The effect of active immunization against adrenocorticotropic hormone on cortisol, β-endorphin, vocalization, and growth in pigs[1]

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ABSTRACT: Because the poor growth performance of intensively housed pigs is associated with increased circulating glucocorticoid concentrations, we investigated the effects of glucocorticoid suppression by inducing a humoral immune response to ACTH on physiological and production variables in growing pigs. Grower pigs (28.6 ± 0.9 kg) were immunized with amino acids 1 through 24 of ACTH conjugated to ovalbumin and suspended in diethylaminoethyl (DEAE) dextran-adjuvant or adjuvant alone (control) on d 1, 28, and 56. The ACTH-specific antibody titers generated suppressed increases in cortisol concentrations on d 63 in response to an acute stressor (P = 0.002; control = 71 ± 8.2 ng/mL; ACTH-immune = 43 ± 4.9 ng/mL) without altering basal concentrations. Plasma β-endorphin concentrations were also increased ($P < 0.001$) on d 63 (control = 18 ± 2.1 ng/mL; ACTH-immune = 63 ± 7.3 ng/mL), presumably because of a release from negative feedback on the expression of proopiomelanocortin in pituitary corticotropes. Immunization against ACTH did not alter ADG (P = 0.120; control = 1,077 ± 25; ACTH-immune = 1,143 ± 25 g) or ADFI (P = 0.64; control = 2,719 ± 42; ACTH-immune = 2,749 ± 42 g) and did not modify behavior (P = 0.681) assessed by measuring vocalization in response to acute restraint. In summary, suppression of stress-induced cortisol responses through ACTH immunization increased β-endorphin concentrations, but it did not modify ADG, ADFI, or restraint vocalization score in growing pigs.

Key Words: Adrenocorticotropic Hormone Immunization, β-Endorphin, Cortisol, Growth, Pig, Vocalization

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Introduction

Pigs raised under commercial conditions are exposed to stressors that result in activation of the hypothalamic-pituitary-adrenal (**HPA**) axis and induce various physiological responses. These responses are then reintegrated within the central nervous system to potentially modify behavior and other neuroendocrine systems to maintain homeostasis (Black et al., 2001). In the pig, ACTH is the primary stimulus for cortisol secretion, although there is some evidence that corticotrophin-releasing hormone (**CRH**) stimulates cortisol release (Lang et al., 2004). Modulation of increased circulating concentrations of cortisol, which is associated with decreased growth rate, muscle wastage, adiposity, and immunosuppression, has the potential to improve production efficiency in commercial hog production facilities. Active immunization against ACTH in sheep chronically suppressed stress-induced cortisol release and resulted in improvements in carcass composition, increased wool growth, and improved humoral immunity (Wynn et al., 1994, 1995). Moreover, active immunization also increased concentrations of β-endorphin, which was associated with decreased fearfulness in grazing sheep (Behrendt et al., 1992). In categorizing pigs as having active or passive temperaments, Hessing et al. (1993) reported that active pigs vocalized more frequently. Using a vocalization score in response to restraint stress, Giles and Kilgour (1999) reported that pigs with low vocalization scores consumed more feed than those with high vocalization scores.

The approach used in the current study was to suppress the activation of the adrenal cortex and the release of glucocorticoids in growing pigs by inducing active immunity to ACTH. This approach provides an experimental tool for understanding the effect of acute stress-induced cortisol responses on pig growth and behavior. Our hypothesis was that active immunization against ACTH would suppress stress-induced cortisol

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responses and increase basal β-endorphin concentrations, thereby improving growth performance. Additionally, to ascertain possible behavioral modifications caused by active immunization against ACTH, we assessed vocalization score during acute, restraint-induced stress.

