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Standard methods of estimating physiological parameters during pig handling and transport

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1 INTRODUCTION

One of the prerequisites for the project »Methods of improving pig welfare and meat quality by reducing stress and discomfort before slaughter« was that all partners should use a standard set of methods for estimating physiological parameters (see »Methods of of assesing meat quality« by BARTON GADE et al. in the proceeding, too).

In the text the following signs will be used as identification marks for both groups by each investigation method:

E = small experimental group (10 - 20 animals)

C = large representative group under commercial conditions (> 20 animals)

Example$_E$: Investigation of a small group of pigs, devided in two or more parts for a special question (showered/ not showered).

Example$_C$: Comparative investigations of transports under commercial (but known) conditions or large numbers of pigs (randomised but basing on giving information about transport and origin) in a slaughterhouse.

PLEASE NOTE:
Not all methods will be used in the given experiment but if they are then the procedures described are to be adhered to.

2 METHODS OF MEASUREMENTS

2.1 Physiological parameters

2.1.1 Heart rate monitoring (E)

The methods used in the investigations described in the following text depends on the number of the animals investigated.

We distinguish between experimental (laboratory conditions) and commercial conditions during transport as well as at the abattoir.

The heart frequency of the pigs to be slaughtered should be recorded on the farm, during loading and unloading, on the transport and during lairage-time until stunning.

Instrument: The new type of Polar-Sport-Tester (»POLAR SPORT TESTER - PROFI«; order number: 900330) with memory, waterproof, modified by Schütte et al.; storage interval: 5 or 15 sec.

Producer: POLAR ELECTRO GMBH Deutschland; Europaring 86; 64521 Gross-Gerau. Price: ECU 273

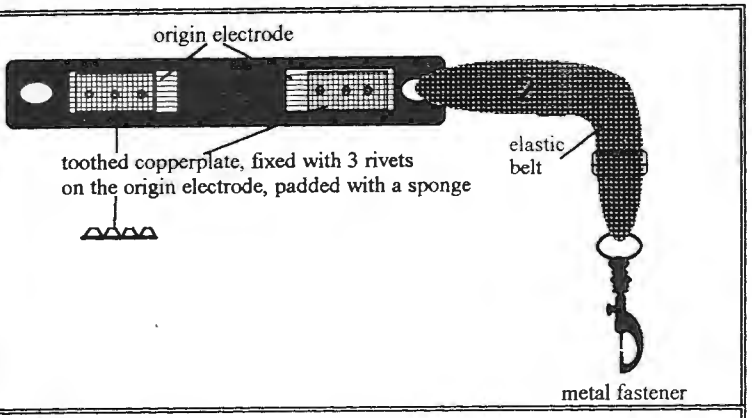

Fig. 1: breast belt, modified with toothed copperplates, sponge and metal fastener

The Polar-Sport-Tester equipment is used as described in the Polar-Sport-Tester prospect. Instead of the plastic fastener we use a small metal fastener for the breast belt.

To increase the conductivity we riveted, by means of copper rivets, a rectangular toothed copperplate on the electrodes inside the breast belt. The copperplates are 6 cm long, 2 cm broad with 3mm teeth in an angle of 90°. Furthermore, we tie up fitted pieces of sponge on the copperplate with twisted thread or elastic and just before using the belt we wet them and put electrode gel onto them (Fig. 1).

