THE INFLUENCE OF STARVATION AND/OR MIXING OF UNFAMILIAR PIGS IN THE PERIOD PRIOR TO SLAUGHTER ON SERUM NEFA CONCENTRATIONS AND PLASMA TRYPTOPHAN DISPOSITION.

Martin S. Burrows[1], Bruce W. Moss[1] and Violet E. Beattie[2].

1. Department of Food Science, The Queen's University of Belfast, Newforge Lane, Belfast. BT9 5PX.
2. Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down. BT26 6DR

Introduction

At all times during an animals life it experiences stress from a range of sources. For a meat producing animal this stress is often maximum at birth and at slaughter. Poor animal husbandry treatments such as the mixing of unfamiliar pigs or the deprivation of food prior to slaughter are frequently responsible for the increase in stress observed during transport and lairage[1]. It has been suggested that the amount of behavioural stress may be related to the amount of fighting that occurs preslaughter.
One possible biochemical link between aggression and preslaughter handling may be brain 5-hydroxytryptamine (5-HT) metabolism. Pharmacological treatments that increase brain 5-HT concentrations at 5-HT synapses have been shown to reduce aggressive behaviours[2], and this is supported by work which indicates that low concentrations of 5-HT in the brain may be involved in the aetiology of aggression[3]. One of the important factors influencing the synthesis of 5-HT in the brain is the availability of its precursor, the essential amino acid L-tryptophan[4].
Under normal conditions plasma tryptophan disposition is such that only 15%-20% of plasma tryptophan is 'free', the rest is found bound to plasma albumin[5]. The alteration of plasma tryptophan disposition induced by stress is of particular interest because if changes in brain tryptophan concentration follow then resultant changes in 5-HT synthesis could have behavioural consequences[6]. Changes in tryptophan disposition have been shown to be caused by stresses induced by food deprivation and 3 hours immobilisation stress in rodents[7]. It is thought that these changes are provoked by the secretion of hormones which effect lipolysis. The resulting increase in non-esterified fatty acid (NEFA) concentrations have been shown to cause an increase in the free fraction of plasma tryptophan, as the free NEFA bind to plasma albumin with much higher affinity than tryptophan[8]. When plasma free tryptophan concentrations rise it appears to be rendered more available to the brain[9].
The purpose of this study was to measure the effects upon plasma tryptophan disposition and NEFA concentrations caused by the starvation and/or mixing of unfamiliar pigs prior to slaughter, both of which are common poor animal husbandry practises.

Method

Experimental Design

48 pigs were obtained from an experimental research station and randomly allocated to the following four treatments:-

	Treatment
Pen 1	not mixed / not fasted
Pen 2	mixed / not fasted
Pen 3	not mixed / fasted
Pen 4	mixed / fasted

Each replicate consisted of 4 groups of 4 pigs penned together in 'familiar' groups one week prior to slaughter. Each pen was fed a pelleted, finishing diet *ad libitum*. The mixing of unfamiliar pigs took place at 1400 hrs on the day before slaughter. This was carried out by exchanging 2 pigs from one of the pens allocated to a 'mixed' treatment (pen 2) with 2 pigs from the other pen allocated to a 'mixed' treatment (pen 4). When mixing was complete the pens allocated to the 'fasted' treatments (pens 3 and 4) were deprived of food. Pigs allocated to the 'not mixed' treatments (pens 1 and 3) remained in their familiar groups of 4 and pigs allocated to the 'not fasted' treatments (pens 3 and 4) had food available until they were taken for slaughter.
At 0800 hrs on the day of slaughter all pigs were removed from their pens and separated on the lorry into their experimental groups before being transported a distance of 75 km to the abattoir. Upon arrival at the abattoir all pigs were penned in the same lairage pen for up to 2 hours, resulting in further mixing. Slaughter occurred at a commercial abattoir using carbon dioxide stunning.
Control samples were taken from the jugular vein of 8 pigs, penned individually, in a fed state on the farm.

Biochemical Procedures

At exsanguination blood samples were collected into heparinised and non-heparinised tubes. These tubes were then stored on ice until returning to the laboratory, where they were centrifuged for 15 minutes at 2,000 g. Plasma samples were stored at -70°C, serum samples were stored at -20°C.
Plasma tryptophan values, both total tryptophan and free (non-albumin bound) tryptophan values were determined by the fluorimetric method of Denckla and Dewey[10]. Serum NEFA values were determined using the commercial NEFA C test kit, ACS-ACOD method (supplied by Wako Chemicals GmbH).

Results and Discussion

There is evidence in rodent research[7] which indicates that stresses induced by food deprivation and 3 hours immobilisation stress have the effect of altering plasma tryptophan disposition. It has been suggested that these changes are due to an increase in NEFA concentration caused by lipolysis[9].

The object of this study was, therefore, to assess whether stress induced by variation in preslaughter handling treatments introduced on the day before slaughter had any effects on NEFA concentrations or on plasma tryptophan disposition.

