

Reducing fat deposition in poultry by immunising against adipocyte membranes: III. Attempts in using eggs for transmission and development of antibodies

(Mengurangkan lemak melalui imunitasi terhadap membran adiposit pada poltri:
III. Percubaan menggunakan telur untuk penyaluran dan penghasilan antibodi)

A. S. Zainur*, B. I. Shukran* and M. Masnizar*

Key words: fat reduction, poultry, passive immunisation, active immunisation, adipocyte membrane antigen, egg antibodies (IgY)

Abstrak

Penyaluran dan penghasilan antibodi terhadap membran sel lemak ke dalam telur dapat mengatasi masalah penyaluran antigen dan sangat berguna untuk pengeluaran ayam yang kurang lemak. Kajian ini menggunakan kaedah imunitasi pasif dan aktif pada ibu ayam yang masih bertelur. Dalam imunitasi pasif, antibodi ayam turki terhadap sel lemak telah disuntik ke dalam ayam yang masih bertelur dan dalam imunitasi aktif pula hanya membran sel lemak sahaja yang disuntik ke jenis ayam yang sama. Pengeluaran telur tidak terjejas bagi ayam yang diimmunisasikan sama ada secara pasif atau aktif, kecuali pada awal imunitasi pasif sahaja. Ini berkemungkinan bahawa antibodi tersebut tidak berkesempatan masuk ke dalam telur, walaupun kepekatan antibodi masih tinggi di dalam darah ayam berkenaan. Dari segi imunitasi aktif pula, pengeluaran antibodi ternyata pada peringkat imunitasi kedua dan ketiga. Kepekatan antibodi juga lebih tinggi berbanding dengan imunitasi pasif. Ini menunjukkan bahawa pemilihan telur untuk pembiakan boleh diambil pada takat ini. Kedua-dua bentuk imunitasi tidak nyata berbeza dari segi berat karkas dan beberapa organ di dalam ibu ayam. Ini menandakan yang proses imunitasi tidak meninggalkan apa-apa kesan sampingan pada ibu yang masih bertelur.

Abstract

The transmission and development of antibodies against adipocyte membranes in eggs were studied. This process overcomes the problem of delivery of the antigen and would be useful for the production of lean chickens. Laying chickens were used for both passive immunisation using turkey antibodies against adipocyte membranes and active immunisation with modified broiler adipocyte membranes. Egg production from immunised chicken was decreased in the early stages after passive immunisation only, but generally there was no significant difference between immunised and control chicken for both passive and active immunisations. There was no build up of antibodies in the eggs in the passive immunisation, even though the concentrations in the blood were high. This was probably because the antibodies did not have enough time to enter the eggs. On the contrary, the active immunisation showed specific antibody build up at the

*Strategic, Environment and Natural Resources Research Centre, MARDI Headquarters, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

Authors' full names: Zainur Alsmi Sharif, Shukran Baki Ismail and Masnizar Mustapha
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second and third immunisations. The concentrations of the antibodies in active immunisation were also higher as compared to passive immunisation. Therefore, eggs from active immunisation could be easily selected for breeding purposes at the peak antibody production. There were also no significant differences ($p > 0.05$) between treatments in terms of weights of carcasses and various organs of layer hens. This is an indication that immunisations caused no side effects on the laying hens.

Introduction

Immunisation against adipocyte membranes has been used to manipulate fat deposition in many animal species (Flint 1990). Its utilisation can either be by passive immunisation of antibodies raised in another species of animal into a target species or it can be by the direct active immunisation of the membranes (from another species or a modified version of the same species) into the target species. In chicken, passive immunisation of sheep antibodies against adipocyte membranes into broiler chicken caused side effects (Butterwith et al. 1989).

In a more recent study (Zainur and Shukran 1999) the active direct immunisation of broilers with modified adipocytes membranes from layer chicken induced antibody production and yielded bigger carcasses with less fat. However, this direct immunisation of the antigen into broilers was not feasible since it was too laborious and would incur too much cost. In this study therefore, attempts were thus made to pass the antibodies to the eggs since each chicken will lay many eggs. The effects of the antibodies on the potential embryos in the eggs will be the topic for future research.