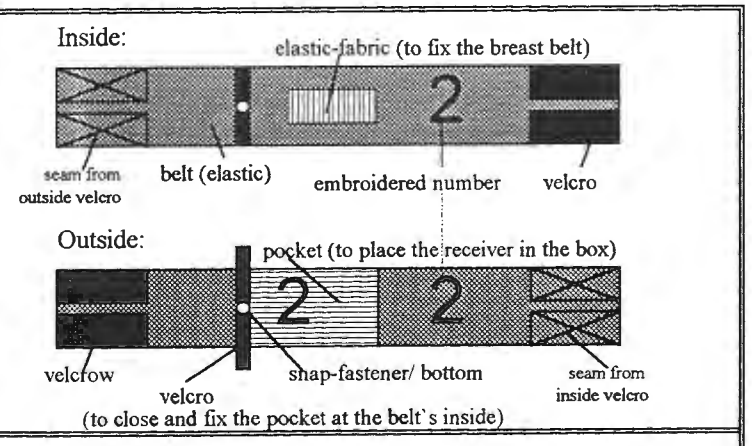

Fig. 2: Elastic belt, inside and outside view

The breast belt is covered by an elastic belt to protect the Polar equipment and to make it possible to place the receiver (Fig. 2).

The elastic belt is made of an elastic fabric (12 cm broad, about 90 cm long; Fa. Skupin, Paul & Co., Nassauische Str. 23, 10717 Berlin, price: ECU 26/ 10 m, order number: 55806850-300) and closed with two stitched pieces of velcro (5 cm broad and about 25 cm long; Fa. Sack & Pack; Brunnenstr. 1, 40223 Düsseldorf, price: ECU 5/ m, order number: 6189903150). So we had the possibility to adapt the equipment to the girth of each pig.

To avoid the breast belt and the elastic belt being displaced against each other we stitched a piece of elastic fabric on the inside of the elastic belt at the level of the receiver between the electrodes so that the breast belt was fixed (i.e. the breast belt has to be shoved through the piece of elastic fabric). On the outside of the elastic belt at the level of the sender we stitched a pocket also made of elastic material (see

above). The pocket was closed by a snapfastener or a bottom and four pieces of velcro.

To protect the receiver and the pocket of the elastic belt we prepared a metal box made of iron with hard rubber on the top („HF-dichte Gehäuse", 53x25x49 mm, Fa. Conrad Electronic GmbH, Klaus-Conrad-Str. 1, 92240 Hirschau, order number 52 16 12-55, price: ECU 4,50/ box). We removed the wristlet of the receiver, welded it in plastic to make it absolutely waterproof and placed the receiver into a metal box.

Each elastic belt, breast belt and receiver is marked with an equivalent number.

30-15 minutes before loading the Polar-Sport-Tester should be put on because usually the animals calm down in the time of 15 minutes.

The breast belt should be put on in the following way: the right electrode lying above the sternum, the other one on the left chest wall (see also the description in the prospect). The belt must be tight.

The receiver has to be started directly before putting on the belt and has to be placed into the metal box which is put into the pocket on the outside of the elastic belt.

The belts will be taken off just after sticking. The receivers should be taken out of the boxes and stopped.

