FALL AND WINTER HORMONE CONCENTRATIONS RELATED TO STRESS IN PIGS IDENTIFIED AS NORMAL AND CARRIER FOR STRESS SUSCEPTIBILITY

Susan A. Roberts, A. L. Schaefer, Austin C. Murray, and Louise Thibault

1School of Dietetics and Human Nutrition, Macdonald Campus of McGill University, 21 111 Lakeshore Road, Ste. Anne de Bellevue, Québec, Canada H9X 3V9
2Agriculture and Agri-Food Research Centre, Lacombe, Alberta, Canada T4C 1W1

ABSTRACT

Stress is associated with significant losses in the swine industry. Seasonal changes have been shown to affect stress resistance in several species of animals. In the present study, we examined the effect of two seasons (fall or winter) on plasma insulin, cortisol, and adrenocorticotrophin hormone (ACTH) levels from fasted normal and carrier pigs for stress susceptibility when submitted to a blood-sampling stressor between 10:00 and 11:00. Comparisons were made of 10 diurnally active normal (NN) and 8 carrier (Nn) pigs reared in the fall to 9 NN and 10 Nn pigs reared in the winter. The light-dark cycle throughout the period of the experiment was 10h light/14h dark, with lights on from 07:00 to 17:00. Feed consisted of the traditional western Canadian swine ration consumed ad libitum. Results revealed significant season-by-genotype interactions for plasma insulin and ACTH concentrations in response to the blood-sampling stressor. The normal pigs displayed a significantly lower plasma insulin level during winter compared to the fall season. Plasma ACTH of normal pigs was 18% lower in the winter compared to the fall season, although this difference was not statistically significant. The car-

Received October 10, 1997; returned for revision October 28, 1997; accepted December 1, 1997.

Address requests for reprints to: Louise Thibault, School of Dietetics and Human Nutrition, Macdonald Campus, McGill University, 21 111 Lakeshore Road, Ste. Anne de Bellevue, Québec, Canada H9X 3V9.

This study was presented in abstract form at the 21st International Society of Chronobiology conference, Québec City, Québec, July 1993.

© 1998 International Society for Chronobiology
rier genotype, on the other hand, demonstrated higher plasma insulin concentration and lower plasma ACTH concentration in the winter compared to the fall season. Furthermore, there was a main genotype effect in plasma cortisol concentration such that the carrier pigs experienced a greater level compared to the normal genotype. Similarly, the carrier pigs had a more acute ACTH response to the blood-sampling stressor compared to the normal pigs during the fall season. Results have demonstrated that these two seasons of the year affect the response of both genotypes to a blood-sampling stressor, with the carrier genotype experiencing a more intense stress reaction in the fall season. These results further contribute to the recent research indicating that carrier pigs for stress susceptibility have a unique stress response. (Chronobiology International, 15(3), 275-281, 1998)

Key Words: Season—Hormones—Stress-susceptible pigs—Blood-sampling stressor.

INTRODUCTION

Stress susceptibility in swine, also known as malignant hyperthermia (MH), is a genetically inherited condition responsible for major economic losses in the swine industry. Pigs that are heterozygous carriers (Nn) of the genetic mutation in the halothane gene are exceptionally sensitive to common stressors such as exposure to high temperature, handling, fighting, breeding, and transport (1). As a result, pork producers are constantly searching for means to control stress in swine.

Research in the area of stress has shown that an animal's stress response is characterized by activation of the neuroendocrine and autonomic nervous systems (2). Adrenocorticotropic hormone (ACTH) and cortisol are released upon stimulation of the anterior pituitary adrenocortical axis and are commonly measured to quantitate the neuroendocrine response to stress. Insulin, on the other hand, is an indirect measure of stress, related to both the sympathetic nervous system (SNS) and adrenocortical axis. Insulin secretion is suppressed by epinephrine released from SNS stimulation and stimulated by elevated glycemia due to cortisol action on hepatic glucose production and release.

Seasonal changes are known to influence the stress response by their action on biochemical peripheral and central pathways, causing the release of pituitary and adrenal hormones and central nervous system monoamines in animals and humans (3-5). The functional relationship between neurotransmitters is greatly affected by the seasons (6) and was correlated to disturbances in animals' stress response (5). The purpose of our study was to examine the effect of two contrasting seasons on stress response as measured by plasma indices of the autonomic and neuroendocrine systems' response to stress when submitted to a blood-sampling stressor in pigs identified as normal and as carriers for stress susceptibility.