The production of antibodies in eggs is increasingly becoming more useful in the field of immunology since the immunoglobulin level of eggs is much higher than that of serum of mammalian species (Akita and Nakai 1993). It is also relatively easy to collect and sample eggs. This type of sampling also has no deleterious effects on the animals concerned (Polson et al. 1980). Chicken eggs have been used for the production of antibodies

against viruses (Burdshall et al. 1990), hormones (Chung-seog et al. 1985) and other peptides (Otani et al. 1989; Schmidt et al. 1989; Pinchasov et al. 1994).

This study hopes to produce antibodies against adipocyte membranes in eggs and therefore to optimise the transmission of these antibodies to as many chickens as possible. This was done by either the passive immunisation of turkey anti adipocyte membranes antibodies into laying chicken or the active immunisation of laying chicken with heated broiler adipocyte membranes. Preliminary assessments of the effects on the layer mother and the antibodies extracted from the eggs are discussed in this paper.

Materials and methods

The transmission of antibodies against adipocyte membranes to eggs were done by two methods:

- 1) Passive immunisation of turkey antibodies against adipocyte membranes into the target species (layer chicken).
- 2) Active immunisation of the adipocyte membranes itself into layer chicken.

Antigen preparation and characterisation

The adipocyte membranes were prepared and characterized following Zainur and Chong (1996). Membranes were collected from various fat depositions but more were obtained from the subcutaneous depositions than other depositions. The membranes contained nine types of proteins. Antibodies developed in turkeys against these membranes were cytotoxic at levels of more than 50% to those of normal serum (Zainur 1999).

Animal trials

Passive immunisation Turkeys that were injected with adipocyte membranes produced good antibody titres to it (Zainur and Chong 1996; Zainur et al. 1999). The antibodies from the turkeys with high titres were pooled and passively injected into laying chickens in two separate trials (I and II). In both trials, the concentration of antiserum and normal serum injected were 53.4 and 51.1 mg protein per kg body weight of chicken. These were in volumes of 1.5 mL each. All injections were done on the brachial arteries for three consecutive days. In trial I the four chickens used were numbered 27, 75, 5, and 23 while in trial II the four chickens used were numbered 8, 15, 44 and 45. The chickens were fed layers' diets throughout the trial. Eggs were collected daily for 30 days after the infusion. In trial II the chickens were slaughtered after 30 days and the carcasses analysed. The blood was also collected for blood metabolite analyses.

Active immunisation This trial consisted of 20 layer chickens divided into two treatments of immunised and control chickens. The control chickens were injected with saline while the immunised chickens were injected with 250 mg of the adipocyte membranes. The immunisations were done 4 times at intervals of 3 weeks each. The immunisations were similar to direct immunisations of broilers (Zainur and Shukran 1999). The chickens were fed layers' diets and the eggs collected daily throughout the trial.

Analyses

Blood Blood samples were obtained after the passively immunised chickens in trial II were slaughtered. Serum was separated from the blood and then used in analyses of antibody titres using similar ELISA systems as Zainur et al. (1998).

Eggs Antibodies from the eggs (now known as IgY) were extracted using the

method of Jensenius et al. (1981). In this method, eggs were broken and the yolk extracted. The yolks of a particular chicken were pooled for 7 days and 10 mL were sampled out and mixed with 90 mL distilled water for freezing. After freezing the sample was thawed and spun at 4 500 rpm for 40 min at 20 °C. The supernatant was taken mixed with 20 mL $(\text{NH}_4)_2\text{SO}_4$, 1 mL K_2PO_4 and pH adjusted to 7.6. The mixture was again centrifuged for the second time at 4 500 rpm for 40 min at 20 °C. The pellet was taken mixed with 5 mL TBS and pH adjusted to 7.3 and again centrifuged for the third time at 4 500 rpm for 20 °C. The supernatant was then mixed with 6.2 mL $(\text{NH}_4)_2\text{SO}_4$ and centrifuged 4 500 rpm for the fourth time for 40 min at 20 °C. The pellet was taken mixed with 3–5 mL TBS and pH adjusted to 7.3. The mixture was finally dialysed with TBS at 4 °C and the sample stored at –20 °C before analyses.

