1:10 The effect of preslaughter stressors on the blood profiles of pigs

B.W. MOSS

Agricultural and Food Chemistry Research Division, Department of Agriculture for Northern Ireland, Agriculture and Food Science Centre, Newforge Lane, Belfast BT9 5PX

Introduction

The amount of stress an animal undergoes before slaughter affects the ultimate meat quality (Lister, 1970). Severe stress or stress just prior to slaughter results in PSE (pale, soft and exudative) meat. Several parameters, including thyroid and adrenal function, serum enzyme levels (CK and LDH), have been implicated in the aetiology of the PSE condition (Fischer, 1974; Bickhardt, 1981). The circulating levels of these is a balance between rate of release into, and rate of removal from, circulation (Moss, 1981).

Increased corticosteroid output in pigs during stress has been well documented (Baldwin and Stephens, 1973; Dantzer, 1981). In stress sensitive pigs, however, the increases are generally not as great (Judge, Briskey, Cassens, Forrest and Meyer, 1968). The apparent adrenal insufficiency of stress sensitive pigs may be due to their greater turnover and clearance of plasma cortisol (Marple and Cassens, 1973). The effect of stress on thyroid function depends on the type of stressor and varies with the species investigated (Brown-Grant, Harris and Reichlin, 1954; Brown-Grant, von Euler, Harris and Reichlin, 1954; Falconer, 1972). Stress senstive pigs show decreases in serum thyroxine and free thyroxine indices when exposed to a specific stressor such as halothane (Hall, Lucke and Lister, 1975). This may be due to increased turnover of thyroid hormones in stress sensitive pigs (Bodart and Francois, 1972; Moss, 1975). Higher serum enzyme levels in stress sensitive pigs may be associated with membrane defects or may be due to increased physical activity (Moss and McMurray, 1979).

The responses of pigs during the preslaughter period will depend on the stress sensitivity of the animal and the amount and type of stressor to which they are subjected. In previous work cortisol and thyroxine levels at slaughter indicated that pigs were less stressed when killed after overnight lairage than after shorter lairage periods (Moss and Robb, 1978). Serum enzyme levels at slaughter, however, indicated that pigs held in lairage overnight were more stressed at slaughter (Moss and McMurray, 1979). The difference in these results may be explained by the rate of response and the type of stress measured by these parameters (Moss, 1981).

The aim of the experiments reported in the present paper is to investigate how various preslaughter stressors affect blood profiles.

Materials and Methods

Blood samples were collected as follows:-
Before slaughter - the pigs were restrained with a noose around the upper jaw and blood was taken from the ear vein (EV) using a 19G or 21G needle.
At slaughter - the blood was collected in universal bottles from the cut made by the slaughterman. The blood collected ran over the muscles on the cut surface of the neck and may be described as 'free bleed' (FB).

Hormone Assays: Total serum thyroxine (T_4) was measured by the method of Seligson and Seligson (1972). Cortisol was measured using a competitive protein binding technique (Bassett and Hinks, 1969), heat denaturation of the endogenous transcortin (Crowley, Garbien and Tuttlebee, 1975) being used as an alternative to the preliminary solvent extraction step.
Serum Enzymes: Creatine kinase (CK) and lactate dehydrogenase (LDH) were measured using standard Biochemica (Boehringer, Mannheim) procedures.

Expt. 1. Effect of Transport and Slaughter

Experimental procedure

Blood samples were taken from nine pure-bred Landrace pigs (83 to 86 kg liveweight) immediately after they had been weighed on the farm of origin at 10.00 to 11.00 h on the day of slaughter. The pigs were transported a distance of 30 km to the abattoir and held in lairage for 2 h before slaughter (carbon dioxide stunning). Blood samples were collected at slaughter (FB).

Results

The mean thyroxine level in samples obtained on the farm was 6.26 µg/100 ml and was signifcantly higher ($p<0.05$) than that in slaughter blood (4.83 µg/100 ml). The mean cortisol level was significantly higher ($p<0.02$) at slaughter, with mean values of 5.9 µg/100 ml compared to 4.1 µg/100 ml on the farm. Serum LDH values were not significantly different between farm (1126 iu/ℓ) and slaughter (1104 iu/ℓ). Serum CK levels increased significantly ($p<0.05$) from farm (686 iu/ℓ) to slaughter (1901 iu/ℓ).

Expt. 2. Effect of Duration of Lairage

Experimental procedure

On arival at the bacon factory, between 10.30 and 11.30 h, twenty commercial crossbred pigs were penned in groups of five, each from a different producer. The distances travelled by the producer groups were 21, 25, 26 and 35 km for groups A, B, C and D respectively. Groups A, B and D were delivered by a commercial haulier and group C by the producer. Blood samples were collected immediately on arrival at the factory (EV_1) and then 3 h (EV_2) and 24 h (EV_3) later. The pigs were stunned by carbon dioxide (Wernberg oval tunnel) 30 minutes after the last sample and blood collected at slaughter (FB). During the 24 hour lairage pigs were not fed but had free access to water.

