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HOUSING, STRESS AND PRODUCTIVITY

studies in growing and reproducing pigs

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ACADEMIC DISSERTATION

To be presented for public criticism,
with the permission of the Faculty of Veterinary Medicine,
University of Helsinki,
in Latokartanonkaari 5, Helsinki, Lecture Room 1,
on December 4th, 2009 at 12 noon.

HELSINKI 2009

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ISBN 978-952-92-6555-8 (paperback)
ISBN 978-952-10-5903-2 (PDF)
Yliopistopaino
Helsinki, 2009

Contents

1. Abstract

Commercial pig production presents the animals with a multitude of potentially stressful challenges. Distress is a threat to animal welfare and may impair productivity in both growing and reproducing pigs. Deleterious effects tend to be neglected when intensifying production.

The objectives of this research were threefold. The first aim was to investigate productivity of pigs in relation to some common practices of pig production known to be stressful, i.e. stall versus group housing of pregnant sows and lack of rooting material in growing pigs. In addition to current housing, effects of early life environment on stress responses later in life were investigated. The second aim was to estimate the amount of stress or the level of welfare of animals. The third objective was to establish relationships between stress or animal welfare measures and productivity.

To address these issues, four studies were carried out. The first and second studies investigated effects of moderate enrichment on behaviour, basal cortisol secretion, health and daily gain in growing pigs. Small groups with siblings were accommodated in pens either barren or enriched with a moderate amount of chopped straw and wood shavings. Six enrichment regimes were used involving a stable environment or changes in enrichment status at 5 and/or 9 weeks of age.

Results from these studies show a higher average daily gain in the nursery as well as a decrease of days in post-weaning diarrhoea in bedded than barren pens. Stress indicators were not associated with productivity.

The early environment (0-4 or 0-9 weeks of age) had significant effects on later stress physiology, as measured by behaviour and basal cortisol secretion. A barren early environment caused signs suggestive of chronic stress that lasted until 21 weeks of age. These effects were evident in response to a fairly small amount of substrate. Previous research indicates that signs of stress will diminish if previously barren-housed pigs gain access to generous enrichment, but this was not the case with the moderate amount of bedding provided here.

The third study compared the fertility of sows housed either individually or in groups on deep litter from weaning to four weeks of pregnancy. Stall housing decreased the odds for early disruption of pregnancy and increased the odds for pregnancy at day 28 post-service substantially. The weaning-to-oestrus interval mediated the effects of housing on the odds of pregnancy.

Group as compared with stall housing post-weaning increased early embryonic death and decreased pregnancy rate substantially. The causes remained undisclosed, but social stress during short periods of time around oestrus and early pregnancy was proposed.

Behavioural indicators showed clear signs of stress in stalls, but behaviour was not associated with fertility. The level or type of stress in stalls may have been insufficient to affect reproduction. Back fat change in early pregnancy was used as a stress measure. No difference existed between treatments, although a larger increase in back fat during early pregnancy enhanced fertility.

In the fourth study, the welfare status of pigs on commercial farms was assessed using an environment-based index comprising several subscales. Welfare scores were regressed on fertility measures. Good-quality floors and stockmanship were the most influential predictors of good fertility, providing some evidence of an association between higher levels of animal welfare (i.e. low levels of distress) and good reproductive performance.

The results of this study support the use of enrichment for pigs in early life, especially if bedding is scarce later in life. During the very first weeks even a small amount will prevent signs of chronic stress. Removal of bedding may, by contrast, increase some harmful behaviours. Group as compared to stall housing of sows is associated with factors that impair reproductive performance. High-quality floors and stockmanship may enhance farm-level reproductive performance in sows.

2. List of original papers

This thesis comprises four original articles, referred to in the text by their Roman numerals.

I Munsterhjelm C, Valros A, Heinonen M, Hälli O, Siljander-Rasi H, Peltoniemi OAT, 2009. Environmental enrichment in early life affects cortisol patterns in growing pigs. doi:10.1017/S1751731109990814 *(published online as First View)* © The Animal Consortium, published by Cambridge University Press, reproduced with permission.

II Munsterhjelm C, Peltoniemi OAT, Heinonen M, Hälli O, Karhapää, M, Valros A, 2009. Experience of moderate bedding affects behaviour of growing pigs. Appl Anim Behav Sci 118: 42–53.

III Munsterhjelm C, Valros A, Heinonen M, Hälli O, Peltoniemi OAT, 2008. Housing during early pregnancy affects fertility and behaviour of sows. Reprod Domest Anim 43: 584–591.

IV Munsterhjelm C, Valros A, Heinonen M, Hälli O, Peltoniemi O 2006. Welfare index and reproductive performance in the sow. Reprod Domest Anim 41: 494-500.

These original publications have been reprinted with the kind permission of their copyright holders. In addition, some unpublished material is presented.

3. Abbreviations

A	Adrenaline
ACTH	Adrenocorticotropic hormone
ADG	Average daily gain
AUC	Area under curve
AVP	Arginine vasopressin
BC24	Change of body fat from 2 days pre-weaning to day 24 of pregnancy (Study III)
CATS	Cognitive Activation Theory of Stress
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CV	Coefficient of variance
DFI	Daily feed intake
DI	Diarrhoea index (Studies I and II)
EDP	Early disruption of pregnancy
EFF	Exploration directed towards floors and fixtures, not including bedding (Study III)
ELI	Ear lesion index (Studies I and II)
F10, F11…F24	10, 11…24 weeks of age, fattening unit (Studies I and II)
FCR	Feed conversion ratio
FL	Finnish Landrace
FSH	Follicle-stimulating hormone
FY	Finnish Yorkshire
GC	Glucocorticoid
GLM	General linear model
GnRH	Gonadotropin-releasing hormone
HPA	Hypothalamic-pituitary-adrenal (axis)
HPO	Hypothalamic-pituitary-ovarian (axis)
LH	Luteinizing hormone
LP	Number of live-born piglets (Study III)
LSY	Litters/sow/year
N5, N6… N9	5,6…9 weeks of age, nursery (Studies I and II)
NA	Noradrenaline
P28	Pregnancy (rate) 28 days post-service
PSY	Piglets/sow/year
PWD	Post-weaning diarrhoea
r	Pearson product-moment correlation coefficient
QBA	Qualitative behavioural assessment
RIA	Radioimmunoassay
SA	Sympathetic-adrenal (axis)
SEM	Standard error of mean
TLI	Tail lesion index (Studies I and II)
xxE, xx0	0=barren, E=enriched, x=barren **or** enriched housing in suckling, nursery

	and fattening phases of Studies I and II, respectively
00, E0, 0E, EE	0=barren, E=enriched housing in suckling and nursery phases, respectively (Studies I and II)

4. General introduction and review of literature

4.1 GENERAL INTRODUCTION

Modern pig production is all about efficiency. Large-scale rearing has pushed input costs to a low level. Society is, however, communicating serious concerns about animal welfare in intensive production systems. Many factors associated with intensive rearing, such as crowding and mixing, are known to be stressful to animals. Although stress has the potential to decrease growth and reproduction in pigs, actions taken to reduce stress are often neglected as an important aspect of economy.

4.2 HISTORY OF STRESS RESEARCH

The basis for our knowledge of the concept of stress was laid more than a century ago by the French scientist Claude Bernard. He was the first to recognize how the body endeavours to maintain a relatively constant internal state in an ever-changing environment (Bernard, 1878). A few decades later, the stress reaction was described by Walter Cannon (1929) and Hans Selye (1936) as actions taken by the body in situations where the internal homeostasis is threatened.

Selye (1936) discovered that a range of stimuli of varying nature increased blood levels of cortisol by provoking activation of the hypothalamic-pituitary-adrenal (HPA) axis. He called this phenomenon the "general adaptation syndrome". Involvement of the autonomic nervous system was described as the sympathetic-adrenal (SA) response a few years earlier by Cannon (1929). Today, anyone intrigued about stress physiology will learn that the topic has inspired scientists to produce enormous amounts of literature (for reviews, see Chrousos, 1997; Salposky *et al*., 2000; and Matteri *et al*., 2000).

4.3 THE STRESS RESPONSE

4.3.1 Acute physiological stress response

The central nervous system (CNS) assesses every stimulus it perceives to identify situations where a significant threat to homeostasis is at hand. If a threat is identified, the hypothalamus will initiate an acute stress response (Moberg, 1985).

Basic understanding of the nature of the stress response has not changed since the days of Cannon and Selye. We still consider it to have a multifactorial and relatively non-specific nature with both physiological and behavioural outcomes. For the physiological response, two major pathways are present: neurovegetative (concerning the autonomic nervous system) and neuroendocrine (Moberg, 1985).

Activation of the **autonomic nervous system** will cause a variety of short-term physiological events. Cannon (1929) collectively calls these the "fightflight response", as they prepare the body for strenuous physical activity. The neurovegetative response includes an increase of catecholamines in plasma, i.e. adrenaline (A) from the adrenal medulla and noradrenaline (NA) from the sympathetic nerve endings. A and NA cause elevations in body temperature, blood glucose levels, heart rate and blood pressure, as well as stimulation of cell-mediated immunity (Dhabhar, 1998).

The **neuroendocrine** pathway includes release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus. CRH and AVP stimulate the pituitary to release adrenocorticotropic hormone (ACTH; Janssens *et al.,* 1995), which stimulates the adrenal cortex to release glucocorticoids (GCs), such as cortisol. AVP is also released from the pituitary to promote water retention in the kidney, thereby concentrating the urine and increasing blood pressure (Eckert, 1988).

GCs amplify and extend the metabolic effects of catecholamines. They act to redirect resources to resolve the threatening situation (Sapolsky *et al*., 2000). At the same time, physiological processes that are not required for immediate survival, e.g. growth and reproduction, are inhibited (Munck *et al*., 1984). GCs will prepare the body for fight or flight by stimulating behavioural responsiveness and rapidly mobilizing energy in the form of available glucose. This is achieved by mobilizing amino acids from muscle and fatty acids from fat tissue as well as by inducing gluconeogenesis and the breakdown of glycogen in the liver. GCs will also suppress cell-mediated immunity, possibly to prevent over-activation of defense mechanisms (Munck *et al*., 1984).

