered to it. then vitelline membrane was punctured and The yolk was carefully poured into 50ml graduated tube. The yolk volume was recorded.

Isolation and determination of IgY :

Isolation of IgY was done by optimizing PEG 6000 (w/v) precipitation method (Nafisa, 2017). Phosphate buffer saline (PBS) was taken twice of the yolk volume (ml) and mixed with the yolk. Thereafter 3.5% of PEG in gram of the total volume was added, vortex and rolled on hand for 10 min and centrifuged (4°C) for 30 min at 4500 rpm. The supernatant was poured through a folded filter paper into a new tube, and 8.5% PEG (w/v) was added to the new volume and centrifuged as above. Supernatant was discarded and PBS was added to a volume of 10ml. The new volume was mixed with 12% PEG (w/v, 1.2) then vortex, and rolled on hand for 10min and centrifuged (4°C) as above. The pellet was carefully dissolved in 2ml PBS. Finally isolated IgY samples were stored in 2ml eppendorf tubes at -18°C. The IgY concentration was measured by spectrometer at 280nm (1:2 dilution with PBS at pH 7.2 and calculated according to the Lambert-Beer Law with and extinction coefficient of 1.33 for IgY.

The Beer- Lambert's law:

$$A = \epsilon * L * C$$

A: is the absorbance of the solution (no unit)

ϵ : is the molar absorptive or the molar extinction coefficient (in l/mol.cm).

L: is the distance the light travels through the solution (in cm).

C: is the concentration

Effects of non- specific IgY on bacteria growth

A total of 24 eggs were collected from three groups. After crack eggs The yolks were separated from the egg white and rolled on paper towels to remove adhering egg white. The vittelline membrane was punctured and the yolk was collected in 50ml graduated tubes and mixed thoroughly. The total volume of yolk was measured, and just a

sample of 1ml was immediately mixed with distilled water for three solutions (1:0.1, 1:1 and 1:10) thereafter the samples were heated at 62°C for 15 minutes and centrifuged at 4500rpm for 16 minutes. The supernatant was filtered through folded filter paper into 15ml new tubes. Nutrient agar media was prepared in 12 Petri dishes and three wells with 5mm in diameter were made in each Petri dish. *E. coli*, *Staphylococcus* and *Pseudomonas* bacteria were inoculated into nutrient agar with spread plate method (36 samples). 25µl of IgY yolk were added into the wells and left for two hours at room temperature. Thereafter the plates were incubated at 37°C for overnight. After that, the diameter of inhibited zone around the wells was measured in (cm) and compared with the measured size of inhibitory zone manual.

Results and discussion

The IgY concentration in the egg yolk has been measured by many investigators, the IgY have varied from (1 to 25mg/g-yolk) (Cutting and Roth, 1973, Carlander, et al., 2001 and Hamal, et al., 2006). In other way Hansen, et al. (1998) and Bizanov, et al. (2003) reported that the IgY concentration varied in the range of (0.4 - 0.9 mg/ml- yolk) in unimmunized birds. We sought the effect of breed and housing system on IgY concentration. The study showed that Hisex produced heavier egg weight compared with Baladi (53.91g, 43.09g), but lower in yolk volume (10.31±1.4ml) than the Sudanese Baladi (13.20±1.5ml). The result revealed significant difference of breed on egg weight, yolk volume, IgY concentration (mg/g-egg, mg/ml-yolk), except on IgY (mg/egg). Hisex produced higher concentration IgY (6.57±0.8 mg/ml-yolk) than the Baladi (5.62±0.7mg/ml-yolk), even so the yolk volume is very low in Hisex, this may be due to selective breed. The result agrees with (Cutting and Roth, 1973,

Carlander, et al., 2001, Bizanov, et al. 2003, Carlander, et al. 2003, Hamal, et al., 2006 Clerici et al., 2006, Ledvinka, et al., 2010) but not agree with (Agrawal et al., 2016) who reported that no significant difference of breed on IgY concentration. The housing system had no significant on yolk volume, IgY concentration (mg/egg, mg/ml-yolk), though a significant on egg weight and IgY (mg/g-egg), the significant found between Hisex (Traditional) in unimmunized and Hisex (Semi close) immunized on IgY (mg/g-egg) might be to variation in vaccinated birds. By our measurement the yolk IgY concentration of two breeds (6.47±0.7 and 5.58±0.7) in the range of (1 to 25mg/g-yolk) which reported by many investigators. And unimmunized bird in our study produced more of IgY concentration than that reported by Hansen, et al. (1998) and Bizanov, et al. (2003).

The result showed that the effect of IgY on different types of bacteria (*E. coli*, *Staphylococcus* and *Pseudomonas*) this result agrees with (Sugita- Konishi, et al. 1996, Kollberg (2015)) they reported that IgY inhibits bacterial growth. On the other hand the study showed that no significant effect of breed and management on antibacterial effect. IgY technology will be expected that IgY will play an increasing role in research and opens the door also for using IgY antibodies as alternative for antibiotic.

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Table (1): Effect of breed and management on IgY concentration in egg yolk.

Main factor	Egg weight (g)	Yolk volume (ml)	Yolk weight (g)	IgY con..(mg/egg g)	IgY con. (mg/g-egg)	IgY con. (mg/ml- yolk)	IgY con. (mg/g-yolk)
Breed ¹	53.91±3.9	10.31 ± 1.4	11.56 ± 1.2	74.29 ±1.5	1.53 ±0.2	6.57 ±0.8	6.47 ±0.7
	43.09 ±2.5	13.20 ± 1.5	13.27 ± 1.4	73.27 ±1.4	1.71 ±0.1	5.62 ±0.7	5.58 ±0.7
Baladi	**	**	**	NS	*	*	*
Sig							
Management ²	53.91 ± 3.9	10.31 ± 1.4	11.56 ± 1.2	74.29 ±1.5	1.53 ±0.2	6.57 ±0.8	6.47 ±0.7
	49.11 ± 4.7	11.44 ± 1.2	11.47 ± 1.0	75.06 ±2.2	1.37 ±0.0	7.05 ±0.5	6.37 ±0.5
Semi close	*	NS	NS	NS	*	NS	NS
Sig							

NS: Not Significant **: Significant differences at (P< 0.001) *: significant differences at (P< 0.01)

1: all breed kept under Traditional system.

