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Patterns of Change of Some Commonly Measured Physiological Indices of Short Term Stress in Pigs

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Abstract
The experiment aimed to map the changes in time of commonly used indices of short term stress in pigs in response to a short term stressor followed by recovery and also to investigate differences in response between individuals. Four pigs were individually driven up and down a ramp and then allowed to recover, on each of three days. Before, during and after being driven measurements of packed cell volume, plasma cortisol, glucose, beta-endorphin, lactate, creatine phosphokinase and heart rate were made. All of the parameters measured changed in response to the treatment in the manner predicted from theory. The graphs shown in the text provide a useful guide to the rise time and the fall time of parameters commonly used in experiments designed to assess animal welfare and also show that some of these parameters are more uniform in the way in which they change over time than others. At the same time the results highlighted that there were readily observable differences between the response of individual animals.

Key words: Pig, physiological indices, stress, welfare

Introduction

Broom (1986) put forward the idea that the welfare of an animal is "the state of an individual as regards its attempts to cope with its environment" and many experiments which attempt to evaluate welfare use this idea. Often the level of a blood constituent is measured and compared before and after a treatment, between treatments, or both. The general assumption is made that the greater the change in the level of the blood constituent the more the animal has had to do to cope with its environment and thus the poorer is the welfare. For instance, Knowles and Broom (1993) compared different handling methods for battery hens and based on level of blood corticosterone at a set time after the start of handling and, with other evidence, drew conclusions about the welfare benefits of one method over another. Ewbank *et al*. (1992) looked at stunning cattle at slaughter with and without head restraint by comparing levels of blood cortisol.

The above types of experiment rely on only a limited number of samples in time because multiple sampling would usually have too much of an effect on the measurements being made. The timing of sampling is thus critical to ensure that the constituents being measured can actually change between the onset of the treatment and sampling and have not decayed by the time a sample is taken. For this reason the following experiment investigated the changes over time of a number of blood constituents which are commonly used as indices of stress in pigs. A series of blood samples were taken whilst pigs were moved up and then down a mock loading ramp and then allowed to recover. Negotiating a loading ramp was used as it was thought it would produce an acceptable sort term stress and it is also something that most pigs are required to do within their lifetime. Subjective assessment suggests that negotiating a ramp is a stressful procedure for pigs, requiring considerable exertion (Warriss *et al*., 1991), causing a rise in heart rate (Van Putten and Elshof, 1978) and an increase in blood lactate (Warriss and Brown, 1985).

Materials and Methods

Four pigs of a commercial white cross weighting approximately 60 kg were surgically prepared with indwelling catheters placed in both external jugular veins. Once in place the catheters were flushed twice a day with heparinised saline. Catheterisation took place three days before the start of the experiment to allow the pigs to recover from the operation and to allow them to become accustomed to approach. Although handling was kept to a minimum the pigs became amenable to people being present in the pen. The pigs were individually penned adjacent to a holding area in front of the experimental ramp. They had free access to food and water throughout.

On the first day of the experiment each pig was individually driven from its pen, into a race and then up and down the ramp once using pig boards. It was then returned to its pen and left, with as little disturbance as possible, to allow recovery. The ramp used was 2.44 m long with cleats at an even spacing of 150 mm and set at an angle of 34° to the horizontal. The angle of 34° is the maximum allowed under legislation for an internal vehicle ramp (Transport of Animals (Road and Rail) Order 1975). Nine blood samples were taken from each pig at different points throughout the procedure:

1. At rest in the pen, approximately 19 min before being driven up the ramp
2. At the bottom of the ramp, approximately 30 sec after leaving the pen
3. At the top of the ramp, approximately 2.5 min after sample 2

4. At the bottom of the ramp after descent, approximately 2 min after sample 3
5. After 15 min of recovery time
6. After 30 min of recovery time
7. After 60 min of recovery time
8. After 120 min of recovery time
9. After 240 min of recovery time

As soon as the first blood sample had been taken radio telemetry heart rate monitoring equipment was attached to the pig and the pig left for 15 min before it was driven from its pen. The equipment picked up a signal from two external electrodes placed on the pig's chest to span the heart and sent it to a receiver where it was recorded using an FM tape recorder (TEAC R-71) for subsequent analysis. After blood samples number 6 had been taken the heart rate monitoring equipment was removed from the pig. The heart rates given in the results were calculated by averaging the recordings for each of 30 sec spanning blood samples 2 to 6. The first heart rate measurement was calculated by averaging the rate over the 30 sec before the pig was first driven from the pen, whilst it was still at rest.

The blood was collected into heparinised monovettes and a subsample immediately analysed for packed cell volume (PCV) using a microhaematacrit method. The remainder of the sample was spun down and the plasma frozen for later analysis for creatine phosphokinase (CPK) (CPK-Randox CK-NAC Kit, Randox Laboratories Limited, Co Antrim), lactate and glucose (Analox GM7 Analyser, Analox Instruments, London), cortisol (Endab Enzyme Immunoassay Test Kit, Immunotech Crop Boston, USA) and beta-endorphin (Peninsula Laboratories Inc. Belmont).

