AN INVESTIGATION OF IMMUNIZATION AGAINST SOMATOSTATIN BY MEASURING GROWTH AND CARCASS PARAMETERS IN GILTS

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Summary

To investigate the effects of immunization against somatostatin (SRIF) on growth rate, feed efficiency and carcass quality; forth-eight Yorkshire gilts (age = 37.5 ± 4.3 d, wt = 8.2 ± 1.6 kg) were randomly assigned to one of the following three treatments (1) control, (2) bovine serum albumin (BSA) and (3) SRIF. Cyclic SRIF was conjugated to BSA as the antigen containing 1 mg of SRIF diluted in 3 ml of saline. The conjugate was injected subcutaneously together with bacterial cell protein (BP) adjuvant on both sides of the neck of each gilt as the initial injection with three subsequent booster injections. Throughout the experiment all pigs were fed *ad libitum* a corn-soy diet containing 20% protein. Body weight and feed intake were measured on a weekly basis. All pigs in the experiment were slaughtered when they approached 101 kg body weight on the weekly weigh day. After slaughter, carcass parameters were analyzed to assess carcass quality. Results revealed that there were no differences among SRIF, BSA and control treatments for average daily gain, feed efficiency and feed intake during the first 5 wk of the experiment and from 6 wk to slaughter. The results for carcass analysis indicated that active immunization against SRIF had no effect on fat content, lean yield, water content and Canadian carcass index. These data, collectively, suggest that the protocol employed in the present investigation for active immunization against SRIF is not an effective method for the enhancement of pig growth and improvement of feed efficiency and carcass quality.
(**Key Words**: Gilts, Immunization, Somatostatin, Growth, Carcass Quality)

Introduction

In modern swine production, improving growth rate, feed efficiency, carcass quality and thus reducing production cost are of utmost improtance. Researchers working on meat animal production have tried to find new ways to enhance animal growth, feed efficiency, and carcass quality. New methods, however, must be convenient, effective, and low cost, or they will not be utilized by the pork industry.

Growth is a very complex physiological process. It is regulated by many factors such as genotype, nutrients and environment as well as hormones. Efficient animal growth is the net result of the ideal integration of all these factors.

Several studies have documented that somatotropin (ST) is very important in controlling postnatal animal growth (Spencer et al., 1986a). Administration of exogenous porcine somatotropin (pST) can increase pig growth rate, carcass quality and feed efficiency (Evans et al., 1989). This method, however, has several disadvantages such as inconvenience, resistance from consumers, and high labour cost. To date the method has not been approved for commercial application. Therefore, the use of pST does not appear to be practical in meat animal production at the present time.

Somatotropin (ST) production is regulated by two hormones. These hormones are growth hormone releasing factor (GRF) and somatotropin release-inhibiting factor (SRIF). As their names imply, GRF can stimulate ST release and SRIF can inhibit ST release (Spencer et al., 1985; Lawrence et al., 1986).

Spencer et al. (1983) and Spencer (1986a) showed that lambs immunized against SRIF had a significant increase in antibody titres and higher growth rates than those of control animals. Similar findings were reported by Laarveld et al. (1986). Piglets born to sows immunized against SRIF

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had heavier birth weights (Osborne and Hacker, 1986). Furthermore, immunization against SRIF has been shown to cause a 20 % increase in growth rate and 5%-10% decrease in backfat thickness in growing Yorkshire pigs (Evans et al., 1988). On the other hand, some studies of immunization against SRIF failed to show increased animal growth rate, or improved feed efficiency (Varner, et al., 1980; Trout and Schanbacher, 1990). Negative results such as reducing animal growth rate (Varner, et al., 1980) have also been reported.

All the studies of immunization against SRIF mentioned above reveal that active immunization against SRIF might be a potential practical approach for enhanced meat animal production. However, the results from the different studies are quite variable indicating that the methods have to be improved before this technique can be used in practical swine production.

