

EFFECT OF TWO DIFFERENT INDUCED MOLTING PROGRAMS ON SERUM, EGG YOLK IGY CONCENTRATIONS AND IMMUNE PARAMETERS IN LAYING HENS.

By

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Received: **25/02/2009**

Accepted: **16/03/2009**

ABSTRACT: *Recent studies have indicated that egg yolk immunoglobulins (Ig) can be used for therapeutic purposes and that yolk Ig levels are increased after molting of laying hens. The present study was performed to compare between the effects of two methods of induced molting namely alfalfa and zinc methods on serum and yolk IgY concentrations as well as their effects on the egg quality and immune status of the molted hens. Three experimental treatments each of 20 Lohman laying hens 80 wks old were fed either control layer ration, 90% alfalfa in 10% layer ration or layer ration containing zinc oxide (20.000 ppm) for 9 days. For the alfalfa and zinc group, photoperiod was reduced to 8h/d. Water was provided ad libitum for all groups. At day 10, hens were returned to basic laying ration and 16 h light/d. Feed intake, birds weight and egg production were monitored during the molting period after which 8 birds from each group were slaughtered and ovaries, oviducts and spleens were excised and weighed. Egg parameters (weight, length, yolk diameter and yolk height) were recorded till resumption to 50% egg production. Serum and egg yolk IgY were measured using ELISA technique and white blood cell count was performed. Results showed that hens in the zinc treatment ceased egg production by 8.4 days, remained out of production until 21.8 days and returned to 50% production after 46.3 days while the values for the alfalfa group were 14.9, 24 and 49.4 days respectively. Egg weight, yolk diameter and yolk height values were increased in alfalfa group. Molting by either method resulted in significant loss in body weight, feed intake and weight of ovaries and oviducts. Spleen weight was slightly increased in the two molted groups.*

Values of serum IgY recorded 35 days post molting induction were 3.42 and 3.3 mg / ml serum in alfalfa and zinc groups respectively as

compared to control value (1.9 mg/ml). Egg yolk IgY levels in control hen eggs ranged from 1.9-2.25 mg /ml yolk . Induced molting significantly increased egg yolk IgY levels up to 3.5 and 3.2 mg/ml in alfalfa and zinc groups respectively .Total leukocyte count was significantly increased in alfalfa and zinc treated groups as compared to control group .Neutrophyl: Lymphocyte ratio recorded at resumption to 50% egg production was 0.16 for control group, 0.41 for alfalfa group and 0.19 for zinc fed group .

It may be concluded that using post molt eggs from hens induced to molt using alfalfa schedule would be advantageous in having larger yolk with higher levels of IgY which has significant application in commercial production of hyper- immune eggs for therapeutic uses.

INTRODUCTION

The transfer of chicken immunoglobulins (Ig) from the hens serum to the yolk and from the yolk to the chick is analogous to cross –placental transfer of IgG from mammalian mother to its offspring (Rose et al., 1974; Kowalczyk et al., 1985). Many reports (Ormrod and Miller, 1991; Ormrod and Miller, 1993; Sharpe et al., 1994) indicated that multiple inoculations of chickens (i.e. hyper-immunization) with inactivated multivalent bacterial vaccines, results in the production of “immune” eggs. Oral consumption of “immune” eggs containing specific immunoglobulins protects against the specific organism(s) with which the hen was stimulated (Fayer et al., 1989; Kuroki et al., 1994; Kullmann et al., 1988). Orally administered immunoglobulins survive passage through the gastro intestinal tract (Hammarstrm et al., 1994).

A recent study by Barua et al. (2001) has indicated that the concentration of yolk IGY is affected by forced feed withdrawal molting. IGY concentrations were increased during early phase of postmolt (4 days) then slightly decreased in the yolk 10 days after relaying. The commercial egg industry commonly uses induced molt procedures to rejuvenate flocks for a second or third laying cycle and to increase profits. Induced molt program can result in a 30% higher profit margin for producers when compared with an all pullet operation (Bell, 2003). In addition to increased profit margins, an induced molt rejuvenates hen’s reproductive tract to produce higher quality eggs, which are more marketable (Keshavarz and Quimby, 2002).