The whole experiment was repeated on two subsequent days using the same four pigs but in a random order within the days. In this way a comparison between any differences in the response of individual pigs was possible as well as an evaluation of the responses across time. A complete set of measurements for one of the pigs on one of the days was lost. The data were analysed by repeated measures analysis of variance in which the sample numbers were within subject repeated measure and pig identity was between subject factor. Where there were nine repeated measurements made (that is all measurements except heart rate) only the first eight were used in the analyses as there were too few degrees of freedom to accommodate all nine, however, all nine points are shown in Figures. The natural logs of the CPK values were used in the analysis in order to normalise the distribution.

Results

The F values and their significance from the test of the effects of time and pig and their interaction are shown in Table 1. The data satisfied the assumptions required for a univariate approach to repeated measures. The mean responses of each of the four pigs across the three days are shown in Figure 1 and 2. There were changes in the levels of PV in times as the pigs were run up and down the ramp and then allowed to recover and there were no detectable

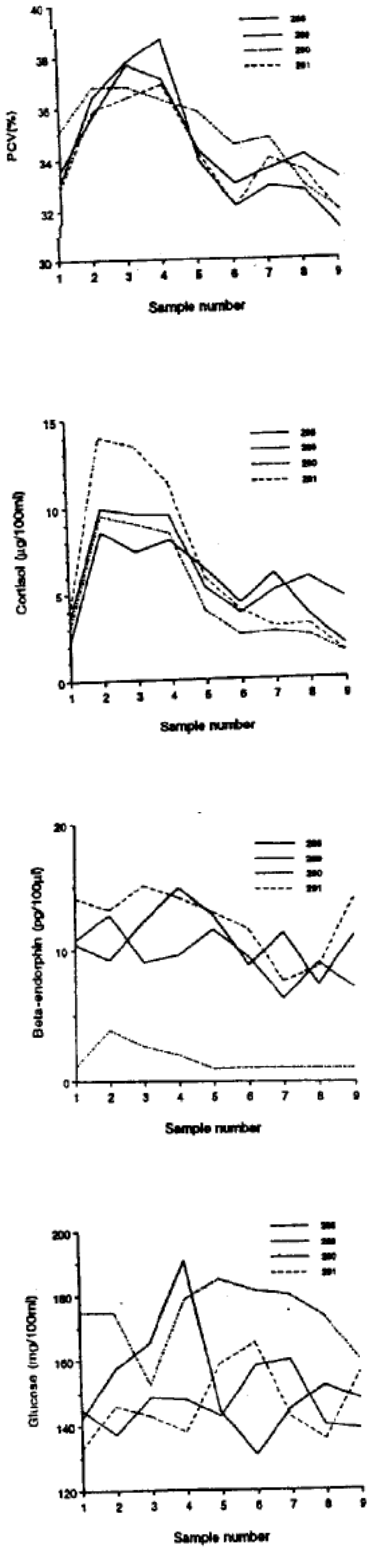

Fig. 1: The means across the three days of changes in levels of PCV, plasma cortisol, glucose and beta-endorphin in each of the four pigs in response to being driven up and then down the ramp

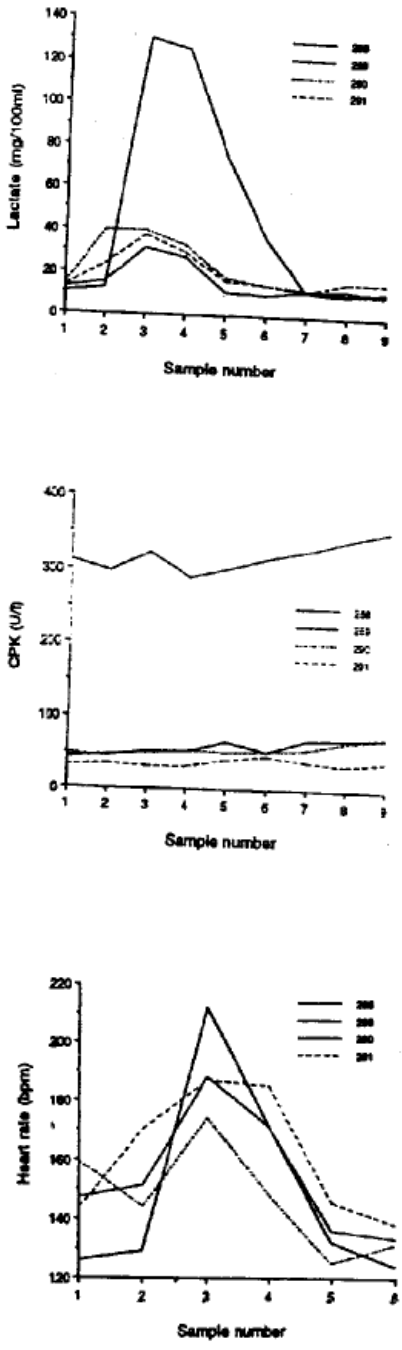

Fig. 2: The means across the three days of changes in levels of plasma lactate, CPK and heart rate in each of four pigs in response to being driven up and then down the ramp

differences between the responses of each pig. The same was true for levels of plasma cortisol and for heart rate (Table 1 and Figure 1 and 2). A significant interaction effect showed there to be changes in the levels of plasma glucose and plasma lactate during the treatment and that the changes in time were different between the pigs. There were changes in the levels of beta-endorphin, lactate and CPK during the treatment and there were difference in the levels of these blood constituents between the pigs, however, there were no detectable differences in the overall patterns of change or the amount of change between pigs.

