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Reducing fat deposition in poultry by immunisation against adipocyte membranes: II. Effects of active immunisation on chicken performance and carcass composition

(Mengurangkan lemak melalui imunisasi terhadap membran adiposit pada poltri: II. Kesan imunisasi aktif terhadap prestasi dan kandungan karkas ayam)

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Key words: poultry, performance, fat reduction, carcass composition, blood metabolites, adipocyte membranes, active immunisation

Abstrak

Kajian ini adalah untuk menentukan sama ada imunisasi aktif boleh menggantikan imunisasi pasif yang memerlukan antibodi yang banyak apabila penyelidikan dijalankan. Ketahanan antibodi pasif yang disuntik ke dalam sistem darah ternakan itu juga tidak lama. Dalam imunisasi aktif yang dijalankan ini antigen dalam bentuk membran sel lemak telah disuntikkan (250 μg sel membran lemak/ekor/suntikan) ke tubuh ayam sebagai ternakan sasaran. Hasil kajian aktif ini menunjukkan bahawa sejumlah 77.8% ayam mengeluarkan antibodi yang maksimum pada tahap imunisasi ketiga. Kepekatan spesifik antibodi adalah dalam kadar pencairan 1:10, 000. Dari segi prestasi, ayam yang diimunisasi kurang makan tetapi karkasnya lebih besar berbanding dengan yang dikawal. Kajian juga menunjukkan bahawa ayam yang diimunisasikan mengalami kekurangan lemak sebanyak 17% berbanding dengan ayam kawalan. Pengurangan ini berbeza antara depo lemak (kekurangan lemak dada 5%, lemak leher 15%, dan lemak lain 41%). Tahap pengurangan lemak juga bertambah mengikut penambahan imunisasi. Selain ini, tidak ada perbezaan antara ayam imunisasi dengan ayam kawalan melalui kajian metabolit darah seperti urea, glukosa, kretinin, trigliserida, kolestrol, GOT dan bilirubin. Ini menandakan bahawa ayam ini tidak mengalami kesan sampingan. Memandangkan kesan kajian ini hampir serupa dengan kajian imunisasi pasif, ini menunjukkan bahawa imunisasi aktif mungkin boleh mengambil alih imunisasi pasif.

Abstract

This study was to find out the possibility of replacing passive by active immunisation. This is because passive immunisation requires too much antibodies for injection and these antibodies were also short lived in the animals' circulatory system. This was done by comparing the effects of active immunisation of chicken to those of passive immunisation as done in many earlier works. Active immunisation involves the injection of 250 μg adipocyte membranes/chicken/immunisation. This study showed that active immunisation produced maximum antibody production after the third immunisation where 77.8% of the immunised chicken produced antibodies. The specific concentration of antibodies was at a

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dilution of 1:10, 000. In terms of performance it was shown that the immunised chicken as compared to controls were eating less while carcasses were bigger. The carcass compositions of these immunised animals as compared to controls showed that there was a reduction of 17% in total fat and these reductions vary between depos (breast fat 5%, neck fat 15% and other fat 41%). Increasing the number of immunisations also increased the reductions of fat in these depos. The immunisation also did not show any adverse effects on metabolism as indicated by values of various blood metabolites like urea, glucose, creatinine, triglycerides, cholesterol, GOT and billirubin. The similarities of these effects to those seen earlier in passive immunisations suggest that there is a possibility of replacement of passive immunisation by active immunisation.

Introduction

Excessive fat deposition in poultry presents many problems and various attempts in reducing it have been done (Futter and Flint 1990). One such method is the manipulation of adipocity by the use of adipocyte (fat) membranes as the antigen for immunisation (Flint 1990). In Part 1 of this paper, study on the extraction and characterisation of the antigen, its antigenicity to produce antibodies and its cytotoxicity to lyse adipocyte membranes were described (Zainur et al. 1999). In most of the studies done earlier the antigen was injected via passive immunisation where the antibodies against adipocyte membranes were injected directly into the blood stream of the animal (Butterwith et al. 1989; Futter and Flint 1990; Nassar and Hu 1991; Kestin et al. 1993). Passive immunisation however, requires big quantities of antibodies for each injection. The retention interval of these antibodies in the circulatory system of the animals too is uncertain. Conversely, in active immunisation, as is done in this study, endogenous antibodies are expected to build up with a minimum amount of the injected antigen and these antibodies will be present in the animals' circulatory system longer than passive immunisation. However, booster immunisations are required in active immunisation. This study therefore, aims to compare some of the differences in effects of active immunisation to those of passive immunisation of adipocyte membranes in broiler breeder chicken.

