

## Original article

# Effects of blood sampling procedures, grouping and adrenal stimulation on stress responses in the growing pig

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**Summary** — Changes in the blood and the behaviour of 14 growing pigs from 4 different litters were evaluated under different experimental conditions of blood sampling, grouping and adrenal stimulation. The results showed that the different techniques of blood sampling influenced lactic dehydrogenase (LDH) and creatine kinase (CK) activities. Cortisol, proteins and CK levels were negatively correlated with social hierarchy after regrouping. Cortisol was also correlated with total activity levels. Adrenal stimulation by adrenocorticotrophic hormone (ACTH) administration caused a sharp increase in plasma cortisol levels. However, plasma glucose, plasma proteins and total leukocyte counts were not affected by the ACTH treatment.

**growing pigs — stress — plasma parameters — behavioural parameters**

**Résumé** — Effets de divers stress sur quelques paramètres sanguins et comportementaux du porcelet. Une expérience portant sur 14 porcelets appartenant à 4 portées différentes a été entreprise afin d'étudier les modifications des principaux indicateurs sanguins et comportementaux provoquées par des stress de diverse nature : prélèvements sanguins par piqûres intraveineuses ou à l'aide d'un cathéter permanent, mélange de porcelets de différentes portées, stimulation surrénalienne par administration d'ACTH.

Les techniques de prélèvements sanguins influencent surtout les activités des enzymes, déshydrogénase lactique (LDH) et créatine kinase (CK). L'ordre hiérarchique des animaux après mise en lots est négativement corrélé avec les taux de cortisol, de protéines et de CK. Le cortisol est aussi corrélé avec négativement avec le taux d'activité totale.

L'administration d'ACTH augmente le taux du cortisol, mais elle n'a pas d'effet sur les taux de glucose et de protéines, ni sur le nombre de leucocytes.

**porcelets — stress — indicateurs sanguins — indicateurs comportementaux**

## Introduction

Several studies have been carried out on stress in livestock in order to standardize the different behavioural, endocrinological and physiological parameters considered to be stress indicators and to define a biological model for the assessment of the adaptive capabilities of farm animals (Dantzer and Mormede, 1983; Moberg, 1985; Wiepkema and Van Adrichem, 1987). Stress response is mediated by many different hormone systems which mainly involve the hypothalamic, pituitary, adrenocortical and sympathetic-adrenomedullary systems (Oliverio, 1987).

The transition between nursing and fattening is known to be a stressful period in pig production. Friend *et al.* (1983) have pointed out that mixing piglets from different litters elicits an increase in agonistic interactions. McGlone and Curtis (1985) showed a negative influence of this practice on health and growth, although Sherritt *et al.* (1974) did not find similar effects on growth. Cortisol levels rise when piglets are grouped at weaning (Blecha *et al.* 1985; Baldi *et al.*, 1987), particularly early-weaned animals (Dantzer and Mormède, 1981); there is a positive correlation among the cortisol levels, aggressivity and non-nutritive behaviour (Worsaae and Schmidt, 1980). Zayan *et al.* (1984) demonstrated an increase in aggressive behaviour and cortisol concentrations after unfamiliar growing pigs were regrouped.

When studies on stress response are carried out in commercial piggeries, a number of environmental stimuli, mostly due to management, must be taken into account; the effect of handling during blood sampling is particularly important if plasma parameters are to be used in assessing stress (Worsaae and Schmidt, 1980; Hemsworth *et al.*, 1987).

Corticosteroid levels are usually measured to assess pituitary—adrenal activation. The levels of some serum biochemical constituents (glucose, protein, urea, CK, LDH) as well as total and differential leukocyte counts have also been used as stress indicators (Gwazdauskas *et al.*, 1980; Barnett *et al.*, 1982).

To study the physiological mechanism of stress response, many researchers use the functional adrenal stimulation test as an experimental model to quantify the degree of adrenal stimulation during chronic stress and to detect physiological changes following the injection of exogenous ACTH (cattle : Wegner and Stott, 1972; pigs : Favre and Moatti, 1978; Rafai and Fodor, 1980; Mormède *et al.*, 1984).

The aim of our work was to study how different procedures of blood sampling might influence cortisol and plasma biochemical parameters, to assess the influence on these same parameters and on the behaviour of unfamiliar pigs when regrouped. We also tried to assess the effect of ACTH administration on levels of plasma cortisol, glucose, and total protein and on the leukocyte profile. The relationships between plasma and behavioural parameters were also studied.

