

AN INVESTIGATION OF IMMUNIZATION AGAINST SOMATOSTATIN BY MEASURING ANTIBODY TITRES, SOMATOSTATIN AND SOMATOTROPIN PROFILES IN GILTS¹

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Summary

The effect of active immunization against porcine somatostatin (SRIF-14) on somatostatin and somatotropin secretion profile in 18 gilts was investigated. Gilts were assigned to the following treatments: control (sham injection, $n = 6$); bovine serum albumin (BSA) (injection of BSA with bacterial protein adjuvant, $n = 6$); SRIF (injection of BSA-SRIF-14 conjugate with bacterial protein adjuvant $n = 6$). Serum SRIF and pST were assayed from the blood samples taken on day 7 after the last immunization injection. Anti-SRIF antibody titres were assayed in weekly samples two weeks after the initial immunization to one week after the last immunization. Results revealed that the immunization protocol used in the present investigation failed to produce antibodies capable of neutralizing endogenous somatostatin. In addition, the porcine somatotropin assay revealed no significant differences in baseline pST concentration, mean peak amplitude and number of peaks during a 24 h secretory period among SRIF, BSA and control treatment. There were also no differences in SRIF baseline concentration, peak amplitude, and number of peaks during a 24 h secretory period among any of the three treatments. Circulating concentrations of pST and pSRIF were highly correlated ($r = -0.09$). Furthermore, anti-SRIF antibody titre was not detected in the serum of the gilts actively immunized against SRIF. These data, collectively, suggest that the protocol employed in the present investigation for active immunization against SRIF is not an effective method for changing SRIF and pST secretion profiles of the gilt and thus to enhance performance.

(Key Words: Gilt, Immunization, Somatostatin, Somatotropin, Profile)

Introduction

Several studies have documented that somatotropin is very important in controlling postnatal animal growth (Spencer et al., 1986). Administration of exogenous porcine somatotropin (pST) can increase pig growth, carcass quality and feed efficiency (Evans et al., 1989). This method, however, has several disadvantages such as inconvenience, hypothesized resistance from consumers, and high labour cost. To date the method has not been approved for commercial application.

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 (1986) demonstrated 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). Pigs immunized against SRIF have been shown to have significantly higher baseline levels of pST than control animals (Dubreuil et al., 1989). In addition, pigs immunized against SRIF have a greater ST release after GRF injection. However, there has been no report on the pSRIF and pST secretion profile after immunization against SRIF in gilts.

On the other hand, some studies of immunization against SRIF failed to show increased antibody titre, increased ST concentration, increase in animal growth rate, or improved feed efficiency (Varner et al., 1980; Trout and Schanbacher,

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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 if active immunization against SRIF can decrease plasma pSRIF concentration and increase plasma pST concentration thus enhancing pig performance.

Materials and Methods

A total of eighteen Yorkshire gilts (weaned at the age of 4 weeks) were used in this study. At approximately five weeks of age gilts were randomly assigned to one of the following three treatments: 1) Sham injection, 2) BSA injection and, 3) BSA-SRIF-14 injection.

The barn used for this experiment was environmentally modified, so that temperature

in the weanling pens was 27 to 30°C throughout the nursery period. The temperature in the rooms of the growing and finishing section varied from 24 (day) to 18°C (night). The humidity averaged 70% and light was provided from 07:00 h to 20:00 h.

Throughout the whole experiment, all pigs were fed a pelleted ration containing 20% crude protein (CP) and 1.06% lysine *ad libitum* (table 1).

The antigen utilized in the experiment was obtained from IAF Bio Chem International Inc. Montreal, Quebec, Canada. In this antigen preparation, cyclic SRIF-14 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 BSA-SRIF-14 conjugate was kept at -20°C.

The SRIF immunization injection solution was prepared as follows: one night before the immunization, BSA SRIF-14 conjugate was taken out of the freezer and stored in the fridge at 4°C to thaw. After thawing, 1 ml antigen (BSA-SRIF-14) containing 1 mg cyclic SRIF-14 was added to 2 ml saline (0.9% NaCl) which contained 0.4 mg bacterial protein (BP: N Acetyl-Muramyl-L-Ala-D-Iso-Gln-oH) adjuvant which was reported to be an effective adjuvant without the adverse side effects of Freund's adjuvant (Evans et al., 1988). The solution was thoroughly mixed by a vortex mixer.

