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Quantitative indicators of stress in stress-susceptible and stress-resistant breeds of pigs

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ABSTRACT

Changes in plasma concentrations of cortisol, ACTH and free fatty acids were evaluated as quantitative indicators of stress in pigs of stress-susceptible and stress-resistant breeds. The changes in these concentrations following loading, transport and subsequent resting in lairage were examined. Resting concentrations of cortisol were higher ($P < 0.01$) in stress-susceptible Pietrain pigs ($44.0 \pm 5.7 \mu\text{g/L}$) than in stress-resistant Gloucester Old Spots ($29.0 \pm 3.8 \mu\text{g/L}$). Plasma cortisol and ACTH concentrations increased two-fold in both breeds following loading on to a lorry, and more than three-fold after 1 hour transportation; two hours after unloading into lairage, levels had returned to near resting levels in all pigs. Free fatty acid (FFA) concentrations were similar in these breeds of pigs and did not change significantly following loading stress. After transport, FFA levels were approximately double resting levels in both breeds, and returned to basal levels in lairage. These data suggest that cortisol is a quantitative marker of stress in pigs and that, despite different resting cortisol concentrations, stress-susceptible and stress-resistant pigs have similar relative responses to stress, although absolute increases are greater in the stress-susceptible Pietrains.

Keywords: Stress, transport, loading, pigs, cortisol, ACTH, free fatty acids

INTRODUCTION

There has long been considerable interest in identifying practical quantitative markers of stress in farm animals. Cortisol has often been suggested and used as a marker of stress, but its value as a quantitative indicator of stress has not been defined. Adrenocorticotrophic hormone (ACTH), as the major cortisol secretagogue, and plasma free fatty acids (FFA) have also been suggested as markers of stress. In previous studies (Spencer, 1980; 1994) it has been shown that plasma free fatty acids alter during the stress response, but there are fewer data on ACTH changes with stress in farm animals.

In the area of farm animal welfare, there is debate as to whether transportation *per se* is stressful to animals, or whether it is the loading-stress that constitutes the major stressor. By comparing the temporal changes of putative stress markers to loading stress, and then to loading followed by transport, it may be possible to evaluate both the relative stress induced by the two procedures, and at the same time identify quantitative measures of stress.

In the present studies the ACTH, cortisol and FFA responses to loading, and to transport have been measured in stress-sensitive and -resistant pigs (Lister *et al.*, 1980).

MATERIALS AND METHODS

Stress-susceptible (SS) Pietrain pigs, and stress-resistant (SR) Gloucester Old Spot (GOS) pork-weight pigs were used in these studies. The pigs were fitted with jugular vein catheters while under thiopentone/N₂O anaesthesia, and the catheters exteriorised between the shoulder blades and secured. After surgery the animals were housed in individual metabolism crates and handled and sampled at least daily to accustom them to the blood sampling procedures.

Loading stress

At least 5 days after surgery, food was removed in the evening and a simulated loading stress (SLS) was applied the

following morning. The SLS and blood sampling have been described in detail elsewhere (Spencer, 1980). Briefly, an initial blood sample was taken while the animals were still asleep (-30 min), the lights were then switched on and a further blood sample was taken (-15 min). A further 15 min thereafter the pigs were unloaded from the crates down a ramp and shepherded around the building prior to reloading into the crates. The SLS procedure took 5 minutes and blood samples were then taken at various intervals.

Transport stress

At least 7 days after surgery, food was again withheld overnight and the following morning the pigs were loaded into a trailer; pigs were loaded and transported in pairs. Five minutes after loading a blood sample was taken, and the pigs transported for one hour. In some cases a blood sample was also taken in mid-journey (back at the laboratory). At the end of the transport, all pigs were again sampled, unloaded, and allowed to rest in lairage for two hours and then sampled again.

Plasma cortisol and FFA were measured as described elsewhere (Spencer, 1980) and ACTH was measured using a commercial radioimmunoassay kit (Amersham).

The pigs were slaughtered by electrical stunning and vena cava puncture. Samples of *M. longissimus dorsi* were taken at 45 min for measurement of pH, and at 48 hours colour and drip loss were estimated (Spencer *et al.* 1983).

Differences between groups were analysed by multiple analysis of variance and significant differences determined by F-test.

RESULTS

Basal concentrations of cortisol in resting animals (Figure 1) were significantly higher ($P < 0.01$) in the SS-Pietrain pigs ($44.0 \pm 5.7 \mu\text{g/L}$) than in the SR-GOS ($29.0 \pm 3.8 \mu\text{g/L}$), but there were no significant differences in resting ACTH or FFA concentrations.