## Materials and methods

### *Animals and housing*

Fourteen male piglets were used; they were a Large White x Landrace x Hampshire cross with a homogeneous birthweight, born on the same day from 4 multiparous sows. At 28 days of age the piglets were weaned, and each litter was moved individually from the farrowing crate to the weaning pen where the pigs stayed until 40 days of age. Each animal was weighed ( $13.1 \pm 1.1$  kg) and identified. Those taken from each litter were put into 2 experimental pens (2 x 2 m) with a concrete floor where they had free access to water and feed. One week after

regrouping the pigs were moved to individual pens (0.5 x 1.0 m) for the adrenal response test.

#### *Blood sampling and adrenal response test*

Blood samples were taken from the same pig by jugular venipuncture and by an anterior vena cava catheter. Jugular venipuncture was carried out with the piglet lying on its back. The time employed for capture and venipuncture was about 2 min for each piglet. Catheterization was carried out in accordance with the method described by Takken and Williams (1981).

Blood samples were collected at 9:00 a. m.

Grouping	1st sample (venipuncture)	Cannulation	2nd sample (catheter)	3rd sample (catheter)	4th sample (venipuncture)
1	4	7	8	9	10 days

according to the following schedule :

Seven piglets (treated group) were tested for the adrenal response 2 days after cannulation. The pigs in each pen were randomly allotted to the treated or the control group. Blood samples were withdrawn from the catheter at 9:00 a.m. (time 0) and after intramuscular administration of ACTH (Synachten Immediat, CIBA; 5 µg/kg) and 0.85 ml of sterile saline to the treated and the control groups, respectively. Blood samples (3—4 ml) were taken 0.5, 1, 3, 5 and 7 h after injection.

#### *Biochemical and hematological measurements*

Blood samples were collected in heparinized tubes; an aliquot of plasma was stored at -20°C until analysis for cortisol by radioimmunoassay (RIA), for total proteins by biuret and for glucose by the glucose—oxidase method. Another aliquot was cooled at 4°C to determine LDH and CK activities using the UV kinetic standardized method; enzyme activities were not determined after the adrenal stimulation test because they are not influenced by ACTH treatment. Additional blood samples were collected on EDTA at 0, 3, 5 and 7 h after ACTH/saline injection for the determination of total circulating leukocytes by electronic Coulter

Wright's stained blood film to count differential leukocytes by optical microscopy.

#### *Behavioural observations*

For one week after regrouping the pigs were observed for 2 min every 10 min. Levels of total activity (TAL) and the dominance value (DV) of each piglet were calculated according to Beilharz and Zeeb (1982) by putting the frequency of the agonistic interactions during the first 24 h into sociometric matrices. Active interactions (active activity levels : AAL) were estimated by quantifying the number of fights which had actually been started by each animal.

#### *Statistical analysis*

The differences between plasma parameters in the samples collected using different techniques were tested by analysis of variance. Paired comparison of the values was carried out by the Newman—Keuls test. Correlations among TAL, AAL, DV and hematologic parameters measured 4 days after grouping in the 2 pens, were performed by Spearman *r*. Differences between blood parameters in the 2 pens were checked previously using analysis of variance.

The results of hematologic measurements after the adrenal stimulation test were analysed by the least-squares method, including the effects of the treatment, time of sampling and their interaction according to the general linear model procedure of SAS (Barr, 1979). Differential counts of leukocytes were submitted to Arcsin transformation before analysis.

## **Results**

#### *Blood sampling*

The means of plasma parameter concentrations and the results of statistical analy-

**Table 1.** Effect of different sampling procedures on some plasma parameter levels.

	<i>Cortisol (ng/ml)</i>	<i>Glucose (mg/dl)</i>	<i>Proteins (g/dl)</i>	<i>LDH (U/l)</i>	<i>CK (U/l)</i>	<i>N</i>
	<i>mean SD</i>	<i>mean SD</i>	<i>mean SD</i>	<i>mean SD</i>	<i>mean SD</i>	
1st sample (venipuncture)	47 ± 20	107 ± 16	5.6 ± 0.43	798 ± 228 <sup>A</sup>	829 ± 820 <sup>a</sup>	14
2nd sample (catheter)	50 ± 22	102 ± 6	6.0 ± 0.46	478 ± 223 <sup>B</sup>	577 ± 729 <sup>ab</sup>	14
3rd sample (catheter)	44 ± 11	95 ± 11	5.7 ± 0.61	347 ± 75 <sup>B</sup>	134 ± 153 <sup>b</sup>	12
4th sample (venipuncture)	45 ± 29	104 ± 8	6.0 ± 0.36	481 ± 190 <sup>B</sup>	267 ± 234 <sup>b</sup>	14

SD = standard deviation.