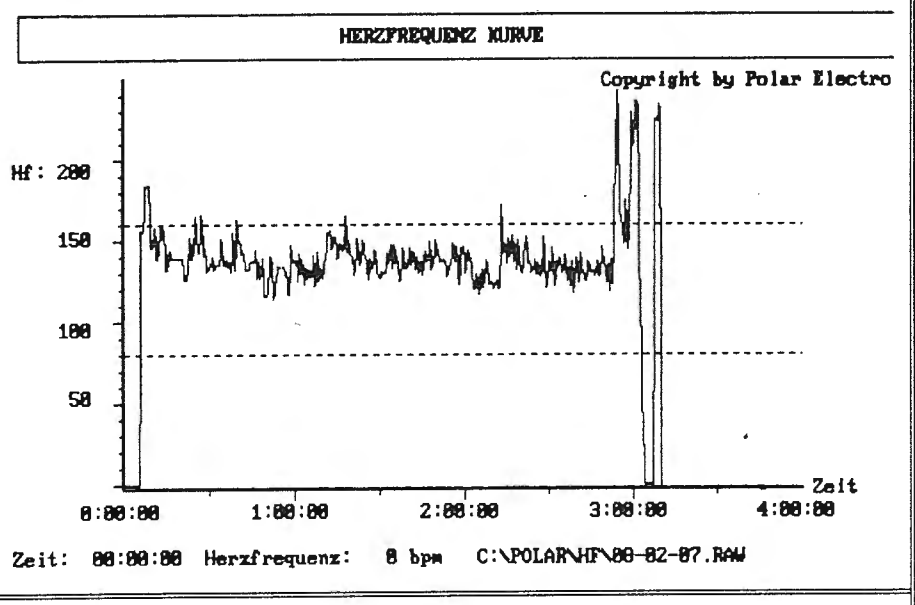

Fig. 3: Original print-out of the course of the heart rate of a major stress susceptibility pig by the POLAR SPORT TESTER software

To transmit the data a special Polar-interface and special software is used (POLAR ELECTRO GmbH, price: ECU 350, order number 925040).

The program transmits the HF-values of each pig into the PC and draws up a plot.

Fig.3 shows the original print-out of one of these plot (original plot from Fig.4).

After this, all events which happened on the transport, during loading, unloading and lairage time have to be entered into a word processing program:

- Transport protocol:
  Time of event and code: e.g. 06 17 L = 6 o`clock and seventeen minutes - beginning of country road, and so on.

- Slaughterhouse protocol:
  Number of pig (= number of the belt), time of event and code: e.g. 01 09 15 nL = pig number one is laying down (without showering) at nine o`clock and fifteen minutes.

In the next step the PC calculates the axis of time and draws up a graphic with the axis of time, heart frequency and the events (Fig. 4 and 6). For each pig and each event the heart frequency will be shown in a box and whisker plot (Fig. 5 and 7).

Fig. 4 represents an example of a minor and Fig. 5 of a major stress-susceptibility pig. The two pigs were transported on the same transport and slaughtered at the same time. Looking at the quartiles it is evident that one pig calms down during transport and lairage (minor stress susceptibility) and that the heart rate of the major stress susceptibility pig is on a high level during the whole time.