Materials and Methods

Animals and Treatments

One week before the experiment commenced, 138 crossbred male pigs (Large White × Landrace) were assessed for temperament using a standardized procedure for the measurement of vocalization in response to restraint (Giles and Kilgour, 1999). Each pig was restrained using a commercial snout snare for 1 min in a separate concrete pen, and the aggregate noise output at intervals of 2 s was recorded with a decibel meter held adjacent to the mouth of the pig. The meter was attached to a laptop computer, which calculated cumulative 1-min recordings. Pigs with the 32 greatest and 32 least restraint vocalization scores were categorized as high and low vocalizers, respectively, and were allocated randomly to treatments in a $2 \times 2 \times 2$ factorial arrangement that was replicated in two rooms. The treatments were immunization (ACTH or control), housing (individual or groups of six pigs per pen), and vocalization (high or low). Vocalization in response to restraint was measured on d 0, 36, and 64.

Immunization Treatments

The antigen was prepared by coupling ACTH 1–24 (Synacthen; Ciba-Geigy, Basel, Switzerland) to ovalbumin (Sigma, St. Louis, MO) with 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (Sigma) in a 1:2 (wt/wt) ratio. The appropriate quantity of ACTH-ovalbumin antigen was dissolved in 300 μL of sterile saline (pH 7.4) containing 20% (vol/vol) diethylaminoethyl (**DEAE**)-dextran (Pharmacia, Uppsala, Sweden). To this was added a mixture of Whittrex-307 (Mobil, Altona, Australia) and Arlacel-80 (Ruger Chemical Co., Linden, NJ) oils at 9:1 (vol/vol). The mixture was then emulsified to provide a formulation to release the antigen slowly.

Pigs were allocated to ACTH-immunized and nonimmunized (control) treatments on the basis of restraint vocalization score and BW to minimize differences between treatment groups. Control pigs (n = 32) received 1 mL of sterile saline emulsified in 1 mL of DEAE-dextran adjuvant for each injection. The remaining pigs (ACTH-immune; n = 32) received 0.5 mg of ACTH (1-24)-ovalbumin (1:2) conjugate dissolved in 1 mL of sterile saline and emulsified in 1 mL of DEAE-dextran adjuvant for each injection. The primary and first and second booster injections (2 mL) were administered i.m. in the side of the neck on d 0, 28, and 56, respectively.

Housing and Feeding

Pigs were housed in two climate-controlled rooms at the Elizabeth Macarthur Agricultural Institute (Camden, NSW, Australia). The rooms were maintained at 22°C, 60% relative humidity, and a 12:12 light:dark cycle, with lights on at 0700. From d 0 to 28, pigs were maintained in group pens (eight pigs per pen) containing equal numbers of ACTH-immune and control animals. Immediately following the first booster immunization (d 28), ACTH-immune and control pigs were allocated to housing treatments (single or group pens) on the basis of BW and vocalization to minimize differences between the four housing × immunization groups. The eight groups [(ACTH or control) × (single or group housing) × (high or low vocalization)] were then allocated to the pens in each of the two rooms. Each room contained eight single and four group pens (six pigs per pen; Figure 1). Flooring (concrete slats) and floor area (1 m^2 per pig) were similar in each room. The four group pens occupied the center of each room flanked by four single pens on each side. For each housing treatment, the four immunization × vocalization groups were allocated randomly to the pens. For single pens, the randomization ensured that each of the four immunization × vocalization groups was allocated one pen in each flank of four single pens.

Feed intake measurements commenced on d 35. Pigs were fed a commercial, pelleted diet based on wheat and sorghum (Vella Stock Feeds, Plumpton, Australia) containing 10 g of available lysine and 13.5 MJ of DE/kg (as fed). Feed was offered ad libitum from an individual feeder in each pen. Water was provided ad libitum from nipple drinkers alongside each feeder. Animals housed in individual pens received a preweighed quantity of feed daily, and at the end of each week, residues were weighed to calculate ADFI. Animals housed in group pens received a preweighed quantity of feed at 1000 each day. Each group feed trough was emptied, and the residue weighed each day to calculate total group feed intake. Each pig was weighed weekly for the 9-wk duration of the experiment.

