Original Research Article

Determination of IgG in Serum and Egg Yolk of Goose

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ABSTRACT

Immunoglobulin G (IgG) refers to animal protein with antibody activity. In poultry, the mother can pass their own immunoglobulin to the offspring through the egg, and play a protective role for the offspring [20]. In this study, 102 samples of Jilin white goose were tested by ELISA. The IgG content of IgG and goose embryo in egg yolk during the development of goose embryo was tested. The results showed that the IgG content in goose serum and egg yolk was 14.707μg / mL and 3967.62μg / mL at 14.5 days, and 1495.6413μg / mL and 2821.363μg / mL at 34.5 days (3.5 days).

KEYWORDS: goose; immunoglobulin G; ELISA

1. Introduction

Egg yolk antibody that is egg yolk immunoglobulin is yolk in the yolk globulin, due to its physical and chemical properties of proteins, immunological properties and other aspects of mammalian immunoglobulin in a certain difference [1, 2], so in the field of immunology the antibody obtained from avian egg yolk is called immunoglobulin of Yolk (IgY) [3]. The use of IgY is very extensive and diverse, and the most important function is the transmission of IgY from the mother to the newborn to provide passive immunity. IgY is prevalent in birds, reptiles and amphibians, functionally equivalent to mammalian IgG, but many of its biological properties have not been found.

Immunoglobulin (Ig) refers to the immune system by antigen stimulation, B cells into plasma cells produced by the antibody activity, with the corresponding antigen specific binding globulin, or chemical structure and antibody similar of globulin. It is widely found in the body's serum, body fluids and mucosal secretion, is the body's body fluids constitute the main material, immunoglobulin is divided into IgG, IgA, IgM, IgD and IgE [4, 5]. There are three types of immunoglobulins identified by birds: IgG (IgY), IgM and IgA [6]. IgG is the most important immunoglobulin, about 70% of animal plasma gamma globulin, molecular weight of about 150,000, sugar 2 to 3%. IgG has to improve immunity, enhance the body resistance and so on. At present, there is no quantitative report of geometric detection of goose IgG. In this paper, the enzyme content of IgG in goose serum and egg yolk was determined by enzyme-linked immunosorbent assay (Enzyme-Linked Immunosorbnent Assay, ELISA). The results are reported below.

2. Materials and methods

2.1. Test material

Test animals

Jilin white goose eggs purchased in Jilin Province, a certain goose farm

Major reagents

Sodium phosphate	Analysis of pure chemical plant	Beijing
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Bovine albumin (biochemical reagent)	Analysis of pure chemical plant	Shanghai emerging biological limited company
Sodium chloride	Analysis of pure chemical plant	Beijing

Saturated Ammonium Sulfate Sephadex-G200	Analysis of pure chemical plant	Beijing Dingguo Biological Co., Ltd
Horseradish peroxidase	Analysis of pure chemical plant	Beijing Dingguo Biological Co., Ltd

Goose IgG, rat anti-goose IgG antibody, rabbit anti-goose IgG antibody labeled for the laboratory homemade

Instruments and equipment

Temperature box, electronic balance; 40-hole polystyrene enzyme standard plate; XYJ80-1 desktop centrifuge; LD4-2A low-speed centrifuge; Beijing Medical centrifuge plant; magnetic stirrer (78-1 type magnetic heating stirrer) Photometer 751C type; enzyme-linked immunosensor DG-3022 type; incubator; refrigerator; pH meter pHS-3C; ELISA plate.

2.2. Experimental methods

Extraction of goose serum IgG

(NH4) 2SO4 saturated solution 10mL, so as to 20% (NH4) 2SO4 solution, after mixing, put it aside for 30min; 3000rpm centrifugation for 20min, discarded, and then mixed with 20ml (NH4) 2SO4 saturated solution (NH4) 2SO4 saturated solution 30 mL, make 50% (NH4) 2SO4 solution, mix well, let stand for 30 min; centrifuge 20 min, discard the supernatant; in the precipitation by adding 20 (NH4) 2SO4 saturated solution 10 mL, make 33% (NH4) 2SO4 solution, mix well, let stand for 30 min; 3000 rpm centrifugation 20 min, discard the supernatant to Remove the albumin, repeat 3 times; with 10mL physiological saline solution precipitation, into the dialysis bag; dialysis desalination, dialysis overnight in normal water, and then in the saline at 4 ℃ dialysis 24h, the exchange of several times, until completely removed SO42- or NH4 + was centrifuged to precipitate; the supernatant was centrifuged to remove (impurity). The supernatant was crude IgG; the G200 gel was activated, flotation, packed column, eluted with 0.01 mol / L pH 7.4 PBS, balance. And the crude IgG sample extracted in the previous step was added, eluted with PBS solution, and the second protein elution peak was collected, and the concentration was fine.