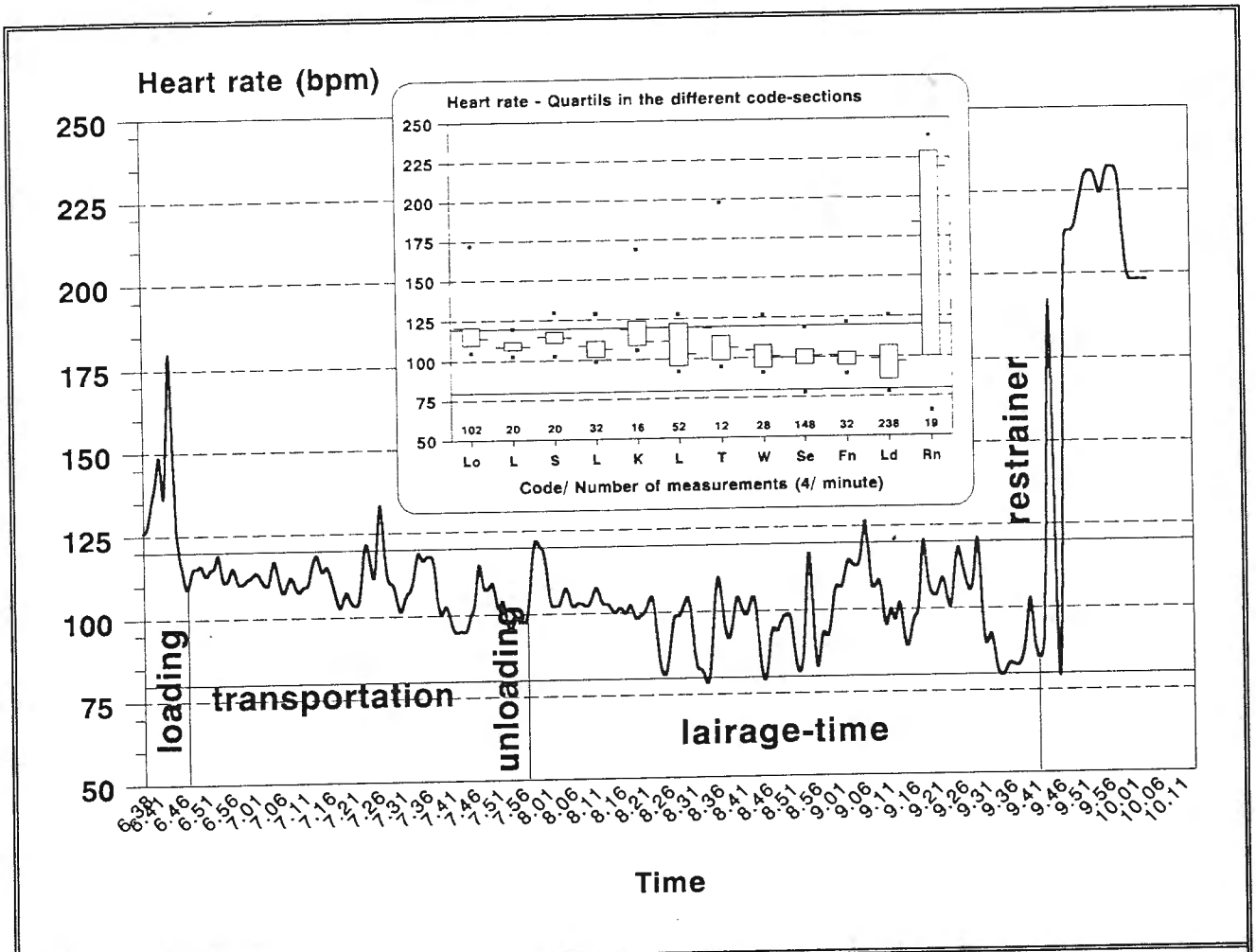

Fig. 4: Course of heart rate of a minor stress susceptibility pig and the statistical summarizing of the heart rate during different code-sections (in form of box and whisker plots) [Code see 2.1.1 & 2.1.3]

The figures with the box and whisker plots show the mean variation of 90% of data in form of box and whisker plots (n = number of measurements = number above the x-axis). The upper and the lower point mark the value above or below each 5% of the data recorded. The vertical box in the middle shows the mean variation of the middle

50% of the data (first and third quartile = X.25 and X.75). The left short horizontal line marks the mean value and the right one the median (X.50).