Sample Collection

On d 0, 35, and 63, each pig was restrained with a nose-snare, and blood samples (5 mL) were collected by vena cava venipuncture using an 18-ga needle. Samples were collected within 1 min of restraint to minimize the chance of acute adrenal activation in response to venipuncture. An additional blood sample was collected on d 63 at 15 min after the blood sampling to determine the magnitude of the acute cortisol response to nose snaring. Blood was collected into tubes containing EDTA (Venoject, Terumo, Tokyo, Japan) and placed immediately on ice until centrifugation at $1{,}500 \times g$ for 15 min at 4°C. Plasma was collected and stored at –20°C until analysis.

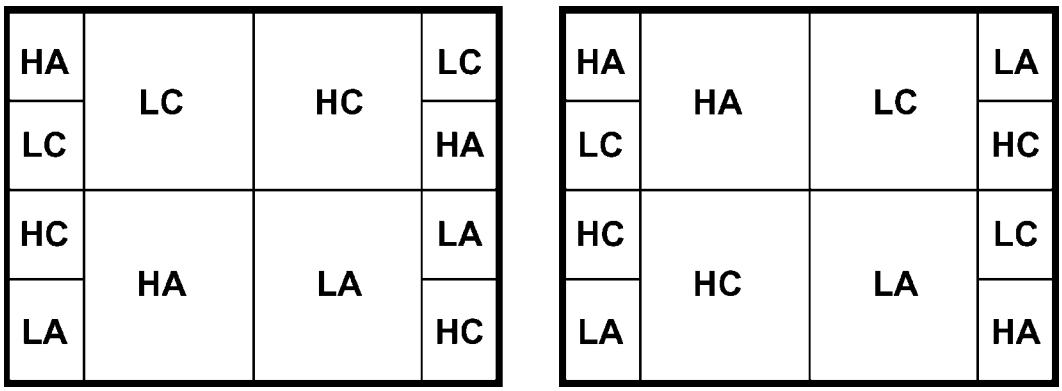

Figure 1. Treatments and room design. Room 1 is on the left; Room 2 is on the right. The larger pens housed groups of six pigs per pen, and the smaller pens housed single pigs. Treatments were vocalization (L = low; H = high) and immunization (A = ACTH; C = control).

Hormone Assays

Cortisol was assessed by RIA (Gallagher et al., 2002). The intra-assay CV for samples containing 11.1 and 35.3 ng/mL were 11.6 and 8.2%, respectively, and the interassay CV for the same samples were 13.0 and 11.3%, respectively. The sensitivity of the plasma cortisol assay was 2.2 ng/mL.

The concentration of β-endorphin in samples was determined using a modified method of Lim et al. (1982). Standards and pig plasma samples (100 μL) were placed in polypropylene tubes (12 mm × 75 mm) and incubated with 100 μL of primary antibody for 24 h at 4°C. On d 2, 100 μL of β-endorphin tracer (9,000 to 11,000 cpm) were added, and the tubes were incubated for 48 h at 4°C. On d 4, 100 μL of normal rabbit serum (1:200 dilution in assay buffer) and 100 μL of donkey anti-rabbit serum second antibody (1:4 dilution in assay buffer) were added. The solutions were incubated for 18 to 24 h at 4°C. On d 5, tubes were centrifuged at 3,000 × *g* for 1 h at 4°C, and the supernatant fraction was aspirated. The precipitates were counted in a gamma spectrometer, and β-endorphin concentrations were calculated using Multicalc software (Wallac, Turku, Finland). The serially diluted porcine plasma samples demonstrated parallelism with the assay standard curve. The mean recovery of added β-endorphin to pig plasma was 92%, and the sensitivity of the assay was 5 pg/mL. The intra-assay CV for samples containing 65.2 and 320.5 pg of β-endorphin/mL were 14.7 and 11.8%, respectively, and the interassay CV for the same samples were 15.3 and 12.7%.