4.3.2 Chronic physiological stress response

Exposure to a stressor will typically cause a pronounced GC response within minutes. The stress response is transitional, and if the stressor is removed, GC levels will drop back to basal levels in minutes or a few hours. This acute, short-span stress response is considered adaptive due to the rapid return to a normal state (Sapolsky, 1992).

If, however, a stressor cannot be eliminated and the reaction persists for more than a few days, harmful consequences will follow (Sapolsky, 1992). Ongoing hypersecretion of GCs will initiate potentially harmful counterregulatory changes at different stages of the HPA axis (Jensen *et al.,* 1996). A number of pathologies may follow, including muscle wasting, gastric ulcers and suppression of growth, immune and reproductive functions (Sapolsky, 1992; Chrousos and Gold, 1992).

During a chronic stress response the acute GC response will be weakened and handling of acute stressors rendered ineffective (Virgin *et al.*, 1997). Several stress-related hormones are known to harm learning and memory processes, thereby impairing the habituation process to the stressor at hand (McGaugh, 1983).

4.3.3 Behavioural stress response

Endocrinological and behavioural reactions to stress are interrelated and neither occurs in isolation (Dantzer *et al.,* 1980), although behavioural changes may be measurable earlier than physiological ones (Keeling and Jensen, 2002). Broom and Johnson (1993) classified behaviour as a sensitive measure of perception.

When housed in (commercial) conditions that prevent escape, an acute stressor will elicit a range of conflict behaviour, as extensively reviewed by Salzen (1991). Conflict actions include intentional movements and agonistic, displacement, interruptive, regressive and redirected behaviours.

Displacement activities are apparently irrelevant and out of context with the current motivational state and may, for instance, involve eating, drinking or grooming inappropriate to the situation (Armstrong, 1950). They are suggested to help the animal to cope with aversive stimuli by decreasing arousal or changing the object of attention (Hinde, 1970).

Examples of redirected behaviours are attack, sexual or parental activities directed at inappropriate recipients such as other animals, fixtures or even humans (Eibl-Eibesfeldt, 1975). Regressive actions involve, for instance, frequent defecation, which is a widely used measure of stress in experimental settings (Fraser, 1974).

If the stressor persists, predictability and controllability of the situation will be lost. In the case of mild to moderate stressors, an ongoing reaction for more than a few hours or days will gradually change conflict behaviour into disturbed behaviour. Stressors of an exceptionally severe nature may cause this transition with a shorter exposure. Disturbed behaviour indicates that the animal can no longer cope and its welfare is seriously at stake (Wiepkema and Koolhaas, 1993).

Examples of disturbed behaviours are injurious actions, such as tail-biting in growing pigs, automutilation or stereotypies, such as chain-biting in tethered sows (Wiepkema and Koolhaas, 1993). Some individuals may go into a state of “learned helplessness”, characterized by pronounced passivity (Seligman, 1972).

4.4 INDIVIDUAL VARIATION IN STRESS RESPONSES

4.4.1 Causes of individual variation in stress responses

Individual variation in stress responses complicates interpretation of experimental data, validation of physiological indicators of stress and welfare assessment of animals. Adrenal and behavioural responses to a given stressor will vary tremendously between individuals, even in homogeneous groups (Ingram *et al.,* 1980). This variation reflects the decisive role of psychological factors, i.e. feelings, as determinants of the magnitude of the stress response. According to Scherer (2001), the very nature of a feeling results from a series of subjective evaluations of the triggering stimulus based on criteria, including novelty, predictability and controllability.

The lower the perceived predictability and controllability of an event, the more stressful it is perceived to be (Weiss, 1970; Wiepkema, 1990). The Cognitive Activation Theory of Stress (CATS) defines predictability and controllability in terms of the individual's expectancies of the outcome of the situation (stimulus expectancy) and the reaction available (response outcome expectancy; Ursin and Eriksen, 2004; Eriksen *et al.,* 2005).
Measures of stress may be influenced by a number of other factors in addition to emotions such as physiological status (e.g. gestation; Douglas *et al.,* 1998a), social rank within a group (McGlone *et al.* 1993a), circadian rhythms, sex and environment (Squires, 2003).

4.4.2 Coping

The individual behavioural response to a stressor is referred to as coping. Two extreme reaction patterns are recognized by Henry and Stephens (1977): fleeing (active coping) and adjusting (reactive coping). The active strategy often involves aggression and is characterized by the release of peripheral catecholamines, whereas the reactive style involves passivity and an increase in adrenocortical activity (Selye, 1950; Koolhaas *et al.,* 1999).

In pigs, coping style can be assessed by a manual restraint test known as the "backtest" (Hessing *et al.,* 1993, 1994a). The extreme responders in the backtest differ in behavioural and endocrine reactions to different stressors (Ruis *et al.,* 2000; Geverink *et al.,* 2002). Housing conditions interact with backtest results (Geverink *et al.,* 2003).

4.5 EFFECTS OF PREVIOUS EXPERIENCE ON STRESS RESPONSES

Previous experience has profound consequences for stress physiology. The most drastic effects are observed in response to early life experience received either pre- or postnatally, i.e. during critical periods of neural and endocrine maturation (Fox, 1970; Gunnar and Vazquez, 2001).

The kind of experience reported to affect adaptive skills includes stressful events of different severity and duration (Weaver *et al.,* 2000; Kanitz *et al.,* 2004), quality of maternal care (Caldji *et al.* 1998), other social contacts, housing environment (De Jonge *et al.*, 1996) and for animals also handling by humans (Levine *et al.,* 1967; general review by Champagne and Curley, 2005). The consequences on reactivity will depend on the period during which the experience is received as well as on type, intensity and duration of the stimuli (Fox, 1970).

Experience of a stressor at any age will affect later stress responses due to habituation. In this case, either stimulus or response outcome expectancy is altered, as defined in CATS (Ursin and Eriksen, 2004; Eriksen *et al.*, 2005).

4.6 DIFFERENT TYPES OF STRESS

In referring to stress, we usually mean an experience perceived as unpleasant, lowering the quality of life and with potential negative consequences for health, i.e. "distress". A physiological stress reaction may, however, be associated with neutral or even pleasurable emotions. In animals, examples of situations involving positively perceived "eustress" could be mating or play-fighting. Hans Selye (1975) clarified the difference in common terms by associating distress with frustration and eustress with fulfillment and victory.

The physiological and behavioural measures currently at hand are not capable of distinguishing the different types of stress. However, to evaluate welfare implications of husbandry procedures and environments for (production) animals correctly, robust and repeatable tools for distress measurement are crucial.

4.7 STRESS AND ANIMAL WELFARE

4.7.1 Definition of animal welfare

The current concern for the welfare of production animals in the Western world was kicked off by Ruth Harrison's book *Animal Machines* in 1964 (Harrison, 1964). Since then, the public has called upon scientists to define good quality of life and its prerequisites for different species. Science has thus faced a very challenging task. As a term expressing societal concerns introduced by philosophers and society critics, animal welfare is a very complex matter, including both ethical and scientific dimensions (Fraser, 1995; Duncan and Fraser, 1997; Dawkins, 1998; Appleby, 1999; Fraser and Weary, 2004).

Animal welfare lacks an unambiguous definition. A number of suggestions have been tendered, most of which have fallen into one of three broad categories (Duncan and Fraser, 1997; Fraser *et al.,* 1997):

The "natural living" school proposes that a high quality of life requires the possibility to express natural behaviour (Webster, 1995) and use other capabilities according to the genetically encoded nature or "telos" (Rollin, 1993). This view is supported by many philosophers and social critics (Singer, 1975; Brambell, 1965; Rollin, 1993).

The biological functioning school views normal functioning of the animal's biological systems as the basis for well-being. Different scientists have underlined different attributes, such as successful coping (Broom, 1991), high levels of growth and reproduction (McGlone, 1993b), behavioural needs (Jensen and Toates, 1993) or health and normal functioning (Taylor, 1972).

The feelings school or subjective experience approach argues that subjective emotions are the key elements in the quality of life of an animal (e.g. Duncan, 1996). According to this view, a high level of welfare requires that animals reasonably free from any unpleasant states experience comfort, contentment and the normal pleasures of life.

None of these three positions can be discarded as scientifically wrong. They merely express different values associated with animal welfare. In practice, these approaches often agree on the welfare status of a given animal, and they can be seen as components of well-being (Fraser, 2008a). However, in certain situations, the aspects conflict to present practical and ethical challenges.

An integrative model for animal welfare assessment combining all three categories has been suggested by Duncan and Fraser (1997). According to this view, animal welfare comprises the state of the animal's body and mind, and the extent to which its nature (i.e. genetic traits) is satisfied (Duncan and Fraser, 1997).

4.7.2 Association between stress and welfare

When drawing conclusions about animal welfare status based on stress indicators, the distinction between different types of stress has to be made clear: *distress* compromises welfare, whereas (pleasurable) eustress has positive effects (Selye, 1975).

By definition, high levels of animal welfare require low levels of distress and vice versa (Broom and Johnson, 1993; Möstl and Palme, 2002). The association between these concepts is, however, not continuous. A number of examples of low correlation between severity of external challenge and physiological responses are given by Veissier and Boissy (2007). The authors conclude that welfare is a state resulting from evaluation of the outcome of a given situation, and that stress may result from this evaluation (Veissier and Boissy, 2007).

4.8 MEASUREMENT OF STRESS AND ANIMAL WELFARE

4.8.1 General approach to stress assessment

As reviewed by Squires (2003), animals can respond to stress by changes in behaviour, in HPA and SA systems, and subsequently also in immune function. Although the stress response is relatively non-specific, all of these systems are not altered in all types of challenging situations. A reliable assessment of stress should involve monitoring of several response systems (Squires, 2003).

4.8.2 Behaviour as a measure of stress and welfare

The use of behaviour in stress and welfare assessment is based on knowledge of normal species-specific behaviour as well as on the nature of deviations in response to different stimuli and emotions (reviewed by Keeling and Jensen, 2002; Squires, 2003). Useful behaviours for welfare assessment include activity levels, posture and movement patterns, vocalization, aggression, sleep patterns and ingestion (Squires, 2003).

