Effect of Sheep and Chicken Antibodies to Rat Adipocytes Plasma Membranes on Rat Carcass Fat*

Jundong Wang, Jianfeng Zhang, Junping Li, Junhu Hao, Shaolin Wang[1] and Jianhai Zhang**

Shanxi Key Lab. of Ecological Animal Science and Environmental Veterinary Medicine

Shanxi Agricultural University, Taigu, 030801 Shanxi, P. R. China

ABSTRACT : Polyclonal anti-sera were collected from sheep and chicken immunized with adipocytes plasma membranes. Thirty two male wistar rats, weighing 185-215 grams, were divided randomly into 4 groups (trial 1: control group and treat group, trial 2: control group and treat group), with 8 rats in each group. The experiment lasted for 7 weeks. Trial one: The control group received four consecutive daily intraperitoneal injections of 1ml of sheep normal sera. The same 4 day daily dose of group sheep anti-rats sera adipocyte plasma membrane anti-sera was administered to the treat group. The results showed that the treatment for treat group increased body weight by 6.35% ($p<0.05$) and food intake by 6.85%, and improved food conversion efficiency (Food intake/gain) by 45.00% ($p<0.05$). Periernal, epididymal and omental adipose deposit weights were decreased by 23.92% ($p<0.05$), 34.45% ($p<0.05$) and 0.98% respectively, while total fat content decreased by 20.92%. Trial two: The control group received four consecutive daily intraperitoneal injections of 1 ml of chicken normal sera, the results of injections of chicken anti-rats sera adipocyte plasma membrane antis-era administered to the treat group indicated that chicken anti-rats adipocyte plasma membranes immunization had an disadvantageous effect on the growth of the wistar rats by the end of 7th wk, compared with the control group. The immunized group decreased in total weight by 40 gram ($p<0.05$) an averagely and in food intake noticeably ($p<0.01$). The deposition of fat and the rates of TG and FFA in serum had no statistical significance. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8 : 1177-1182)***

Key Words : Adipocyte Plasma Membranes, Chicken, Fat Content, Passive Immunization, Sheep, Wistar Rat

INTRODUCTION

Researchers have long been searching for safe methods for decreasing the fat sediment in livestock, in order to satisfy consumers' need for high-protein and low-fat animal food. The existing methods include genetic breeding (Wang, 2003), food supplementation (Xi et al., 2002; Shim, 2004), metabolism modification (Kim, 2002; Loh et al., 2002; Lien, 2003), and the application of hormone and adipocyte plasma membranes immunization. Hormones have been forbidden owing to safety concerns. The genetic breeding can obviously increase the amount of lean meat in some species, but the spread of species with high-lean meat percentages results in the fact that many local species with excellent production performance confront the danger of extinction only for the low-lean meat performance (Marks, 1997). This is the case in China. Thus, it is necessary to look for non-inheriting methods of protecting biological diversity and excellent local species. Our researches have indicated that betaine, L-carnitine, or herbal medicine can produce the effect of decreasing fat to some degree (Wang, 1993; Wang, 1994). However, every additive substance is exotic, and not absolutely safe.

Decreasing fat by passive immunization on the APM (adipocytes plasma membrane) is a better idea, for antigen and antibodies are natural materials from the animal itself and are safe. There were a few studies which obtained some success in decreasing fat on experimental animals and some species of livestock (Moloney, 1989; Panton, 1990; Nassar, 1991; Kestin, 1993), but there are still some problems to be solved on regulating animal fat through APM immunization. This experiment aims to study: 1) The effect of sheep antibodies on rat APM immunization. 2) The effect of chicken antibodies on rat APM immunization. 3) The mechanism for decreasing fat through passive immunization, with the purpose of providing good methods for decreasing the fat of some local swine species.