Plasma ACTH-Specific Antibody Titers

Plasma ACTH antibody titers were measured by ELISA (Paull et al., 2004). The intra-assay CV was 18.3%, and the interassay CV was 21.0%. The specificity of the ACTH antibodies was tested against porcine amino acids 1 to 39 of ACTH ($ACTH_{1\text{-}39}$), β-endorphin, corticotropin-like intermediate lobe $peptide_{18\text{-}39}$, $ACTH_{1\text{-}24}$, and alpha-melanocyte-stimulating hormone (α-$\mathbf{MSH_{1\text{-}10}}$) for concentrations ranging from 4.8 to 5,000 pg/mL using (^{125}I) Phe_2-$Nle_4ACTH_{1\text{-}24}$ as the radioligand in an ACTH RIA procedure (Behrendt, 1998). The antibodies were specific for the carboxyl terminus of $ACTH_{1\text{-}24}$ because cross reactivity was not observed with α-MSH, which corresponds with $ACTH_{1\text{-}13}$, nor was cross reactivity observed with $ACTH_{18\text{-}39}$.

Statistical Analyses

Pigs were allocated to immunization and housing treatments, which, along with high or low vocalization, were allocated to pens within each room. Housing treatments were not randomized within each room. To provide approximate inference for the effects of housing treatment and its interactions, a pseudo-factor called "section" was used, which took one of three values: the left flank of single pens, the middle section of four group pens, or the right flank of single pens.

Mixed model analyses were conducted using the schematic ANOVA representation of the terms in the statistical model shown in Table 1. Cortisol and β-endorphin were analyzed on a logarithmic scale, as there was a strong mean variance relationship. Univariate, mixed model analyses were conducted for ADFI, ADG, and vocalization change. Repeated measures mixed model analyses were performed on the three post-treatment measurements of cortisol and the two post-treatment measurements of β-endorphin. As in Table 1, each mixed model comprised the fixed effects of the treatment factors, housing (single or group), vocalization (high or low), and ACTH immunization (absent or present), and their interactions. For the repeated measures analysis, the mixed model also included the interactions of each of these listed fixed effects with "time," where "time" represented the measurement [5 or 9 wk for β-endorphin or 5, 9 (0 min), and 9 wk (15 min) for cortisol]. For the cortisol, β-endorphin, and vocalization analyses, the d 0 measurement also was considered as a covariate to decrease the residual variation. The effects

Table 1. Schematic representation of the terms in the ANOVA

Source	Decomposition	df	Fixed or random
Room			
	Mean	1	Fixed
	Error	1	Random
Sections within rooms			
	Housing	1	Fixed
	Error	3	Random
Pens within sections			
	Vocalization	1	Fixed
	Immunization	1	Fixed
	Housing × vocalization	1	Fixed
	Housing × immunization	1	Fixed
	Vocalization × immunization	1	Fixed
	Housing × vocalization × immunization	1	Fixed
	Room × vocalization × immunization (single pens)	3	Random
	Error (group pens)	3	Random
	Error (single pens)	6	Random
Pigs within pens			
	Error	39	Random

of rooms, sections within rooms, pens within sections within rooms, and their interactions with time for the repeated measures analyses were fitted as random, allowing two separate variance components for group and single pens, respectively. For the repeated measures analyses, the residual error was modeled as an unstructured covariance matrix. All analyses were performed in ASREML (Gilmour et al., 2004). *F*-statistics and the denominator degrees of freedom for testing fixed effects were calculated using Kenward-Roger adjustments (Kenward and Roger, 1997). One pig, a high vocalizer allocated to ACTH immunization and group housing, was omitted from the analysis because it had lost 2 kg of BW over the 4-wk period, and it was found to be suffering from acute pleuropneumonia.

Results

ACTH-Specific Antibody Responses

No ACTH-specific antibodies were detected in any of the control pigs, whereas pigs immune against ACTH displayed significant antibody titer responses ($P < 0.001$; Figure 2). These increased from d 0 to 35 in response to the first booster injection (d 28; $P < 0.001$) and were maintained on d 63 in response to the second booster injection (d 56; $P = 0.088$). Housing did not affect antibody titers, as there were no differences between ACTH-immune pigs housed singly or as a group ($P = 0.510$). When assessed on d 35, restraint vocalization score was related to antibody titer. High vocalization pigs had greater ($P = 0.050$) ACTH-antibody titers than did low vocalization pigs; however, this difference did not persist, as it was not evident on d 63 ($P = 0.700$).