The solution was transferred into a 3 cc sterile plastic syringe fitted with a 22 gauge needle and kept on ice until utilized. A total of three ml antigen solution was administered in 14 sites on both sides of the pig neck area subcutaneously. Animals in the control treatment received a sham injection (14 sites on both sides of the 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. After the initial immunization injection, three booster injections were followed at three week intervals, for a total of four injections.

Blood samples were obtained by suborbital sinus puncture on a weekly basis for the anti-SRIF antibody test. Initial blood samples (ap-

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 D₃: 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 B₁₂: 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.

proximately 5 ml) were collected from all the pigs before the first SRIF immunization injection. Approximately 5 ml of blood from each pig in the experiment was collected into a glass tube which contained EDTA (1.0 mg/ml blood) and aprotinin (1,000 KIU/ml blood) weekly, starting two weeks after the initial injection and ending one week after the last immunization injection.

One week after the last immunization injection (at 15 wks of age), all 18 gilts were cannulated via the ear vein. Cannulation was conducted by inserting a 14-gauge needle, through which plastic tubing (inner diameter 0.86 mm, outer diameter 1.27 mm) was introduced into the vein. Blood samples were collected at hourly intervals for 24 hours to determine the profile of circulating somatotropin and SRIF of gilts in the three treatments. Approximately 9 ml of blood was collected from each pig hourly and placed into two glass tubes: 4 ml of blood into one tube containing EDTA and aprotinin, and the remaining 5 ml of blood into another glass tube which contained only EDTA. All blood samples in the tubes were kept on ice and immediately centrifuged at 2,500/rpm for 20 min in a refrigerated room at 4°C. After centrifugation plasma was harvested and placed in plastic vials and kept in a freezer at -20°C until assay. All weekly blood samples collected from the experiment were used to determine anti-SRIF antibody titres. Blood samples collected from the 24 h bleeding were used to determine the circulating profiles of pSRIF and pST.

Somatostatin concentration was measured by using the radioimmunoassay procedure of Gerich et al. (1979) with modifications. Tyrosine-somatostatin (Sigma, St. Louis, Mo) was used for preparation of ^{125}I -labelled-SRIF. To 45 μl of 0.5 M phosphate buffer (pH = 7.5) were added 5 μl of [tyr 1]-SRIF dissolved in 0.1 M acetic acid (1 $\mu\text{g}/\text{ml}$) and 5 μl of Na ^{125}I (0.5 mCi; New England Nuclear, Boston, MA). All the reagents were mixed at the presence of 2 μg Iodogen. After 3 min of vortexing, 100 μl of 10% BSA (w/v) in 0.1 M acetic acid were added to stop the reaction. The contents of the reaction were transferred to a Sephadex G-25 (fine) column (1 by 27 cm) and then eluted with 0.1 M acetic acid. The eluate was collected in 1 ml fractions and a total of 30 such fractions were collected. Radioactivity was measured in a gamma counter

(Bioscay Qc. 2000).

The concentration of SRIF in the plasma samples was assayed by utilizing 0.05 M phosphate buffer [pH = 7.5, containing 0.1% BSA, 0.25% EDTA and 5,000 Kallikrein Inhibitor Unit (KIU)/ml of aprotinin (Sigma, St. Louis, Mo)] in the assay system. The plasma samples were diluted at a 1:10 proportion, 100 μl diluted plasma sample were added to 100 μl of the first antibody (Rabbit anti-porcine SRIF, 1:200,000 dilution, Sigma, St. Louis, Mo). After 24 h incubation at 4°C, 100 μl of ^{125}I -labelled [Tyr 1]-SRIF (5,000 cpm) was added to the reaction solution. It was then incubated for another 24 h at 4°C before adding 100 μl of the second antibody [Goat anti-rabbit IgG (Calbiochem) diluted at 1:20 in the assay buffer] and 1% Normal Rabbit Serum (NRS) (Elastoplast). After 24 h incubation, all the samples were centrifuged at 3,000 rpm for 30 min at 4°C. Supernatant was decanted immediately after centrifugation and the tubes were counted in a gamma counter machine (LKB 1212).