Behavioural indicators of welfare are generally more important than physiological measures. Behavioural data are easier to obtain and presumably far more sensitive than physiological information (Keeling and Jensen, 2002). Behaviour is thought to reflect an animal's first attempts to cope with a stressor and thus indicate a situation where welfare is at risk earlier than any known measure of physiology or pathology (Dawkins, 1998). The same is true for health problems, many of which become evident as behavioural deviations, e.g. disturbed biorhythms, at an early stage when other symptoms are sub-clinical (Dawkins, 2004).

A wide variety of tests have been developed for measurement of behavioural signs of stress (reviewed by Ramos and Mormède, 1997). Perhaps the most widely used is the open field test, used for assessing emotionality, fearfulness, temperament, stress-susceptibility or coping style by observing an animal in a semi-standardized open arena (Hall, 1934).

Dawkins (2004) states that behavioural data can be used to answer two key questions in animal welfare assessment: 1) is the animal physically healthy and 2) does it have what it wants? Behaviour can be used for both clinical and pre-clinical diagnoses in answering the first question. Choice and preference tests address the second question by giving an animal an opportunity to choose between different environments, handling treatments, etc. Preference tests measure the value an animal puts on preferred resources according to a method adopted from human consumer demand theory (Keeling and Jensen, 2002; Squires, 2003).

Effects of certain housing systems on animal welfare are traditionally investigated by comparing the behaviour of animals in different conditions. In such studies, the interpretation of the observed differences is based on knowledge about motivational mechanisms of the behaviours of interest. Failure to perform a behaviour indicates a threat to welfare only if the animal is intrinsically motivated to perform this behaviour (reviewed by Keeling and Jensen, 2002).

4.8.3 Physiological variables in stress and welfare assessment

4.8.3.1 Cortisol

In pigs, cortisol is the main GC secreted (Bottoms *et al.,* 1972). A rapid increase in serum cortisol levels is a widely used measure of acute stress. For correct interpretation of results, several factors known to affect basal secretion have to be considered, the most important being the time of day.

Basal GC secretion follows a circadian (about 24 h) rhythm, where plasma concentrations peak in the morning and decline to reach a nadir in the evening (in pigs: Becker *et al.*

1985; Evans *et al.* 1988; Griffith and Minton, 1991). An additional concentration peak has been found in some studies in the afternoon (Evans *et al.* 1988; Griffith and Minton 1991).

The secretion of cortisol is not rhythmic in very young pigs (Evans *et al.,* 1988). Maturation takes place during early development, but reported ages vary. Gallagher *et al.* (2002) noted a distinctive secretion pattern as early as the age of 6-10 days. A cosinor rhythm was found at 8 weeks by Ekkel *et al.* (1996), but not until 20 weeks by Ruis *et al.* (1997).

Chronic stress causes flattening of the basal secretion pattern. The rhythm changes either due to a diminishing morning surge (i.e. low concentration throughout the day) or continuously high values (Becker *et al.,* 1985; Janssens *et al.,* 1995). Chronic stress has also been evaluated by measuring cortisol secretion in response to ACTH (in pigs: Mormède *et al*., 1984). An exaggerated response is thought to indicate chronic activation of the HPA-axis.

To identify abnormal cortisol patterns, several working definitions of a normal rhythm have been applied. Unfortunately, different analysis methods applied to the same data will yield widely differing results (de Weerth *et al.,* 2003). Ekkel and colleagues (1996), among others, have used cosinor analysis, a chronobiological tool described by Halberg (1969). Krieger *et al.* (1971) and Santiago *et al.* (1996) have characterized a normal rhythm by a sufficient decline from morning to afternoon and/or evening.

Several non-invasive methods for cortisol assessment are available. Cortisol diffuses to saliva, urine, faeces and milk. Saliva concentration is a good indicator of the amount of free, i.e. biologically active, cortisol in plasma (Kirschbaum and Hellhammer, 1989; Cook *et al.,* 1996).

The use of stress indicators in animal welfare assessment requires distinction between eustress and distress. David Fraser (2008b) states that cortisol data can be used as a welfare measure only if the stressor is known to negatively affect welfare. As an example of a situation when cortisol data indeed measures suffering, Fraser (2008b) describes dehorning of calves. An increase in cortisol, without knowledge about the effects the stressor has on body function or affective state, should not be interpreted in terms of animal welfare.

4.8.3.2 Implications for the use of back fat changes as a measure of stress and welfare

Most sows lose body weight when lactating and should replenish these reserves during pregnancy. Back fat gain during gestation is used as an indicator of feeding efficiency on commercial farms, but it probably contains information about the welfare status of the animals as well. In a sow group with a sufficient feeding level failure of an individual to gain back fat during pregnancy may reflect serious welfare problems such as disease or low social status. Large variation in back fat gain within a group would raise the same concerns.

Some support for the inclusion of information on distress in back fat data is given based on observations on commercial Danish farms. Kongsted (2006) found low but significant negative correlations between back fat change from weaning to 3 weeks post-service and not eating at feeding time and the number and severity of skin lesions. Skin lesions have previously been associated with stress (Barnett *et al.,* 1992). As gestating sows are commonly fed according to a severely restricted scale (Douglas *et al.,* 1998b), not eating at feeding time would certainly cause distress and likely be the result of severe illness or fear. Kongsted's (2006) observations on aggressive behaviours did not, however, support the theory of back fat change as a stress indicator.

4.8.4 On-farm animal welfare assessment

Any attempt to assess the welfare status of animals must be based on a clear definition. Given the diversity of views on the definition, a multitude of approaches for assessment have emerged.

Based on the "biological functioning" approach to animal welfare, assessment should emphasize measurable parameters of biological functioning such as health and productivity (Broom, 1991). The major drawback of this view is the failure to recognize situations where deviations from normality and feelings do not match, e.g. in the case of an individual with a symptomless pathology feeling perfectly happy (Mason and Mendl, 1993). The feelings-based definition of animal welfare calls for emotional states being the major component of assessment (Duncan, 1996). This view is challenged with how to measure feelings.

Fortunately, as reviewed by Broom (1991), a general consensus appears to exist on many properties relating to animal welfare. Animal welfare is considered a characteristic of animals, not the environment. The welfare state can range from very poor to very good (Brambell, 1965; Broom and Johnson, 1993).

Most herd-level animal welfare assessment systems are based on a multitude of parameters. These can be divided into environmental or indirect and animal-based parameters (reviewed by Johnsen *et al.,* 2001). The former group includes easily measured attributes such as physical dimensions of accommodation, feeding and husbandry routines and access to pasture. Animal-based factors fall within the categories of behaviour, health and physiology. They measure the outcome in a given system and may thus be considered more appropriate than indirect measures that evaluate input. However, animal-based parameters require costly and time-consuming recording, and their interpretation may be problematic (Johnsen *et al.,* 2001).

Several index systems have been developed for on-farm welfare assessment in different domestic species (Johnsen *et al.,* 2001). Indices consist of selected attributes that are assigned scores based on knowledge of the needs of the animals, and finally summated for an overall score or prerequisite for animal welfare. Generally, indices are highly repeatable and flexible, as they allow for compensation of weaker items (Schatz *et al.,* 1996; Amon *et al.,* 2001).

An innovative technique referred to as qualitative assessment of animal behaviour (QBA;) allows observers to create their own descriptive terminology to communicate an animal's behavioural expression (Wemelsfelder *et al.,* 2000). QBA utilizes the natural ability of the observers to integrate a multitude of perceived behavioural information into a "feeling" of the emotional state of the subject. QBA is useful in farm conditions and has proven to be both reliable and repeatable (Wemelsfelder & Lawrence, 2001)

Very recently, European standards for on-farm welfare assessment have been published (for pigs: Welfare Quality® consortium, 2009) as a result of a comprehensive scientific project. Until now, the most widely used index system has been the Animal Needs Index TGI (Tiergerechtheitsindex) 35L developed in Austria for use in pigs, cattle and lying hens (Bartussek, 1999). The TGI assigns points to easily identifiable attributes of the housing system, whereas management, health and behaviour parameters are noted only superficially. Attributes belong to one of the six categories locomotion, social interaction, floor quality, stable climate and health and stockmanship.

4.9 STRESS AND WELFARE IN COMMERCIAL PIG PRODUCTION

4.9.1 Overview

Modern pig production challenges the animals with a cascade of stimuli, all requiring adaptation. If adaptation fails, a state of distress will follow. The challenges, or stressors, are a mixture of social, physical and psychological factors in any given situation.

One approach to the relative importance of different factors affecting pig welfare can be adopted from the constitution of the TGI 35L, an environment-based animal welfare index (Bartussek, 1999). For weaners and fattening pigs, 23% of the maximal score is assigned to outdoor access or rearing, 15% to measures of animal health and husbandry, 11% to space allowance and 9% to the use of bedding. For dry sows, most points (26%) are given for social contacts, followed by 13% for use of bedding, 13% for type of housing and 10% for details on feed and the feeding system (Bartussek, 1999).

Housing of gestating sows and lack of rooting substrate in general are conditions investigated in this thesis. They will be discussed as important examples of stressors in commercial pig production.

4.9.2 Lack of rooting material

Rooting has evolved into a behavioural necessity for pigs, as it is a prerequisite for survival in a natural environment. Pigs will root even if their nutritional needs are met (Beattie and O'Connell, 2002). In barren environments, the need for rooting will be redirected towards penmates in different manipulative behaviours, such as tail- and ear-biting (Schouten, 1986; Fraser *et al.,* 1991; Day *et al.,* 2002; Bolhuis *et al*., 2005, 2006).
Bedding material, particularly straw, in an otherwise poor environment is known to relieve stress due to its multifaceted use as a recreational stimulus, a nutritional substrate and a provider of thermal and physical comfort (Fraser *et al.,* 1991).

4.9.3 Housing of gestating sows

Welfare in stall versus group accommodation for dry sows has been a matter of debate since the 1960s (Bäckström, 1973). Attempts to rank these housing systems in terms of welfare have yielded inconclusive results (e.g. Scientific Veterinary Committee 1997 vs. Barnett *et al.,* 2001). The divergence is due to different prioritization of factors affecting sow welfare.

A recent review by an international task force organized by the US Council for Agricultural Science and Technology concludes that scientific findings do not currently exist to allow for firm decisions on which type of housing is in the best interests of the sows (CAST, 2009). Both accommodations are associated with obvious drawbacks.