MATERIAL AND METHOD

Material

Forty two male wistar rats (185-215 g), from, Shanxi Medicine University's Experiment Animal Center; an emasculation sheep, and 10 hens (without immunity) were employed. Food for the rats came from Shanxi Medicine University's experiment animal center. Food for the chickens was provided by Zhengda Food Company. BCG vaccine came from Taigu Disease Prevention Station. Collagen enzyme II (No. C6885) came from Sigma Company. PBS solution was used for decomposing solutions of collagen enzyme (1 mg/ml).

* This work was financially supported by Shanxi Natural Science Foundation (20011089).

** Corresponding Author: Jundong Wang. Tel: +86-354-6288206, Fax: +86-354-6222942, E-mail: wangjd@sxau.edu.cn

[1] College of Veterinary Medicine, China Agricultural University, Beijing, 100094, P. R. China.

Received December 2, 2003; Accepted April 29, 2004

Experiment method

Preparation of adipose cell and adipose cell membrane : Ten male wistar rats (weight 180-210 g) were used for this purpose. Periernal fat tissue was quickly obtained by operation with blooding from the heart. After washing residue blood with PBS at 37°C it was put in a conical flask with 50 ml of decomposing solution and 0.5 ml of sheep serum. Fat tissue was decomposed for 3-5 h at a constant temperature of 37°C, while checking the effect every 30 minutes. This mixed solution was filtered through a 250 μm filter bed, and centrifuged for 5 minutes at 400 g in order to discard surface triaclyglycerol. The middle level solution, full of adipose cells, was collected and washed two times with PBS, resulting in a final volume of 35 ml. Finally the number of adipose cells were calculated by haemacytometer with the result of 8×10^6/ml.

The adipose cells were broken down by ultrawave (50 HZ, 5 min), and centrifuged for ten minutes at 37°C at 2,800 g. After discarding surface triaclyglycerol, the adipose cells sediment was collected. The concentration of membrane protein determined by ultraviolet spectrometry was calculated to be 0.1506 mg/ml by the experience formula (albumin as the control).

The Serum preparations of sheep antibody applied to rats adipocyte membrane and Chicken antibody applied to rats adipocyte membrane : Preparation of vaccine: Complete freud adjuvant vaccine was derived of 11 ml antigen+11 ml complete freud adjuvant, and incomplete freud adjuvant vaccine was derived of 22 ml antigen+22 ml incomplete freud adjuvant.

Immunizing animals : 2 ml of complete freud adjuvant vaccine was injected under the skin of the sheep at four points along two sides of the spine, and two points on two sides of the cervix muscle for the first immunity. Three weeks later, the second immunization was conducted with incomplete freud adjuvant vaccine. Blood was taken on the 7th day for the agar diffusion experiment with positive results (APM as the antigen). Finally, incomplete freud adjuvant vaccine was used for the third immunization after two weeks. Blood was taken from the cervix vein, from which the serum was collected.

The first immunization for the chickens was conducted by injecting 1ml of the chicken complete freud adjuvant vaccine. Ten days later, the incomplete freud adjuvant vaccine was used to strengthen the immunity. On the 7^{th} day, blood was taken for the agar diffusion experiment, which had a positive results. After another 10 days, an incomplete freud adjuvant vaccine was used for the third immunization. Blood was taken from the heart for serum. The serum was bathed at 56°C water for 30 min to make the alexin inactive, and kept in a 4°C refrigerator.

Measuring the concentration of IgG

Grade salt out by ammonium sulfate : First, 5 ml of PBS (0.01 M pH 7.0) and 5 ml of serum were blended, and put on a magnetic mixer. 10 ml saturated ammonium sulfate was slowly added to this solution, and mixed for 15 minutes. After centrifuging for 20 minutes at 3,000 rpm, and discarding the superstratum liquid, the sediment was collected. Second, 10 ml of PBS (pH 7.0, 0.01 M) and 5 ml of saturated ammonium sulfate solvent were added to the sediment with 33% of the concentration of ammonium sulfate. Third, the pH of the solution was adjusted to 7.0, kept in a 4°C refrigerator for 15 minutes, and the centrifuged for 15 minutes at 3,000 rpm. After discarding the superstratum liquid, initial IgG was collected, resulting in a volume of 5 ml. Finally, salt ion was removed by dialyse.