Hormones

There were no significant effects caused by housing or restraint vocalization score measured on d 35 and 63 for concentrations of cortisol ($P = 0.896$ for housing; $P = 0.771$ for vocalization) or β-endorphin ($P = 0.102$ for housing; $P = 0.787$ for vocalization), and there were no significant interactions with time (cortisol: $P = 0.683$ for housing and $P = 0.726$ for vocalization; β-endorphin: $P = 0.914$ for housing and $P = 0.378$ for vocalization). Plasma cortisol and β-endorphin concentrations for each main effect are shown in Table 2. There was an interaction between ACTH immunization and time for cortisol ($P = 0.004$) and a significant main effect of ACTH immunization on β-endorphin concentrations ($P < 0.001$). On d 63, cortisol concentrations were increased in control pigs compared with ACTH-immune animals when measured 15 min after nose snaring ($P = 0.002$), but there were no significant ACTH immunization ef-

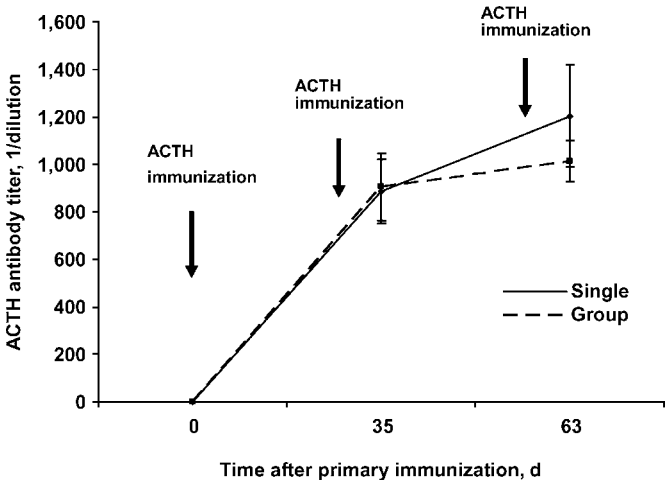

Figure 2. Mean (±SEM) ACTH-specific antibody titer for growing pigs housed in single and group pens (six pigs per pen) immunized with amino acids 1 to 24 of ACTH conjugated to ovalbumin and suspended in diethylaminoethyl dextran-adjuvant.

Table 2. Mean plasma cortisol and β-endorphin concentrations (g; ±SEM) for growing pigs immunized with ACTH adjuvant or adjuvant alone (control) on d 0, 28, and 56, housed in single or group pens (six pigs per pen), and classified as having high or low vocalization

	Immunization			Vocalization[a]			Housing			*P*-value		
Item	Control	ACTH	SEM	Low	High	SEM	Single	Group	SEM	Immunization	Vocalization	Housing
	Log cortisol[b]											
d 0	4.14 (63)	4.22 (68)	0.10	4.15 (64)	4.21 (68)	0.10	4.06 (58)	4.31 (74)	0.16	0.58	0.66	0.12
d 35	3.94 (52)	3.47 (32)	0.17	3.63 (38)	3.78 (44)	0.16	3.70 (41)	3.71 (41)	0.25	0.07	0.55	0.98
d 63/0 min	3.58 (36)	3.54 (35)	0.07	3.54 (34)	3.58 (36)	0.07	3.52 (34)	3.60 (37)	0.15	0.92	0.78	0.60
d 63/15 min	4.27 (72)	3.74 (42)	0.10	4.03 (56)	3.99 (54)	0.10	4.04 (57)	3.98 (53)	0.16	<0.001	0.75	0.71
	Log β-endorphin[b]											
d 0	3.09 (22)	3.08 (22)	0.07	3.09 (22)	3.08 (22)	0	3.09 (22)	3.07 (22)	0.07	0.90	0.90	0.84
d 35	3.07 (22)	3.99 (54)	0.14	3.50 (33)	3.56 (35)	0.19	3.35 (28)	3.71 (41)	0.14	0.004	0.76	0.12
d 63	2.97 (19)	4.07 (58)	0.21	3.57 (36)	3.46 (32)	0.29	3.35 (28)	3.69 (40)	0.21	0.01	0.71	0.27
Average	3.02 (20)	4.03 (56)	0.15	3.54 (34)	3.51 (33)	0.21	3.35 (28)	3.70 (40)	0.15	<0.001	0.89	0.12