¹These studies were undertaken at the Meat Research Institute, Langford, Bristol, UK.

Plasma cortisol concentrations rose slightly upon waking (Figure 2), but following SLS, cortisol concentrations doubled ($P<0.01$) in both breeds, with peak levels occurring at 10 min and levels returning to basal by 60 min. (Figure 2). A similar increase was seen following loading in the transport experiment but after 1 hour of transport, levels had increased further ($P<0.01$, compared with post-loading levels) in both breeds (Figure 1). Levels had returned to basal in all pigs after two hours in lairage.

Initially, FFA concentrations marginally decreased (in Pietrain pigs) following SLS but later rose to be significantly elevated ($P<0.05$) by 75 mins (Figure 3). In the transport experiment, no significant increase in FFAs was seen after loading, but levels were double basal following transport ($P<0.01$) and they had returned to near basal after lairage (Figure 4).

ACTH was measured only in 3 SS and 5 SR pigs. In both breeds basal concentrations, and stress responses, were not significantly different and the data have been pooled. There

was a doubling of ACTH levels following loading ($P<0.01$), and a trebling ($P<0.001$) after transport (Figure 5).

In those animals where mid-transport samples were taken, plasma concentrations of all stress indicators were similar to the post-transport levels (data not shown).

All the Pietrain pigs exhibited low 45 min muscle pH (<5.8) and had pale, soft exudative (PSE) meat as determined by colour and drip measurements. The meat data from the GOS pigs showed they all had a 45 min pH >6.0 and did not show PSE characteristics.

DISCUSSION

Plasma cortisol has been widely advocated as a practical indicator of stress, but its use as a quantitative indicator has not been adequately evaluated in farm animals. This is because it has been difficult to determine different degrees of stress independently of the indicator being examined. In the present studies, the comparison of temporal changes in load-

FIGURE 1: Plasma concentrations of cortisol in Pietrain (□) and Gloucester Old Spot pigs (■) at rest, after loading, after 1 hour of road transport, and 2 hours lairage after transport. Values are means ± sem. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared with appropriate resting values. † $P<0.01$ compared with appropriate post-loading values.

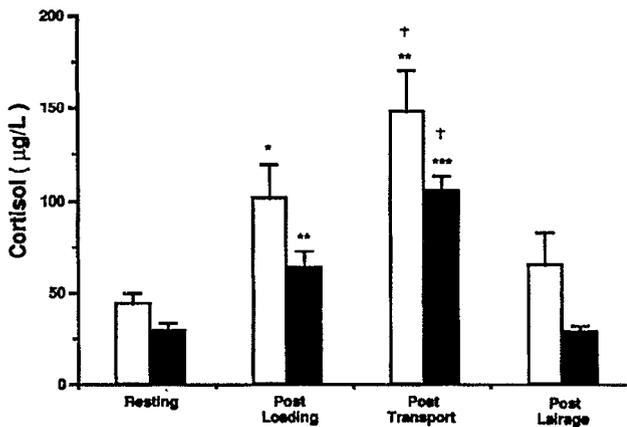


FIGURE 2: Plasma cortisol concentrations in Pietrain (- - -) and Gloucester Old Spot (—) pigs before and after a simulated loading stress (SLS-▨). Values are means ± sem. * Values are significantly different from mean pretreatment values $P<0.05$ or less.

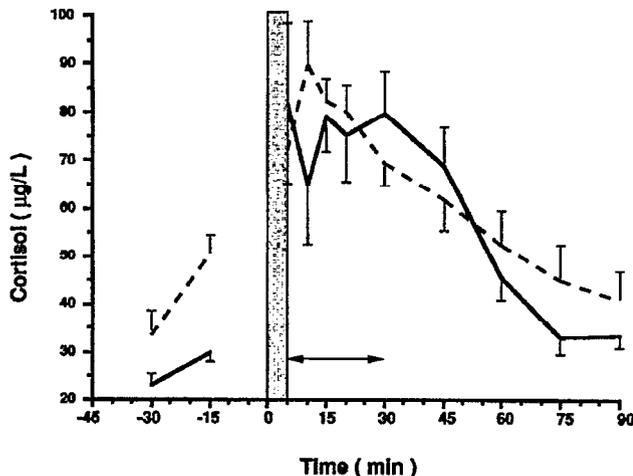


FIGURE 3: Plasma free fatty acid concentrations in Pietrain (- - -) and Gloucester Old Spot (—) pigs before and after a simulated loading stress. (SLS-▨). Values are means ± sem. * significantly different from mean pretreatment values $P<0.05$.