The concentration of the antibody was measured by ultraviolet spectrometry using the standard curve method. The concentration of the sheep antibody in the rats was 8 mg/ml, and the concentration of the chicken antibody in the rats was 5 mg/ml. The blank sera of sheep and chicken were taken as control groups.

Animal experiment

Thirty two (185-215 g) male wistar rats, were divided into 4 groups randomly, which were weighed 1 week before experiment, each group having eight rats. Two groups were for used the sheep antibody research, and the other two groups are for the chicken antibody research. Every group was immunized, the control group by the celiac injection of 1 ml/h/d of blank serum and the test groups by the celiac injection of 1 ml/h/d of antibody serum. Within the first four days of immunity, the animals were given a limited level of food, and took food freely. The feeding period was 7 weeks. Body weight at the end of every week, and the quantity of food consumed every day were routinely recorded. At the end of 2^{th} and 7^{th} weeks, the animals were slaughtered, and the weights of the carcass, periernal, epididymal and omental adipose depot, and the weights of liver, spleen and kidney were recorded. The concentration of serum triaclyglcerol and FFA were also measured (reagent was purchased from Jiancheng company of Nanjing). The numbers of adipocytes was calculated with the haemacytometer, and the volume of adipocytes was reckoned by the diameter of the adipocytes (the diameter was measured with the scaled object lens).

Data analysis

All data were analysed by an SAS statistical software program. Then the data showing significant differences were repeatedly compared using TTEST`s method, with the standards of 0.01 and 0.05.

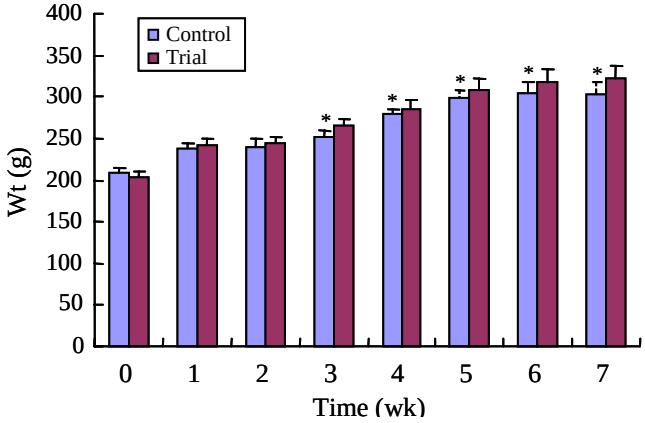

Figure 1. Effect of sheep antibodies to rat APM on rat body weights. * Indicated the differences are significant.

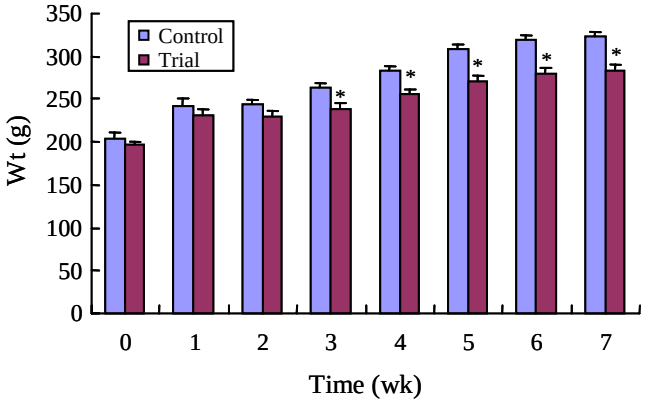

Figure 2. Effect of chicken antibodies to rat APM on rat body weights. * indicated the differences are significant.