Results

The effect of duration of lairage on cortisol and thyroxine levels is shown in Figure 1 for the four producer groups A, B, C and D. The group which was owner delivered had higher cortisol levels on arrival than the other groups and exhibited a different pattern of cortisol response to lairage than the other producer groups. The only marked consistent trend was the decrease in thyroxine (T_4) levels from 24 h sample (EV_3) to that obtained at slaughter some 30 min later. When the results of all four groups were analysed using the paired t-test it was found that the T_4 level at slaughter was significantly lower than the 24 h sample ($p<0.01$), 3 h sample and that on arrival ($p<0.05$ see Table 1).

Mean cortisol level of all pigs was significantly higher on arrival than af
24 h in lairage ($p<0.05$) or slaughter ($p<0.01$). The cortisol levels 3 h af
penning in the lairage were of a similar level to those on arrival
although higher than cortisol levels after 24 h lairage and at slaughter
were only significantly higher than slaughter samples ($p<0.05$).

Table 1 Thyroxine and cortisol levels during lairage and at slaughter

Time in lairage	Thyroxine (µg/100 ml)	Cortisol (µg/100 ml)
On Arrival (EV_1)	4.35 [a]	5.61 [de]
3 h after Arrival (EV_2)	4.30 [b]	5.58 [f]
24 h after Arrival (EV_3)	3.99 [c]	4.61 [d]
At Slaughter (FB)	3.18 [abc]	4.18 [ef]

means with the same superscript are significantly different (paired t-
$p<0.05$, c,d,f; $p<0.01$, a,b,e.

Expt. 3. Effects of Mixing and Fasting

Experimental procedure

Blood samples were obtained at between 10.00 h to 11.00 h from a group of
pigs (group A) and a group of 9 pigs (group B) on 3 successive days in each
3 weeks. The pigs were fed *ad libitum* and had free access to water. Dur
the experimental period the pigs were subjected to the stressors of fast
and mixing as outlined below.

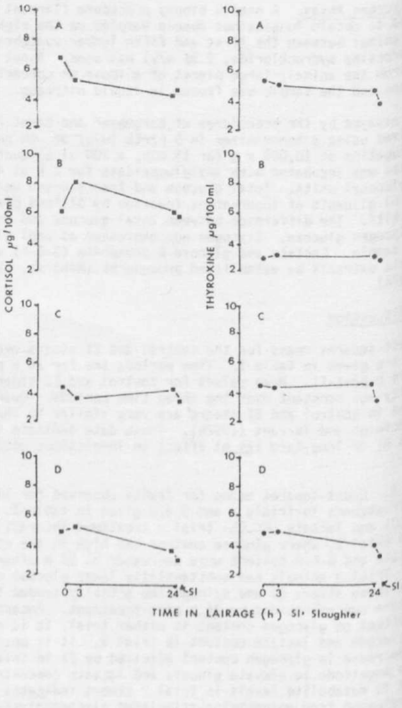
Week 1. On day 2 group B were withheld feed from 10.00 h until after bl
samples had been taken on day 3.
Week 2. On day 2 group A pigs were withheld feed from 10.00 h until af
blood samples had been taken on day 3.
Week 3. The two groups of pigs were mixed together at 09.00 h on day 2
blood samples were taken 1 h after mixing. The pigs were then penned as
groups, one containing 5 group A and 5 group B pigs (group C) and the ot
containing 5 group A and 4 group B pigs (group D). Group C pigs were with
food until after 10.00 h on day 3 and group D pigs were fed.

Results

The mean thyroxine and cortisol values for the sampling days are given
Table 2. Considerable day-to-day variation within animals was observ
Statistically significant differences between sampling days were obtained
pigs in both group A and group B on days when no stressor had been appli
Thus it is difficult to assign the significantly lower thryoxine and cort
levels after fasting in group B (see Table 2, week 2, day 3) to the fast
per se and not sampling stress. In both groups A and B marked increase
cortisol levels from 2.57 and 2.42 to 4.99 and 3.38 µg/100 ml 1 h after mix
were observed. Cortisol levels in both groups decreased significan
($p<0.05$) 24 h after mixing, whether pigs had been fed or fasted after mix

Fig.1

Effect of Duration of Lairage on Cortisol and Thyroxine levels in Pigs



Thyroxine levels 1 h after mixing although lower than premixing values, were not statistically significantly different.