Concentration of somatotropin in the plasma was measured using a double-antibody radioimmunoassay procedure similar to that described by Marple and Aberle (1972) with modifications. Five μg purified porcine somatotropin (USDA-pGH-B-1) in 25 μl 0.5 M PBS was reacted with 25 μl chloramine T (Sigma, St. Louis, Mo) (1 mg/ml column buffer) and 5 μl ^{125}I (0.5 mCi). After vortexing for 30 seconds, the reaction was stopped with 50 μl sodium metabisulfite (2.5 mg/ml in the column buffer). ^{125}I -labelled porcine somatotropin was separated from free ^{125}I by gel filtration chromatography (Sephadex-G75). Standards were made in 1% BSA-PBS (0.01 M) to give 0.5-150.0 ng/ml porcine somatotropin (USDA-pGH-B-1). Monkey anti-porcine somatotropin (1:100,000) was used as the first antibody and goat anti-monkey globulin (1:16) (Terochem Scientific) was used as the second antibody. The maximum and nonspecific binding was 30 and 2.13% respectively. Increasing volumes of plasma (25-300 μl) displaced ^{125}I -porcine somatotropin from the antiserum to produce a binding curve that was parallel to the standard. The recovery of known concentrations was 95%. Sensitivity of the assay was 0.45 ng/ml. All samples were quantified in one assay.

Anti-somatostatin antibody titre was deter-

mined using a radioimmunoassay procedure. Plasma from blood samples collected weekly was diluted at 1:100 dilution. A one hundred μ l diluted sample was reacted with 100 μ l 125 I labelled SRIF (5,000 cpm). After 24 h incubation at 4°C, the second antibody (Rabbit anti porcine IgG 1:20) was added to the reaction. After a second 24 h incubation at 4°C, all tubes were centrifuged at 3,000 rpm for 30 min at 4°C. The remainder of the procedure was the same as the somatostatin assay procedure outlined above.

In order to do the statistical analysis for somatostatin and somatotropin profiles, the following parameters were calculated after the radioimmunoassay:

Mean of somatotropin: the mean of all 24 somatotropin concentration values from hourly blood samples.

Initial peaks of pST: the mean of somatotropin + 1 standard error (SE)

Baseline of pST: the mean of somatotropin concentration omitting initial peak values

Peaks of pST: baseline + 2 SE

The calculation of somatostatin was the same as for somatotropin except that the peak of somatostatin was the baseline of somatostatin + 1 SE.

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.

Results

The hourly plasma samples from the 24 h bleeding were assayed to determine the circulating pST profile. Table 2 shows the baseline, mean height of peaks and the number peaks of pST and pSRIF during the 24 h secretion period. The results in table 2 showed that the baseline of somatotropin in the gilts was about 1.1 ng/ml; the average of the peak values in the different treatments varied from 8.5 to 11.3 ng/ml. During 24 h, there were 3 somatotropin secretion peaks observed. The pST was secreted in a episodic pattern with example profiles shown in figure 1. These profiles clearly indicated that pST secretion is quite variable among individual pigs. The number of peaks varied from two to four within a 24 h period. The variation in peak height ranged from 5 ng/ml to 28 ng/ml. The height of peaks within each pig was also quite variable (for example: 8 to 16 ng/ml in BSA-M gilt). Most pST peaks occurred between 01:00 h and 12:00 h. Peaks occurring during this period accounted for 67.6% of the total peak numbers within 24 h. The highest frequency of peaks appeared in the early morning from 01:00 h to 09:00 h, although there were some exceptions among the gilts tested. pST baseline was very stable around 1 ng/ml among the gilts. Table 2 shows that although there were some small differences in the baseline, the mean height of the peaks and the number of pST peaks, there were no significant differences between any of the three treatments ($p > 0.05$). In this experiment, active immunization against SRIF failed to change

TABLE 2. PORCINE SOMATOTROPIN AND SOMATOSTATIN PROFILE PARAMETERS*

Item	Treatment			SE
	T1	T2	T3	
Somatotropin				
Base line (ng/ml)	1.14	1.16	1.14	0.05
Mean peak height (ng/ml)	8.52	11.32	9.19	1.72
Number of peaks per day	3.20	3.33	3.33	0.34
Somatostatin				
Base line (pg/ml)	10.83	9.50	10.63	0.68
Mean peak height (pg/ml)	15.25	12.69	15.40	0.96
Number of peaks per day	6.50	6.60	5.33	0.79

T1 = Sham injection.

T2 = BSA-BP.

T3 = SRIF-BSA-BP.

* Values are least squares means; SE is the pooled standard error.

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the pST secretion profile.

The results for somatostatin are also shown in table 2. The baseline concentration of SRIF varied from 9.50 to 10.83 pg/ml plasma and the mean height of the peaks varied from 12.69 to 15.40 pg/ml plasma. The number of SRIF peaks

within 24 h varied from 5.33/d in the somatostatin immunized gilts to 6.50 and 6.60/d in the control and BSA treatments respectively. The SRIF secretion profiles during the 24 h secretion period is also shown in figure 1.