The objective of this study was to examine the effects of immunization against SRIF on:

1. Gilt growth performance and feed efficiency and

2. Gilt carcass quality (carcass fat content and carcass index)

Materials and Methods

A total of forty eight Yorkshire gilts (weaned at the age of 4 weeks) were used in this study. At approximately five weeks of age (37.5 ± 4.3 days, wt = 8.2 ± 1.6 kg) gilts were randomly assigned to one of the following three treatments: 1) Control, 2) BSA injection and, 3) SRIF injection, and housed in groups based on their treatment with four gilts in each nursery pen. At eleven weeks of age, all the gilts were transferred from the nursery pen to the grower and finisher section in the barn and housed in individual pens until the completion of the experiment. The experiment consisted of two groups of 24 gilts put on trial at a two week interval.

The barn used for this experiment was environmentally controlled. The temperature in the weanling pens was 27℃ to 30℃ throughout the nursery period. The temperature in the rooms of the growing and finishing section varied from 24℃ (day) to 18℃ (night). The humidity averaged 70% and light was provided from 07:00 h to 20:00 h.

Throughout the whole experiment, all pigs in the trial were fed a ration containing 20% crude protein (CP) and 1.06% lysine *ad libitum*. The experimental diet was fed as pellets. All pigs in the experiment had free access to water from a nipple waterer. One week before the experiment started, all selected piglets were fed the experimental ration so as to become accustomed to the new diet. Feed intakes were measured on a weekly basis throughout the experiment. Diet formulation and analyzed nutrient content results are shown in table 1.

TABLE 1. COMPOSITION OF DIET

Item	%
Ingredient	
Corn 2# dried	69.10
Soybean meal (48%)	28.40
Dicalcium phosphate	1.20
Ground limestone	0.81
Sodium chloride	0.25
Vitamin premix[a]	0.25
Trace mineral premix[b]	0.10
Analyzed composition	
Dry matter	89.05
Crude protein	20.45
Digestible energy (kcal)	3.96

[a] Vitamin premix provided per kg of diet: Vitamin A: 10,000 IU; Vitamin D_3: 1,500 IU; Vitamin K: 2.2 mg; Riboflavin: 5 mg; Vitamin E: 30 IU; Pantothenic acid: 16 mg; Niacin: 25 mg; Choline: 300 mg; Vitamin B_{12}: 15 μg; Biotin: 0.2 mg; Pyridoxine: 1.5 mg; Thiamine: 1.5 mg; Folic acid: 1.0 mg.

[b] Trace mineral premix provided per kg of diet: Se: 0.3 mg; Mn: 59.9 mg; Zn: 100.0 mg; Cu: 10.1 mg; Fe: 70.0 mg.

Somatostatin is a very small molecule (MW = 1639.88 g/mole) and therefore can not be antigenic. In order to enhance its antigenicity, somatostatin has to be conjugated to a large protein molecule, such as bovine serum albumin (BSA). In this experiment, the antigen was ordered from IAF BioChem International Inc. Montreal, Canada. In this antigen preparation, cyclic SRIF was conjugated to BSA using the coupling agent Sulfo-Smcc. The conjugate was dialysed in phosphate buffered saline (PBS) buffer (pH = 7.2, 0.01% thimerosal). The final concentration of SRIF in the conjugate was 1.0 mg/ml

and the final concentration of BSA in the conjugate was 1.34 mg/ml. Prior to antigen injection, the SRIF-BSA conjugate was kept at 20℃.

The SRIF immunization injection solution was prepared as follows: one night before the immunization, SRIF-BSA conjugate was taken out of the freezer and stored in the fridge at 4℃ to thaw. After thawing, 1 ml antigen (SRIF-BSA) containing 1 mg SRIF was added to 2 ml saline (0.9% NaCl) which contained 0.4 mg bacterial protein (BP) adjuvant. The solution was thoroughly mixed by a vortex mixer.