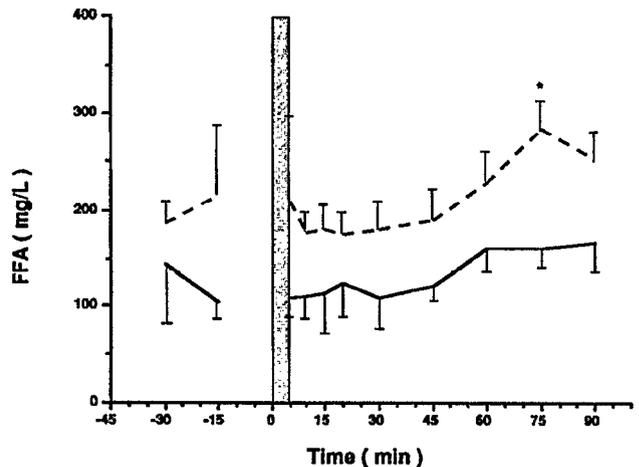
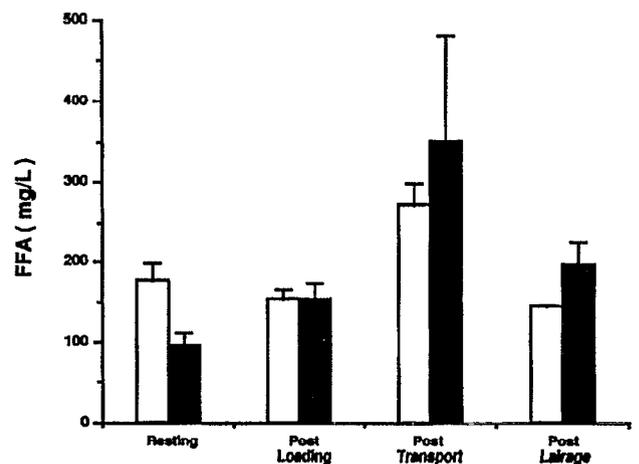


FIGURE 4: Plasma concentrations of free fatty acids in Pietrain (□) and Gloucester Old Spot pigs (■) at rest, after loading, after 1 hour of road transport, and 2 hours lairage after transport. Values are means ± sem.



ing stress, followed by the addition of transport stress, allows the relative stressors to be evaluated. The SLS data shows that cortisol levels reach a maximum at 10 min after stress. Thus in the transport experiment, the immediate post-loading samples are peak levels. If transport provided no further stress, levels would either have returned to basal or have been maintained after 1 hour of transport. However, cortisol levels were elevated further in the post-transport sample indicating that transport provides an additional stress to that induced by loading. Further evidence of quantitative changes in cortisol may be inferred from the apparent elevation in cortisol upon waking, which is likely to induce a slight stress. Although ACTH levels were not measured in the SLS experiment, the step-wise increase in ACTH (which precedes cortisol release) with loading followed by transport, is further evidence of a quantitative increase in stress with transportation.

The possibility that a single exposure to loading onto a trailer may have accustomed the pigs to the procedure was avoided as the SLS did not involve the use of a trailer. Furthermore, the similarity of the cortisol levels in response to loading in both the SLS and loading/transport studies suggest there was no acclimatisation to the procedure.

The changes in cortisol and FFA after loading stress agree both temporally and in magnitude with those previously reported (Baldwin & Stephens, 1973; Spencer, 1980, 1994; Becker et al, 1985). It has been suggested that SS pigs may suffer from adrenal insufficiency (Judge et al, 1968; Marple & Cassens, 1973) and have an impaired adrenocorticoid response to stress. The similar magnitude of the cortisol response among breeds in this study to SLS and transport stresses does not support this hypothesis, although different breeds of pigs were used in the two studies.

It has been indicated that the metabolic response to stressors differs among SS and SR pigs (Wood et al, 1977), particularly in regard to lipolytic responses to exogenous catecholamine infusion. The role of plasma FFA in protecting against decreased meat quality from stressed animals has been shown (Lister and Spencer, 1983; Spencer et al, 1983), and may influence the meat quality in SS and SR pigs. In the

present experiments it was not possible to find a significant difference between SS and SR pigs in terms of their basal FFA levels or their FFA response during stress. Thus the availability of energy from fat depots by lipolysis would not seem to contribute to the meat quality differences among the breeds in this study.

From these experiments it appears that cortisol and ACTH can be used as quantitative indicators of stress and that transport provides an additional stress to that imposed by loading.

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FIGURE 5: Plasma concentrations of adrenocorticotrophic hormone in pigs at rest, after loading, after 1 hour of road transport, and 2 hours lairage after transport. Values are means \pm sem. ** P<0.01; *** P<0.001 compared with resting values.