There are several molting methods, feed withholding has been the most popular (Keshavarz and Quimby, 2002; Bell, 2003). Induced molting have been conducted using diets mixed with high zinc concentrations (Bell, 2003), thyroxin (Keshhavarz and Quimby, 2002), and low sodium

concentrations (Berry and Brake, 1985). A second general approach has incorporated the use of insoluble plant fibers such as alfalfa (Kwon et al.,2001; Landers et al.,2005). Alfalfa is well balanced in amino acids and rich in vitamins, carotenoids, and xanthophylls that give poultry carcasses their desirable yellow color (Sen et al., 1998; Ponte et al., 2004). Alfalfa also contain high level of saponins ,which have been shown to have hypo-cholesterolemic ,anti-carcinogenic, anti-inflammatory, and antioxidant properties (Klita et al.,1996; Ponte et al.,2004).

The aim of the present study was to compare between the effects of two methods of induced molting namely alfalfa and zinc methods on serum and yolk IgY concentrations as well as their effect on the immune status of the molted birds. Also, some of the egg quality parameters were monitored during the experimental period of the study.

MATERIALS AND METHODS

A total of 60 White Single Comb Lohmann laying hens 80 wks of age were used in the present study. Birds were housed individually one per cage and were fed a complete layer ration (Table -1) *ad libitum* and allowed full access to water. Egg production was monitored to ensure that all hens were healthy and actively producing. After acclimation for 2 weeks the birds were exposed to the molting procedure. The hens were divided into 3 treatment groups (Table- 2) with 20 birds per treatment: non molted control given full layer ration feed, group 2 hens fed on 90% Alfalfa and 10% layer ration for 9 days (Kwon et al., 2001) while in the third group (zinc molt group) hens were fed on layer ration containing 20.000 ppm of zinc as zinc oxide for 9 days according to North and Bell (1990).

Hens were placed on an artificial lighting program of 8hour L:16D during molt induction period (9 days) then returned to control layer ration and 16h L: 8h D /d.

Feed intake was measured by weighing each diet prior to the start of molt and after the 9-days molt period. During molt, birds weights were monitored at 2, 5 , 10 and 35 days.

At the end of the molt, 24 birds were slaughtered (8 birds from each group), and the ovaries, oviducts and spleens were excised and weighed and expressed as relative weights (% of B.W.). Egg production was measured daily (% of hen-day), whereas egg quality parameters were measured twice per week. Egg weight (recorded to the nearest 0.01g) , egg length, , yolk height and yolk diameter were measured with a caliper and recorded to the nearest 0.1mm.

Yolk samples were collected before the induction of molting (pre-molt), and 5 and 10 days after relaying and at return to 50% egg production.

Table.1: Ingredients of experimental diets

Ingredient%	Layer diet	Alfalfa-layer diet	Zinc diet
Corn	56.10	5.61	56.10
Barely	9.0	0.90	7.00
Soybean meal	21.65	2.165	21.65
Fish meal	3.00	0.30	3.00
Limestone	9.00	0.90	9.00
Dicalcium Phosphate	1.00	0.10	1.00
Vit.-mineral Premix	0.25	0.25	0.25
Alfalfa	0.00	90.00	0.00
Zinc oxide	0	0	2.00
Crude protein	17.3	17.48	17.11
ME K cal /Kg diet	2693.65	1349.37	2641.25
C/P ratio	155.70	77.20	154.37

Composition of premix per 2.5 Kg: 12,000 IU of Vit A, 2,400,000 IU of Vit.D3, 30 gm of vit.E, 25 g of vit.K3, 2.5 g of vit.B1, 6 gm of vit.B2, 4 g of Vit.B6, 20 mg of vit.B12

Blood sampling

All hens were bled via the wing vein, and 4 ml of blood was collected using a 5-ml syringe with a 23-gauge needle. Two ml of blood for each hen was centrifuged to separate serum which was collected and stored frozen until analysis, while heparin was added to the remaining 2 ml blood to be used for estimation of total leukocyte count and differential leukocyte count. Blood sampling was done on the 2nd, 5th, 10th and 35 days after molt induction and at the return to 50% of pre molt egg production.

Purification of IgY from egg yolk :

To extract immunoglobulins from the egg yolk, a chloroform-based method described by Polson (1990) was used. The egg yolk was taken out of the eggshell and placed in a clean Petri dish. The egg yolk membrane was washed with distilled water and then cut with the forceps. The yolk was allowed to run into a measuring cylinder, and its volume was noted. Twice the volume of phosphate buffer saline (PBS) was added, and the contents were mixed thoroughly by shaking. Chloroform equal to the volume of egg yolk and PBS was then added, and the contents were mixed vigorously, which resulted in the production of a thick emulsion. The emulsion was then

centrifuged at 3000 rpm for 30 min at room temperature. After centrifugation, the mixture was separated into 3 distinct layers in the centrifuge tube: an orange-colored solution at the bottom, a semisolid emulsion of yolk in chloroform in the middle, and a watery phase of chicken serum protein on top. The watery phase on the top containing the Ig was removed, aliquoted, and stored at -20°C until analysis.