Materials and methods

The antigen in the form of adipocyte membrane was extracted as described previously (Zainur and Chong 1996a) and characterised (Zainur and Chong 1996b; Zamri and Zainur 1996; Zainur and Shukran 1997). A total number of 84 layer breeder (6 months old) chicken were fed standard breeder's ration for the 4 months trial. They were divided into two equal treatments of controls and immunised groups. The immunised chicken were injected subcutaneously with the antigen every 3 weeks at a rate of 250 mg of protein per chicken. Immunisation which started when the chicken were 6 months old were done for 4 months. The feed intake of the chicken was monitored daily. At the end of each immunisation, 8 chicken from each group were slaughtered and their blood and tissues sampled and analysed. Blood samples were taken for the determination of antibody titres (Zainur and Chong 1996a) and for the analyses of various blood metabolites following dry chemistry techniques as developed by the manufacturer of the Reflotron machine (Boehringer Manheim). Adipocyte tissues were manually dissected from the breast region, neck region and the whole body while the meat and the bones were dissected from the whole body. These tissues were weighted and statistically analysed following standard covariate analyses using the average carcass weight as the standard covariate.

Results and discussion

Antibody production

In this study the antibody production of the actively immunised chicken are as shown in *Figure 1*. Antibody production increased with increasing number of immunisations with the third immunisation having maximum amount of antibodies where 77.8% of the birds were producing antibodies. The maximum specific antibody concentration was at a dilution of 1:10,000. However, the antibody production of the same antigen in turkeys had been examined earlier where 100% of the turkeys were producing antibodies (Zainur and Chong 1996b). The antibody production of the turkeys was also maximum after the third immunisation with a concentration of 1:100,000. The differences in antibody production between chicken and turkeys can be explained by the ability of the antigen to induce antibody production (immunogenecity). The antibody concentration dropped slightly after the fourth immunisation probably because of many reasons including the pooling of the different chicken serum sample, the antigen used may be excess causing self tolerance and the many manual errors involved during the analyses. The lower immunogenecity of this antigen when injected into chicken as compared to turkeys must be due to the process of self-protein recognition since the antigen used were also chicken protein. However, there were still responses in the chicken, probably because some of the membrane proteins might have been denatured during the process of extraction. Unfortunately, in this study other species membranes could not be used since they cross reacted to the other membranes as had been noticed earlier by Butterwith et al. (1989).

Feed intake and feed conversion

Increasing number of immunisations seemed to have decreasing trend on the feed intake *(Figure 2)* of both control and immunised chicken and without any overall differences. However, differences occurred at the different immunisations where immunised chicken ate less (p <0.05) than control chicken, except at immunisation 1. The decrease in feed intake of immunised birds was more than controls in immunisations 2 and 3 (p <0.05). This decrease, however, did not jeopardise the performance of the birds as indicated by the increase in carcass weights during these immunisations *(Figure 2).* This therefore, suggests that the immunised chicken were more efficient in converting feed into meat than control chicken *(Table 1).* Similar results were observed in passive immunisation studies in rats. Animals treated with antibodies against adipocyte membranes had better growth than

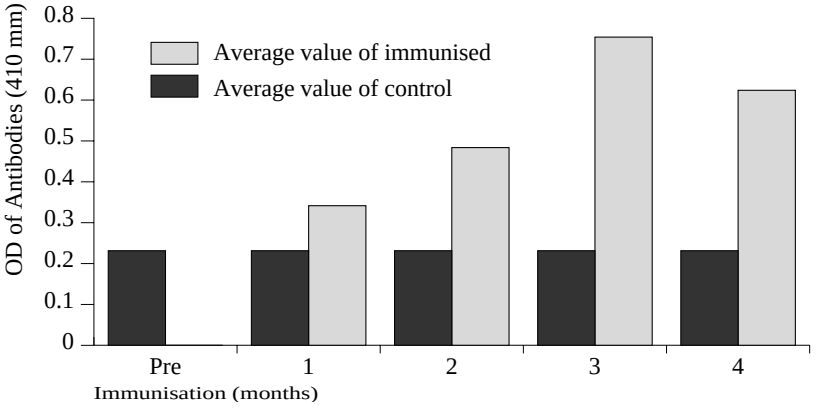

Figure 1. Antibody titres (in optical densities) of control and positive chicken after consecutive immunisations with adipocyte membranes

controls resulting in a better feed conversion efficiency by about 15% after 8 weeks of treatment (Panton et al. 1989). The difference in carcass weights of immunised birds were greater than controls at increasing number of immunisations This is similar in studies of active immunisations of hormones where the live weights of sheep became greater at increasing number of immunisations (Spencer et al. 1983a; Zainur et al. 1990; Zainur and Tan 1995).