The solution was transferred into sterile 3 cc plastic syringes fitted with 22 gauge needles and placed on ice until injected. The injection area of the neck was thoroughly sterilized using 70% ethanol before injection. A total of three ml antigen solution was administered in 14 sites on both sides of the pig's neck area subcutaneously. Animals in the control treatment received a sham injection (14 sites on both sides of neck area) and animals in the BSA treatment group were injected as the animals in the SRIF immunization treatment omitting SRIF in the injection solution.

Body weight and feed intake were measured weekly throughout the experiment. Body weights were determined by using an electronic scale (Sterling Scale, 6000 plus), and feed intakes were measured by using an electronic scale which was adapted specifically to weigh feeders (Detecto, 10 k, 8701-24).

To investigate the effect of SRIF immunization on pig carcass quality, parameters were measured both before and after slaughter. Backfat thickness was measured (at the second last rib, both sides 7 cm from the middle line) by ultra sonic equipment (Ultra Sonomatic, Medimatic U-76A) at a live weight of 60 and approximately 101 kg. All pigs were slaughtered once they approached a live weight of 101 kg. The final pig weight was obtained the night before slaughter. After slaughter, the eviscerated and longitudinally split hot carcass was weighed (including the head). Carcass backfat was measured by using a steel ruler at the second last rib. Carcasses were graded by an electronic grading probe (Destron Hog Grading Probe) according to the Canadian Hog Carcass Grading/ Settlement System (1986). The electronic grading probe was inserted into the carcass between the third and the fourth last ribs 7 cm from the middle line. After probing, the following carcass parameters were measured: lean yield, maximum loin fat, loin depth, and carcass index.

Loin and belly samples were cut from between the second and the third last ribs to facilitate fat and water measurements. All samples were ground with an electric grinder after being taken from the carcass, and were put into aluminum trays. After weighing, all samples were freeze dried.

Water content of belly and loin samples was measured as the difference between the weights before and after the samples were dried (fresh meat sample weight-dried meat sample = water weight).

Fat content of both belly and loin sample was measured by using ether extraction. Each sample was duplicated and extracted until all the fat in the sample was removed. After extraction, all samples were removed from the extracting socklet and weighed after the ether in the samples was totally evaporated. The fat content of the samples was calculated as the weight difference before and after the extraction.

The General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) Institute (SAS, 1988) was used for statistical analysis of data collected in this experiment. Growth, carcass, and feed efficiency data were analyzed with the following overall statistical model:

$$Y_{ijk} = \mu + B_i + T_j + W_{ijk} + \sigma_{ijk}$$

where Y_{ijk} = Response variable
μ = overall mean
B_i = replicate (where i = 1, 2)
T_j = treatment (where j = 1, 2, 3)
W_{ijk} = initial body weight covariates (where k = 1~48)
σ_{ijk} = error term

For the nursery period, the experimental unit was the pen and for the growing and finishing period the experimental unit was the individual pig.

Results

The results for feed intake, feed efficiency and growth rate are shown in table 2. During the first five weeks of the experiment, there was no difference ($p > 0.05$) in feed intake (FI), average daily gain (ADG) and feed efficiency (FE) among the three treatments.

Feed intake, feed efficiency and growth rate

TABLE 2. THE EFFECT OF TREATMENT ON PIG FEED INTAKE, AVERAGE DAILY GAIN AND FEED EFFICIENCY[a]

Item/Time	Treatment			SE
	T1	T2	T3	
First 5 wks:				
FI (kg/d)	1.07	1.01	1.00	0.03
ADG (kg)	0.52	0.54	0.53	0.02
FE	1.99	1.89	1.90	0.04
Six wk to slaughter:				
FI (kg/d)	2.35	2.29	2.25	0.05
ADG (kg)	0.91	0.83	0.87	0.02
FE	2.57	2.77	2.62	0.04

T1 = Sham injection. T2 = BSA-BP. T3 = SRIF-BSA-BP.
FI = Feed intake. ADG = Average daily gain. FE = Feed efficiency.
[a] Values are least squares means; SE is the pooled standard error.

from six weeks to slaughter were analyzed on an individual pig basis. During this period, the feed intake in the SRIF immunization treatment remained lower than in the other two treatments, but again this difference was not statistically significant ($p > 0.05$). In addition, there were no significant differences ($p > 0.05$) in ADG and FE. The results demonstrate that in this experiment active immunization against SRIF failed to exhibit any positive effects on feed intake, feed efficiency and growth rate in gilts from five weeks of age until market weight (approximately 101 kg).