N = number of observations.

Means in the same column with different superscripts differ (A, B :  $P < 0.01$ ; a, b :  $P < 0.05$ ).

sis are shown in Table 1. The treatment had no significant effect on cortisol, glucose and total protein levels, while the activities of LDH and CK varied significantly with sampling conditions. Serum enzyme activities were higher after grouping than in all the other samples.

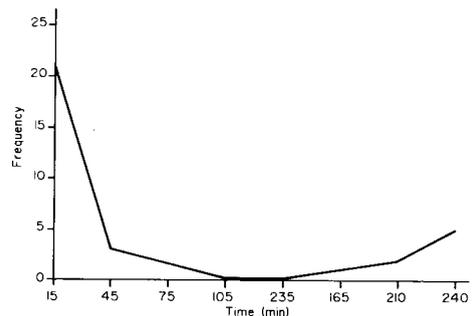
#### *Behavioural observations and behavioural-physiological relationships after grouping*

The frequencies of agonistic interactions during grouping in the 2 pens are given in Figure 1. Figure 2 shows the proportions of AAL and TAL in decreasing order of the dominance value.

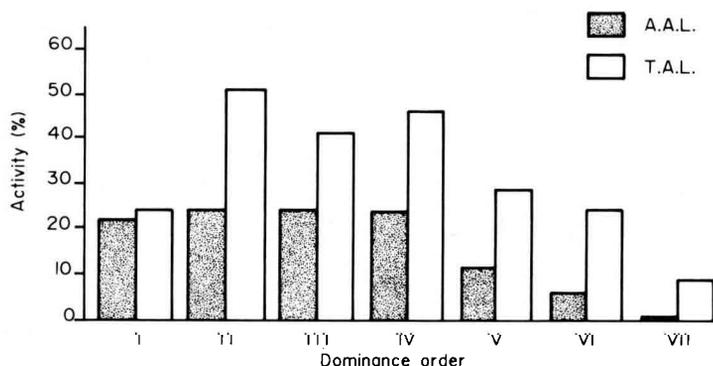
In both pens DV and AAL were positively correlated ( $r = 0.71$ ;  $P < 0.10$ , pen 1;  $r = 0.75$ ,  $P < 0.05$ , pen 2), while no relationship was demonstrated between DV and TAL.

In pen 1, cortisol and TAL showed a negative correlation ( $r = 0.72$ ,  $P < 0.10$ );

in pen 2, DV and CK were inversely related ( $r = 0.81$ ,  $P < 0.05$ ); in both pens, total proteins and DV ( $r = -0.78$ ,  $P < 0.05$ , pen 1;  $r = -0.90$ ,  $P < 0.01$ , pen 2) were inversely related. There were no differences between the means of the hematologic parameters in the 2 pens 4 days after regrouping.



**Fig. 1.** Frequencies of total interactions among piglets during the first 240 mn after regrouping (mean of the 2 pens).

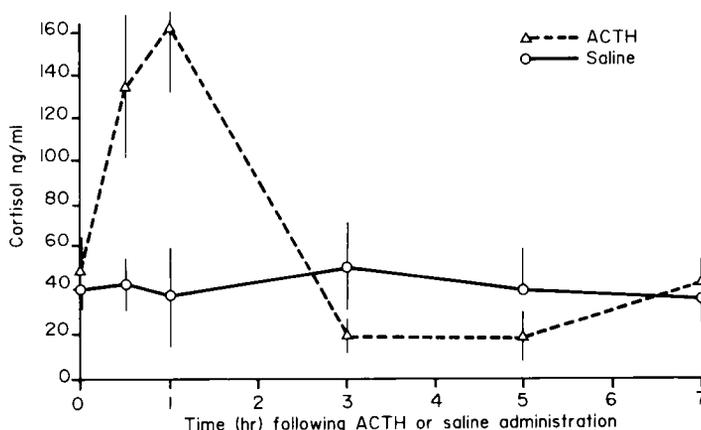


**Fig. 2.** Frequencies (%) of active activity interactions (AAL) and of total active interactions (TAL) among piglets of different dominance order after grouping (mean of the pens).