Sow groups are usually formed at weaning and kept stable throughout gestation. At mixing, vigorous fighting will take place for 2-3 days to establish a dominance hierarchy. Commercial group housing conditions provide only limited space for these fights, making mixing an important source of social stress (Mendl *et al.,* 1992; Pedersen *et al.,* 1993; Tsuma *et al.,* 1996) and fear (Hemsworth and Barnett, 1990; review by Kongsted, 2004). Depending on the physical characteristics of the pen, the incidence of injuries may be high. Stress responses, as measured by peripheral GCs, are usually higher in losers than winners (Mendl *et al.,* 1992)

The post-mixing fights will subside once a hierarchy forms in the group. If the number of individuals is higher than 3-7, difficulties in establishment and maintenance of this pecking order may follow (Bracke *et al.,* 2002). Low social status has been associated with low levels of welfare (O´Connell *et al.*, 2003).

Continuous competition for resources is a very potent cause of stress not only in newly formed, but also in established sow groups. As a consequence, feed intake may be extremely variable (Kongsted, 2005, 2006). Feeding during gestation is usually severely restricted relative to the amounts sows would choose to eat (Douglas *et al.,* 1998b). If fed *ad libitum*, sows eat small amounts up to 15 times a day (van der Peet-Schwering *et al.,* 2004), as opposed to the common regime of only one daily feeding.

The occurrence and extent of fighting in any group housing system are influenced by housing system, group size, stocking density, feeding method and mixing practices (Edwards and Riley, 1986). Compared with stalls, group housing of sows does substantially increase the risk for body lesions caused by biting (Gjein and Larssen, 1995; Mendl *et al.,* 1992). Overall aggression is more frequent in groups, whereas unresolved aggression is a characteristic of gestation stalls (Broom *et al.,* 1995).

Stall housing of sows has been associated with several signs of compromised health relative to group housing, including reduced cardiovascular fitness (Marchant *et al.,* 1997), muscle weight and bone strength (Marchant and Broom, 1996). Locomotor pathology (Harris *et al*., 2006) and morbidity (Tillon and Madec, 1984) may be increased. Confinement in stalls in conjunction with feed restriction has been implicated in the development of oral stereotypies (Vieuille-Thomas *et al.,* 1995).

Abnormal inactivity and unresponsiveness in confined sows have been reported repeatedly (e.g. Zanella *et al.,* 1996). This type of behaviour has parallels with clinical depression in man (Seligman, 1972).

Fertility effects of dry sow housing are debated. McGlone *et al*. (2004) concluded in a meta-analysis that fertility is equal to or better in stalls than in group housing, but this seems to require expertise in managing sow groups (Arey and Edwards, 1998).

4.10 STRESS EFFECTS ON PIG HEALTH AND PRODUCTIVITY

4.10.1 Stress effects on health

Generally, stress is thought to increase morbidity in the presence of a pathogen. Research on stress effects on immunity in animals has, however, revealed widely differing results. Salak-Johnson and McGlone (2007) conclude in their review that the way stress affects immunity is at least partly dependent on factors such as characteristics of the stressor, genetics, age, social status and aspects of the immune system investigated. Social status seems to affect immunological responses to a stressor more than the stressor itself (McGlone *et al*., 1993a).

According to Radostits and co-authors (1994), psychosomatic diseases as they occur in man are almost unknown in animals. One exception to this statement is, however, oesophagogastric ulcers in pigs. Stress is an important predisposing factor in the aetiology of this disease, although several other factors also have a significant influence (Radostits *et al.,* 1994).

Coping style may be associated with health parameters. In man, individuals with active coping styles may be healthier than those reacting more passively (Nowack, 1989). Causalities remain, however, to be determined.

4.10.2 Stress effects on reproduction

Appropriate timing of reproductive events requires an ability to respond to changes in social and environmental conditions. This is the most probable reason for the development of complex hormonal regulative mechanisms (Dobson and Smith 2000). Female reproduction is controlled by the hormonal interactions of the hypothalamic-pituitary-ovarian (HPO) axis. Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus, causing the anterior pituitary to secrete follicle-stimulating hormone (FSH), which stimulates the ovaries to release sex steroids. Ovulation is caused by a peak of luteinizing hormone (LH) from the anterior pituitary (Hafez and Hafez, 2000).

Successful reproduction requires precise timing of these endocrine events as well as behavioural changes at the appropriate time to assure fertilization of a sufficient number of oocytes. Following fertilization, a delicate interaction between the offspring and the reproductive tract of the dam must occur for pregnancy to be established and maintained (Andersson, 2000).

A variety of stressors are capable of affecting reproduction through a number of endocrine, paracrine and neural systems (Rivier and Rivest, 1991; Tilbrook *et al.,* 2000). Stress has been shown to suppress reproduction in a number of species, including the pig (reviewed by Varley and Stedman, 1994; von Borell, 1995; Einarsson *et al.,* 1996).

Activation of the HPA axis inhibits actions of the HPO axis. Several hormonal components of the HPA axis act at the hypothalamic level to inhibit GnRH release and at the pituitary level to decrease GnRH responsiveness and subsequently LH release (Liptrap, 1970; Dobson and Smith, 2000). At the ovarian level, gonadotropin responsiveness is then cut back, leading to reduced oestradiol secretion by growing follicles (Liptrap and Cummings, 1991; Dobson and Smith, 2000).

Effects of stress on reproduction in an individual vary depending on factors such as health, immune and reproductive statuses (Moberg, 1991). The pre-ovulatory period (Moberg, 1985), implantation and early pregnancy are suggested to be stress-susceptible time frames (Van der Lende *et al.,* 1993). Stress before ovulation may delay the onset, suppress the activity or completely abolish oestrus (Liptrap, 1970; Liptrap and Cummings, 1991). Hormonal disturbances during implantation and early pregnancy can increase embryo mortality, reducing conception rate and/or litter size (Van der Lende *et al.,* 1993).

Although acute stress has been shown to affect hormone profiles in sows (Razdan *et al.,* 2004), short-term stressors of a non-social nature often fail to affect reproduction, even after repeated application (Turner *et al.,* 2002, 2005; Soede *et al.*, 2007). Acute stressors may even have stimulatory effects, e.g. the well-documented effect of transport and mixing stress in puberty induction in gilts (du Mesnil du Buisson and Signoret, 1962).

Turner and colleagues (2005) conclude in their review that stress must be severe and prolonged (i.e. cortisol needs to be elevated for more than four days) before reproduction in female pigs is suppressed. Even under these circumstances, great individual variation exists, with some individuals being unaffected even by long-term stress. Chronic stress effects are mediated by ACTH and cortisol (Varley and Stedman, 1994).

Intensive pig production comes with a number of factors capable of inflicting stress serious enough to impair reproduction in female pigs. These concern the social environment: group size, stocking density, housing systems and human–animal interactions (Varley and Stedman, 1994).

4.10.3 Stress effects on farrowing and piglet performance

In sows, failure to perform highly motivated nest-building activities shortly before farrowing is associated with both endocrinological and behavioural signs of stress (Jarvis *et al*., 1997). These stress responses seem, however, not to persist after farrowing.

Prevention of nest-building has been associated with prolonged farrowing (Oliviero *et al*., 2008) and disturbances in maternal behaviour (Cronin and Smith, 1992; reviewed by Barnett *et al*., 2001), to the extreme degree of savaging (Jarvis *et al*., 1998), and an increased number of still-born piglets, increased pre-weaning mortality (Svendsen *et al*., 1986) and slower piglet growth (Cronin and Smith, 1992). The importance of maternal stress as a mechanism between nest-building, maternal behaviour and piglet performance is yet to be determined.

Effects of stress on milk yield are more clear. Stress-induced catecholamines and opioids are capable of decreasing the synthesis of oxytocin, leading to reduced milk ejection and lower milk production (Leng, 2000).

4.10.4 Stress effects on growth

Chronic GC treatment (Squires, 2003) as well as many stressors of a social or environmental nature (Hessing *et al.,* 1994b; Hyun *et al.,* 1998a, 1998b; Wellock *et al.,* 2003) are reported to have the potential to impair growth in pigs. During stress, muscle tissues are low in the hierarchy for nutrient availability (Hammond, 1952). At the same time, efficiency in the use of nutrients for growth is decreased, and increased amounts of energy are needed for maintenance (Elsasser *et al.,* 2000)

The effects of individual stressors (heat, crowding and social stress) are shown to be additive (Hyun *et al*., 1998a). Wellock and colleagues (2003) have quantified the effects of different social stressors (i.e. group size, space allowance, feeder space allowance and mixing) and incorporated them into a general growth simulation model.

Stress effects on daily gain seem to be mediated by different mechanisms under different circumstances. Cortisol is catabolic and may affect growth by increased gluconeogenesis and reduced protein incorporation into tissues (von Borell *et al.,* 1992). Daily feed intake and nutrient availability are lowered by decreased appetite, gut function and nutrient absorption (Elsasser *et al.,* 2000; Squires, 2003).

5. Aims of the study

This thesis comprises research on stress measures, animal welfare and productivity in relation to some common practices of pig production. Attempts have been made to clarify if stress or welfare status may mediate effects of the environment on productivity.

Specific aims were as follows:

1. To test the hypothesis that behavioural and physiological signs of chronic stress (basal cortisol, ACTH-challenged cortisol) in growing pigs kept in barren, but adequately spaced pens can be prevented with provision of moderate amounts of bedding (Studies I and II).

2. To research long-term effects of environmental conditions early in life as described in #1 in fattening pigs (Studies I and II).

3. To investigate effects of moderate substrate-enrichment on productivity of weaned and fattening pigs (daily gain, feed conversion ratio, health; Study II).

4. To investigate effects of housing system during early pregnancy (stall versus group) on reproductive performance of sows (odds for pregnancy at day 28 post-mating, incidence of early disruption of pregnancy, litter size and number of stillbirths; Study III).

5. To look into relationships between stress measures and productivity in growing pigs (Study II) and sows housed in stalls or groups during the first four weeks of pregnancy (Study III). For growing pigs, the stress measures used are given in #1. and productivity traits in #3. For sows, the stress measures used are behaviour and change in back fat, and productivity measures as described in #4.