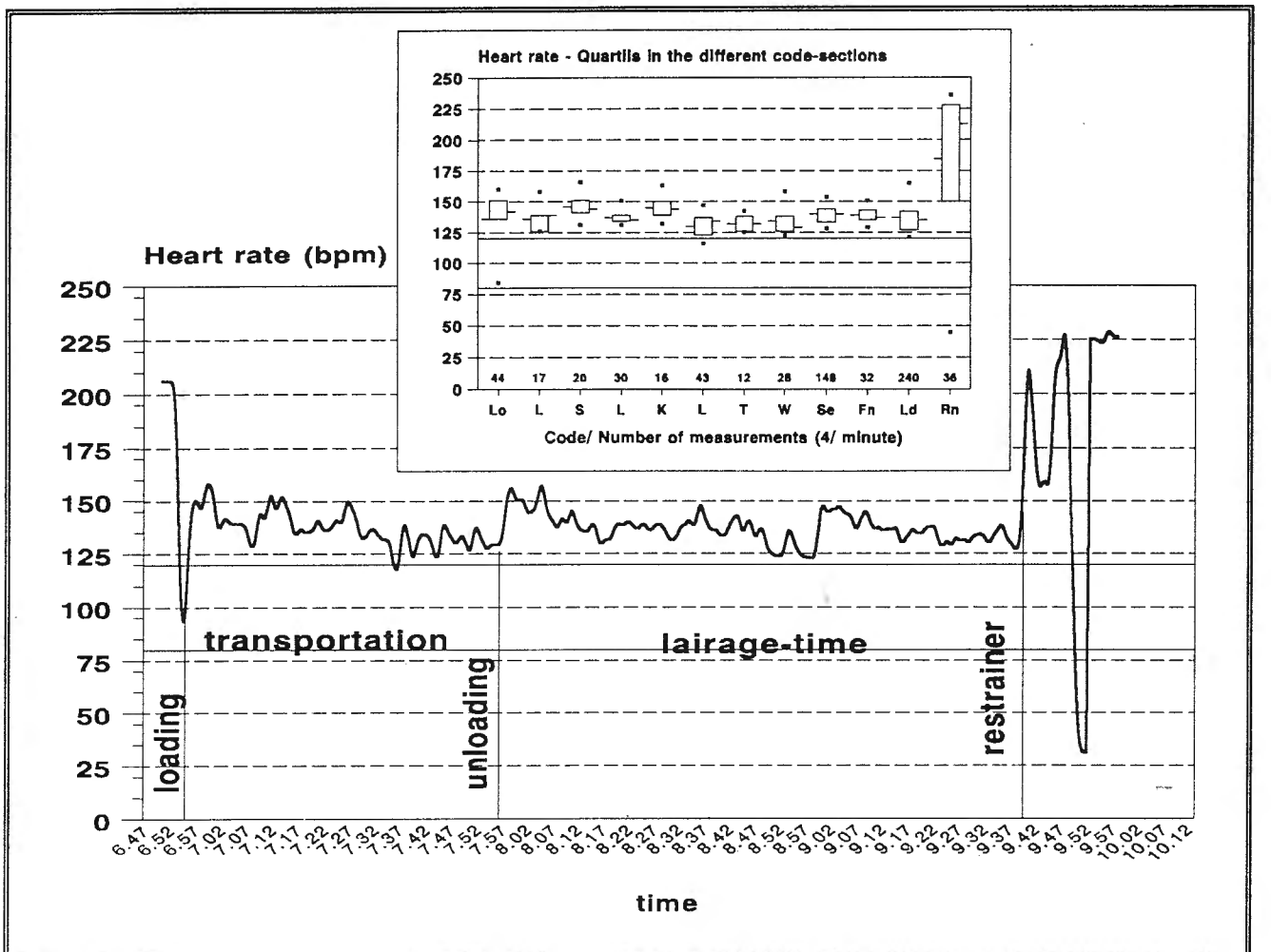

Fig. 5: Course of heart rate of a major stress susceptibility pig and the statistical summarizing of the heart rate during different code-sections (in form of box and whisker plots) [Code see 2.1.1 & 2.1.3]

Both figures show that there are distinct differences in the heart frequeny while driving on a country road or through town and that the highest heart rate is at the time when the pigs went into the restrainer (heart rate > 200/ min.). As well while »loading, driving curved road« and »stop and go« the heart frequency of both pigs increased.

The considerable decrease at the end of both plots shows the bradycardia under carbon dioxide stunning.

The complete data preparation and the plots are running automaticall.

2.1.2 Body temperature

To find out changes of the body temperature we use the pillbox-logger (Type 0-50°C - 8 sec.; Fa. Driesen & Kern, Bad Bramstedt; ECU 400,--). We install them intra-vaginally in fixed combination with a balloon-catheter (20 cm/ CH. 22; Fa.

Eickemeyer, Art. No. 180105; ECU 6,--). After installing, the balloon is blown up with a syringe (20 - 30 ml) and if the end of the catheter hangs out it has to be turned intra-vaginally, too. After this the pillbox-logger can't be drawn without letting out the air of the balloon. The logger stores the body temperature every 32 seconds. Immediately before or after stunning the logger has to be drawn. The data of the body temperature are managed like the data of the heart-frequencies.