Table 1. Effect of sheep antibodies to rat APM on rat growth performance (*X±SE*)

	BW (g/wk/h)	FI (g/d/h)	I/G
0-3 wk			
Control	22.56±2.65	17.69±0.83	6.00±0.49
Trial	26.63±2.67	18.73±0.19	6.82±0.60
3-7 wk			
Control	14.13±0.98	17.64±0.58	10.36±0.93
Trial	14.34±0.87	18.98±0.21*	17.33±1.14**
0-7 wk			
Control	17.74±1.08	17.66±0.44	8.58±1.05
Trial	19.60±1.02	18.87±0.14**	12.47±1.27*

* p<0.05, ** p<0.01.

Table 2. Effect of chicken antibodies to rat APM on rat growth performance (*X±SE*)

	BW (g/wk/h)	FI (g/d/h)	I/G
0-3 wk			
Control	27.94±3.76	19.02±0.12	5.85±0.87
Trial	21.81±2.79	16.87±0.72*	8.20±1.51
3-7 wk			
Control	15.00±2.07	18.60±0.09	9.64±0.98
Trial	12.31±1.15	15.86±0.17*	11.7±1.16
0-7 wk			
Control	20.54±2.07	18.78±0.11	8.01±1.24
Trial	16.81±1.95	16.29±0.35**	10.92±1.73

RESULT

The effect of APM passive immunization on the weight and growth performance

From Figure 1 In the first experiment passiveimmunization with sheep antibody applied to rat AMP_increased the weight of rats to some degree. The weights for the test group by 4th, 5th and 6th weeks were higher than those for control group. The trend showed a greater and greater increase in wetght (3.47, 4.85 and 6.35%) with feeding time. Passiveimmunization with chicken antibody applied to rat AMP had an adverse affect on the weight of the rats, and the weights of the test group were lower than those of the control group by the end of 2nd, 3rd, 4th, 5th, 6th and 7th weeks. The trend showed a perpetually wide decrease in the weights of the test group (5.77, 10.28, 11.15, 13.77, 14.31 and 13.92%) with feeding time (Figure 2).

From Table 1, passiveimmunization with sheep antibody applied to rat AMP promoted food intake, the feed conversion rate, and the growth of the animal. The increasing rates of these three indexes were 6.85, 45.00 and 10.48%. From Table 2, passiveimmunization with chicken antibody applied to rat AMP had an adverse effect on growth performance and food intake, but a less adverse affect on the food conversion rate.

The effect of APM passive immune on the content of fat

From Table 3, passiveimmunization with sheep antibody applied to rat APM decreased the content of fat depots. The weight of perirenal, epididymal and omental fat depots

Table 3. Effect of APM passive immunization on rat fat depot weights (*X±SE*), u:g

		Sheep to rat		Chicken to rat	
		Control	Trial	Control	Trial
2 wk	Perirenal fat	5.04±0.32	3.79±0.30**	3.03±0.30	2.98±0.30
	Epididymal fat	3.39±0.29	2.88±0.23	2.62±0.11	2.41±0.14
	Omental fat	4.90±0.53	3.94±0.54	3.69±0.24	3.83±0.29
7 wk	Perirenal fat	5.77±0.22	4.39±0.43*	4.87±0.55	5.23±0.58
	Epididymal fat	4.76±0.69	3.12±0.23*	3.72±0.30	4.93±0.52*
	Omental fat	4.10±0.21	4.06±0.29	4.48±0.36	4.76±0.46

Table 4. Effect of sheep antibodies to rat APM on the volume and quantity of fat cell (7 wk)

	Control	Trial
Perirenal fat,		
Cell volume (μm)	88.21±3.37	83.37±2.71*
1 g cell No. (105/ml)	6.3±0.4	5.8±0.5*
Epididymal fat		
Cell volume (μm)	76.21±5.52	79.27±4.21*
1 g cell No. (105/ml)	8.2±0.7	7.3±0.6*
Omental fat		
Cell volume (μm)	63.38±4.27	67.47±5.39*
1 g cell No. (105/ml)	14.2±1.7	12.6±1.1*

declined 24.80, 15.04 and 19.59% respectively by the end of the seventh week; and 23.92, 34.45 and 0.98% by the end of seventh week. Generally, body weight increased 19.25 g (6.35%), and the weight of fat depot declined 3.06 g (20.92%) by the end of the experiment. Passiveimmunization with chicken antibody applied to rat AMP reduced the weights of perirenal, epididymal and omental fat depots to some degree by the end of the second week, but increased those fat depots by the end of the experiment to some degree. The body weights of the test group were lower than that of the control group during the period of the experimental.