2: all birds are Hi sex

Table (2): effect of IgY on bacterial growth

Main factor		E. Coli	Staphylococcus	Pseudomonas
Breed	Hisex	8.75 ± 2.2	7.40 ± 1.1	9.25 ± 1.6
	Baladi	9.00 ± 1.7	9.00 ± 1.4	8.00 ± 2.8
	Sig	NS	NS	NS
Management	Traditional	9.40 ± 1.9	8.40 ± 1.4	8.83 ± 1.8
	Semi close	8.25 ± 2.6	5.60 ± 3.2	9.25 ± 2.1
	Sig	NS	NS	NS

NS: Not Significant

Values mean are inhibition zone measured in cm.

Table (3): IgY concentration reported by some authors

Author	IgY concentration	Units	Remarks
Shimizu <i>et al.</i> , (1988)	1.0 – 3.2	mg/ml	Varied in tow groups.
Hansen, <i>et al.</i> , (1998)	0.6	mg/ml	Ammonium sulphate (60% v/v)
Carlander, <i>et al.</i> , (2001)	3 - 7	mg/ml	IgY concentration varies significant among individual
Bizanov and Jonauskiene (2003).	1-3.8	mg/ml	varied in tow groups
	1.6-2.0	mg/ml	Ammonium Sulphate method.
	1.6-1.8	mg/ml	Ammonium Sulphate method.
	0.4 – 0.9	mg/ml	Unimmunized birds.
Hamal, <i>et al.</i> , 2006)	1.5	mg/ml	Lower concentrate in line chicken
	2.6	mg/ml	Lower concentrate in other line chicken
Carlander, <i>et al.</i> , (2010)	2.21	mg/ ml	Compare between three breeds.
	1.95	mg/ml	Three individual of Rode Island Red have very
	1.62	mg/ml	low IgY concentration
	0.33,0.35 and 0.40	mg/ml	
Gadde, <i>et al.</i> , (2015)	8 - 25	mg /ml	PEG
Agrawal, <i>et al.</i> , (2016)	2.4	mg/ml	Concentration of IgY between four breeds
	2.3	mg/ml	
Nafisa (2017)	2.6	mg/ml	Varied between different lines of chicken.
	2.5	mg/ml	
	16.7- 30.9	mg/ml	
Cutting and Roth (1973)	1 -25	mg/g -yolk	Range variation of (IgY) concentration.
Carlander, <i>et al.</i> , (2001)	42 - 105	mg/g- yolk	
Hamal, <i>et al.</i> , (2006)	22.5 – 43.9	mg/g- yolk	
Kitaquchi, <i>et al.</i> , (2008).	12.2	mg/g-yolk	PNP/DO strain
	6.2	mg/ g- yolk	DeKalb strain
	5.7	mg/g- yolk	Nagoya strain
Ulmer- Franco, <i>et al.</i> ,(2012)	9.3 – 11.3	mg/g- yolk	Higher level among different strain
Nafisa (2017)	4.03 – 6.8	mg/g-egg	Varied between different lines of chicken.
	206.01 -392.03	mg/ egg	
Rose <i>et al.</i> , (1974)	100 - 150	mg/egg	zinc sulphate. ammonium sulphate.

أثر السلالة ونظام التربية علي بعض الخصائص الخارجية والداخلية للبيضة

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المستخلص

هدف البحث دراسة أثر سلالة (الهايسكس والبلدي السوداني) ونظام التربية (شبه المغلق والتقليدي) علي بعض الخصائص الخارجية (وزن البيضة، جم البيضة، محيط السطح، وزن القشرة، سمك القشرة، مؤشر القشرة والثقل النوعي) والداخلية (ارتفاع البياض ، وزن البياض ، وحدة الهوف ، وزن الصفار ، حجم الصفار ونسبة الصفار الي البياض). عدد (204) جمعت لهذه الدراسة ، سلالة الهايسكس تربت تحت نظام شبه مغلق والأخري تربت تحت نظام تقليدي ، بيض البلدي السوداني اشترى من سوق حلة كوكو. ميزان كهربائي رقمي. فيرنا كاليبر ومعادلات استخدمت لتحديد المقاسات. الدراسة اظهرت ان السلالة لها اثر معنوي علي وزن البيضة ، حجم البيضة ، محيط البيضة ، وزن القشرة ، سمك القشرة ، الثقل النوعي ومؤشرة القشرة. الجودة الخارجية للبيضة قيمها اعلي في الهايسكس مقارنة بالبلدي السوداني ، في المقابل نظام التربية له اثر معنوي علي محيط البيضة ، وزن القشرة ، مؤشر القشرة والثقل النوعي ماعدا وزن البيضة ، حجم البيضة ، وسمك البيضة . ايضا الدراسة اظهرت اثر معنوي للسلالة علي ارتفاع البياض ، وزن البياض ، وحدة الهوف ، وزن الصفار ، حجم الصفار ، ونسبة الصفار الي البياض . في حين ان نظام التربية له اثر معنوي علي ارتفاع البياض ، وزن البياض ، وحدة الهوف ، وزن الصفار ونسبة الصفار الي البياض إلا انه ليس له اثر علي حجم الصفار .