Discussion

All of the parameters measured changed in response to the treatment in the manner predicted from theory. The figure provide a useful guide to the rise time and the fall time of parameters commonly used in experiments designed to assess animal welfare and also show that some of these parameters are more uniform in the way in which they change over time than others. The result highlighted that there were readily observable differences between the response of individual animals with plasma glucose and plasma lactate the actual pattern of responses varied between individuals, whilst with beta-endorphin and CPK it was just the overall levels that were different between animals.

As part of a stress response red blood cells are expelled from the spleen into the blood steam. The graph in Figure 1 shows PCV at sample number 1 to be roughly 34 percent. This was probably already slightly elevated above normal resting levels as this would have been the first occasion that a pig had been approached on a given day. There was a marked rise in PCV as a pig was driven to the bottom of the ramp and this rise continued is most pigs between samples 2 and 4 when the pig was driven up and then down the ramp. It should be remembered that some of the rise seen between samples 1 and 2 could have been due to the handling required to fit the heart rate monitoring equipment, despite the 15 min recovery period. Additionally, although the best precautions were taken the levels of all parameters may have been influenced to some extent by the sampling procedure itself. During the recovery phase between samples 4 to 6 PCV decreased to below the initial values found in each pig. After blood samples number 6 had been obtained the pigs were disturbed by the removal of the heart rate monitoring equipment and this affected PCV levels as shown by the subsequent small rise in PCV by sample number 7, after which PCV fell again, probably to resting levels by sample number 9. It should be remembered that the horizontal scale is not liner in time and is more akin to a long scale. A very similar pattern of response can be seen with levels of plasma cortisol and a similar explanation holds.

Levels of, and changes in the levels of plasma glucose were different between pigs the main difference in level probably due to high plasma glucose in pig 290. The reasons for the changes in terms of what was happening to the pigs during the treatments are difficult to ascribe. It is tempting to draw relationships between difference seen in plasma glucose between individual pigs and the other parameters that differed between individuals, however, this would be probably be unwise given the small scale of the experiment.

Table 1: The F values and their significance from the test of the effects of time and pig and their interaction

	Pig		Time		Pig Time	
Parameters	F	p	F	p	F	p
PCV	0.355	0.787	17.315	0.000***	0.879	0.616
Cortisol	1.010	0.433	29.162	0.000***	1.720	0.060
Glucose	0.842	0.513	1.339	0.252	2.538	0.004**
Beta-endorphin	15.383	0.003**	3.169	0.009**	1.126	0.316
Lactate	6.531	0.019*	23.721	0.000***	9.359	0.000***
In (CPK)	54.244	0.000***	4.110	0.001***	1.350	0.191
Heart rate	1.367	0.329	24.205	0.000***	1.927	0.055

*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

Beta-endorphin is secreted in the brain and blood level rise during and after a stressful episode. In all pigs there was an increase in response to being driven up the ramp and levels were seen to fall during recovery until about sample number 7. The changes seen in beta-endorphin were not as marked as in many of the other parameters measured. Pig number 290 had very much lower overall levels of plasma beta-endorphin than the other pigs.

An increase in plasma lactate level reflects an increase in muscular activity, the patterns of change of lactate over time were similar between the pigs except that the response of pig 288 was greatly amplified. On each of the three days of the experiment pig 288 was easily the most difficult pig to drive up the ramp, the other three pigs being driven mostly, without baulking. It can also be seen from the figures that pig 288 showed the highest heart rate and also seems to have displayed the grates change in blood glucose and perhaps even beta-endorphin. The greatest change in lactate levels in all pigs occurred as a result of muscular activity whilst being driven. Recovery to resting levels had occurred by sample number 5 except in pig 288 which required an hour to recover. In contrast to PCV, cortisol and beta-endorphin there was little change in level in response to the removal of the heart rate monitoring equipment.

CPK is leaked into the bloodsteam form muscle following muscular activity. In all pigs there was a gradual rise in plasma levels throughout the experiment including the recovery period, reflecting the slow clearance rate. Pig 289 had much higher levels of plasma CPK than the other three animals (Fig. 2). The changes in heart rate were similar between the four animals. The largest increase occurred when they were driven up the ramp a reflection of the physical exertion required. Heart rate fell during the descent despite evidence that pigs find it more difficult to decent a steep ramp than they do to ascent; they take longer to drive down than to drive up a ramp (Warriss *et al*., 1991).

The work described here shows that the response and recovery times of physiological parameters are quite varied. Care is, therefore, needed when interpreting physiological data, collected at only one time, especially from animals whose previous handling history in unknown.

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