From Table 4, passiveimmunization with sheep antibody applied to rat APM decreased the number of adipose cells. The number of perirenal, epididymal and omental adipose cells declined 8.62% ($p<0.05$), 10.98% ($p<0.05$) and 11.27% ($p<0.05$) respectively. The volume of perirenal adipose cells decreased by 5.49% ($p<0.05$), but the volume of epidiymal and omental adipose cells increased by 4.02% ($p<0.05$) and 6.45% ($p<0.05$) respectively.

The effect of APM passive immune on the content of blood biochemical indexes

Table 5 shows the increasing trend in the level of blood triaclyglycerol and FFA by the end of the second week, and by the end of the seventh week to some degree, but this has no statistical meaning. Passiveimmunization with chicken antibody applied to rat AMP has no obvious effect on the levels of triaclyglycerol and FFA in the blood.

The effect of APM passive immune on the liver, spleen and kidney

From Table 6, Passiveimmunization with sheep antibody applied to rat APM increased the weight of the liver, spleen and kidney by the end of the second week, but not by the end of the seventh week. Passiveimmunization with chicken antibody applied to rat AMP had no constant effect on the rats.

DISCUSSION

The effect of APM passive immunization on the weight and growth performance

The results of some researches indicated that the effect of APM passive immunization on food intake, body weight and food conversion efficiency of the animals was different after injecting APM polyclonal antibody. The weight of some animals declined during the early days following the injection, and then the speed of growth accelerated and finally the body weight surpassed that of the control group. Others think that the weight of other experiment animals is lower than that of the control groups the entire time (Dulor, 1990). In this experiment immunization with chicken antibody applied to rat APM decreased the weight ($p<0.05$)

Table 5. Effect of APM on the serum FFA, TG of Wistar rat ($X\pm SE$)

	Sheep to rat		Chicken to rat	
	Control	Trial	Control	Trial
2 wk				
FFA (mmol/L)	2.29±0.17	2.37±0.21	3.46±0.32	3.37±0.25
TG (mg/dL)	106.29±11.61	113.50±9.42	123.23±10.52	129.21±8.53
7 wk				
FFA (mmol/L)	1.82±0.17	1.97±0.23	3.08±0.24	3.12±0.31
TG (mg/dL)	99.25±9.80	102.37±10.26	123.00±10.82	119.27±14.49

Table 6. Effect of APM on the liver, spleen and kidney of Wistar rat ($X\pm SE$), u:g

	Sheep to rat		Chicken to rat	
	Control	Trial	Control	Trial
2 wk				
Liver	8.28±0.54	9.59±0.36**	8.67±0.52	7.3±0.79*
Spleen	0.53±0.04	0.61±0.07*	0.57±0.02	0.65±0.03
Kidney	1.89±0.16	2.05±0.11	2.01±0.07	1.78±0.10
7 wk				
Liver	9.75±0.41	9.81±0.55	9.86±0.54	8.91±0.28
Spleen	0.62±0.04	0.64±0.05	0.61±0.01	0.61±0.03
Kidney	1.94±0.12	1.98±0.14	2.13±0.11	2.10±0.18

and food intake of the rats, possiblely as a result of the strong crossing reaction between species. Panton (1990) reported that after immunization with APM, the appetites of the animals were not influenced, and that the greater and greater increase in the weight of the animals were 17 percent during the first 3 weeks, and 40-50 percent from the third to the seventh week. The food conversion efficiency increased 15 percent during the first two months. Hu (1992) indicated that there was small weight difference between the rats with APM polyclonal and the control group. In this study, immunization with APM anti-sera increased the weight of the rats ($p<0.05$), and though food intake declined from 18 g/d to 10 g/d during the first day following the injection, it surpassed that of the control group by the third day and remained higher than the control group for the rest of the experiment. Theoretically, the antibody, as a heterogenous protein, lead to the stress reaction, and to the decomposition of fat at the same time; subsequently, the rise of triaclyglycerol in the blood will result in a rapid decline in food intake. Following that, the animals begin to grow proportionally with the increase in food while also increasing in weight.