6. To establish relationships between sow welfare status, assessed on-farm using an environment-based index, and herd level fertility measures (Study IV).

6. Materials and methods

6.1 OVERVIEW

Basic information about the studies comprising this thesis is given in Table 1. Studies I and II were carried out in a controlled environment on an experimental farm, and Studies III and IV on commercial farms. An overview of materials and methods is given here. For details, see the original publications.

Table 1. Basic information about Studies I-IV.

No. of study	Type of study	No. of animals/ experimental units	Treatment	Main indicators of stress	Main productivity parameters
I[a],II	Clinical/ experimental	252 animals /63 groups of 4 animals	Barren housing (vs. moderate bedding)	Basal cortisol, behaviour	Post-weaning diarrhoea, daily gain
III	Clinical	275 animals[b] /10 replicates of 20+20 animals per treatment	Stall housing (vs. group)	Behaviour, change in back fat during early pregnancy	Pregnancy rate (not including rebreeders)
IV	Observational	28 herds	None	Welfare index	Piglets/sow/year

[a]The number corresponds to the original paper.
[b]400 weanings entered the study, 285 inseminations in 275 animals completed it

6.2 CLINICAL TRIALS (I-III)

6.2.1 Animals and management

Data for Studies I and II were obtained from the same experiment with predominantly purebred Finnish Yorkshire (FY) or Landrace (FL) pigs and a minority of crosses of these with or without ¼ Duroc. The animals were kept in spacious partially slatted pens at all times. The farrowing pen measured 5.4 m^2. Stocking density was 0.7 and 1.2 m^2 per pig in the nursery (5-9 weeks of age) and fattening (10-24 weeks of age) stages, respectively. Average weaning age was 29.5 days. At weaning four littermates, two gilts and two castrates, were weight-matched to form the experimental unit. Feeding was *ad libitum* in the nursery and restricted in the fattening unit. No additional roughage was provided.

In Study III, 2/3 of the sows were FY and 1/3 FYxFL hybrids. Only sows bred within 9 days after weaning participated in the study. Animals were fed a standard ration twice a day in a stall. Oestrus was detected in the stalls after each feeding using manual stimulation and a boar. Sows showing a standing reflex were inseminated, and those still in standing heat after the next feeding were re-mated.

6.2.2 Experimental design

In Studies I and II pens were either enriched or not enriched in each of the three growing phases. Thus, five enrichment treatments were designed to be compared with barren housing from birth to slaughter (Table 2.). The enrichment material consisted of wood shavings and chopped straw that was topped up twice daily to achieve the amount indicated in Table 2. All sows farrowed in bedded pens, but the material was removed two days after farrowing in barren groups.

Table 2. Experimental treatments and the amount of bedding used in Studies I and II.

			Treatment					
			1)[1]	2)	3)	4)	5)	6)
			000	E00	EE0	00E	0EE	EEE
Number of groups in suckling and nursery/fattening phase			10/7	11/9	12/9	10/10	10/10	10/9
Rearing phase (age in weeks)	Amount of wood shavings/ m^2 solid floor	Amount of chopped straw/ pen						
Suckling (weeks S0–S4)	2.2 litres	50 g	0[2]	E[3]	E	0	0	E
Nursery (weeks N5–N9)	1.8 litres	50 g	0	0	E	0	E	E
Fattening (weeks F10-F24)	0.8 litres	50 g	0	0	0	E	E	E

[1]control, [2]0=barren, [3]E = enriched with wood shavings and chopped straw

In Study III, 40 sows were weaned every 3 weeks. Group housing was practiced on the farm before the experiment, thus at least some of the animals were familiar to each other. Sows were moved to a deep-straw area before noon, and in the afternoon half of the sows in each replicate (n=20) were randomly allocated to individual gestation stalls. The other half (n=20) formed one group in the deep-straw area (stocking density approximately 4.3 m^2 deep litter + 0.8 m^2 slatted area per sow) with free access to individual feeding stalls. The stalls locked up when a sow entered, but if an animal from the group treatment left her place or did not take it at all another sow had an opportunity to get extra feed. No bedding or roughage was provided in the stalls. Treatment groups were divided only by stall gates, allowing for close sow-to-sow contact. The experiment ended when most sows were in their 28th day of pregnancy. Sows returning to oestrus before day 28 were diagnosed not pregnant and excluded from the study, but remained in the group.

6.2.3 Saliva sampling (I, III)

Saliva samples were taken for cortisol (Study I) and progesterone (Study III) analyses by allowing each animal to chew on a cotton swab (Salivette®, Sarstedt, Germany) until thoroughly wet (from 30 seconds to 1 minute). In Study I, saliva was extracted by centrifugation before freezing for storage, whereas in Study III the complete Salivette® was frozen prior to centrifugation.

In Study I, saliva samples for cortisol analysis were taken at the end of the nursery and finishing phases (weeks N9 and F21, “C” in Figure 1.) every hour from 07:00 h to 19:00 h (13 + 13 samples). One barrow per group was randomly chosen as a focal animal.

In Study III, each sow was sampled for progesterone analysis at days 17, 20, 24 and 27 of pregnancy (“P” in Figure 2.) for diagnosing early disruptions of pregnancy (EDP).

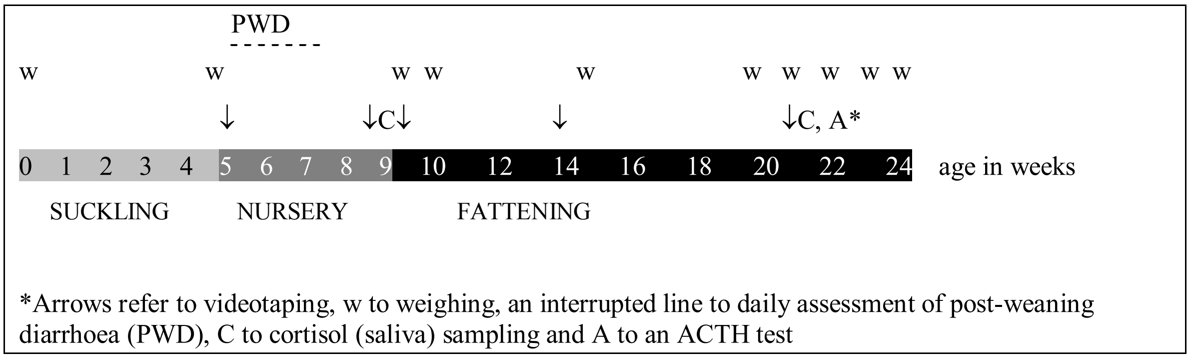

Figure 1. Timetable for data collection in Studies I and II.

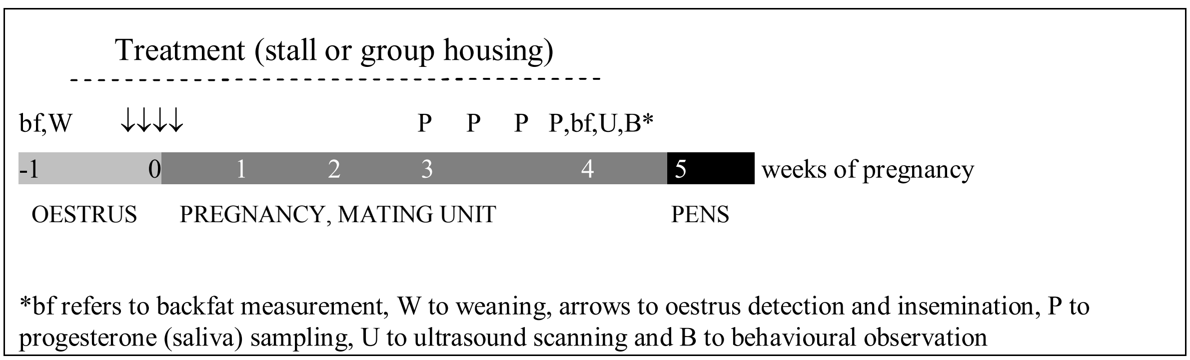

Figure 2. Timetable for data collection in Study III.

6.2.4 ACTH challenge test (I)

In Study I an ACTH test was conducted on F21. The focal animals used for cortisol sampling were used. Immediately after a basal sample at 09:00 h, animals were injected intramuscularly with 500 IU synthetic ACTH (Synacthen®, 0.25 mg/ml, Novartis, Sweden). Thereafter, a total of five saliva samples were taken every 30 min.

6.2.5 Hormone assays and validation of progesterone RIA for pig saliva (I, III)

Salivary cortisol concentration in Study I was analysed by radioimmunoassay (RIA; Coat-A-Count Cortisol, OrionDiagnostica, Turku, Finland), with modifications for use with pig saliva as described by Ruis *et al.* (1997). Inter- and intra-assay coefficients of variation were 9.6 and 10.9%, respectively.

Salivary progesterone in Study III was analysed by RIA (Spectria Progesterone RIA, Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions for serum. The assay was validated for use in pig saliva (Grotjan and Keel, 1996). Recovery of exogenous progesterone was determined by adding known amounts of progesterone to charcoal-stripped pig saliva. These preparations with different concentrations (n=5) had progesterone concentrations almost identical to calculated values (Pearson's correlation coefficient r=0.997), indicating that increasing amounts of progesterone added to pig saliva can be recovered quantitatively with accuracy (Grotjan and Keel, 1996).

To investigate parallelism, samples with known amounts of progesterone were pooled to yield samples with high (n=4), medium (n=4) and low (n=4) concentrations. Samples were serially diluted (1:1, 1:2, 1:4 and 1:16) using stripped plasma from the recovery procedure. The resulting displacement curves were found to be parallel, indicating that saliva does not affect the progesterone concentration estimate at the tested progesterone concentrations (Grotjan and Keel, 1996). Inter- and intra-assay coefficients of variation were <10%.

6.2.6 Observations of behaviour (II, III)

Behaviour was recorded according to ethograms modified from Beattie *et al.* (1995; Tables 3. and 4.). The animals in Study II were monitored with a time-lapse video-recording device for 24 h starting at 07:00 h over five days indicated by an arrow in Figure 1. Agonistic and exploratory behaviours were extracted by continuous focal sampling at 13:00-13:10 h, 14:00-14:10 h and 15:00-15:10 h at each recording (Martin and Bateson, 1993). Behaviour was recorded separately for each animal to calculate a pen mean for percentage of observation time engaged in each behaviour. The number of bouts of each behaviour was expressed as the sum for the pen.