Organs All the chickens from trial II of the passive immunisation were slaughtered at the end of the trial. The carcasses and the main organs were weighed and statistically analysed following covariate analyses. Values were standardised to a standard carcass weight of 1 173.98 g as the covariate.

Results and discussion

Animal trials

Egg production from passive

immunisation The percentage of eggs produced from the passively immunised chicken of trial II is as shown in *Figure 1*. There was no difference ($p > 0.05$) between immunised and control chickens. However, the immunised chickens showed a dropping trend in egg production at the start of the infusion, but increased later to around 65% production capacity. This must be the effect of the immunisation and not due to the injection itself since the pattern in egg production of control was almost similar to the immunised. Like the immunised the egg production of control was maintained at

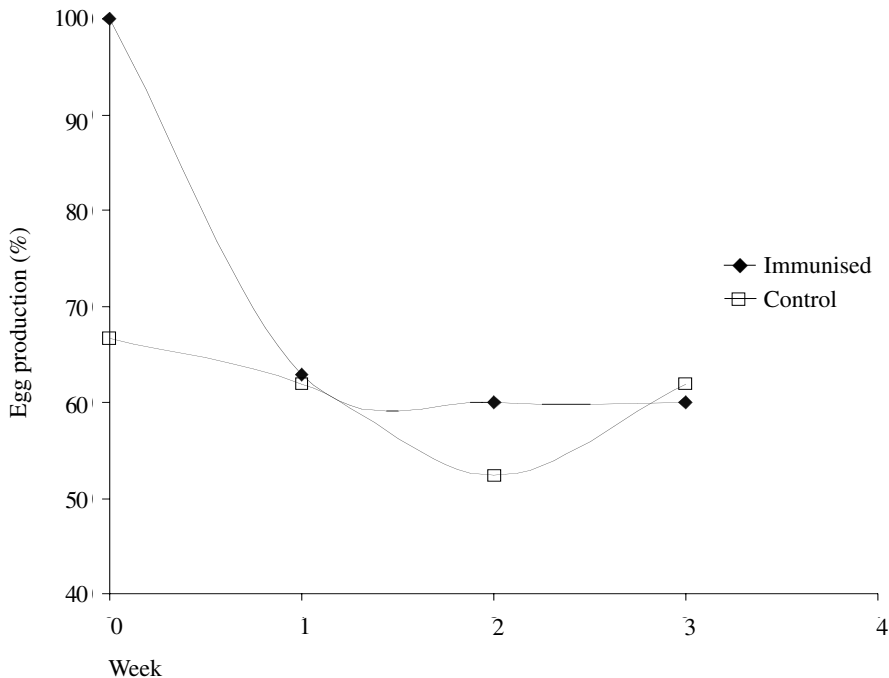


Figure 1. Egg production of passively immunised and control layer chickens

around 65% production capacity. This observation was similar to the egg production of chicken immunised with plasma apolipoprotein VLDL-II, which was around 65–85% (Pinchasov et al. 1994).

Egg production from active immunisation

Similarly in the active immunisation (Figure 2), the egg production dropped after the initial immunisation, but stabilized later. It is possible that chickens became insensitive at later injections and were thus able to maintain their egg production. As seen in both trials I and II the immunised chickens were affected only after the first immunisation and the egg production increased to levels similar to the control. These phenomena enhanced the recommendations of the ECVAM workshop as edited by Schade et al. 1996, for the suitability of this method (using eggs to develop antibodies) to animal welfare groups.

Antibody transmission and production

Passive immunisation The antibody titre of the IgY extracted from the eggs from the passive immunisation trial I and trial II are shown in Figure 3 and Figure 4 respectively.

In trial I the antibody titre increased after 10 days of immunisation for chickens no. 75 only, but no such trends were seen in the others. This was later confirmed in trial II, where no fixed pattern of antibody production was seen.