Using the pillbox-logger for only a small number of animals (25-50%) seems to be sufficient and it is suggested that the body temperature of the others be recorded at three different times during the investigation:

- before loading (rectal)
- after unloading (rectal)\ before showering\ after showering
- in the sticking blood (see point 2.2.3.1)

2.2 Reports - Protocols

2.2.1 **Transport-report** (E) (see also Measurement protocol for characterising vibration on transporters, ARFC Silsoe Research Institute)

All important incidents and the distances have to be noted as a code with the exact time from the begining until the end of the transport, for example:

- country road / motorway (L)
- town (S)
- curved road (K)
- stop and go (T)
- one stop and going on (A) (for example at the traffic lights)

Doing this it has to take care that the clock used and the heart frequency-receivers are contemporaneous.
Examples of the report to record road type, distance and typical speeds for experimental journeys from Schwarzenbek to the abattoir are shown as following:

Road code 1,2, etc.	Distance, km	Speed, km/ h
2	3	40
1	3	70
2	4	50
3	0,5	50
1	9	60-80

2.2.2 Registration of the physical excitation and state (see also Handling and Environmental Conditions, IVO-DLO)

For small experimental groups:

During loading and unloading as well as every 15 minutes at the slaughterhouse the following parameters have to be recorded for each animal:

a) Breathing rate
- inconspicous =..... 0
- increased =..... 1
- gasping for breath =..... 2

b) Muscular tremor
- inconspicous =..... 0
- just tail =..... 1
  - + ham =..... 2
  - + flank =..... 3

c) Foaming
- no =..... 0
- yes =..... 1

d) Lameness
- no one =..... 0
- slight =..... 1
- severe =..... 2
- unability to walk =..... 3

e) Aggression score (MOSS, 1978) [♣= pig No. 1, ♠ = pig No. 2]
- 1 = ♣ threatening movement of the head, ♠ no retaliation
- 2 = ♣ threatening movement of the head, ♠ mild retaliation
- 3 = ♣ thrust firmly, ♠ submits or retaliates
- 4 = ♠ retaliates strongly, causing ♣ to reinforce its threat and escalation to occur
- 5 = severe fight, looser being chased and bitten

For large representative groups under commercial conditions:

Assessment of the behaviour with a video or directly observation during:

a) Unloading:
Detail about the percentage of animals which are trembling, foaming, having an increased breathing rate, serious lameness, inability to walk, escape/ following.

b) Resting at the slaughterhouse:
ba) Assessment of the behaviour with the video during the whole time.
bb) Direct observation: recording changing behaviour for five minutes 4 times/ hour or every ten minutes the temporary behaviour like sitting, standing, lying.

2.2.3 Recording of the physical activity in the holding pen (E)

Additional to the data reported in point 2.2.1 the activity of each animal will be recorded continuously during the whole time of lairage in the holding pen. These incidents will be assigned to the heart frequency with the help of a code.

The following activities will be taken into account:

- lying ..... (L)
- standing ..... (G)
- sitting ..... (Z)
- walking ..... (S)
- fighting ..... (K)
- escaping ..... (F)
- guiding to another pen ..... (U)
- going into the restrainer ..... (R)
- entering the stunning-system ..... (O)

A »d/ n« before each code should be used to assign whether the animal is showered during this activity or not.

- showering .....d
- no showering .....n
- no showering whilst an other group get showered .....m

In general the begining and the end of showering and the watertemperature *striking the pigs* is to be noted.

2.2.3.1 Sticking blood temperature (E and C)

After stunning the blood temperature will be measured as the last bod temperature measurement. To measure the sticking blood temperature a plastic mug can be used to catch the blood as close as possible to the wound just after sticking. The plastic mug should be coated with isolating material. We use the measuring instrument PT-star/ CPU (Fa. R. Matthäus, Ebenried 33, 86554 Pöttmes; exactness of measuring: +/- 0,1°C). The PT-instrument has an internal data store. To receive the temperature values of the first pig more quickly or if the time between sticking of two pigs is too long, the instrument is warmed up in water of about 40°C. After measuring the stored data are transmitted into the computer by a commercial software.