Assay of serum and Yolk IgY :

Serum and egg yolk IgY were determined by rapid enzyme immuno assay method according to Blais and Yamazaki (1991). A commercial ELISA kit was used and the instructions of the manufacturer were followed. Absorbance at 492 nm values was measured using an ELISA plate reader. Results were expressed as the ratio between the optical density (OD) generated by the serum or yolk sample being tested (S) and the OD in a well containing a positive-control sample (P). Values were expressed in mg/ml sample.

Statistical analysis: Data were analyzed using the GLM procedure of SAS software (2001). Differences in parameters among treatment groups, when significant, were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS

Hens subjected to Zn treatment ceased egg production by 8.4 days of the experiment and remained out of production until day 21.8. Values for Alfalfa treated hens were 14.9 and 24 days respectively. During this period few birds lost little plumage at the neck and at the back. Feed intake in alfalfa and zinc treated groups was much reduced during the molting induction period. Up to 50 % reduction in feed intake was observed in both groups as compared to feed intake of control group. Layers lost an average of about 13.9 % of their initial body weight due to Alfalfa and 18.4% due to Zn treatment as compared to 4.9% loss in control hen body weight during the same period.

The pre-molting weight was reached 1.5 wk after the start of re-feeding on control layer ration and 16h light/day in both treated groups.

Hens in zinc treatment reached 50% production by 46.3 and alfalfa group by 40.4 days following the return to full feed layer ration and 16 h light/day (Table- 3).

Average egg weight, yolk diameter and yolk height were significantly higher in alfalfa group. Yolk diameter in eggs of zinc molted hens was also higher than that of control group but close to alfalfa values.

Adoption of molting schedule resulted in significant increase in reproductive weight loss (ovaries and oviducts) which was more marked in Zn treated hens. However, the variation between Zn and alfalfa groups was not significant. Spleen weight was slightly increased in both induced molting groups as compared to spleen weights of control birds, but the values were not different significantly (Table- 5).

Serum IgY values were elevated in hens exposed to either alfalfa or zinc induced molt regimens as compared to premolt values or those of control birds. Values of serum IgY recorded 35 days post molting induction were 3.42 and 3.3 mg / ml serum respectively as compared to 1.9 mg/ml recorded in serum of control non treated hens (Table- 6).

Egg yolk IgY levels in control hen eggs ranged from 1.9-2.25 mg /ml yolk. Induced molting significantly increased egg yolk IgY levels up to 3.5 and 3.2 mg/ml in alfalfa and zinc groups respectively with intermediate values recorded at day 25 post molt (Table -7).

Total leukocytic count was significantly increased in alfalfa and zinc treated groups as compared to control group ($14.243, 12.554$ and $7.553 \times 10^3/\text{mm}^3$ respectively) (Table 8).

Heterophyl: Lymphocyte ratio recorded at 50% of egg production was 0.16 for control group, 0.41 for alfalfa group and 0.19 for zinc fed group (Table-9).

Mean values of neutrophil: lymphocyte ratio (N/L) was higher in alfalfa and Zn molted hens at 35 days post molt (0.60 and 0.31 respectively) as compared to N/L ratio in control hens (0.21). On return to 50% egg production the ratio was still higher for alfalfa group compared to controls (Table9).

Table 2: Effect of different induced molting programs on feed intake, body weight loss and % of body weight loss during a 9-d molting period of Lohmann laying hens

Group	Feed intake (g/bird)	Body weight loss (g/bird)	Body weight loss (%)
Control	543.5 ± 15.5^a	51.9 ± 12.8^a	4.9 ± 1.0^a
Alfalfa90%	256.5 ± 22.5^b	194.6 ± 11.5^b	13.9 ± 0.5^b
Zinc	210.5 ± 10.5^b	230.0 ± 10.3^c	18.4 ± 0.2^c

Values are Mean \pm SEM

Means in the same column bearing different superscript are significantly different ($P \leq 0.05$)

Hens were returned to control ration and 16 h light on day 10.

