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Effects of monoclonal antibody on fat tissue development, carcass composition, growth performance and fat metabolism of pigs by subcutaneous injection

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ABSTRACT

This study was to investigate the effects of monoclonal antibody against a porcine 40-kDa adipocyte-specific plasma membrane protein on fat tissue development, carcass composition, growth performance and fat metabolism in pigs. Forty-eight Landrace×Saba pigs were randomly divided into eight groups, the control group was given 16 mL saline and the treat groups were given the monoclonal antibody (McAb) with 0.1, 0.5 or 1.0 mg/kg body weight at 30 kg or 60 kg body weight respectively by subcutaneous injection. The results showed that the injection of 1.0 mg/kg monoclonal antibody at 30 kg or 60 kg could increase the lean meat percentage by 3.04% or 4.25% ($P<0.05$) respectively, decrease fat percentage by 10.50% or 9.32% ($P<0.05$) and backfat thickness by 16.28% or 18.65% ($P<0.05$) respectively under the condition of unchanged growth performance. Moreover, the injection of 1.0 mg/kg monoclonal antibody at 30 kg body weight could significantly increase serum LPL activities by 13.79%, 25.00%, 43.91%, 21.77% and 11.61%($P<0.05$) at 1 to 5 weeks respectively after injection, increase serum TG contents by 7.83%, 11.08% and 5.37% ($P<0.05$) at 2 to 4 weeks respectively after injection, but reduce serum NEFA levels by 6.58%, 7.80% and 8.15%($P<0.05$) at 1 to 3 weeks respectively after injection. The injection of 1.0 mg/kg monoclonal antibody at 60 kg body weight could significantly increase serum LPL activities by 10.02%, 18.77% and 14.26%($P<0.05$) at 1 to 3 weeks respectively after injection, increase serum TG contents by 9.23%, 9.70% and 8.67%($P<0.05$) at 2 to 4 weeks respectively after injection, but reduce serum NEFA levels by 15.46%, 16.64%, 13.96% and 10.85%($P<0.05$) at 1 to 4 weeks respectively after injection. Meanwhile, this antibody could restrain subcutaneous fat tissue development by reducing the diameter and triglyceride contents of subcutaneous fat cells. This suggested that this McAb could improve the carcass composition by reducing fat percentage which was regulated by increasing fat mobilization and suppressing the fat deposition in adipose tissue.

1. Introduction

Excessive fat in domestic animals has been recognized as detrimental to lowering production costs and a health risk to human consumers. Current meat animal production systems produce a large amount of fat in their carcasses. Finding effective methods to suppress excessive fat deposition in the production of livestock such as pigs is of utmost importance.

Various approaches have been attempted to solve the excessive fat deposition problem. Recently, studies have used polyclonal and monoclonal antibodies to depress the development of adipose tissue and great progress has been achieved. Flint et al. (1986) first showed that polyclonal antibodies raised in sheep against rat adipocyte plasma membranes were cytotoxic *in vitro* and caused a decrease of rat body fat *in vivo*. In other studies, polyclonal antibodies raised against fat cells or

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Table 1
Treatment on group I–VIII of pigs

Group	I	II	III	IV	V	VI	VII	VIII
Body weight (kg)	30	30	30	30	60	60	60	60
Dose of injection (mg/kg)	0.1	0.5	1.0	Control	0.1	0.5	1.0	Control

adipocyte plasma membrane proteins of different animals had been reported (Panton et al., 1990; Hu et al., 1992; Dulor et al., 1990; Cryer et al., 1984; Moloney and Allen, 1989; Nasser and Hu, 1992; Kestin et al., 1993; Gao et al., 2007a, b; Dong et al., 1991; Butterwith et al., 1992). These studies revealed that those antibodies could lead to fat cell lysis *in vitro* or *in vivo* in different animals, therefore decrease body fat deposition considerably. However, these polyclonal antibodies not only specially act on fat cells, but also produce serious side-effects to several other tissue cells for cross-reaction. In order to improve the specificity of anti-adipocyte antibodies, a few studies have focused on the production of monoclonal antibodies against fat cell surface proteins for some animals such as pigs (Wright and Hausman, 1990; Killefer and Hu, 1990a, b; Wright and Hausman, 1995; Gao et al., 2007a, b) and chicken (Wu et al., 2000). These researches indicated that the depressive effect of monoclonal antibody on adipocytes and pre-adipocytes was revealed in primary porcine and chicken stromal-vascular cultures and could significantly reduce the fat mass weight of pigs, chickens and rats by injection (De clercq et al., 1997; Wright and Hausman, 1995; Nassar and Hu, 1992). These studies suggested that the immunological approach, using the specific monoclonal antibody against porcine adipocyte plasma membrane protein, might be a potential method to suppress excessive fat deposition in the production of pigs. However, the mechanisms by which these antibodies reduced fat deposition is unclear.