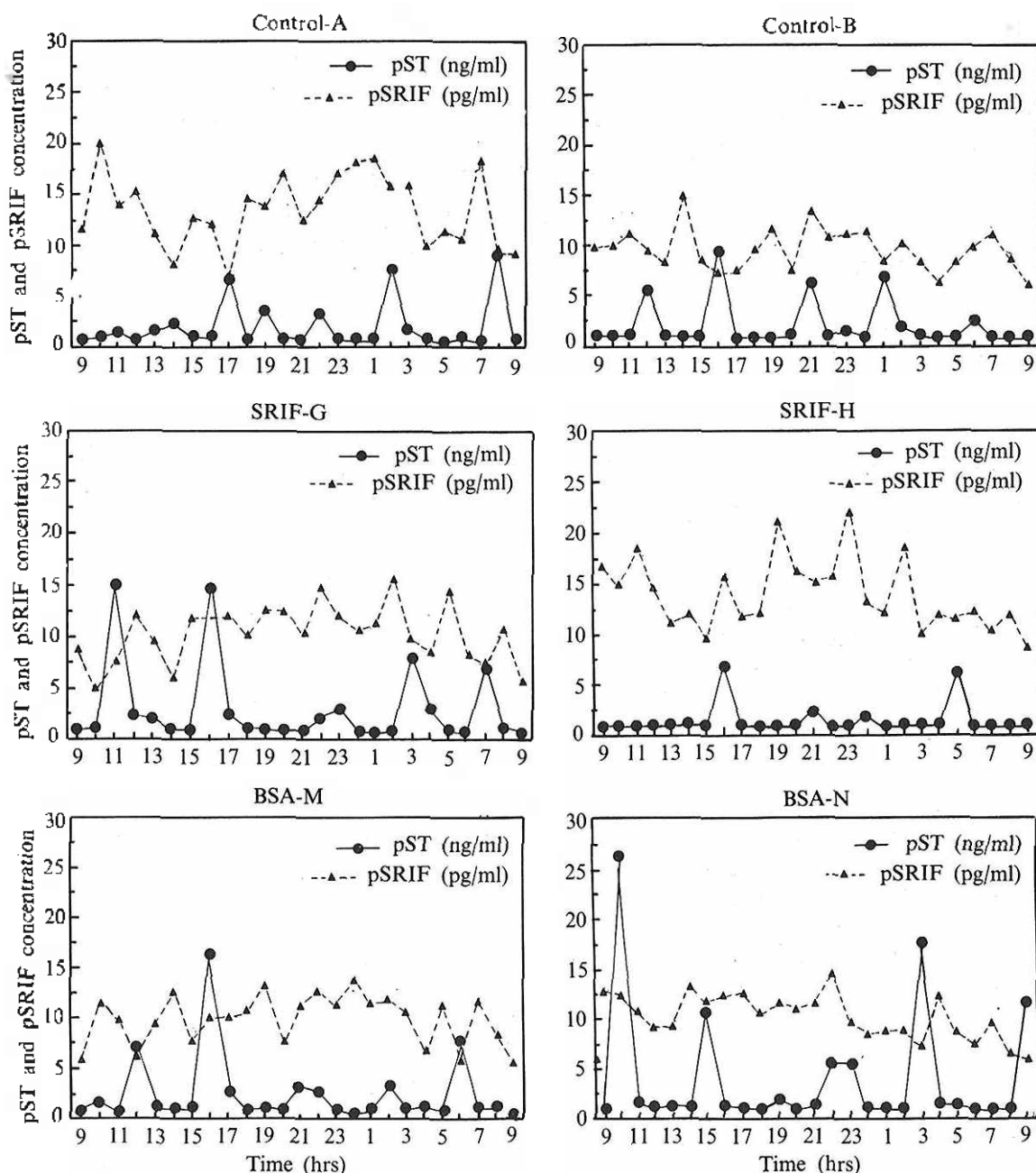


Figure 1. Circulating porcine somatotropin (pST) and somatostatin (pSRIF) profiles during a 24 h period. A and B are animals from sham injection (control) treatment: G and H are animals from SRIF treatment: and M and N are animals from BSA treatment.