Carcass composition

The effects of active immunisation against adipocyte membranes on the carcass composition of chicken are as shown in *Table 2*. There was a 17% reduction in the amount of total fat in immunised animals as compared to controls. This reduction, however, varies between the various depos where the breast fat had 5%, the neck had 15% and the other depos had 41% reduction in amount of fat. Futter and Flint (1990) suggested that the reaction of the antibodies against adipocytes membranes resulted from the decrease in fat cell numbers or by the reduction in fat cell volumes. Similarly Truscott et al. 1983 found that in cattle the increase in cell volume (hypertrophy) or in cell number (hyperplasia) vary between depos. The perirenal depo grew almost exclusively by hypertrophy while the subcutaneous depo grew up to 13 months by mainly hypertrophy. After this the cells of the subcutaneous depo then grew by both hypertrophy and hyperplasia. The same explanation may be true for the chicken. The fat reduction in the different depos seen in this study must be a balance between these two processes resulting in different number of cells in the different depos.

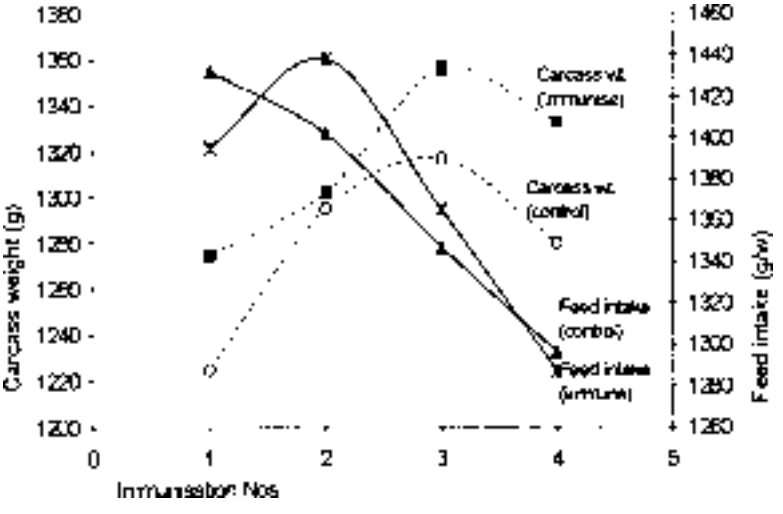

Figure 2. Feed intake and carcass weight of control and actively immunised chicken

Table 1. Effects of active immunisation on feed intake (g) per weight of carcass (g)

Immunisation	Feed intake (g)/carcass weight (g)	
	Control	Immunised
1	1.17	1.10
2	1.08	1.10
3	1.02	1.01
4	1.01	0.97

The effects of passive immunisation as done in other studies also showed long term effects in fat reduction as were seen in rats (Futter and Flint 1987), sheep (Moloney and Allen 1989) and pigs (Kestin et al. 1993; De Clercq et al. 1997). In these studies the mechanisms of reactions might have been where the exogenous (injected) antibodies acted directly on the antigen (fat cell membranes) with the help of compliments (Flint et al. 1986).

In terms of immunisations, the results of this study *(Table 3)* showed that the percentage decrease between the first and the fourth immunisation were 17% for total fat, 15.5% for neck fat, 4.7% for breast fat and 40.8% for other fat. Similarly, in studies on percentage involving active immunisation of hormones, the percentage reduction of body fat also increased with greater number of immunisations as seen in sheep (Spencer et al. 1983a) and cattle (Zainur and Zainab 1994). This was due to the increasing neutralising effects of the increased amount of endogenous hormones. Thus, the responses of animals seen in the different immunisations of this study could be similarly explained. The build up of endogenous antibodies with increasing number of immunisations caused greater reduction in fat deposition of immunised animals as compared to control animals as