[a]Pig restraint with a nose snare and vocalization (decibels) recorded at intervals of 2 s and aggregated over 60 s.
[b]Data are expressed on a log scale, with back-transformed means (ng/mL) in parentheses.

fects at the other times ($P = 0.109$ for d 35; $P = 0.893$ for d 63 at 0 min). The ACTH-immune pigs displayed increased β-endorphin concentrations compared with controls on both d 35 ($P < 0.001$) and d 63 ($P < 0.001$). In control pigs, β-endorphin concentrations changed little for the 9-wk experimental period.

Intake, Growth, and Vocalization

There were no significant effects of ACTH immunization and no significant interactions on ADFI ($P = 0.658$), ADG ($P = 0.119$), or vocalization ($P = 0.681$; Table 3). The ADFI by group-penned pigs was less than that by single-penned pigs ($P < 0.001$). Pigs with low restraint vocalization scores had greater ADFI than those with high restraint vocalization scores ($P = 0.045$).

Discussion

The induction of circulating ACTH-specific antibodies suppressed the increase in cortisol concentrations in response to acute physical stress on d 63, while maintaining basal circulating cortisol concentrations. Despite the effectiveness of the antibodies in stimulating β-endorphin and suppressing stress-induced cortisol concentrations, no effect of ACTH immunization on the growth of pigs was observed. These animals, however, were not subjected to a pathological or environmental challenge to activate the HPA axis to a level that compromised growth chronically. Our previous studies, in which we found a strong correlation between high stocking density and adverse air quality in a commercial hog production facility with increased cortisol status (Lee et al., 2005), were conducted under much more adverse environmental conditions. The anti-ACTH antibody titers reported here are an order of magnitude less than those achieved with the same antigen-adjuvant system in sheep, in which production responses were observed (Wynn et al., 1994, 1995). In the current study, there might have been insufficiently high ACTH-specific antibody titers to exert any changes in growth. Limitations associated with immunoneutralization of a hormone make it difficult, as the endocrine system compensates and chronic high titers of high-affinity antibody are required to remove much of the hormone (Reeves et al., 1989). In sheep, ACTH immunity resulted in decreased carcass fatness when the sheep were subjected to the long-term psychosocial stress through disruption of

Table 3. Mean daily weight gain, daily feed intake (d 35 to 63), and vocalization (±SEM) of male grower pigs housed in either single (n = 16) or group pens (n = 8; six pigs per pen), immunized against either ACTH or adjuvant (control), and with high or low vocalization.

	Immunization			Vocalization[a]			Housing			*P*-value		
Item	Control	ACTH	SEM	Low	High	SEM	Single	Group	SEM	Immunization	Vocalization	Housing
ADG, g	1,077	1,143	25	1,130	1,089	25	1,167	1,052	51	0.09	0.29	0.18
ADFI[b], g	2,719	2,749	42	2,804	2,664	42	2,965	2,504	53	0.66	0.03	<0.001
Vocalization on d 0, dB	2,538	2,550	21	2,186	2,901	21	2,554	2,534	24	0.42	<0.001	0.54
Vocalization change, dB[c]	–9	7	32	–31	29	119	–73	71	43	0.68	0.58	0.14

[a]Pig restraint with a nose snare and vocalization (decibels) recorded at intervals of 2 s and aggregated over 60 s.
[b]ADFI on an as-fed basis.
[c]Change in restraint vocalization score between d 0 and 64.