The effect of APM passive immunization on the sediment of fat of rats

The passive immunization experiments with mammal APM indicated that the rate of the accrual of fat sediment decreased, and the protein content increased to some degree.

The study discovered that the color of perirental adipose depot in the group injected with anti-sera was brown-yellow (khaki), and very different from that of the control group during the second week, and this may be due to cell infiltration caused by the antibody. By the seventh week, the weights of periernal, epididymal and omental adipose depots were decreased by 23.92% ($p<0.05$), 34.45% ($p<0.05$) and 0.98% respectively, and the total fat content decreased by 20.92%. However, the weight of rats rose. Some reports showed that there was no significant change in the material extracted by aether from the tissues of the animal that received passive immunization (Hu, 1992; Kestin, 1993; Moloney, 1998), so it is inferred that the increasing weight was caused by the protein sediment, which meliorated the quality of the carcass. At the same time, the quantity and value of fat cells measured in this study elucidated that the declining weight of perirental adipose depot was due to the decrease in volume and quantity of adipose cells, but that the declining weights of the epididymal and omental adipose depots were due only to the decreasing quantity of adipose cells, suggesting that passive immunization has the tissue particularity, that is to say, that the antibody produced by passive immunization with perirental adipose cells has a greater effect on perirental adipose depot. In the second experiment, the weights of periernal, epididymal and omental adipose depots did not changed significantly by the end of the second and the seventh weeks, indicating that the method using chicken as the animal producing the antibody is not satisfactory.

The effect of APM passive immunization on the biochemical indexes of blood lipid metabolism

The experiments on big animals showed that the content of TG and FFA in the blood did not change significantly change by the end of the experiment (Nassar, 1991; Kestin, 1993). The present experiment showed that the content of TG and FFA in the blood rose by the end of the 2^{th} and 7^{th} weeks, but that the difference was not significant, which is in congruent with the results of Futter and Hu's experiments on rats. It may be due to the length of the experiment, for the length of experiments on rats are generally about seven weeks, whereas the length of experiments on big animal are at least three months. Quickly after passive immunization, the content of TG and FFA went up, for fat storage in adipose tissue is restricted due to the direct elimination of the antibody. It is beneficial to the animal to use fat as energy, promoting the sediment of protein. When the ability to store fat in the adipose tissue recovers in later stage, the levels of TG and FFA return to normal. In the second experiment, anti-sera from chicken antibody applied to rat APM had little effect on the content of TG and FFA in the blood.

The side effect of APM passive immunization on the rats

During normal conditions, the side effect of polyclonal antibody applied to APM on animals exhibits a sedative effect over a short period, which may last 12 to 24 h. The weights of the kidney and spleen rise by the end of the 3^{rd} wk after the injection of anti-sera, but fall back to normal by the end of the 7^{th} week (Panton, 1990). In this experiment, the weights of the liver and spleen of the rats rose by the end of the 2^{nd} wk ($p<0.05$), which may be due to the immune reaction after the injection of anti-sera. But their weights were similar to those of the control group by the end of the 7^{th} wk, indicating that the side effect was disappearing.

CONCLUSION

Anti-sera passive immunization with Sheep antibody applied to rat APM improves the growth performance of rats, decreases the sediment of fat and meliorates the quality of the carcasses. Anti-sera from chicken antibody applied to rat APM has no a significant effect on the rats. The mechanism for regulating fat during the short term depends upon the direct relegation to adipose tissue of anti-sera.