Selection and preparation of reference negative and reference positive for goose serum and yolk

2.2.2.1 Preparation of goose serum reference negative

Take the Jilin white goose serum 5 copies of the full mixing (4ml / copies), take this mixture 20mL, add 20mL of saline, mix thoroughly, add 35% saturated ammonium sulfate, add side while stirring, mixing and standing for 30 minutes, Then 3000 r / min centrifuged for 20 minutes, discard the precipitate, the supernatant into the dialysis bag, water flow dialysis for 12 hours, and then dialysis 24 hours of saline, during the exchange of several times. Finally, the extract was concentrated to the original volume and repeated three times. Frozen in -20 ℃ refrigerator.

2.2.2.2 Preparation of goose serum reference positive

Take Jilin white goose serum 5 points, mixed (the serum from the preparation of reference negative serum Jilin white goose), sub-frozen at -20 ℃ refrigerator.

2.2.2.3 Preparation of Goose Egg Reference Negative

Sterile take yolk 5 copies, fully mixed, take this mixture 20mL, 10 times dilution of distilled water, adjusted with dilute acid to PH5.2, 800 / min centrifugation. Distilled water diluted to the yolk original volume, the egg yolk, saline, chloroform by 1: 2: 2 mixed, mixed room temperature 2h, 2 000 g centrifugal centrifugation 20min, divided into three phases, oil phase, Discard the water phase, and then the oil phase and solid phase fully mixed at room temperature volatile chloroform, distilled water diluted to the original volume. Distilled water diluted 7 times, sub-frozen with -20 ℃ refrigerator [8].

2.2.2.4 Preparation of goose egg yolk reference positive

Take 5 portions of sterile egg yolk, mix well, 7 times dilute with distilled water (the yolk is taken from the preparation of reference negative yolk eggs), and frozen in a refrigerator at -20 ° C.

2.2.3 Determination of the reaction conditions for double antibody sandwich ELISA assay

2.2.3.1 Determination of the concentration of coated antibody

The mouse anti-goose IgG was diluted to 5 μg / mL, 10 μg / mL, 20 μg / mL, 30 μg / mL, 40 μg / mL, 50 μg / mL, 60 μg / mL with 0.01 mol / L pH 9.5 buff er, 70μg / mL, 80μg / mL were coated with enzyme-labeled plate, the goose serum 100 times dilution ELISA test.

2.2.3.2 Determination of the optimal dilution factor of goose serum

The antigen was diluted with the antigen concentration of the antigen, coated with the enzyme plate, and then mixed with goose serum, respectively, with 0.1% bovine serum albumin PBST liquid for 40,50,60,70,80,90,100,200 , 400-fold dilution, each dilution plus five holes, ELISA test and calculate the average OD of each dilution (490nm). The optimal dilution factor of the goose serum was determined as the dilution factor of the goose serum when the OD value was closest to 1.

2.2.3.3 Determination of the best dilution factor of goose egg yolk

The antigen was diluted with the antigen concentration of the antigen, coated with the enzyme plate, and then mixed with goose egg yolk, respectively, with 0.1% bovine serum albumin PBST liquid for 4,8,10,16,20,32,64,100 Times dilution, each dilution plus five holes, ELISA test and calculate the average OD of each dilution (490nm). When the OD value is closest to 1, the dilution factor of goose egg yolk is taken as the best dilution factor of goose serum.