In trial II antibody transmissions did not follow any specific pattern. They were random throughout the trial and were not related to the time of laying of the eggs. Antibody infusions into the eggs must therefore be random amongst the newly formed eggs and the matured eggs. These results thus suggest that it is difficult to select eggs that contained peak antibody for breeding purposes when laying mothers were passively immunised with antibodies against adipocyte membranes.

Analyses of the sera of the passively immunised chicken at slaughter showed that

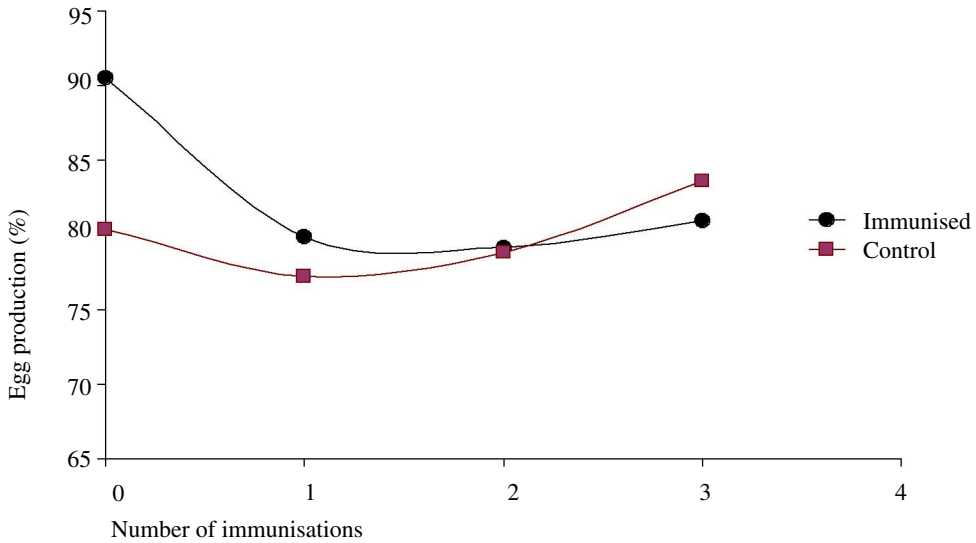


Figure 2. Egg production of actively immunised and control layer chickens at different immunisations

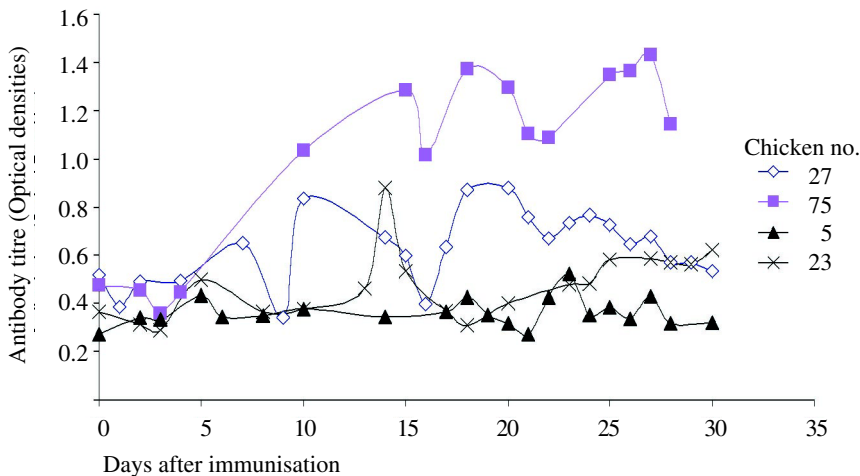


Figure 3. Antibodies (IgY) in eggs from different chickens after passive immunisation (Trial I)

the optimum specific concentrations of the antibodies were around 1:100 000 dilutions as shown in *Figure 5*. This concentration was similar to the optimum specific concentration of the original turkey antibodies used to passively immunised these chickens. This therefore, suggests that the infused IgG from turkeys might not have entered the eggs and were still in the circulatory of the chickens after about 30 days of being infused. This explained the low antibody titres in eggs observed in both

trial I and trial II of this passive immunisation.