Table 3: Effect of different induced molting programs on egg production parameters during and after molting of Lohmann laying hens

Time (days)	Alfalfa 90%	Zinc oxide
1 st day out of production from start of treatment	14.9	8.4
Days to first egg Postmolt	20.5	28.8
Days to return to 50% egg production	40.4	46.3

Table 4: Effect of different induced molting programs on egg parameters post molting of Lohmann laying hens

Egg parameter	Control group	Molting program	
		Alfalfa 90%	Zinc oxide
Weight(g)	60.78 ± 0.3 ^a	62.8 ± 0.8 ^b	61.2 ± 0.42 ^a
Length (mm)	57.45 ± 0.22 ^a	58.6 ± 1.2 ^a	58.2 ± 0.15 ^a
Yolk diameter (mm)	37.56 ± 0.14 ^a	39.3 ± 0.4 ^b	38.22 ± 0.2 ^b
Yolk height (mm)	18.16 ± 0.07 ^a	19.0 ± 0.1 ^b	18.1 ± 0.1 ^a

Values are means ± SEM

Means in the same row bearing different superscript are significantly different (P ≤0.05)

Table 5: Effect of different induced molting programs on organ weights (as % of body weight)of Lohmann laying hens

Organ	Control group	Molting program	
		Alfalfa 90%	Zinc oxide
Ovary %	2.10 ± 0.21 ^a	0.82 ± 0.12 ^b	0.62 ± 0.03 ^b
Oviduct %	3.91 ± 0.3 ^a	2.10 ± 0.80 ^b	1.76 ± 0.50 ^b
Spleen %	0.09 ± 0.006 ^a	0.10 ± 0.005 ^a	0.10 ± 0.005 ^a

Values are means ± SEM

Means in the same column bearing different superscript are significantly different (P ≤0.05)

Table 6: Effect of different induced molting programs on serum IgY (mg/ml) of Lohmann laying hens

Time of serum IgY evaluation	Control group	Molting program	
		Alfalfa 90%	Zinc oxide
Serum IgY (pre-molt)	2.25 ± 0.2 ^a	2.05 ± 0.2 ^a	1.9 ± 0.10 ^a
Serum IgY Day 2 molt	2.14 ± 0.25 ^a	3.26 ± 0.28 ^b	2.5 ± 0.23 ^{ac}
Serum IgY Day 10 molt	2.22 ± 0.24 ^a	3.63 ± 0.3 ^b	3.36 ± 0.27 ^b
Serum IgY Day 35 postmolt	1.900 ± 0.2 ^a	3.420 ± .22 ^b	3.30 ± 0.32 ^b

Serum antibody levels are means ± SEM

Means in the same row bearing different superscript are significantly different (P ≤ 0.05)

Table 7: Effect of different induced molting programs on egg yolk IgY (mg/ml) of Lohmann laying hens .

Time of sampling	Control group	Molting program	
		Alfalfa 90%	Zinc oxide
Egg yolk IgY/ml (pre-molt)	2.25 ± 0.11 ^a	2.02 ± 0.3 ^a	2.30 ± 0.25 ^a
Egg yolk IgY/ml Day 25 postmolt	2.03 ± 0.21 ^a	2.92 ± 0.25 ^b	2.7 ± 0.21 ^b
Egg yolk IgY/ml Day 35 postmolt	1.90 ± 0.30 ^a	3.5 ± 0.15 ^b	3.2 ± 0.31 ^b

Egg yolk antibody levels are means ± SEM; means are based on 30 eggs from each group.

Means in the same row bearing different superscript are significantly different (P < 0.05)

These antibodies were extracted from egg yolk (4 to 5 eggs/ hen) using chloroform.

The antibody levels were determined per milliliter of egg yolk.

Table 8: Effect of different induced molting programs on total leukocyte count ($10^3/\text{mm}^3$) during and after molting of Lohmann laying hens

Time of sampling	Control group	Molting program	
		Alfalfa 90%	Zinc oxide
2 nd day	9.765 ± 0.150 ^a	8.435 ± 0.12 ^b	7.322 ± 0.9 ^b
5 th day	7.739 ± 0.094 ^a	7.654 ± 0.03 ^a	6.754 ± 0.09 ^b
10 th day	7.950 ± 0.028a	7.545 ± 0.05 ^b	6.542 ± 0.25 ^c
35 day	7.764 ± 0.140a	8.656 ± 0.14 ^b	8.774 ± 0.2 ^b
At 50% of pre molt egg production	7.553 ± 0.045a	14.243 ± 0.15 ^b	12.554 ± 0.3 ^c

Values are means ± S.E.M of 6 samples per group.