2.2.3.4 The working concentration of the coated antigen

Dilute the antigen, coated with the enzyme plate, and then were five copies of yolk mixed, respectively, with 0.1% bovine serum albumin PBST solution for 1, 2, 4, 8, 10, 16, 20, 32, 64, 100 times Dilution, each dilution plus five holes, ELISA test and calculate the average OD of each dilution (490nm). When the OD value is chosen to be closest to 1, the dilution factor of the goose egg yolk is the best dilution factor for the tested egg yolk.

2.2.3.5 Determination of the optimum antigenic coating time

The mouse anti-goose IgG was coated overnight at 37 ° C for 3 hours, at 4 ° C overnight, and then at 37 ° C for 3 hours overnight at 4 ° C, and the results were observed.

2.2.3.6 Determination of the optimum reaction time of antigenic antibody

The antigen and antibody were treated at 37 ℃ 60min, 90min, 120min, the determination.

2.2.3.7 Determination of the time of antibody activity

The time of antibody labeling was 37 ℃ 60min, 90min, 120min, respectively.

2.2.3.8 Determination of the time of action of the optimum substrate

The reaction time was 37 ℃ for 15min, 30min, 45min, and the results were observed.

Determination of positive judgment value

ELISA test method in the results to determine the qualitative determination of the specimens were tested with 'positive', 'negative' said. According to the national epidemiological survey requirements, positive OD value should be negative 4 times.

ELISA test method of testing

2.2.5.1 Sensitivity test

The mixed solution of 5 adult goose serum was diluted with 0.1% bovine serum albumin PBST solution at 50, 60, 70, 80, 90, 100, 200 and 400 times. The OD value was measured according to the established ELISA. Determine the sensitive response titers of goose serum.

2.2.5.2 Repetitive test

Several goose serum and goose egg yolk were selected and tested five times according to the established ELISA. The OD value was measured and the results were observed.

2.2.5.3 Specificity test

Twenty copies of adult goose serum were coated with mouse anti-goose serum and compared with the mouse serum coated plate without immunization with goose IgG.

3. Results

3.1. Preparation of goose IgG and its antibody

Goose was purified by saturated ammonium sulfate method. Sephadex-G200 chromatography was used to collect the liquid. The protein content was calculated by 751 spectrophotometer. The curve was obtained according to the OD value (280nm). The curve is shown in the following figure:

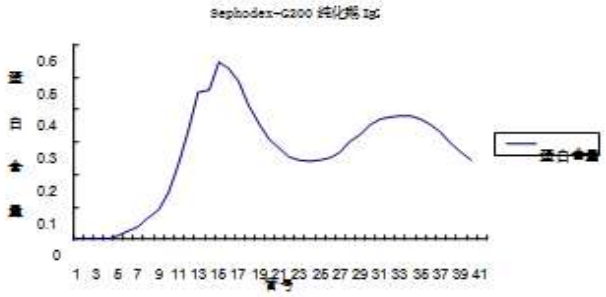

Fig 1. Purification of goose's IgG with SephodexG200

The curve obtained by Sephadex-G200 purification of goose IgG shows that the first peak and the second peak appear very small interval, so when collecting eluent, only from the second peak on the left side of the protein OD value of more than 0.25 wash Dewate the eluent with an OD value greater than 0.2 on the right side of the second peak. The concentrate was then concentrated and the protein content was calculated [9,10].

The OD280 and OD260 values were measured with a 751 spectrophotometer and the protein concentration was calculated as follows: Protein concentration (mg / mL) = (OD280 x 1.45-OD260 x 0.74) x dilution factor. Calculate the goose IgG protein content of 10.7318mg / mL.

3.2. Establishment of double antibody sandwich ELISA assay

Determination of the optimal dilution factor of the quasi-goose serum reference positive and the goose yolk reference negative

Table 4 Determination of serum dilution

Hole number	1	2	3	4	5	6	7	8
Dilution factor	4	8	10	16	20	32	64	100
Serum mean OD	1.31	1.26	1.27	1.26	1.24	1.16	1.10	0.98
Serum reference negative mean OD	0.31	0.29	0.29	0.28	0.24	0.24	0.21	0.19

As can be seen from the above table, the serum 100 times dilution, the OD value of the closest 1.0, the reference negative 0.19, in line with P / N> 4, therefore, the detection of 100 times the serum diluted.