their social hierarchy (Wynn et al., 1994). The positive relationship between stress hormone status and carcass fatness has been reported in both female and castrated male Large-White × Duroc pigs (Foury et al., 2005). Similarly, breeds with a propensity for high fatness, such as the Duroc (Smith and Pearson, 1986) and the Meishan (Bidanel et al., 1993), also secrete greater amounts of cortisol. Sillence et al. (1992) passively immunized rats with ACTH-specific antibodies, which resulted in a 37% increase in BW gain and a 59% decrease in peak plasma concentrations of the key glucocorticoid in rodent species, corticosterone. Shahneh (1995) was unable to induce changes in growth in ACTH-immune crossbred sheep; however, these animals were maintained on pasture at low stocking density with access to a concentrate pelleted diet ad libitum. Hence, stress and, therefore, activation of the HPA axis were not major factors in that study. The present findings suggest that the efficacy of ACTH immunity on growth should be assessed in a commercial hog production facility where animals are subjected to a repertoire of acute and chronic physical, social, and environmental stressors. Although manipulation of adrenal responses may result in improved growth of pigs under stressful conditions, a more ethical approach, in terms of animal welfare, may be to remove the source of the stress.

In sheep, very high ACTH antibody titers resulted in increased abundance of proopiomelanocortin (**POMC**) mRNA in sheep skin and pituitary gland, which was accompanied by a 10-fold increase in circulating β-endorphin concentrations (Porter et al., 1999). The functional relationship between POMC expression and adrenal function is most dramatically illustrated by the undetectable glucocorticoid status of transgenic POMC knock-out mice (Coll et al., 2004). The maintenance of basal glucocorticoid status in immune animals also may be assisted by the presence of anti-idiotypic antibodies, which activate adrenocortical ACTH receptors. We have evidence regarding the presence of such ACTH antibody clones in the sheep (A. Shahneh and P. C. Wynn, unpublished data), and these may be important in ensuring that ACTH-immune sheep do not lapse into a state of adrenal insufficiency.

The increased cortisol concentrations measured on d 0 may reflect the stress of mixing and regrouping, which have been reported as potent adrenocortical activators (McGlone, 1985). On d 63, the first blood sampling occurred rapidly, within 1 min of restraint, reflecting basal cortisol concentrations. The second blood sample was taken 15 min after the acute stress of nose snaring, and the effectiveness of the ACTH antibodies was displayed by the suppression of the cortisol response in ACTH-immune pigs. The timing of these blood samples was consistent with the chronology of the release of hormones following the initial perception of the stressor within the limbic network; thus, the 1-min sample precedes adrenal activation, whereas the 15-min sample provides a measure of the extent of adrenal cortisol release in response to the stress (Sapolsky et al., 2000). A similar time course is reported for the release of ACTH and cortisol in response to chronic restraint stress in pigs (Klemcke, 1994). Although ACTH immunization results in suppression of restraint, stress-induced release of glucocorticoids, it is important for the animal to maintain the basal concentrations of these hormones required for normal metabolism. This becomes a challenge for the biologist because most studies of the role of the glucocorticoids, as indeed with most hormones, involve the ablation of the source tissues by either surgical adrenalectomy (McFarlane et al., 1995) or transgenic approaches (e.g., POMC knock-out mice). In the latter case, POMC gene knock-out in mice resulted in an obese phenotype caused by hyperphagia (Smart and Low, 2003), and subsequent POMC rescue experiments suggest a role for glucocorticoids in augmenting this obese phenotype (Smart et al., 2003), which is undesirable in animal production. Nevertheless, it is desirable to suppress the high stress-induced concentrations responsible for the catabolic responses in skeletal muscle and immune tissues (Munck et al., 1984; Sapolsky et al., 2000).