Active immunisation The IgY titres of the eggs from the actively immunised chicken is shown in *Figure 6*. There was a distinct increase in the titre of the immunised chicken after the second immunisation. This has been seen by many other workers working with antigen either from virus origin (Burdshall et al. 1990), short peptides (Otani et al. 1989; Schmidt et al. 1989;

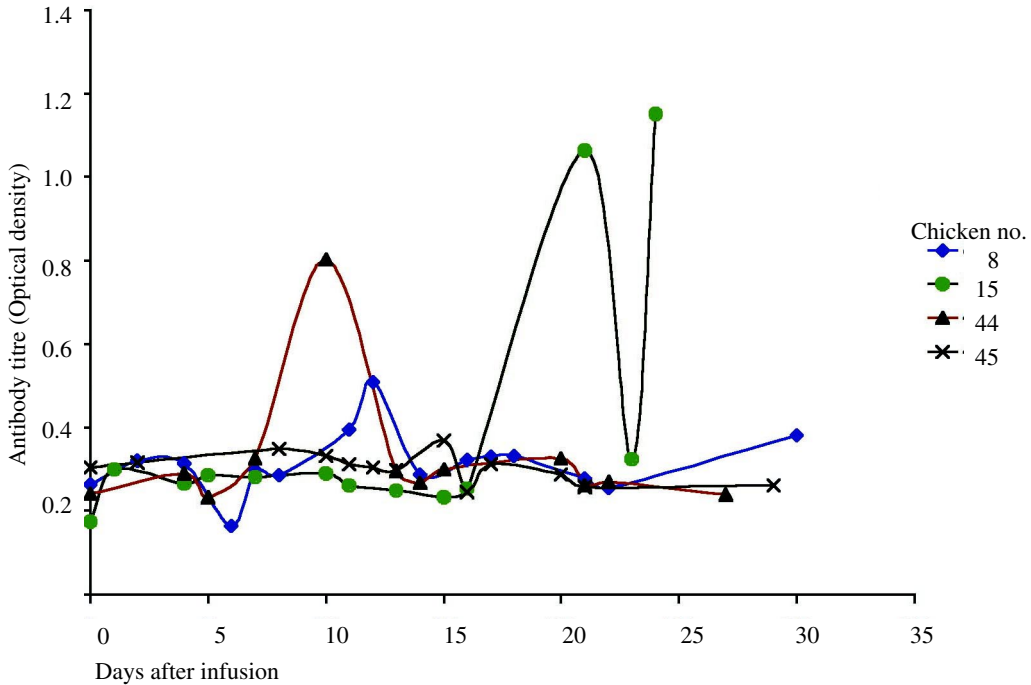


Figure 4. Antibody titre (IgY) of eggs from different chickens after passive immunisation (Trial II)