Determination of the optimal dilution factor of the tested goose egg yolk reference positive and the goose yolk reference negative

Table 5 Determination of yolk dilution

Hole number	1	2	3	4	5	6	7	8
Dilution factor	4	8	10	16	20	32	64	100
The average OD of egg yolk	1.2	1.09	1.06	0.1	0.97	0.73	0.67	0.44
Yolk reference negative mean OD	0.36	0.31	0.25	0.24	0.21	0.15	0.12	0.09

From the above table, it was found that when the yolk was diluted 20 times, the OD value was closest to 1.0 and the reference negative was 0.21, which was P / N> 4, so that the egg yell was diluted 20 times.

Determination of the optimum substrate time

Table 6 Results of various incubated time of substrate

Hole number	1	2	3
Treatment	20 min	30 min	45 min
The average OD value	0.83	1.02	1.22

The results showed that the OD value of the colostrum was 1 when the substrate had a reaction time of 30 min and 37 min for 15 min, 30 min and 45 min, respectively. So the substrate action time is determined to 30 min.

IgG sensitivity test results

In serum, the OD value no longer changes when the known amount of goose IgG is diluted to 0.0683594 μg / mL.

Table 7 Results of sensitivity experiment for serum

IgG content (μg / mL)	8.750	4.375	2.188	1.094	0.547	0.273	0.138	0.068	.0342	0.017
Average OD	0.76	0.68	0.62	0.46	0.41	0.4	0.39	0.23	0.2	0.21

In serum, when the known amount of goose IgG was diluted to 0.0341767 μg / mL, the OD value was no longer changed, that is, the sensitivity of the detection method was 0.0341767 μg / mL.

Table 8 Results of sensitivity experiment for egg yolk

IgG content (μg / mL)	70	60	50	40	30	20	10	5	1	0.5
Average OD value	0.3701	0.34	0.33	0.295	0.28	0.26	0.25	0.23	0.235	0.23

In egg yolk, when the known content of yolk diluted to 5μg / mL, OD value no longer changes, that is, the sensitivity of the method is 5μg / mL.

Repetitive test results

The results of ELISA showed that the double antibody sandwich ELISA method established in this study had relatively good stability and reproducibility.

Package Specific Test Results

The normal serum of mice was diluted 1: 100 times, and the serum of mice immunized with goose IgG was diluted 1: 100 times. The results showed that the serum OD value of normal mice was significantly different from that of immunized mice.

Table 9 results of coating antibody specificity test

Hole number	Mouse serum	Immunized goose IgG mouse serum
Dilution factor	1 : 100	1 : 100
The average OD	0.13	0.98

3.3. Protein standard content curve test results

Double antibody sandwich ELISA OD value and egg yolk IgG content of the standard curve

The results showed that the regression equation was y = 145.42ln (x) +214.77, and the regression equation was y = 145.42ln (x) +214.77

Table10 double antibody sandwich ELISA OD and content of IgG in egg yolk

IgG (ug / mL)	OD value
140	0.605
120	0.515
110	0.485
90	0.425
60	0.0.34
50	0.33
40	0.295
30	0.28
20	0.26
10	0.25
5	0.23
1	0.235
0.5	0.23

Double antibody sandwich ELISA OD and serum IgG content of the standard curve

Double antibody sandwich ELISA OD value and serum IgG content by doing the figure to see the power function between them, so the regression equation is y = 23.865x5.6726

Table11 double antibody sandwich ELISA OD and content of IgG in serum

IgG (ug / mL)	OD value
140	1.4
70	1.17
35	1.05
17.5	0.98
8.75	0.82
4.375	0.74
2.1875	0.68
1.09375	0.56
0.546875	0.48
0.2734375	0.49
0.0683594	0.36

Fig.2 double antibody sandwich ELISA OD and content of IgG

3.4 Comparison of IgG content in goose serum and changes of IgG content in goose egg yolk

Table14 Comparison of IgG in serum and egg yolk (with error)

Daytime	Egg yolk IgG content (ug / mL)	Serum IgG content (ug / mL)
14.5d	3967.62±23.8664	0.1717±3.362E-02
34.5d	2821.363±41.5072	1495.6413±80.9186

Figure 3

It can be seen from Fig. 4 that the IgG antibody in goose embryo serum increased from 14.7 days at age 0.1717μg / mL to 34.5 days (3.5 days) 1495.6413μg / mL, the diff erence was signifi cant (P <0.05) (P <0.05), and the diff erence was signifi cant (P <0.05) at 34 days. (1495.6413μg / mL) in the serum did not exceed the lowest value of the egg yolk antibody concentration, but the serum antibody content in the serum was higher than that of the egg yolk antibody (2821.363μg / mL), the antibody concentration in egg yolk was much higher than that in serum.