METHODS

Pigs were bred at the Agriculture and Agri-Food Canada Research Station (Lacombe, Alberta, Canada). They were kept in pens with cement floors of dimensions 2.5
m by 5.0 m and were provided daily with wood shavings as bedding. Feed consisted of a barley-based swine diet commonly used in western Canada (Table 1) and was designed to meet or exceed the National Research Council recommendations for nutrient intake (7). Feed and water were provided ad libitum. The light-dark cycle throughout the period of the experiment was 10h light/14h dark, with artificial lights on from 07:00 to 17:00. This lighting protocol was the usual schedule at the research station. To maintain study conditions constant, all animals were reared in one facility, which was kept at an ambient temperature of 20°C; therefore, the animals were not exposed to seasonal differences in external environment. The specific genotypes of the diurnally active animals were determined when they were between 7 and 8 weeks of age; the determination was based on their sensitivity to a halothane challenge (8). The two genotypes with respect to the MH mutation were (1) NN (homozygous normal), represented by pigs of the Lacombe breed shown to be free of the MH defect, and (2) Nn (heterozygous carrier), derived from specific matings of NN and a line of nn (homozygous carrier) pigs developed from Pietrain–Lacombe crossbred pigs as described by Sather and Murray (9). Following geno-

Table 1. Diet Composition and Nutrient Analysis of the Western Canadian Swine Diet*

<table>
<thead>
<tr>
<th>Composition</th>
<th>%</th>
<th>Analysis</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>58.90</td>
<td>DE (Mj·kg⁻¹)</td>
<td>13.08</td>
</tr>
<tr>
<td>Hard wheat</td>
<td>25.00</td>
<td>ME (Mj·kg⁻¹)</td>
<td>12.37</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.50</td>
<td>Crude protein (%)</td>
<td>15.54</td>
</tr>
<tr>
<td>Canola meal</td>
<td>4.00</td>
<td>Crude fiber (%)</td>
<td>4.37</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.40</td>
<td>Fat (%)</td>
<td>1.70</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.35</td>
<td>Calcium (%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.50</td>
<td>Phosphorus (%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Premix</td>
<td>0.25</td>
<td>Sodium (%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Pellaid</td>
<td>0.05</td>
<td>Potassium (%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.06</td>
<td>Magnesium (%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.01</td>
<td>Manganese (mg·kg⁻¹)</td>
<td>57.71</td>
</tr>
</tbody>
</table>

*Prepared at Quality Feeds Mill, Lacombe, Alberta, Canada.

11.5% crude protein
13.5% crude protein (red, winter)
46.5% crude protein (dehulled, solvent)
36.0% crude protein (prepressed, solvent)
38% calcium
18% calcium, 21% phosphorus

Ionized

Premix provided the following per kg: vitamin A, 160,000 IU; vitamin D, 240,000 IU; vitamin E, 10,000 IU; potassium, 1000 mg; thiamine, 400 mg; riboflavin, 1200 mg; niacin, 8400 mg; pyridoxine, 440 mg; vitamin B₁₂, 8 mg; pantothenic acid, 6800 mg; folic acid, 240 mg; biotin, 80 mg; iron, 60,000 mg; zinc, 60,000 mg; manganese, 12,000 mg; copper, 10,000 mg; iodine, 80 mg; cobalt, 160 mg; selenium, 40 mg

Nonnutritive pelleting agent

DE = digestible energy; ME = metabolizable energy.
type identification, 8-week old pigs were randomly selected according to gender and weight into two groups of 8–10 pigs per genotype. Comparisons were made of 10 diurnally active normal (NN) and 8 carrier (Nn) pigs reared in the fall to 9 NN and 10 Nn pigs reared in the winter. At 18 weeks of age, plasma indices of stress were determined from one group of each genotype in the fall. This protocol was repeated in another group of 18-week-old pigs during the winter season.

The blood-sampling stressor was performed after 10–12h of food deprivation. Blood samples were drawn from the jugular vein while the pig was secured with a snare. Samples were taken between 10:00 and 11:00; blood was immediately placed on ice and centrifuged within 1h. Plasma samples were stored at −30°C until biochemical analyses were performed. The plasma for ACTH analysis was stored at −80°C. Plasma was analyzed for insulin, cortisol, and ACTH by radioimmunoassay (10), with kits supplied by Stanbio, Incorporated (Bio-Endo, Montréal, Canada).