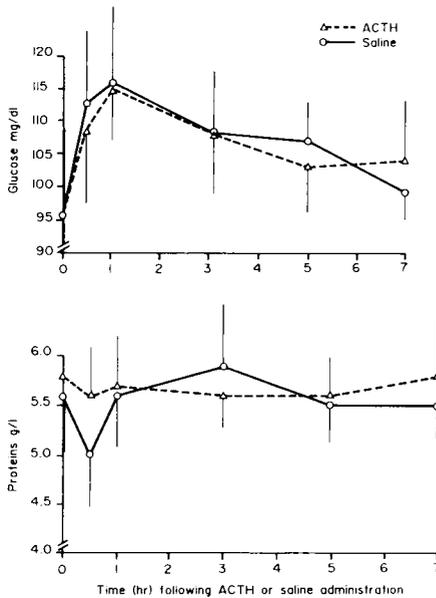
**Adrenal response test**

Plasma cortisol levels increased sharply 30 min after ACTH injection ( $P < 0.001$ ), reaching a peak after 1 h (Fig. 3). After 3 and 5 h, they decreased to below baseline levels ( $P < 0.001$ ), and then returned to normal values 7 h after injection. Plasma cortisol levels were significantly affected by the treatment ( $P < 0.001$ ), time of sampling ( $P < 0.001$ ) and their interaction ( $P < 0.01$ ). Significant changes in plasma cortisol were not detected after the saline injection.

Neither glucose nor total proteins (Fig. 4) was influenced by the treatment, but glucose levels were affected by the time of sampling. Total and differential leukocyte counts are shown in Figure 5. Total circulating leukocytes and the percentage of lymphocytes remained unchanged in both the treated and the control groups, and they did not vary with sampling time, while neutrophils increased and eosinophils decreased significantly with sampling time 3 and 5 h after ACTH or saline injection.



**Fig. 3.** Plasma cortisol response in pigs following injection of ACTH and saline (means  $\pm$  SD).



**Fig. 4.** Plasma glucose and protein levels following injection of ACTH and saline (means  $\pm$  SD).

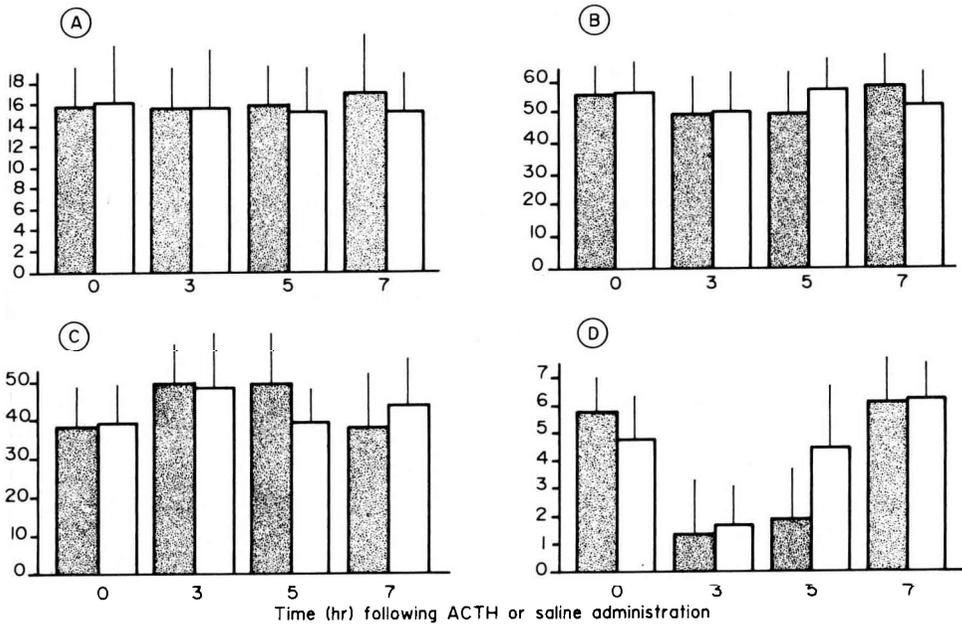
## Discussion

In this study the levels of the direct (cortisol) or indirect (glucose, proteins) indicators of adrenal response were within the range of values reported in animals of the same age (Rafai and Fodor, 1980; Baldi *et al.*, 1987) and they did not change significantly with the sampling procedure.