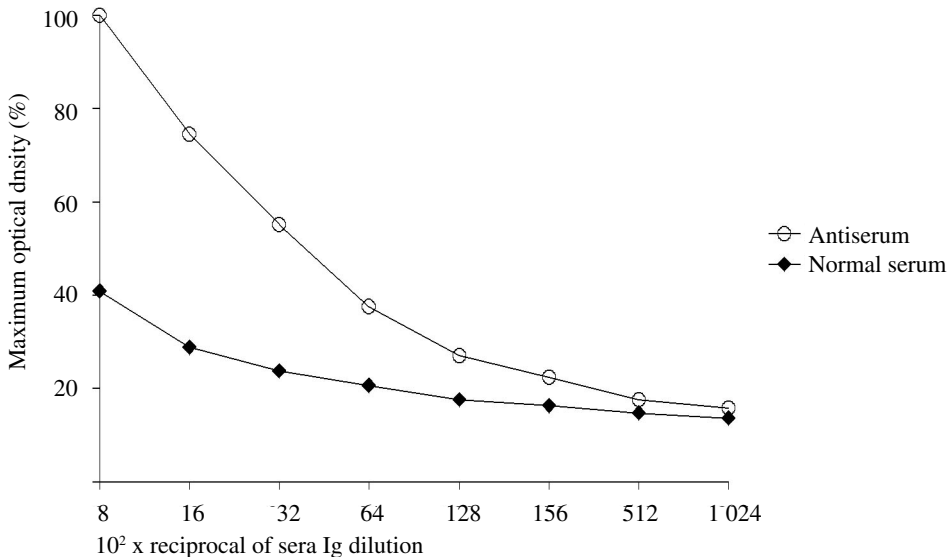


Figure 5. The specific concentrations of antibodies (IgG) from the blood of passively immunised chicken (Trial II)

Pinchasov et al. 1994) or hormones (Chung-Seog et al. 1985). The antibody production peaked at the third immunisation with a maximum specific concentration of 1:100 000 dilutions. This suggests that eggs

could be collected around the third immunisation for breeding purposes. This concentration of antibody is higher than the antibody concentration of the sera of broiler chickens which were actively immunised

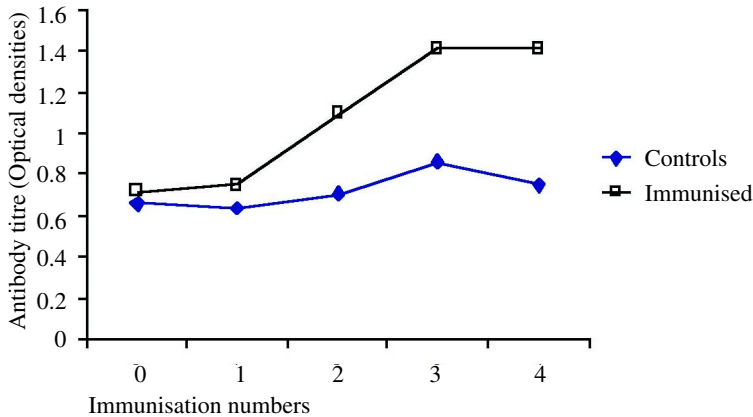


Figure 6. Antibody titre of eggs (IgY) after active immunisation

Table 1. Transformed weights of organs of passively immunised and control layer chickens (Trial II) following a standard covariate carcass weight of 1 173.98 g

Parameters	Immunised	Control
Carcass dressing (%)	68.48 ± 0.672	70.15 ± 0.868
Liver weights (g)	36.60 ± 2.173	34.33 ± 2.807
Heart weights (g)	7.26 ± 0.245	7.17 ± 0.317
Kidney weights (g)	11.19 ± 0.585	11.31 ± 0.756
Gizzard weights (g)	44.19 ± 2.760	42.98 ± 3.567
Spleen weights (g)	4.08 ± 0.104	3.95 ± 0.135

using the same antigen (Zainur et al. 2000). Similarly Akita and Nakai (1993) found that the concentration of antibody in egg yolk is higher than that found in the serum of either chickens or rabbits or humans. This may be probably due to the role of eggs in the conferment of immunity to the young. Laying chickens also formed antibody at a higher rate, which is equivalent to that of cows or horses (Gassmann et al. 1990; Larsson et al. 1993). The effects of these antibodies on the future embryos from the eggs will be further investigated.

Organ weights of passively immunised chicken

There were no statistical differences ($p > 0.05$) between the control and immunised chickens in terms of the organ weights of the laying mothers, even though there were increasing trends (Table 1). As discussed earlier the presence of the

antibodies in the circulatory system suggest that there was not enough time for the antibodies to enter the organs and will not cause harm to the animals.

Conclusion

Antibodies against adipocyte membranes could be transmitted to the eggs in laying chicken. This was better achieved by active immunisation with the adipocyte membranes than by passive immunisation with turkey antibodies against adipocyte membranes. Active immunisation developed greater concentration of antibodies and clearly showed the peak antibody production than passive immunisation. This thereby facilitated the selection of eggs for breeding purposes. The effects of the antibodies on future embryos in the eggs is the subject of our current investigations.

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