Data were analyzed by a general linear model (11), including genotype, season, and their interaction. Means were compared by a Student's two-tailed t-test.

**RESULTS**

The cortisol concentration of the two genotypes is presented in Fig. 1. Only a genotype effect on cortisol was found to be significant ($F(1,31) = 6.83, p < .013$), with a

![Cortisol (ug/mL)](image)

**FIGURE 1.** The effect of genotype on plasma cortisol concentration in stress-susceptible pigs.
**HORMONE CONCENTRATIONS RELATED TO STRESS**

Table 2. Effect of Genotype and Seasonal Variation on Plasma ACTH and Insulin in Pigs Submitted to a Blood Sampling Stressor

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fall</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACTH (pg/mL)</td>
<td></td>
</tr>
<tr>
<td>NN</td>
<td>63.37 ± 9.16</td>
<td>52.06 ± 13.31</td>
</tr>
<tr>
<td>Nn</td>
<td>107.95 ± 24.71</td>
<td>62.96 ± 14.23b</td>
</tr>
<tr>
<td></td>
<td>Insulin (uU/mL)</td>
<td></td>
</tr>
<tr>
<td>NN</td>
<td>15.39 ± 1.42a</td>
<td>12.85 ± 0.76b</td>
</tr>
<tr>
<td>Nn</td>
<td>14.65 ± 1.76b</td>
<td>18.64 ± 2.46kce</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
NN = homozygous normal; Nn = heterozygous carrier.
*Means with different letters are significantly different within genotype.
Means are significantly different from normal genotype within season.

greater level in carrier pigs compared to normal genotype pigs. ACTH and insulin levels demonstrated a significant genotype-by-season interaction ($F(1,31) = 5.65, p < .025$ and $F(1,32) = 16.25, p < .001$, respectively) (Table 2). Carrier pigs experienced a significantly higher plasma ACTH ($p < .002$) and significantly lower plasma insulin level ($p < .035$) during the fall compared to the winter season. The normal pigs did not differ in their ACTH response between seasons, but experienced a significantly lower ($p < .0001$) plasma insulin response during the winter season (Table 2). In the fall season, the carrier pigs experienced a greater ACTH response ($p < .0001$) than normal pigs. Finally, in the winter season, the carrier pigs had a higher insulin response ($p < .0001$) than did normal pigs.

**DISCUSSION**

The genotype effect of cortisol observed in this study is consistent with the existing literature available in stress-susceptible pigs (12). Carrier pigs for the stress-susceptible genetic defect are known to have a greater sensitivity to stressors.

Studies that have examined the response of the classical stress hormones in normal, carrier and stress-susceptible pigs have demonstrated that both the carrier and stress-susceptible pigs are more sensitive to stressors than are normal pigs (12–14). The lower ACTH response observed during the fall season in the normal compared to the carrier genotype in conjunction with the significantly greater plasma ACTH levels observed in the carrier pigs during the fall compared to the winter season support this observation.

During each season, pigs were maintained under constant living conditions of temperature, lighting, feeding, penning, and handling, yet they experienced seasonal differ-
ences in their endocrine response. The carrier pigs’ ACTH and insulin responses seem to reflect an increased level of stress in the fall season. A greater stress response in the fall season compared to winter has been previously demonstrated in rats (15), cats (4,5), and humans (3,16). With respect to ACTH, normal pigs do not seem to show seasonal variations in their stress response.

Results of this study support the contention that the carrier pigs have a unique stress response and a greater sensitivity to season. These data also underscore the need for ensuring use of predetermined genotypes when examining the stress response in normal and carrier pigs for stress susceptibility. Finally, it should be noted that, due to the similarities between the human and the porcine condition of MH and their metabolic, physiologic, and anatomic systems (17,18), the stress-susceptible pig may serve as a good model for its human counterpart, malignant hyperthermia.

ACKNOWLEDGMENTS

The study was supported by funds from the Canada-Québec subsidiary agreement on Agri-Food Development. Susan Roberts was supported by a scholarship from the Conseil de Recherches en Pêches et Agro-Alimentation du Québec (CORPAQ).

The authors thank Agriculture and Agri-Food Canada for providing the pigs used in the study. We also would like to thank Penny Johnson for her technical expertise. The piggery staff at the Agriculture and Agri-Food Canada Research Centre, Lacombe, Alberta, is much appreciated for their help with the care of the animals and good advice throughout the study.

REFERENCES

HORMONE CONCENTRATIONS RELATED TO STRESS