Time budgets in Study II were investigated by instantaneous scan sampling of each animal with a 10-min interval for 5 x 24 h (Martin and Bateson, 1993). Each behaviour was expressed as the percentage of total scans in which the behaviour occurred.

In Study III, behaviour was recorded directly on day 27 using scan sampling with a 10-min interval (Martin and Bateson 1993). Sows were observed individually for 120 min from feeding at 14:00 h onwards.

Table 3. Definitions of behaviours for scan sampling in Study II. Behaviours for continuous sampling in Study II are indicated with an asterisk (*). Additionally, the ethogram for scan sampling included the behaviour "Submissive", described as "Being the recipient of Pushing or Fighting, responding passively or by fleeing, not fighting back".

Behaviour	**Description**
<u>Location</u>	Where the largest part of the animal is viewed from above
Slatted floor	
Solid floor	
Trough[a]	
Missing	Location not identifiable
<u>Body position</u>	
Standing	Standing on all four legs
Sitting	Dog-sitting on the tail with both forelegs stretched underneath
Lying	Lying on the belly or on the side
Other	Any other position
Missing	Position not identifiable
<u>Activity</u>	
Inactive	Performing no activity
Explore	
a) Exploring the ground*	Nosing or manipulating any part of the floor or substrate
b) Exploring fixtures*	Nosing or manipulating any part of the pen other than the floor, the water nipple, or inside of the feeder or through
c) Exploring outside*	Observing, nosing or manipulating any object outside the pen
Social	
a) Mounting/ mounted	Both front hoofs on or resting on sternum on penmate´s back/ being the recipient
b) Pushing*	Pushing penmate with any part of the body in order to displace him, no biting
c) Nosing*/ nosed	Nosing other body part of penmate than the tail, distance from snout to skin less than 5 cm/ being the recipient
d) Tail-nosing*	Nosing penmate's tail, distance from snout to tail less than 5 cm
e) Tail biting*/ tail bitten	Taking penmate´s tail in the mouth/ being the recipient
f) Ear biting*/ ear bitten	Taking penmate´s ear in the mouth/ being the recipient
g) Fighting*/ attacked	Mutual pushing parallel or perpendicular, ramming or pushing penmate with the head accompanied by biting, lifting by pushing the snout under penmate's body/ being a passive recipient
Locomotion and comfort ("LOCOCOM")	
a) Locomotion	Walking or running across pen, scraping the ground with one or both forelegs, jumping, frisking, shaking of head
b) Comfort behaviour	Rubbing body against any object, stretching, yawning, laying on back moving body from side to side
Ingestive	
a) Feeding	Head in the feeder or trough or chewing feed (not substrate)
b) Drinking	Water nipple in the mouth
c) Elimination	Urinating or defecating
Other activity*	Any other activity
Missing	Unidentified activity or pig missing

[a] Noted only in the fattening unit.

Table 4. Ethogram used in Study III

Behaviour	**Description**
Inactivity 1. Sitting inactive 2. Lying inactive 3. Standing inactive	1. Sitting on the tail with the forelegs stretched straight under the body, performing no activity 2. Lying down on the belly or on one side, performing no activity 3. Standing on all four legs, performing no activity
Exploratory 1. Exploring or manipulating the floor or pen fixtures 2. Exploring or manipulating substrate	Nosing, sniffing, touching, licking, chewing, sucking or rooting 1. any part of the pen or floor 2. substrate
Ingestive 1. Feeding 2. Drinking 3. Eliminative	1. Head in the feeder or chewing feed (not substrate) 2. Use of water nipple for drinking 3. Defecating or urinating
Other behaviours 1. Social 2. Agonistic 3 .Locomotory 4. Comfort and grooming 5. Other activity	1. Mounting / mounted (both front hoofs on or resting on sternum on other sow′s back/ being the recipient); Pushing (pushing another sow to make lying space, to get to the feeder etc - not aggressive or agonistic behaviour); Nosing/ nosed (Sniffing or touching any body part of other sow with the snout/ being the recipient) 2. Mutual pushing parallel or perpendicular, ramming or pushing other sow with the head with or without biting. Lifting penmate by pushing the snout under its body. Trying to bite another sow between the bars. Being the recipient of these behaviours. 3. Scraping the ground with one or both forelegs, w alking or running across the pen, jumping, frisking, tossing head (rapid horizontal shaking movement of head) 4. Stretching, yawning, rubbing the body against pen fixtures, lying on back and moving from side to side 5. Performing any other behaviour

6.2.7 Collection of health and productivity data (I-III)

The animals in Studies I and II were weighed at times indicated by "w" in Figure 1. The weight of served food and leftovers was also recorded.

In Study II, the consistency of faeces was scored as follows to investigate the occurrence of clinical post-weaning diarrhoea (PWD): 0 = normal, solid faeces, 1 = soft faeces, 2 = moderate diarrhoea (soupy faeces) and 3 = severe diarrhoea (watery faeces). The assessment was performed daily for 18 days after weaning (interrupted line in Figure 1.).

In Study II, occurrence of body lesions was recorded on a pen basis once a week throughout the experiment. Skin (located anywhere on the body, except for the legs, ears or tail) and ear lesions were scored as follows: 0 = no, 1 = only mild or 2 = at least one severe lesion. Tail lesions were scored according to the following categories: 0 = no lesions, 1 = wounds, but no inflammation (mild lesion), or 2 = piece of tail missing and/or inflammation (severe lesion).

In Study III, back fat thickness was measured using a Renco Lean-Meter ultrasound device (Renco Corp., MN, USA) two days before weaning and in the 4th week of pregnancy (indicated by "bf" in Figure 2.). The measurement was performed 60-70 mm from either side of the backbone at the 12th rib. Production data were stored in WinPig software (AgroSoft Oy, Seinäjoki, Finland). A-index scores (see *6.3.1*) for the breeding unit were estimated separately for stall and group housed sows.

6.2.8 Pregnancy diagnosis and early disruption of pregnancy (III)

Sows were examined for pregnancy by real-time ultrasound (5 MHz sector probe, Agroscan 7, ECM Ltd., Angouleme, France) on day 28. A sow was considered pregnant on this day if the ultrasound result was positive and the salivary concentration of progesterone on day 27 was at least 5.0 ng/ ml (Moriyoshi *et al.*, 1996). A diagnosis of EDP around days 17-26 required a drop of progesterone concentration from above 5.0 ng/ ml to below this limit during the sampling series.

6.3 OBSERVATIONAL STUDY (IV)

6.3.1 Modification of the TGI 35L Animal Needs Index

The Animal Needs Index TGI 35L for dry sows (Bartussek, 1999) was modified for use in Finnish commercial pig production, with national pig protection legislation as a baseline. The resulting "A-index" was complemented with a category for feeding based on TGI 200, a longer version of TGI 35L (Sundrum *et al.*, 1994). Finally, a welfare assessment system for lactating sows with litters was developed based on the TGI 200. For both production stages (dry sows and lactating sows with litters) the A-index comprised six categories, each of which was assigned a score based on 3-6 attributes evaluated on-farm. The categories were locomotion, social interaction, floor quality, stable climate, feeding and finally health and stockmanship. Scores for the six categories were summed to give an overall welfare estimate.

6.3.2 Farms, welfare assessment and production data

Data were collected on commercial piglet-producing (n=10), gilt-producing (n=8) and integrated (n=10) herds. The farms participated in a voluntary slaughterhouse-financed sow nutrition programme and were thus included by nonprobability sampling. Welfare was assessed according to the A-index.

Farm characteristics and production parameters calculated by production surveillance software for one year preceding the visit were collected. Back fat measurements of all sows in the batches nearest to weaning and farrowing (2 batches/ farm) were carried out on a single day within one year before the herd visit, as explained in *6.2.7*. To compare results with those from Study III, average reproductive performance was calculated separately for farms with stall and group accommodation in the breeding unit.

6.4 STATISTICAL ANALYSES

6.4.1 Cortisol data (I)

SPSS software (SPSS Inc., Chicago, IL, USA; versions 12.0.1 and 16.0), SAS 8.02 (SAS Institute Inc., Cary, NC, USA) and the MLwiN 2.2 package (Centre for Multilevel Modelling, University of Bristol, UK) were used for the analyses.

The assessment of rhythmicity of salivary cortisol was based on a definition by Santiago *et al.* (1996). A circadian cortisol secretion rhythm was characterized by a decline of at least 32.7% (three times the mean intra-assay CV) from the morning concentration (average of the 07:00 h and 08:00 h samples) to both the afternoon (average of the 15:00 h and 16:00 h values) and evening values (average of the 18:00 h and 19:00 h samples). Effects of treatments on the odds of a circadian rhythm were investigated using binary logistic regression.

Treatment effects on salivary cortisol concentration were analyzed with a repeated measures general linear model (GLM) with age and time of measurement as within-subject factors. Post-hoc comparisons were performed using the Bonferroni adjustment.

For each focal animal, the total response of the HPA axis to the ACTH challenge was expressed as the area under the response curve (AUC) above the baseline value at t=0 min. Treatment effects on the total response were investigated by Mann-Whitney U, and Kruskal-Wallis tests.

6.4.2 Behavioural data (II, III)

The treatments in Study II were pooled in order to investigate effects of the environment during certain rearing phases. Among the abbreviations of the pooled treatments, “x” refers to either bedded or barren conditions in the corresponding phase.

Groups enriched pre-weaning (Exx, i.e. treatments E00, EE0 and EEE pooled) were compared with groups barren at this time (0xx, i.e. 000, 0EE and 00E pooled), groups enriched in the nursery (xEx: EE0, 0EE and EEE) with the corresponding barren treatments (x0x: 000, E00 and 00E pooled) and finally groups enriched in the fattening phase (xxE; i.e. 0EE, 00E and EEE pooled) with barren groups (xx0, i.e. 000, E00 and EE0 pooled).