Table 2. Effects of active immunisation against adipocyte membrane on the carcass composition of chicken (Value adjusted to average body weight by using 1791.5±129.8 g as the standard covariant)

Parameters	Control	Immunised
Carcass weight (g)	1295.48±14.380	1319.19±16.277
Dressing (%)	72.33±0.811	73.58±0.919
Breast fat (g)	16.92±1.079	15.33±1.222
Neck fat (g)	12.07±0.824	10.59±0.933
Other fat (g)	7.03±1.506	3.79±1.705
Total fat (g)	36.02±2.204	29.71±2.495
Meat (g)	540.09±7.027	552.28±7.954
Bone (g)	443.88±7.529	462.19±8.523
Skin (g)	*127.48±3.08	136.15±3.48
Meat : Bone ratio	1.22±0.02	1.21±0.02
Meat : Fat ratio	19.25±1.402	21.24±1.587

$^{*}p$ <0.05

Table 3: Effects of the different immunisations against adipocyte membranes on the carcass compositions of chicken (using body weight of 1791.5±129.8 g as the standard covariant)

Parameters	Immunisations			
	1	2	3	4
Carcass weight (g)	^{A}1274.5±35.97	^{A}1302.1±40.00	^{A}1355.8±33.01	^{A}1332.8±32.85
Dressing (%)	^{A}70.9±2.03	^{A}72.5±2.26	^{A}75.5±1.86	^{A}74.4±1.85
Breast fat (g)	^{A}19.7±1.87	^{B}10.9±2.07	^{AC}17.2±1.71	^{B}12.4±1.70
Neck fat (g)	^{A}11.5±1.59	^{A}8.2±1.77	^{A}10.7±1.46	^{A}11.2±1.45
Other fat (g)	^{A}3.5±0.78	^{A}4.2±0.86	^{A}3.7±0.71	^{A}3.8±0.71
Total fat (g)	^{A}34.8±3.58	^{B}23.3±3.98	^{AB}31.6±3.29	^{AB}27.5±3.27
Meat (g)	^{A}528.6±16.51	^{AB}522.9±18.35	^{AC}570.6±15.14	^{C}574.1±15.07
Bone (g)	455.4±23.34	486.2±25.95	479.5±21.42	434.6±21.32
Meat:bone ratio	^{A}1.16±0.044	^{AB}1.07±0.049	^{AC}1.22±0.041	^{C}1.32±0.041
Meat:fat ratio	^{A}16.61±2.754	^{B}26.43±3.062	^{AB}19.58±2.527	^{AB}23.56±2.515

Different superscript represents $p < 0.05$

were seen in immunisations 3 and 4 *(Figure 3)*. Alternatively, the effects may also be due to the increased neutralisation of the endogenous membranes (antigen) by the increase in endogenous antibodies.

There was little difference in dressing percentage, total meat and total bones of immunised as compared to control chicken *(Table 2).* The immunised chicken however, had more (p <0.01) skin than control birds.

Similar effects were seen on feed utilisation and carcass composition of both active immunisation and passive immunisation. This thus suggests that active immunisation as had been done in this study may form a good alternative method for passive immunisation, provided that the frequent injection does not jeopardise the health of the animals or incur excessive input costs. The dosage used in this study of 250 ug/immunisation/bird was lower than passive immunisation of 500 mg/bird/day for 3 days. The immunogenecity of the antigen can also be further improved by using turkey instead of layer chicken adipocyte membranes.

Blood metabolites

The effects of the immunisations on some blood metabolites are as shown in *Table 4.* There were no significant differences between immunised or control chicken in terms of metabolites like GOT, glucose, urea, creatinine and billirubin indicating that the metabolism of the chicken were not upset by the immunisations.

Looking at the metabolites which are more related to fat metabolism such as triglycerides (TG) and cholesterol, as shown in *Figure 4*, the patterns of TG (simple lipids) and cholesterol (complex lipids) showed little differences between control and immunised chickens. This suggests that the reduction in fats might not be through the process of lipolysis or fat breakdown and which would have resulted in increases of triglycerides and glycerol in the blood (Nassar and Hu 1991). Instead it could be due to a reduction in the process of lipogenesis or fat build up in chicken that were actively immunised against adipocyte membranes.