4. Discussion

4.1. Double antibody sandwich ELISA

Over the past two decades, immunological analysis has developed rapidly, especially after the use of labeled antigen and antibody analysis techniques, so that many of the original classical analytical methods in terms of sensitivity and specifi city cannot be compared. Following the 50 years of immunofl uorescence (IFA) and 60 years of radioimmunoassay (RIA) analysis techniques, Engvall and Perlmann published an enzyme linked immunosorbent assay (ELISA) for the quantifi cation of IgG in 1971, So that in 1966 began to use for antigen targeting antibody technology developed into liquid samples of trace substances in the determination of the establishment of the enzyme to mark the antigen or antibody analysis technology. It is an immunoenzyme technique developed after immunofl uorescence and radioimmunoassay, and is a method of labeling antigens or antibodies with enzymes. Due to the effi cient biocatalytic activity of the enzyme, an enzyme molecule can catalyze the reaction of several tens of hundreds of substrate molecules in a few minutes, resulting in an amplifi cation that makes the original extremely minimal antigen or antibody recognized in a few minutes The

ELISA test is a highly sensitive, specifi c and reproducible experimental diagnostic method. The specifi city of the antigen, the specifi city of the antibody immune response and the effi cient catalysis of the enzyme are organically combined to sensitively detect trace amounts of specifi c antibodies or antigens in the body fl uid. Since the advent of the early 1970s, this technology has developed rapidly and has been widely used in many fi elds such as biology and medical science because of its stable, easy to keep, easy to operate, and objective and other factors.

4.2. Goose serum antibody and egg yolk antibody changes

In the yolk antibody and serum antibody comparison results, can be found in egg yolk antibody and serum antibody growth and decline in the concentration does not correspond. It seems that the egg yolk antibody concentration

has not decreased, and the serum concentration of the highest concentration of antibody did not exceed the lowest value of egg yolk antibody concentration, but the overall trend tends to rise. This phenomenon has the following explanation: First, although the concentration of yolk antibodies does not change much, but the egg yolk antibody volume has undergone great changes in the 14.5 days after the change is more obvious, and in 34.5 days after the egg yolk the volume of the antibody was 0. Thus, although the content of the egg yolk antibody did not change significantly, the egg yolk antibody (total amount) was also transferred to the goose embryo sera due to the change in volume; secondly, the egg yolk antibody In the whole incubation process, in addition to passive transfer to the goose embryo serum, there are other functions, such as the provision of energy to provide the formation of tissue raw materials, therefore, goose embryo serum antibodies in the total less than the egg yolk antibodies The total amount of antibody in serum is also one of the possible reasons for the concentration of antibody in egg yolk.

5. Conclusions

5.1. IgG content in goose serum and goose egg yolk

The IgG antibody in goose embryo serum increased from 14.7 days at age 0.1717μg / mL to 34.5 days (3.5 days), and the difference was significant (P <0.05). IgG antibody from goose was from 14.5 days old 3967.62μg / ML to 34.5 days (3 days after crusting) to 2821.363μg / mL, the difference was significant (P <0.05). At present in the country has not yet seen the determination of goose IgG content of the relevant reports.

5.2 Changes of IgG content in goose serum and goose egg yolk

The results showed that the IgG antibody content in goose embryo showed a general trend (0.1717μg / mL-1495.6413μg / mL), and the content of goose egg was the highest in the range of 14.5 days and 34.5 days (3.5 days) (397.62μg / mL-2821.363μg / mL), but the highest value of antibody concentration (1495.6413μg / mL) in serum did not exceed the lowest value of egg yolk antibody concentration (2821.363μg / mL), Antibody concentration is much higher than serum antibody concentration.

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