Effects of treatment on agonistic and explorative behaviours (I) were tested with a repeated measures mixed GLM. Effects of treatments on behavioural time budgets were analysed with a repeated measures mixed GLM followed by Tukey’s LSD test.

In Study III, behavioural variables were considered as group means and coefficient of variance (CV) of the number of observations in which sows were observed performing each behaviour. Variables were modelled using linear or Poisson regression with replicate as the random factor according to distribution.

6.4.3 Welfare and productivity data (I-IV)

In Study II a diarrhoea index (DI) was calculated as the sum of the daily diarrhoea scores in each pen. Average daily weight gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR), piglet mortality, litter size, DI, and days in PWD were statistically analysed using the MIXED procedure of SAS, version 8.02 (SAS, 1999), with Tukey-Kramer as the *post-hoc* test.

Lesion indices for the skin (SLI), ear (ELI) and tail (TLI) in each growing phase were calculated as the average lesion score. Treatment effects were investigated using one-way ANOVA, Student's t-test and the Mann-Whitney U-test.

In Study III, the existence of pregnancy at day 28 post-insemination (P28) and EDP were analyzed twice, with both sow and group as observation levels. To predict P28 and EDP at sow level, logistic models were built with random factors replicate (level 2) and sow (level 1). For analyses on P28 at group level, the rate of pregnant animals was assigned as the response variable in a mixed linear model. For EDP at group level, effects of treatment and replicate were analyzed with Mann-Whitney U, and Kruskal-Wallis tests. Culling reasons were analysed using a Chi-square -test.

In Study IV, reproductive performance and A-index scores on farms with different housing in the breeding unit were compared by t-tests. In order to investigate the usefulness of back fat data as a welfare indicator Pearson correlation coefficients were calculated between back fat levels (at weaning and farrowing, and the difference between them) and welfare scores. In order to research associations between back fat levels and productivity Pearson correlation coefficients were calculated between back fat and productivity data.

6.4.4 Associations between stress or welfare and productivity

Associations between stress indicators in Studies I and II (cortisol rhythmicity and agonistic behaviours) and ADG were investigated by linear regression. Associations between stress indicators in Study III (passive sitting, passive standing and back fat change -indicated stress level) and productivity (pregnancy on day 28, litter size) were investigated by mixed logistic and linear regression models, respectively, with replicate as a random factor.

To investigate effects of overall welfare assessment scores and subscores in Study IV on performance traits (LSY, litter size, PSY and piglet mortality) were regressed on total A-index scores for farrowing, breeding and gestation units using a linear regression model.

Applicability of the final models from Study IV predicting LSY and PSY for data from Study III was tested by calculating A-index scores for both treatments and inserting these into the models. The outcomes were compared with LSY and PSY values calculated based on actual WOI, P28, culling rate and number of live-born piglets in each treatment in Study III.

7. Results

7.1. RHYTHMICITY OF SALIVARY CORTISOL (I)

Of focal animals, 43% and 61% displayed a circadian rhythm at 9 and 21 weeks of age (weeks N9 and F21), respectively. The odds for rhythmicity at N9 was affected by birth weight (OR=6.7 for every additional 100 g, p=0.02) and treatment. In pair-wise comparisons between the treatments, enrichment in suckling and nursery phases (EEx) increased the odds (OR=30.0, P<0.01) as compared with barren housing (00x).

The final model predicting treatment effects on rhythmicity at F21 is given in Table 3. Cross-tabulation with Chi-squared tests of the significant interactions indicated that nursery stage enrichment promoted cortisol rhythmicity significantly only in animals from barren farrowing pens, and fattening phase enrichment affected cortisol rhythmicity significantly only in animals without a rhythm at N9.

Table 3. Effects of moderate bedding in different growing phases on the odds of a circadian secretion rhythm of cortisol at 21 weeks of age.

Predictor (versus)	p-value	OR
Enrichment weeks 0-4 (barren housing)	>0.1	
Enrichment weeks 5-9 (barren housing)	0.07	23.4[a]
Enrichment weeks 10- (barren housing)	>0.1	
Rhythmic cortisol at 9 weeks of age (no rhythm)	0.08	9.5
Enrichment w. 0-4 x Enrichment w. 5-9	0.02	0.01
Enrichment w. 10 x rhythmic cortisol at 9w	0.05	0.02
Replicate	>0.1	

[a]The ratio of the odds of a circadian secretion rhythm in groups enriched at the age of 5-9 weeks to the odds in groups barren housed at this age

7.2. SALIVARY CORTISOL CONCENTRATION AND ACTH CHALLENGE (I)

Significant effects in the final repeated measures GLM are given in Table 4. *Post-hoc,* all other treatments were compared with the control (000). At N9 a few effects were significant, but the analyses lacked adequate power (≥ 0.8) leaving the full extent of the effects undisclosed. In the 12:00 h sample on F21, treatment EEE and its interaction with weaning age affected the cortisol concentration as compared with treatment 000 (p<0.05). E00 affected the cortisol concentration significantly at 9 of 13 sampling occasions, as indicated in Figure 3. The interaction of E00 and weaning age was significant at all the same samplings.

Table 4. Effects of treatment on salivary cortisol concentration in a repeated measures GLM with age and time of measurement as within-subject factors. Only significant factors and interactions are given.

	p-value[a]
Within-subject effects	
Age[b] x time[c]	0.04
Age x treatment	0.02
Age x time x treatment	0.048
Age x treatment x weaning age	0.03
Time x replicate	0.045
Between-subject effects	
Treatment	0.01
Treatment * weaning age	0.01

[a]Subjected to Huynh-Feldt correction
[b]Age at sampling (9 or 21 weeks)
[c]Time of sampling (hourly between 07:00 and 19:00, n=13)

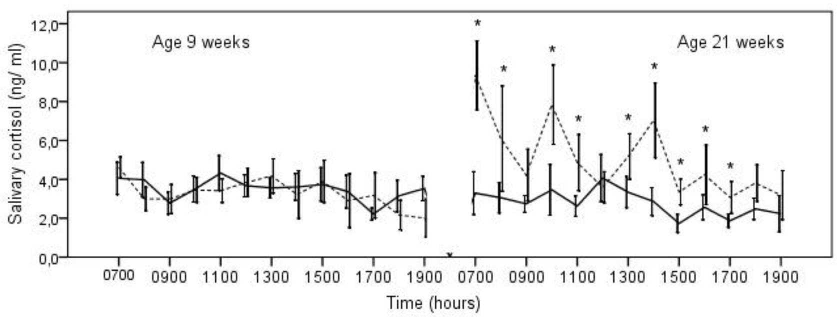

Figure 3. Average cortisol concentration (±SEM) in treatments E00 (interrupted line) and 000 (solid line) at 9 and 21 weeks of age. Asterisks indicate a significant difference at a given time.

To investigate the significant interactions of treatment and weaning age, the data were split in two subsets based on weaning age: "low" (average 26.0 ± SEM 0.15 days, 50% of cases) and "high" (31.1 ± 0.38 days, 50% of cases). Repeated measures GLMs were built for both subsets with time as a within-subject factor, and a two-level variable for treatments 000 and E00 as a between-subject factor. Although the number of subjects in each combination of weaning age and treatment was very low, early enrichment seemed to affect cortisol concentration (prevent flattening of the curve) only in groups with a "low" weaning age.

The overall response of the HPA axis in response to ACTH was unaffected by treatment.

7.3 EFFECTS OF CURRENT ENVIRONMENT ON BEHAVIOUR (II-III)

In Study II the current environment affected continuously sampled agonistic and explorative behaviours only on the first day after relocation to the fattening unit on F10. Animals in treatment xxE explored the floor (including bedding) more than xx0 ($p<0.05$), and groups enriched from birth (EEE) spent more time in total explorative behaviours than treatment 000 ($p<0.01$). Scan-sampled time budgets were affected by current environment only shortly after weaning at N5, when enriched groups were spent more time active ($p=0.05$) and exploring the surroundings ($p<0.01$) than barren groups.

In Study III, total passivity and total exploratory behaviours were unaffected by treatment (stall vs. group housing). Group housing increased exploration directed towards floors and fixtures (EFF, not including bedding). The larger the percentage of rebreeders was in the group, the more EFF was detected. Stall as compared with group housing increased the odds of inactive sitting 10.2 times ($p<0.01$), mean overall ingestive behaviour performed in the group as well as the CV ($p<0.001$ and $p<0.05$, respectively). Group housing increased the odds of "other behaviours" (social, agonistic, comfort, locomotion and other behaviours pooled) 18.0 times ($p<0.001$).

The smaller the change in back fat from 2 days before weaning to day 24 of pregnancy (BC24), the more active or restless the sow. Controlling for housing, a decreasing BC24 decreased overall passivity and ingestion to increase EFF and the pooled "other behaviours".

7.4 EFFECTS OF EXPERIENCE OF ENRICHMENT ON BEHAVIOUR (I-II)

Experience of pre-weaning enrichment (Exx), as compared with barren housing (0xx), had several effects on agonistic and explorative behaviours. Time spent exploring pen fixtures was increased ($p=0.04$). For this behaviour, interactions with age at recording were significant at weeks F10 ($p<0.0001$) and F14 ($p<0.01$). At F14, groups in E00 explored fixtures non-significantly more than controls ($p=0.09$). At F14, Exx was associated with less bouts of agonistic behaviours than 0xx ($p=0.01$). The total time spent in agonistic behaviour at this age was not affected by treatment, although it was twice as long in 0xx than Exx ($p>0.1$).

7.5 HEALTH AND GROWTH PERFORMANCE (II)

During the last weeks in the nursery from, N7 to N9, ADG was higher in enriched than barren groups (Figure 4.). The effect seemed to be mediated by DFI (Figure 4.). Treatment did not affect any production parameters in the suckling or fattening stages. PWD was most severe in barren groups 000 and E00, as measured by both days and DI (Table 5.).