2.3 Procedure for taking blood samples etc. (E and C)

Two possible procedures dependent on feasibility of catheterising pigs on farms (probably in the ear vein). Otherwise, venepuncture will have to be used.

The third alternative is to take the blood samples after sticking.

Plasma samples will be assayed in duplicate for cortisol, corticosterone, lactate and lysine vasopressin (LVP). It may be desirable to monitor other hormones, e.g. ACTH, catecholamines, ß-endorphin prolactin and thymulin as well as the enzymes LDH, LDH_5, choline kinase and choline phosphokinase.

For cortisol and corticosterone it is well known that they are released in quantity following a stressful stimulation. It is important to realise that both these hormones are likley to increase as a result of „human approach". The threshold time between approach and sample collection is believed to be 2 minutes. In the plasma ≈ 90% of each of these hormones is bound to proteins. Free cortisol and corticosterone levels can be measured in the plasma, but as the % bound is not known to be affected by stress, it may be unnecessary to take such measurements. Similarly, free cortisol and corticosterone can be detected in the saliva. Although this suggests that blood sampling may not be necessary, we do want to measure LVP levels to investigate the hypothesis that supraphysiological concentrations of LVP are associated with „travel sickness", i.e. nausea in pigs.

The other hormones mentioned have all been implicated as being released during stress. However, it is not clear if showing this will add any weight to data gained on the corticosteroid levels during the experiment.

The enzyme LDH_5 is released from muscle tissue into the blood during stress, the ration between LDH_5:LDH offering another index of stress. However, there is thought to be a 5-6 hour delay between the stressful stimulus and the release of the enzyme (following glycogen depletion). CAMBAC found significant differences in both these enzymes in animals slaughtered at different abattoirs, even after very short journeys. Therefore, the delay may be less than first envisaged. In any case, levels of these enzymes could be measured after slaughter, possibly providing an indication of the overall state of stress of the animal resulting from the journey (An initial sample would be of value in this case).

2.3.1 Sampling via implanted catheters

Things to note:

i) Catheters must be filled with heparinised saline
ii) Air bubbles in the catheters must be avoided

Catheters will be „capped" with a stopcock:

Syringes required:

1. 2 ml syringe containing 1 ml heparinsed saline (A)
2. 10 ml heparinised sampling syringe (B) (from Sarstedt)
3. 2 ml syringe containing 2 ml heparinised saline (C)

1. Fill region A with heparinised saline from Syringe (A) whilst stopcock is on OFF position
2. Insert (A) into stopcock on OFF position
3. Turn stopcock to ON position
4. Withdraw fluid (heparinised saline) from catheter until blood appears in the syringe
5. Turn stopcock to OFF position
6. Remove (A) from stopcock and discard
7. Insert (B) into stopcock
8. Turn stopcock to ON position and withdraw 10 ml blood
9. Turn stopcock to OFF position
10. Remove (A) from stopcock and place on ice
11. Insert (C) into stopcock
12. Turn stopcock to ON position and flush in the saline
13. Turn stopcock to OFF position
14. Remove (C) from stopcock and discard

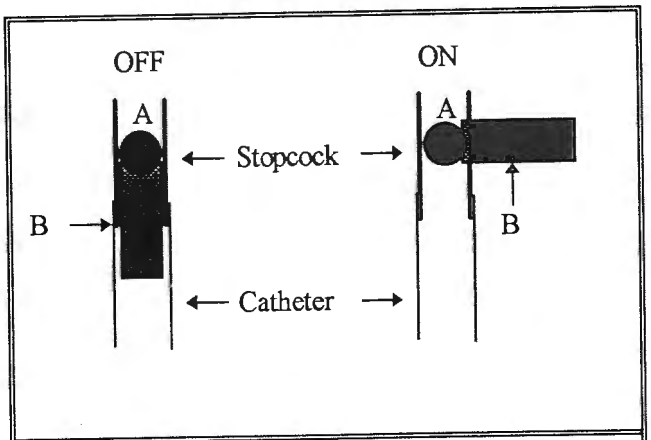

Fig. 6: Pattern of implanted catheter

2.3.2 Sampling by venepuncture

This only requires a 10 ml heparinised sampling syringe with a 21 guage needle attached. The needle is inserted into the jugular vein (practice and familiarisation will be necessary) and 10 ml blood ist withdrawn and placed on ice.