Means in the same row bearing different superscript are significantly different (P < 0.05)

Table 9: Effect of different induced molting programs on neutrophil: lymphocyte ratio during and after molting of Lohmann laying hens.

Time of sampling	Control group N/L ratio	Molting program N/L ratio	
		Alfalfa 90%	Zinc oxide
2 nd day during molt.	0.17	0.17	0.17
5 th day during molt.	0.20	0.46	0.32
10 th day during molt.	0.18	0.37	0.42
35 day post molt.	0.21	0.60	0.31
At 50 % egg production	0.16	0.41	0.19

Values are means of 6 samples per group.

DISCUSSION

There have been many studies regarding the isolation and purification of egg antibodies, especially considering the easy access to this source of antibodies and the high levels of specific antibodies present in the egg.

The present experiment points out to the preferable use of hens exposed to alfalfa induced molt for the production of IgY enriched egg yolk .Wang et al.(2007) extracted

61mg IgY against *Schistosoma japonicum* from one egg, an amount that is much higher than levels obtained in the present work. This difference may

be attributed to differences in the immunization schedule , immune response of the bird and method of extraction adopted. The delipidization method followed in the present work was reported (Svendsen et al., 1995) to be superior in the yield of Ig isolated from the egg yolk than other methods used previously such as using caprylic acid (Mc Laren et al.,1994), ammonium sulphate precipitation (Wallman et al.,1990) or chromatography (Hassl and Aspöck,1988). Indirect enzyme linked immunosorbent assays (ELISAs) have been applied by many investigators to detect egg yolk IgY immunoglobulins (Barua et al.,2001; Murase et al.,2006). The present results showed that serum as well as egg yolk immunoglobulin levels were almost parallel.

The present results indicate that alfalfa method for molt induction has more advantages over the zinc method in terms of time to return to 1st egg production, egg size , % loss in body weight , reproductive organs loss and amount of egg yolk IgY .

The use of alfalfa mixed with layer ration proved to be effective in molt induction, increasing postmolt egg quality and postmolt egg production when compared with the zinc method. Contradictory to our findings, Alodan and Mashaly (1999) reported that different induced molting programs (Zn, CAL, On-Off) had no effect on egg weight. Previous studies of molting methods have often shown conflicting results in hen responses because of different conditions. Alfalfa-induced molting, also offers advantages in that it is readily available in Egypt as a common feed for farm animals. The loss in body weight in alfalfa fed group was attributed to the low palatability of alfalfa by hens (Sen et al., 1998), decreased feed intake due to delayed emptying of the crop and by its slow passage rate in digestive tract as suggested by Ueda et al. (2002).

Decreased feeding stimulation with reduced daylight hours (Andrews et al., 1987) is a common factor in alfalfa and zinc groups causing loss in body weight.

In the present study , although hens in the zinc group lost body weight significantly more than the alfalfa group , both groups laid eggs of nearly similar parameters postmolt. This result could be because the zinc hens stayed out of production longer than hens in the alfalfa group and that resulted in the same egg production as previously reported by Buhr and Cunningham (1994). These authors suggested that the longer the cessation period , the better the postmolt egg production.

The enlarged size of the spleen may coincide with the greater number of lymphocytes observed in the differential leukocytic count test.

The higher values of IgY in sera and in egg yolk of induced molt birds confirm the previous work of Barua et al.(2001) using feed withdrawal method in 515 –days-old hens .

One disadvantage of using zinc as molt inducing factor was its tissue and egg residue.

Hassanabadi and kermanshahi (2007) reported that postmolt internal zinc concentration in molted hens was higher than values in hens induced to molt by feed withdrawal method. The present finding that egg parameters were not significantly different between the molted hens is in agreement with results of Christmas et al. (1985) who found that different induced molting programs did not significantly affect egg weight when compared to the non molted birds.

In the present work molted hens in either method had involuted reproductive organs which was proportional to the loss in body weight. This finding is in accordance with the results of Brake (1992) who suggested that the rebuilding of the reproductive tract would lead to the removal of fat accumulation and therefore increased tissue efficiency and improved egg production.

The present results showed a significant increase of the neutrophil to lymphocyte ratio in molted hens being more pronounced in alfalfa group. This result coincides with the results of Alodan and Mashaly (1999) who demonstrated an increase in N/L ratio after forced molting.

In the current study, our main concern was the amount of IgY in the egg yolk which proved to be significantly higher in the second egg production cycle and in the alfalfa molted group. . This finding suggest the adoption of alfalfa molting procedure in experiments designed to use egg yolk IgY in therapeutic purposes or other serological techniques.