Table 1:- ***Concentrations of various parameters after 4 different preslaughter handling treatments (n=12). Values expressed as mean (± s.d.).***

Treatment	NEFA (mEq/L)	Total Tryptophan (mg/dL)	Free Tryptophan (mg/dL)	% Free Tryptophan
not mixed / not fasted	**0.43** (0.297)	**1.53** (0.725)	**0.44** (0.225)	**29.8** (11.21)
mixed / not fasted	**0.47** (0.240)	**2.07** (0.809)	**0.63** (0.189)	**34.2** (12.97)
not mixed / fasted	**0.57** (0.308)	**2.11** (1.350)	**0.71** (0.245)	**41.6** (20.31)
mixed / fasted	**0.51** (0.234)	**2.27** (1.632)	**0.64** (0.336)	**35.4** (16.09)

There were no significant differences in NEFA concentration, total plasma tryptophan concentration, free plasma tryptophan concentration and % free tryptophan due to the variations in preslaughter handling treatments introduced the day before slaughter (***Table 1***). It may be that the stress which the pigs were subjected to after leaving the farm i.e. transport, mixing in the lairage and stunning, had a greater influence on the parameters studied than the 4 treatments imposed on the farm. The NEFA concentration, free tryptophan concentration and % free tryptophan in the blood collected from pigs at slaughter were significantly greater ($p < 0.05$) than those taken from the jugular vein of pigs penned individually in a fed state on the farm (***Table 2***).

Table 2:- ***Concentrations of various parameters obtained from pigs individually penned on the farm (n=8). Values expressed as mean (± s.d.).***

NEFA (mEq/L)	Total Tryptophan (mg/dL)	Free Tryptophan (mg/dL)	% Free Tryptophan
0.09 (0.123)	**1.49** (0.230)	**0.25** (0.107)	**16.8** (6.58)

There are significant differences ($p < 0.05$) in NEFA concentration, free tryptophan concentration and % free tryptophan when the values from Table 1 and Table 2 are compared. Thus, although no significant difference was observed between the variation in preslaughter handling treatments introduced on the day before slaughter, the overall stress associated with slaughter, including the 2 hours mixing in lairage, appeared to have significant effects on tryptophan disposition and NEFA concentration. The effects observed here agree with the results obtained from rodent research, where the introduction of a stress increases NEFA concentrations which is accompanied by an increase in free tryptophan concentration leading to a higher % free tryptophan[7]. Presumably this is due to the extra NEFA, released due to lipolysis induced by stress, competing with tryptophan for binding to plasma albumin and displacing it, due to its higher binding affinity.

The resultant increase in % free tryptophan leads to a somewhat paradoxical conclusion. The increase in plasma free tryptophan should increase the availability of tryptophan to the brain[9], which should lead to an increase in brain 5-HT synthesis[4]. Such an increase in 5-HT synthesis, if accompanied by 5-HT release should lead to a reduction in aggressive behaviour[6], yet this is the opposite of what is normally observed, as the mixing unfamiliar pigs prior to slaughter is usually associated with an increase in aggressive behaviour[11].

It may be concluded that either, the increased free plasma tryptophan did not result in increased brain 5-HT or if it did, that the latter did not result in neuronal firing. Although it is well established that administration of tryptophan leads to an increase in brain 5-HT synthesis it has been more difficult to show that the increase in brain synthesis is accompanied by a facilitation of brain 5-HT neurotransmission, and some have argued that the increased amount of 5-HT produced by tryptophan may be metabolised without becoming functionally active[12].

References

1) Moss, B.W. (1992). In *The Chemistry of Muscle-Based Foods: Lean Meat, Animal Welfare and Meat Quality*. Ed. by D.A. Ledward, D.E.Johnston and M.K.Knight. pp. 62-76.
2) Fachinelli, C., Sargo, R., Bataller, R. and Rodriguez Echandia, E.L. (1989). Behavioural Brain Research, **35**, pp. 265-270.
3) Young, S.N. (1991). Canadian Journal of Physiology and Pharmacology, **69**, pp. 893-903.
4) Cowen, P.J. (1994). Human Psychopharmacology, **9**, pp. 371-376.
5) McMenamy, R.H. and Oncley, J.L. (1958). Journal of Biological Chemistry, **233**, pp. 1436.
6) Chamberlain, B., Ervin, F.R., Pihl, R.O. and Young, S.N. (1987). Pharmacology, Biochemistry and Behaviour, **28**, pp. 503-510.
7) Knott, P.J. and Curzon, G. (1972). Nature, **239**, pp. 452-453.
8) Curzon, G., Friedel, J. and Knott, P.J. (1973). Nature, **243**, pp. 198-200.
9) Knott, P.J., Hutson, P.H. and Curzon, G. (1977). Pharmacology, Biochemistry and Behaviour, **7**, pp. 245-252.
10) Denckla, W.D. and Dewey, H.K. (1967). Journal of Laboratory Clinical Medicine, **69**, pp. 160-169.
11) Moss, B.W. (1978). Applied Animal Ethology, **4**, pp. 323-339.
12) Grahame-Smith, D.G. (1974). In *Advances in Biochemical Psychopharmacology*. Ed. by E. Coster, G.L. Gessa and M. Sandler. pp. 83-91.