2.3.3 Treatment of samples

Once all the samples have been collected proceed as follows:

1. If haematocrit (HCT) is required, take up a volume of the blood into a microhaematocrit tube (, 25µl) by capillary action. Seal the end of the tube with Crystaseal and spin in the microhaematocrit centrifuge for 5 minutes. The HCT can be calculated by dividing the red cell content by the total content of each tube.
2. Spin the remaining blood at 3.00 g for 10 minutes in a bench centrifuge set to 4°C.
3. Withdraw the plasma using a pasteur pipette and distribute in 1-2 ml aliquots into plastic vials. Approximately 4-5 ml plasma should be collected from a 10 ml blood sample.
4. Store the plasma in a freezer at -48°C, for monitoring catecholamines in a freezer at -80°C.

NOTE:IF YOU ACCIDENTLY WITHDRAW SOME RED CELLS, DO NOT ADD IT TO PLASMA FOR STORAGE AS THIS MAY DISTURB THE HORMONE ASSAYS. YOU MAY RE-SPIN THE REMAINING BLOOD OR DISCARD IT IF THERE IS MINIMAL PLASMA LEFT TO BE COLLECTED.

DON'T FORGET TO LABEL EVERYTHING FOR LATER IDENTIFICATION!!

2.3.4 Choice of sampling procedure - pros and cons of each method

Method	Pros	Cons
Catheters	Multiple samples Less likely to invoke „approach" stress	May not remain patent throughout journey Need to be inserted at farm Initial sample may be influenced by surgical stress
Venepuncture	No surgical procedure required Initial sample not influenced by surgery	Possible maximum of 4 samples Possible „approach" stress Requires expertise

In addition, venepuncture requires second person to restrain the animal, although this may also be necessary for catheterised animals.

Below is a list of the hormones and enzymes, the necessary assay-volume and addresses where these can be order:

- Cortisol.....requires 0,4 ml plasma
- LDH.....requires 0,25 ml plasma
- Creatine kinase.....requires 0,25 ml plasma
- Lactate.....requires 0,20 ml plasma
- Corticosterone.....requires 0,20 ml plasma
- [Lysine Vasopressin .....requires 2 ml plasma]
- Oxytocin.....requires 1 ml plasma
- ß-endorphin.....requires 0,2 ml plasma

Therefore, a total of > 4 ml plasma is required to carry out all these assays (sufficient dor duplicate samples). This requires a blood sample of > 10 ml. Heparinised tubes and syringes have to be used.

Order-addresses:

CK NAC-activated
Randox Labs Ltd.
55 Diamond Road
Crumlin
Co. Antrim
N. Ireland BT29 4QY

Beta-endorphin (Cat No. RIK-8616)
Peninsula Laps (Europe)
Box 62
17K Westside Industrial Estate
Jackson Street
St. Helens
Merseyside
England WA9 3AJ

Corticosterone RIA ^{125}I
ICN Biomedicals Ltd.
Eagle House
Peregrine Business Park
Gomm Road
High Wycomb
Bucks
England HP13 7DL

Lactate
Analox Industries Ltd.
8 Goldhawk Industrial Estate
Brackenbury Road
Hammersmith
London
England W6 0BQ

Cortisol -Immunotech Corp. (Cat. No. 107)
Biogenesis Ltd.
12 Yoemens Park
Yoemens Way
Bournmouth
England BH3 0BJ