Plasma concentrations of β-endorphin were greater in ACTH-immune pigs than in control pigs ($P < 0.001$). The suppression of stress-induced peaks in secretion of β-endorphin most likely resulted in a release from cortisol negative feedback in the prefrontal cortex. Specific glucocorticoid receptors have been localized in this brain region in both the pig (Weaver et al., 2000) and rodent species (Diorio et al., 1993; Akana et al., 2001) and directly in the pituitary gland of both species (Hauger et al., 1987; Weaver et al., 2000). The release from cortisol negative feedback most likely increased the expression of CRH, its receptor, and then expression of the POMC gene and subsequent translation and processing to yield increased circulating concentrations of β-endorphin. Similar responses have been noted with adrenalectomy in the rat for CRH and its receptor (Wynn et al., 1985) and for POMC in both the pituitary gland and hypothalamus (Wardlaw et al., 1998). Potentially, the elevation in β-endorphin status would increase feed intake because the stoichiometric processing of the POMC protein to form β-endorphin provides an opioid stimulus for caloric intake. Pharmacological administration of opioid agonists results in hyperphagia (Glass et al., 1999); however, increased β-endorphin in our study was not associated with increased feed intake by these pigs. Although little is known of the interactions of the components comprising the orexigenic and anorexic pathways of the porcine hypothalamus, it is likely that they are similar to that of other species. For example, the evolutionary conservation of the receptor effector system of the key feeding stimulant, ghrelin, from the puffer fish to the human is well documented (Palyha et al., 2000). Some differences exist, for example, in the way POMC expression is regulated by feeding rodent and ruminant species (Henry, 2003), suggesting that it is important that these mechanisms be elucidated for the pig. The fact that peripheral

β-endorphin stimulates feeding, whereas α-MSH derived from the same precursor protein POMC is anorexic, points to the complexity of the role of this prohormone and its constituent peptides in regulating feed intake (Heisler et al., 2003). Despite the increased β-endorphin concentrations measured in ACTH-immune pigs, the behavioral response to restraint stress as measured by vocalization score in our study was not affected. Behavior was modified in ACTH-immune sheep subjected to an approach-avoidance paradigm or "arena test" (Fell and Shutt, 1989; Adams and Fell, 1997) designed to assess fearfulness by assessing the conflict between approaching flock mates and avoiding an unfamiliar person placed strategically between the flock and the test sheep. Sheep immunized against ACTH disregarded the unfamiliar person and lost their natural flocking instinct behavior completely (Behrendt et al., 1992). These responses were attributed to activation of central opioid mechanisms presumably associated with the increased circulating β-endorphin concentrations induced in these sheep. Similarly, an opioid-induced decrease in vocalization was observed after restraint stress with a snout roping procedure in pigs, providing evidence of a behavioral role for β-endorphin in this species (Janssens et al., 1995). Despite this evidence, the current study failed to find changes in vocalization in response to ACTH immunization.

Hessing et al. (1993) discriminated between active and passive pigs by measuring physical activity in response to a restraint or "back test." In their study, the more active pigs also squealed most frequently. Passive pigs also have been shown to consume more feed (Giles and Kilgour, 1999), which is consistent with the negative relationship between restraint vocalization score and feed intake observed in both single- and group-penned animals in the current study; however, this result was independent of ACTH antibody status. This finding has important implications for pig production, as the vocalization test may facilitate selection of pigs with passive temperaments and potentially greater growth performance. A point to note on the measurement of vocalization in the current study is that it was not recorded out of ear-shot or sight of other pigs, and this might have influenced the response of other pigs tested. In future studies, vocalization will be measured out of range and sight of other animals. Further investigation is required of the relationships among vocalization, back test, and position in the dominance hierarchy, perhaps focusing on the age range of 3 to 8 wk, when temperament seems to be stable (Hessing et al., 1994).

In conclusion, pigs that were maintained in a clean environment and immunized against ACTH maintained cortisol concentrations in the normal or unstressed range, but they showed a suppressed cortisol response when the HPA axis was challenged by acute restraint stress. Despite increased β-endorphin concentrations in ACTH-immune pigs, immunization was not associated with changes in the restraint vocalization score of pigs. Vocalization was, however, related to growth performance, with low-restraint vocalization pigs having greater ADFI than high-restraint vocalization pigs. There was no effect of ACTH immunization on growth or feed intake, however. Based on our observations, the manipulation of glucocorticoid and opioid status through the induction of low-level immunity to ACTH was ineffective in boosting feeding behavior and growth in growing pigs. Perhaps our experimental conditions provided insufficient stress challenges to the animals, thereby negating any potential growth and behavioral advantages that might result from ACTH immunization.

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