REFERENCES

Dulor, J. P., Y. Reyne and J. Nougues. 1990. *In vivo* effects of a treatment with antibodies to adipocytes plasma membranes in the rabbit [J]. Reproduction Nutrition Development 30:49-58.

Hu, C. Y., A. Suryawan and J. Killefer. 1992. Effect of antiserum to rat adipocytes on growth and body composition of the rat. Comp. Biochem. Physuil. 101A:669.

Kestin, S., R. Kennedy, E. Tonner and M. Kiernan. 1993. Decreased fat content and increased lean in pigs treated with antibodied to adipocytes plasma membranes [J]. J. Anim. Sci. 71:1486-1494.

Kim, Y. M., J. H. Kim, S. C. Kim, H. M. Ha, Y. D. Ko and C. H. Kim. 2002. Influence of dietary addition of dried wormwood (*Artemisia sp.*) on the performance, carcass characteristics and fatty acid composition of muscle tissues of Hanwoo heifers. Asian-Aust. J. Anim. Sci. 15(4):549-554.

Lien, Tu Fa, Jan Derfang and T. F. Lien. 2003. Enzyme activities realted to lopid metabolism in the liver and adipose tissue of Tsaiya ducks underfasting and ad libitum feeding condition. Asian-Aust. J. Anim. Sci. 16:403-408.

Loh, T. C., H. L. Foo, B. K. Tan and Z. A. Jelan. 2002. Effects of palm kernel cake on performance and blood lipids in rats. Asian-Aust. J. Anim. Sci. 15(8):1165-1169.

Moloney, A. P., P. Allen and W. J. Enright. 1989. Growth and weight of abdominal and carcass fat in sheep immunized against adipose cell membranes [J]. Proc. Nutr. Soc. 48:14A.

Marks, H. L. and K. W. Washburn. 1997. Divergent selection for yolk cholesterol in laying hen [J]. Br. Poult. Sci. 18:179-188.

Moloney, A. P., P. Allen and W. J. Enright. 1998. Passive immunization against adipose tissue: Effect on metabolism growth and body composition [J]. Livestock Production Science 56:233-244.

Nassar, A. H. and C. Y. Hu. 1991. Growth and carcass characteristics of lambs passively immunized with antibodies developed against ovine adipocytes plasma membranes. J. Anim. Sci. 69:578-586.

Panton, D., C. Futter and S. Kestin. 1990. Increased growth and protein deposition in rats treated with antibodies to adipocytes [J]. Am. J. Phy. 258:E985-989.

Shim, K. S., G. H. Park and C. J. Choi. 2004. Decreased triglyceride and cholesterol levels in serum, liver and breastmuscle in broiler by the supplementation of dietary codonopsis lanceolata root. Asian-Aust. J. Anim. Sci. 17:511-513.

Wang, Jundong, Du Rong, Qin Jian Wang Shaolin, Wenkui Wang, Hongquan Li and Quanhai Li. 2003. Effect of L-carnitine and Yeast Chromium on Lipid Metabolism of Broiler Chickens. Asian-Aust. J. Anim. Sci. 16:1809-1815.

Wang, Jundong, Li Junping, Zhang Jianfeng and Hao Junhu. 2004. Effect of Betaine Supplement in Different Growing Stages on Broiler Performance and Fat Distribution. Chin. J. Vet. Med. 24:87-91.

Wang, Jundong, Li Shuqin, Shen ruiling and Hao Junhu. 2003. Effects of Different Traditional Chinese Medicine Compounds on Lipid Metabolism, Production Performance and Meat Quality in Broiler. Chin. J. Vet. Med. 23:377-379.

Wang, Wenjun, Huang Lusheng and Gao Jun. 2003. Polymorphism of growth hormone gene in 12pig breeds and its relationship with pig growth and carass traits. Asian-Aust. J. Anim. Sci. 16:161-164.

Xi, G., Z. R. Xu and P. Xiao. 2002. Growth associated hormones response and fat metabolism change in finishing pigs fed with n-methyl-d, l-aspartate. Asian-Aust. J. Anim. Sci. 15(7):1026-1030.