Conclusion

The antigen developed in this study was immunogenic and antibody development increased with increasing immunisations. Active immunisation of adipocyte membranes tends to decrease feed intake but resulted in bigger carcasses. Like passive immunisation, active immunisation here reduced fat deposition of different depos in chicken without any obvious increase of blood metabolites associated with problems

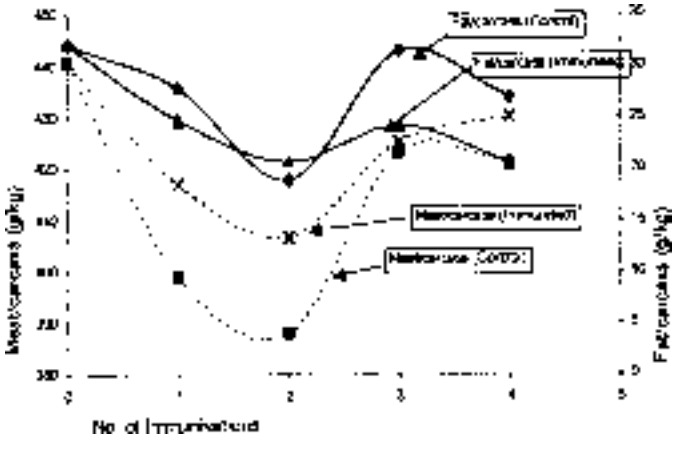

Figure 3. Effects of immunisations on the deposition of fats and meat per kg carcass weight in the carcass of chicken

Table 4. Effects of active immunisation of chickens against adipocyte membranes on blood metabolites

Metabolites	Immunisations							
	1		2		3		4	
	I	C	I	C	I	C	I	C
Triglyceride (mg/dl)	1334±491	1446±469	*1501±235	1419±476	1196±524	831±548	826±329	949±322
HDL cholestrol (mg/dl)	24.3±12	31.3±16	30.1±11	33.1±9	36.8±9	32.8±11	15.8±7	24.6±12
GOT (mg/dl)	156.9±43	140.2±22	119.6±15	169.4±110	121.6±53	94.5±37.6	186.9±40	144.4±49
Glucose (u/l)	205.9±14	200.7±10	201.4±13	191.1±27	189.1±37	207.8±15	205.5±19	212.6±21
Urea (mg/dl)	<20	<20	<20	<20	<20	<20	<20	<20
Creatine (mg/dl)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Billirubin (mg/dl)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

*p <0.05
I: Immunised C: Control

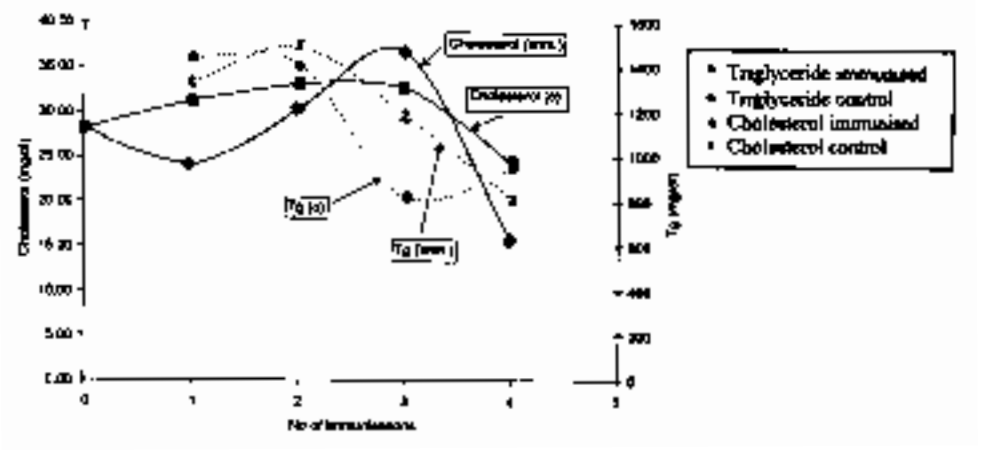

Figure 4. Effects of immunisations on the triglycerides (TG) and cholesterol levels in blood of chicken

in fat metabolism. Active immunisation may therefore be a good alternative to passive immunisation since less antigens were used and presence of antibodies were longer in the circulatory system than passive immunisation. This technology would be feasible for chicken provided a proper delivery system is developed. It may be possible if the embryos in the eggs from the broiler breeders were used as the target animal since a single breeder will lay many eggs. This angle of investigation is currently being looked at. This study can also be a model for the use of this technology in bigger and more expensive meat producing animal species.

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