Table 2 Effect of fasting and mixing on thyroxine and cortisol levels

	Group A			Group B		
	Treatment	Thyroxine (μg/100 ml)	Cortisol (μg/100 ml)	Treatment	Thyroxine (μg/100 ml)	Cortisol (μg/100 ml)
Week 1						
Day 1	Fed	5.60	2.55 [b]	Fed	5.35	1.82
Day 2	Fed	4.69 [a]	1.35 [bc]	Feed withheld	4.85	1.18
Day 3	Fed	5.13 [a]	2.09 [c]	Fasted 24 h	5.19	1.32
Week 2						
Day 1	Fed	5.56 [df]	1.78	Fed	6.13 [h]	2.21 [i]
Day 2	Feed withheld	4.92 [de]	1.81 [g]	Fed	5.53	1.76 [i]
Day 3	Fasted 24 h	4.15 [ef]	1.07 [g]	Fed	5.26 [h]	1.95
Week 3						
Day 1	Fed	4.54 [1](4.42)	2.10 (2.57[k])	Fed	4.59 (4.71)	2.93 (2.42)
	Group C			Group D		
Day 2	Mixed Feed Withheld	4.89	4.99 [kl]	Mixed and Fed	4.08	3.38 [m]
Day 3	Mixed Fasted 24 h	4.64	2.84 [l]	Mixed for 24 h	4.87	1.62 [m]

[1] Figures in parentheses indicate mean values corresponding to animals comprising mixed groups on following day.
Significance of differences calculated using paired t-test, means with identical superscripts are significantly different
$p<0.05$; a, c, d, h, i, l, m $p<0.01$; b, e, k $p<0.001$; f, g

Discussion

Before slaughter pigs experience a series of stressors:- mixing and loading for transport on the farm; transport; unloading at the factory; lairage and finally stunning. These stressors may affect cortisol, thyroxine and serum enzymes in different ways. In obtaining blood samples some stress is inevitable and in experiment 3 both cortisol and thyroxine levels were significantly different on days when no stressor treatment had been deliberately applied. The stress of restraint and sampling would, however, appear to be less than that of mixing since large statistically significant increases in cortisol were observed after mixing. According to Dantzer (1981) these increases are related to the amount of fighting and the social rank of the pig.

In experiment 1 the pigs were subjected to all the routine preslaughter stressors (eg transport, mixing, lairage and stunning). The significant increases in cortisol and serum CK, and the significant decreases in thyroxine, could be due to these preslaughter stressors or to the fact that the site of sampling was different. Lower T_4 levels have been reported in Large White pigs but not Landrace pigs when subjected to similar preslaughter handling (Moss, 1981) and by Hall, Lucke and Lister (1975) when subjected to a specific stressor. Although the high serum CK and LDH values at slaughter could be due to local tissue damage, the leakage of tissue enzymes due to stress may override local cut tissue damage (Moss and McMurray, 1979). Similarly, it could be argued that the lower T_4 and cortisol levels at slaughter in experiment 2 compared to those in ear vein blood samples taken 30 minutes previously are due to the site of sampling and not the stress associated with stunning. Other factors, such as previous stress history and differences in response to stressors between thyroid and adrenal systems, are also important.

Different groups of pigs exhibited different patterns of cortisol response during lairage and this is dependent both on the amount of stress between farm and factory and how they settled down during lairage. Behaviour studies indicate that over 70% of pigs are resting after 1 h in lairage (Moss, 1978). In two groups (A and C) in experiment 2 the cortisol levels 3 h after penning in lairage would indicate that these pigs may have partially recovered from previous stressors. Overall, it would appear that after 24 h in lairage the pigs are less stressed, ie lower cortisol levels, and is in agreement with previous studies (Moss and Robb, 1978). The total T_4 levels 24 h after arrival, however, were lower than on arrival and previously lower T_4 levels have been suggested after stress (Hall, Lucke and Lister, 1975). In the 24 h lairage situation the association of decreases in T_4 with decreases in cortisol, when previously these tend to be inversely related (Moss, 1981), may reflect a differential response of these hormone systems to the different stressors or to differences in the time course of the response (Dantzer, 1981, Moss, 1981). Although the time course of pituitary adrenal stimulation has been studied in pigs (Sebranek et al., 1973; Lister, Lucke and Perry, 1972) little information is available on the time course of the pituitary-thyroid response in pigs. Thyroid responses to various stressors in sheep have been shown to be rapid and possibly related to sympathetic nervous stimulation (Falconer, 1972).

Fasting is one stressor involved in overnight lairage and in man starvation has been shown to result in higher T_4 levels due to a reduced peripheral conversion of T_4 to triiodothyronine (Merimee and Fineberg, 1976). There was, however, no marked evidence for any effect of fasting on T_4 levels in experiment 3. Differences in T_4 levels at slaughter after overnight or normal lairage (Moss and Robb, 1978) may then be associated with stressors other than fasting. In young pigs, T_4 excretion rate shows a diurnal fluctuation with peak excretion just prior to the morning feed (Moss and Jordan, 1980). Such diurnal fluctuations, which tend to anticipate the normal daily routine, are likely to continue for some time after interruption of the daily routine and interact with responses to other stimuli and stressors.

From the studies reported here it would be difficult to suggest any one blood parameter that could be used to indicate a pig was under stress. Several parameters may lead to a more meaningful interpretation of the stress response. Since the stress involved in sampling may be variable, and to some extent depend on the stress sensitivity of the animal, the stressor under measurement must cause a greater response than the sampling stress in order to be assessed. Further work is needed on the time curve of response of various blood parameters to different stressors to enable blood profiles to be used to assess whether an animal is, or has been, stressed.

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