Backfat thickness was measured at 60 kg live body weight and again before slaughter to test if active immunization against SRIF had any effects on fat deposition. The results are shown in table 3. Active immunization against SRIF did not have a significant effect on backfat thickness, although backfat thickness was lower in the SRIF before slaughter than in the sham injection and BSA treatments.

Table 4 shows that both hot carcass weight and carcass index were higher in SRIF treatment than in the other two treatments, however, there

TABLE 3. THE EFFECT OF TREATMENT ON PIG BACKFAT THICKNESS MEASURED ULTRASONICALLY AT 60 KG LIVE WEIGHT AND BEFORE SLAUGHTER[a] (mm)

Time	Treatment			SE
	T1	T2	T3	
60 kg live body weight	9.18	10.37	9.57	0.48
Before slaughter	12.50	13.00	12.06	0.59

T1 = Sham injection. T2 = BSA-BP. T3 = BSA-SRIF-BP.
[a] Values are least squares means; SE is the pooled standard error.

TABLE 4. THE EFFECT OF TREATMENT ON PIG LIVE SLAUGHTER WEIGHT, HOT CARCASS WEIGHT AND CARCASS INDEX[a]

Item	Treatment			SE
	T1	T2	T3	
Live slaughter weight (kg)	101.50	98.91	100.37	1.02
Hot carcass weight (kg)	81.25	80.46	81.62	0.77
Carcass index	109.62	109.18	109.87	0.61

T1 = Sham injection. T2 = BSA-BP. T3 = BSA-SRIF-BP.
[a] Values are least squares means; SE is the pooled standard error.

were no significant differences between the SRIF treatment and the other two treatments. Table 5 shows that maximum loin fat and fat thickness were lower in the SRIF treatment than in the other two treatments and lean yield was higher in the SRIF treatment than in the other two treatments. There was no significant difference between SRIF treatment and the other two treatments for the parameters measured.

Because administration of exogenous pST can increase protein retention and decrease fat content of the pig carcass to produce a leaner carcass, the data for moisture and fat content of the carcasses from the pigs in the three treatments was collected and analyzed. The results are shown in table 6. The moisture content in the belly was a little higher in the SRIF treatment than in the sham injection and BSA treatments. However, this small difference was not statistically significant ($p > 0.05$). The moisture content in the loin was slightly higher in the SRIF treatment than in the BSA treatment and was lower than in the sham injection treatment. There were no statistically significant differences among any of the three treatments. Fat content in the belly was lower in the SRIF treatment than in the other two treatments and fat content in the loin was lower in the SRIF treatment than in the BSA treatment but higher than in the sham injection treatment. There were no significant differences among the three treatments.

TABLE 5. THE EFFECT OF TREATMENT ON MAXIMUM LOIN FAT, FAT THICKNESS, LOIN DEPTH AND LEAN YIELD[a]

Item	Treatment			SE
	T1	T2	T3	
Maximum loin fat (mm)	24.93	25.50	24.87	1.09
Fat thickness (mm)	17.09	17.25	16.62	0.92
Loin depth (mm)	46.70	52.65	49.15	1.93
Lean yield (%)	51.00	51.26	51.30	0.36

T1 = Sham injection. T2 = BSA-BP. T3 = BSA-SRIF-BP.
[a] Values are least squares means; SE is the pooled standard error.