Higher CK and LDH activities have been recorded in samples collected by venipuncture than by other methods (ear vein, venipuncture with needle flushing) because of tissue contamination during sampling (Bickardt and Richter, 1980; Bruss and Becker, 1981). Catheterization was also used to minimize animal handling during sampling and to measure resting CK activities (Watson *et al.*, 1976; Hallberg *et al.*, 1979). In the present study, the first sample, taken by venipunc-

ture when the pigs were regrouped, showed higher activities than the samples collected by catheter, but, in the last sample (taken when the pigs were individually housed), no difference from the catheter samples was seen. This indicates that sampling sites and techniques are not the only factors to consider when evaluating enzyme activities. Thus, it is reasonable to assume that the fights which followed the mixing of piglets caused tissue damage with subsequent long-term enzyme release, as reported by Moss (1979) and Baldi *et al.* (1985). This confirms that such enzyme activities are good indicators of previous stress.

In both pens the correlation observed between the DV and the levels of active activity indicates that the dominant piglets established social hierarchy by beginning the fights, while the submissive pigs acquired their position in the hierarchy without actively taking part in the fights. On the contrary, the lack of correlation between DV and TAL could denote that the former piglets were not only aggressive towards the others but were also enterprising in other activities such as play and exploration. In rats, Ely and Henry (1978) reported that corticosterone levels seemed to be lower in dominant animals than in those with low environmental control. Mormède *et al.* (1984) showed that high adrenocortical activity was associated with low motor activity in pigs during an open-field test. We did not find any relationship between DV and cortisol levels, probably because the hierarchic order was established 4 days after regrouping. There was a negative relationship between cortisol and total activity levels in pen 1 where fighting ended earlier and social order was established sooner than in pen 2, as already reported by Canali *et al.* (1986). This would confirm that agonist activity might be an effective way of coping with a new environment



**Fig. 5.** Total and differential counts of leukocytes following injection of ACTH (  ) and saline (  ) (means  $\pm$  SD). Panel A : total leukocytes ( $\times 1000/\text{mm}^3$ ); panel B : lymphocytes (% total leukocytes); panel C : neutrophils (% total leukocytes); panel D : eosinophils (% total leukocytes).

and thus corticosteroid levels would be lower in animals with the high activity levels.

Recent studies suggest caution in using the stimulation test with ACTH to obtain a qualitative and quantitative evaluation of stress; it is necessary to take into consideration the various physiological and endocrinological factors involved in stress response (Ladewig, 1987). In our study, the endocrine response, measured as cortisol output, agreed with the results of other studies (Mormède and Dantzer, 1978) as the compensatory drop to below resting values found after the initial peak (Rafai and Fodor, 1980). We did not observe any significant variations in the other parameters considered.

According to the present data, some of these parameters actually changed significantly and to the same extent in both the

treated animals and the controls. In particular, a similar trend in leukocyte profiles was seen in both groups. As found in other studies (Müller *et al.*, 1969), the eosinophils diminished and the neutrophils increased more markedly in pigs treated with ACTH. Nevertheless, differences between the 2 groups did not emerge here, perhaps because of high individual variability. The trend in the plasma glucose levels was the same in the 2 pens. Thus, other factors which are side-effects of the treatment seemed to be involved; these include the activation of the adrenomedullary system due to a reaction to injection procedures with a subsequent rise in plasma glucose.

From the present study, it appears that different sampling techniques do not extensively affect plasma parameters in

pigs. However, enzyme activities seemed to be influenced by the stress stimulations to which the pigs were subjected before sampling.

The relationship between behaviour and hematologic parameters suggests the hypothesis of a main adrenocortical activation in animals which reacts to the change of environment with low motor activity. Although adrenal stimulation by ACTH injection produced a remarkable increase in plasma cortisol, in this case, it did not cause significant changes in the metabolic and hematological parameters considered.

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