Discussion

During the last decade, active immunization against SRIF has been studied in many species including cattle, poultry, goats, sheep and swine. However, the effect of active immunization against SRIF is still controversial. Administration of antibodies against somatostatin, or passive immunization, in rats has been shown to increase somatotropin concentrations (Ferland et al., 1976), but there is little evidence for an effect of active immunization against SRIF on somatotropin concentration in farm animal species (Varner et al., 1980; Spencer et al., 1983).

Antibody titre against somatostatin was not detected in the plasma of gilts immunized against somatostatin in this experiment, nor in several other investigations (Varner et al., 1980; Evans et al., 1988; Osborne, 1989). In the plasma samples from immunized gilts a binding rate between 3 and 5% was observed, which is similar to the plasma samples from gilts in the sham injected and BSA treatments. One explanation for this is that gilts immunized against somatostatin failed to produce the specific antibody, or to produce enough antibody to be capable of neutralizing endogenous SRIF. A second possibility is that the gilts might have produced antibodies, but they were not detected. This is because the antibodies produced were bound to endogenous somatostatin in the plasma samples and were not stripped of SRIF prior to the assay.

In comparison with pST secretion, SRIF secretion had higher number of peaks and lower peak values than pST during the 24 h period. The baseline of SRIF was not as clear as the pST baseline and the concentration of SRIF was lower than that of pST. Table 2 demonstrates that the baseline and the mean height of the peaks of circulating somatostatin were slightly different among the three treatments. These small differences were not statistically significant. The number of peaks during the 24 h period were lower in the SRIF treatment than in the sham injection and BSA treatments, however, this difference was again not statistically significant. Immunization against SRIF did not change the SRIF secretion profile in this experiment.

The 24 h circulating pST profiles of growing gilts analysed in this experiment revealed that somatotropin concentrations in gilts immunized

against somatostatin were similar to those of gilts in both the sham injected and BSA treatments. All pST was secreted in an episodic manner as reported by other investigators (Evans et al., 1988).

The pST profiles in the present study were from gilts at 15 weeks of age. The baseline concentrations of pST varied from 1.13 to 1.15 ng/ml plasma in the gilts in the three treatments. The baseline concentrations were lower in this study than the concentrations (3.12 ± 0.62 ng/ml plasma) of Klindt (1986). This is because the pigs in Klindt's experiment were younger (18 days) than the pigs in the present study. This probably indicates that the pST concentration continues to decline after 18 days of age although not as dramatically.

The data of Klindt (1986) and Klindt and Stone (1984) for swine and Klindt et al. (1985) for sheep indicated that the amplitude of pST peaks was also declined with maturity. The mean peak amplitude of pST in this study varied from 8.5 to 11.3 ng/ml plasma. It was again lower than the amplitudes (13 ± 1.85 ng/ml plasma) of Klindt (1986). Thus it appears that both the baseline concentration and the amplitude of peaks are higher in younger than in older pigs.

The observation of three peaks of pST in this experiment during a 24 h period is in agreement with the results of Evans et al. (unpublished data), who utilized the same breed of gilt as in the present study. The number of peaks were again lower than in Klindt's experiment (Klindt, 1986), probably due to animal age, sex, and breed. Gluckman and Parsons (1985) proposed that the high concentration of ST in the ovine fetus was due to immaturity of the negative feedback control system. A part of this immaturity may be a lack of hepatic response to ST.

Circulating somatostatin profiles of gilts, during a 24 h period, were studied in this experiment. Somatostatin secretion was episodic, with about eight peaks of SRIF during 24 h. The amplitude of the SRIF peaks relative to baseline concentration was lower than that of pST peaks. The baseline somatostatin concentration was much lower than the baseline somatotropin concentration. The profiles of the somatotropin and somatostatin showed that somatostatin had a negative effect on somatotropin secretion (figure 1). When somatostatin concentration increased in the blood,

the somatotropin concentration was decreased. There was a significant negative correlation of circulating pST and pSRIF concentrations ($r = -0.09$). The data from the present study failed to show immunoneutralization of SRIF because there were no significant differences in the baseline concentrations, numbers of peaks, and amplitudes of peaks of somatostatin between any of the three treatments. Growth hormone-releasing factor is responsible for the amplitude of ST peaks, and SRIF controls the baseline concentration of ST (Tannenbaum and Ling, 1984; Wehrenberg et al., 1982; Dubreuil et al., 1987). This may explain why active immunization against SRIF had no effect on the amplitude of pST peaks in the present study.

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.

In summary, immunization against SRIF in swine did not affect pST secretion or neutralize endogenous SRIF. 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 antibody response in pigs immunized against SRIF.

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