TABLE 6. THE EFFECT OF TREATMENT ON MOISTURE AND FAT IN BELLY AND LOIN SAMPLES[a] (%)

Item		Treatment			SE
		T1	T2	T3	
Belly :	Moisture	50.29	50.01	51.25	0.01
	Fat	66.94	68.06	65.32	1.31
Loin :	Moisture	73.32	71.95	73.11	0.05
	Fat	4.97	5.27	5.12	0.62

T1 = Sham injection. T2 = BSA-BP. T3 = BSA-SRIF-BP.
[a] Values are least squares means; SE is the pooled standard error.

Discussion

Active immunization against SRIF has been studied in cattle, poultry, goats, sheep and swine. However, the effect of active immunization against SRIF is still controversial. Some studies showed positive effects on growth, feed efficiency, and carcass quality, however, others did not. It is still questionable whether this technique can promote growth and improve performance, particularly in swine.

This study indicated that active immunization against SRIF had no effect on daily gain, feed efficiency, feed intake, or carcass quality. Active

immunization against SRIF in steers failed to show any significant effects on growth and feed efficiency (Trout and Schanbacher, 1990). Active immunization against somatostatin in sheep decreased growth and had no effect on feed efficiency (Varner et al., 1980). There are few studies of active immunization against SRIF in swine, testing growth and feed efficiency from weaner to finisher. Trout and Schanbacher (1990) showed that carcass weight, length, and quality were not affected by active immunization against somatostatin in steers. Their results are in agreement with the results of the present study.

Active immunization against SRIF in sheep and goats has been more successful. However, there are differences among species in immune response. There are species differences in antibody response, and it is believed that it is more difficult to get good antibody titres in pigs than in sheep, although scientific evidence for this is lacking. Within species and within breeds, there can be marked variations in the magnitude and speed of antibody response (Spencer et al., 1985). Poor or slow antibody response may well be responsible for much of the difficulty encountered in obtaining reproducible effects with active immunization. Hormone assays in this study showed that pST secretion profile were not changed in gils immunized against SRIF (Du and Hacker, 1992, in press). This might be one of the reasons why growth performance was not improved.

Hoskinson et al. (1988) found that active immunization against SRIF had no effect on weight, or on growth of two groups of the immunized lambs having different antibody titre levels. They concluded that there was no relationship between antibody titre and growth rate of immunized lambs. Bass et al. (1987) suggested a nutrition/immunization interaction for carcass weight, and studies of this effect may resolve conflicting reports in this field.

In the present study, a new adjuvant (BP, bacterial cell protein) was employed instead of Freund's adjuvant, which was previously used by most investigators. This study showed that BP adjuvant had no side effects, including ulceration or abscesses in the injection area. This is consistent with the results of Evans et al. (1988) who also demonstrated good immunogenicity promotion activity.

In lambs, conflicting results exist where active immunization against SRIF either did not affect (Laarveld et al., 1986) or increased ST concentrations (Varner et al., 1980; Spencer et al., 1983). Similarly, in cattle active immunization against SRIF did not influence (Lawrence et al., 1986) or increased ST concentrations (Petitclerc et al., 1988). These results indicate that the success of active immunization against somatostatin is influenced by many factors, including genotype, nutrition, species, age, dosage, conjugate, adjuvant, administration procedure, and stress.

ST secretion is regulated by a host of factors. Once the hormonal homeostasis is disturbed, the hormonal homeostasis will be re-established via an endogenous feedback mechanism (Spencer, 1986b).

Deligeorgis et al. (1988) reported that there was no significant effect of immunization against SRIF on milk production in ewes or on birth and weaning weight of their progeny. The physiological mechanism responsible for improved growth performance after SRIF immunization has not been determined.

In conclusion, active immunization against SRIF in swine did not affect pST secretion or neutralization of endogenous SRIF. It did not increase growth rate, feed efficiency, or carcass quality during the weaning to finishing period. In order to develop this technique for practical application, more research is needed into antigen dosage, coupling agents, conjugate, adjuvant, animal age, and administration procedure, to get good continuous antibody response in pigs immunized against SRIF.

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