In our previous studies, a 40 kDa porcine adipocyte-specific plasma membrane protein had been identified and prepared (Hao et al., 2004), and subsequently one mouse monoclonal antibody (McAb) was raised against this 40 kDa protein (Gao et al., 2005). This McAb showed desirable specificity to subcutaneous fat cells. Western blot identification revealed that the antibody could only bind to the 40 kDa protein of cell membrane fractions in subcutaneous dorsal and abdominal adipose tissue, but not to any protein of cell membrane fractions in mesenteric and parietal adipose tissue, heart, liver, spleen, lung, kidney, skeletal muscular or red blood cells(Gao et al., 2005).

This implied that this monoclonal antibody could display cytotoxicity only to the adipocytes from subcutaneous and abdominal adipose tissues and produce a suppressive effect to these fat cells and tissues, but would not produce side-effect to other tissues. In the present experiment, we investigated the effects of this McAb on the growth performance, carcass composition, fat metabolism and adipocyte tissue development to explore the possible mechanism of McAb to restrain the fat deposition and improve the carcass composition.

2. Materials and methods

All experimental procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of Yunnan Agricultural University. The experimental protocol was approved by the Departmental Animal Ethics Committee of Yunnan Agricultural University.

2.1. Production and characterization of McAb

In our previous studies, a 40 kDa porcine adipocyte-specific plasma membrane protein had been prepared and identified (Hao et al., 2004) and subsequently one mouse monoclonal antibody (McAb) of the IgG1 subclass which was raised against this 40 kDa porcine adipocyte-specific plasma membrane protein was developed (Gao et al., 2005). Briefly, immunization of four BALB/c mouse was performed by four injections of 1 ml purified 40 kDa porcine adipocyte specific plasma membrane protein antigen (1.0 mg/ml) emulsified with an equal volume of complete or incomplete Freund's adjuvant (sigma) by intercutaneousl injection at 2-week intervals. Twenty-four hours after the last injection, spleen was used to prepare single-cell suspensions. The spleen of immunized mice were aseptically excised and washed in serum-free RPMI 1640. Spleen were cut and agitated to free cells from connective tissue. Immune spleen cells were fused with an equal number of SP2/0 myeloma cells by slowly adding polyethylene glycol to the mixture of spleen and myeloma cells. The mixture of cells was incubated. Containing positive hybrids were cloned twice by limiting dilution to obtain monoclones. Positive clones were further tested for cross-reactivity with porcine other tissue cell plasma membrane proteins (liver, kidney, and muscle) to examine the specificity of McAb against porcine adipocytes.

Table 2
Composition of basic diet

Ingredients (%)	30–60 kg	60–90 kg
Corn	62.50	66.44
Wheat bran	19.00	20.00
Soybean meal	11.36	7.80
Fish meal	5.00	3.50
Soybean oil	0.03	0.16
Limestone	0.81	0.75
Lysine	0.00	0.05
Salt	0.30	0.30
Premix*	1.00	1.00
Calculated nutritional composition		
Dry matter	88.00	88.00
Digestive energy(MJ)	12.97	12.68
Crude protein	16.00	14.00
Calcium	0.46	0.37
Total phosphorus	0.21	0.14
Available phosphorus	0.79	0.69
Lysine	0.55	0.46

*Premix composition: Vitamin premix provided the following per kilogram of diet: vitamin A, 8267 IU; vitamin D2, 2480 IU; vitamin E, 66 IU; menadionine (as menadionine pyrimidinol bisulfite complex), 6.2 mg; riboflavin, 10 mg; Ca d-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B12, 45 μg; d-biotin, 331 μg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamine, 3.31 mg; vitamin C, 83 μg.
Trace mineral premix provided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg, as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, ethylenediamine dihydriodide, respectively, with calcium carbonate as the carrier. Provided 0.3 mg Se per kilogram of diet.