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الملخص العربي

تأثير أحداث القلش بطرق مختلفة على مناعة الدجاج البياض وعلى تركيز الجلوبيولين المناعي في المصل وصفار البيض.

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أظهرت الدراسات الحديثة ان الجلوبيولين المناعي في صفار البيض يمكن الاستفادة منه في الأغراض العلاجية وأن مستواه يزيد في المح بعد عملية القلش في الدجاج البياض. وفي هذا البحث استخدمت طريقتين لأحداث القلش وهما التغذية على البرسيم أو إضافة الزنك لدراسة تأثيرهم على مستوى الجلوبيولين المناعي في كل من مح البيض ومصل الدم، كما تم دراسة التأثير على مواصفات البيض والحالة المناعية للدجاج البياض المعامل.

لهذا الغرض تم تغذية ثلاث مجموعات كل من 20 دجاجة بياضة من سلالة اللوهمان عمر 80 اسبوع على عليقة متوازنة للدجاج البياض (المجموعة الضابطة) او على 90% برسيم + 10% عليقة متوازنة (مجموعة البرسيم) أو التغذية على عليقة متوازنة تحتوي على أكسيد الزنك 20.000 جزء (مجموعة الزنك) وذلك لمدة 9 أيام تم خلالها تخفيض ساعات الإضاءة لكل من المجموعتين (البرسيم والزنك) إلى 8 ساعات إضاءة فقط لأحداث القلش. وفي اليوم العاشر تم إعادة تغذية جميع المعاملات على العليقة المتوازنة وإعادة ساعات الإضاءة إلى 16 ساعة. تم حساب معدلات التغذية ووزن الطيور ومتابعة إنتاج البيض أثناء فترة أحداث القلش ثم تم ذبح عدد 8 دجاجات من كل مجموعة لأخذ عينات من الدم الطحال والمبيض وقناة البيض وذلك لتسجيل أوزانهم وعمل عد لكرات الدم البيضاء. تم متابعة إنتاج البيض في الدجاجات المتبقية حتى عودتها إلى إنتاج 50% من الإنتاج قبل أحداث القلش.

أظهرت النتائج ان الأمهات المعاملة بالزنك قد توقفت عن وضع البيض بعد 8.4 يوم من المعاملة وبقيت كذلك حتى يوم 21.8 ثم عادت تدريجيا إلى إنتاج البيض ووصلت إلى 50% من الإنتاج السابق لأحداث القلش بعد 46.3 يوم وكانت هذه القيم في المجموعة المعاملة بالبرسيم هي 14.9 يوم & 24 يوم و 49.4 على التتابع.

كانت قيم أوزان البيض وقطر المح وارتفاع المح مرتفعة في المجموعة المعاملة بالبرسيم ، بينما تسبب أحداث القلش في انخفاض معنوي في وزن الأمهات المعاملة ومعدلات التغذية ووزن المبيض وقناة البيض كما لوحظ ارتفاع نسبي في وزن الطحال في كلا المجموعتين المحدث بهما القلش.

سجلت قيم الجلوبيولين المناعي في مصل الأمهات المعاملة بعد 35 يوم من عملية القلش متوسط 3.42 و 3.3 مجم لكل مل مصل في الأمهات من مجموعة البرسيم ومجموعة الزنك على التوالي . تراوحت قيم الجلوبيولين المناعي في مح بيض الأمهات من المجموعة الضابطة بين 1.9-2.25 مجم لكل مل من صفار البيض. كما احدث القلش زيادة معنوية في مستوى الجلوبيولين المناعي ووصل إلى 3.5 و 3.2 مجم / مل في مجموعة البرسيم ومجموعة الزنك على التوالي.

زاد عدد كرات الدم البيضاء الكلى في كلا المجموعتين المحدث بهما القلش بينما كانت نسبة الخلايا المتعادلة الى الخلايا الليمفاوية المسجلة عند الوصول الى 50% من إنتاج البيض حوالي 0.16 للمجموعة الضابطة و 0.41 لمجموعة البرسيم و 0.19 لمجموعة الزنك.

تخلص هذه الدراسة الى تفضيل استخدام أمهات الدجاج البياض بعد عملية القلش في إنتاج البيض المرتفع المناعة تجاريا للأغراض العلاجية حيث أن عملية القلش تزيد من حجم صفار البيض وكمية الجلوبيولين المناعي به.