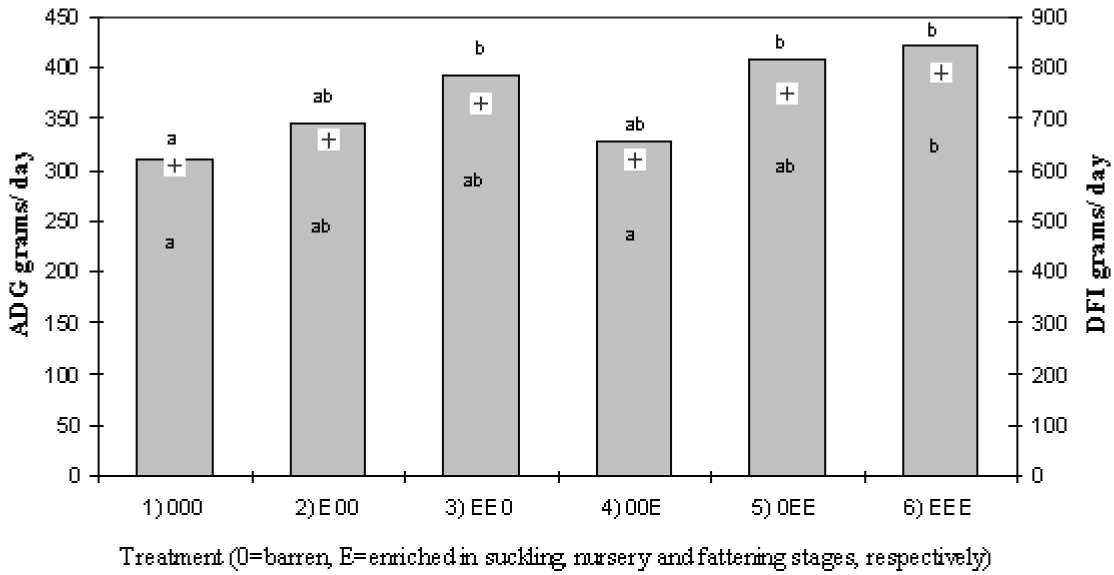

Figure 4. Average daily gain (ADG, bars) and daily feed intake (DFI, crosses on lighter background) per treatment. Different letters indicate a significant difference. Letters above the bars correspond to ADG, letters below to DFI.

Table 5. Treatment effects on weaning age and occurrence of post-weaning diarrhoea. Values given are averages for pens. Within a row, averages with different superscripts differ significantly.

	Treatment							
	1) 0[1]00	2) E[2]00	3) EE0	4) 00E	5) 0EE	6) EEE	SEM	p-value
n (pens) in suckling and nursery/fattening period	10/7	11/9	12/9	10/10	10/10	10/9		
Weaning age, days	27.3	31.3[a]	29.2	26.9[b]	27.3[b]	28.9	0.85	0.002
Days in diarrhoea /pen	8.6[a]	8.1	3.6[b]	6.2	3.8	5.7	1.20	0.011
Diarrhoea index[3]	13.9[a]	12.9[a]	5.1[b]	8.1	4.6[bc]	8.9[a]	2.00	0.002
Feed conversion ratio[4]	17.6	18.5	16.9	17.5	16.8	17.4	0.64	>0.05

[1)]0=barren, [2)]E = enriched pen in suckling, nursery and fattening stages, respectively
[3)] Sum of daily diarrhoea scores during the first 18 days post-weaning
[4)]Megajoule net energy/ kg gain

7.6. BODY LESIONS (II)

Lesion indices are given in Table 6. SLI and ELI were affected by treatment in the nursery phase only. TLI was decreased by current enrichment in the fattening unit. All of the severe tail lesions detected during the experiment occurred in barren groups (treatments 000 and EE0). Experience of enrichment in the farrowing pen increased fattening stage TLI, probably as a consequence of loss of early enrichment (treatments E00 and EE0 pooled), which increased TLI as compared with moving from a barren to an enriched environment (treatments 0EE and 00E pooled, p=0.003).

Table 6. Body lesion indices (average ± SEM) in each pooled treatment and growing stage.

Lesion index[1] (LI)	Pooled treatment					
	Enrichment weeks 0-4[2]		Enrichment weeks 5-9[3]		Enrichment weeks 10-24[4]	
	no	yes	no	yes	no	yes
Suckling weeks 0-4	n=30	n=33				
Skin LI	0.36 ± 0.06	0.45 ± 0.05				
Ear LI	0.34 ± 0.05	0.04 ± 0.04				
Tail LI	0.00 ± 0.00	0.00 ± 0.00				
Nursery weeks 5-9	n=30	n=32	n=30	n=32		
Skin LI	0.22 ± 0.04	0.28 ± 0.05	0.17 ± 0.04*	0.33 ± 0.05 *		
Ear LI	0.05 ± 0.02	0.12 ± 0.03	0.06 ± 0.02†	0.11 ± 0.03†		
Tail LI	0.03 ± 0.02†	0.00 ± 0.00†	0.02 ± 0.02	0.01 ± 0.01		
Fattening w. 10-24	n=30	n=31	n=29	n=32	n=31	n=30
Skin LI	0.6 ± 0.05	0.57 ± 0.05	0.56 ± 0.05	0.61 ± 0.05	0.53 ± 0.05	0.64 ± 0.05
Ear LI	0.14 ± 0.02	0.1 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02
Tail LI	0.01 ± 0.01 *	0.06 ± 0.02 *	0.04 ± 0.02	0.03 ± 0.02	0.07 ± 0.02 *	0.01 ± 0.00 *

[1]Average lesion score (0=no lesions, 1= at least one mild lesion, 2= at least one severe lesion in the pen)
[2]Treatments 000 (0=barren, E = enriched pen in suckling, nursery and fattening stages, respectively) 00E, and 0EE pooled compared with treatments E00, EE0 and EEE pooled
[3]Treatments 000, E00, and 00E pooled compared with treatments EE0, 0EE and EEE pooled
[4]Treatments 000, E00, and EE0 pooled compared with treatments 00E, 0EE and EEE pooled
Within a treatment, means with superscript (*) differ significantly ($p<0.05$) and means with superscript (†) nearly significantly ($0.05 \leq p<0.1$)

7.7. REPRODUCTIVE PERFORMANCE (III, IV)

Fertility traits in stalls and groups in Study III are given in Table 7., and salivary progesterone concentrations in Figure 5. According to progesterone concentration limits by Moriyoshi *et al.* (1996), most early disruptions of pregnancy seem to have taken place between days 20 and 24 of pregnancy.

Factors affecting P28 were investigated with both sow and group as observation levels. Stall housing and a high back fat gain were the most beneficial factors (Table 8.) WOI was an intervening factor in the sow-level analysis.

Early disruptions of pregnancy (EDP) were diagnosed in 10 of the 12 replicates of group-housed animals (8.5% of animals on an average), but in only 3 of the replicates of stalled animals (average 1.8% of animals). The most important factor increasing the odds of EDP at sow level was WOI (Table 9.).

In Study III, group-housed sows had a 23% higher A-index score in the breeding unit ‘feeding’ section. Assuming that all other subscores included in the final models in Study IV were equal, these models predicted 0.07 LSY and 2.2 PSY more in stalls than groups. Using actual data from Study III, LSY was 0.12 and PSY 0.61 higher in stalls than groups.

In Study IV, the change in back fat thickness during pregnancy was moderately correlated with the average total A-index score on the farm (farrowing, mating and dry sow sections; $r=0.44$, $p<0.05$). Reproductive performance did not differ between farms with stall and group housing in the breeding unit (Table 10.). Most of the farms with stalls were small even in comparison with the Finnish average at the time of assessment. The A-index score in the breeding unit was significantly lower on the farms with stalls than with group housing (57.7 ± 10.5 vs. 67.7 ± 13.2, $p=0.04$), but the only item differing significantly was 'locomotion'.

Table 7. Reproductive performance in relation to housing in Study III. Values are given as average ± SD.

Parameter	Stall housing	Group housing
Parity	2.4 ± 1.0	2.4 ± 1.3
Weaning-to-service interval (days)	5.1 ± 0.7	5.3 ± 1.0
Rebreeding rate (%)	10.7	18.1
Irregular returns to oestrus (25−35 days post-service; % of rebreedings)	45%	70%
Backfat 24 days post-service (mm)	12.2 ± 2.8	13.3 ± 2.7
Backfat change weaning − 24 days post-service (mm)	+ 0.4 ± 2.2	+ 0.6 ± 2.1
Live-born piglets / litter	11.7 ± 3.0	12.1 ± 2.9
Stillborn piglets / litter	1.2 ± 2.0	1.0 ± 1.3

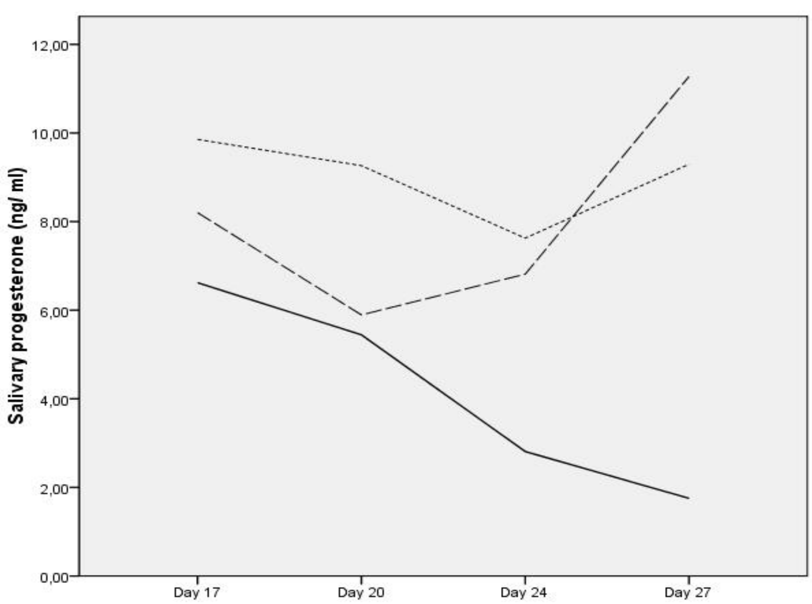

Figure 5. Median salivary progesterone concentration in sows diagnosed as pregnant at day 28 (interrupted line, short distance), EDP (solid line) or not pregnant, no EDP (interrupted line, long distance). Note: the rising progesterone concentration in the not

pregnant, no EDP-group was probably caused by return to oestrus around day 21 and successful insemination.

Table 8. Factors affecting the odds of pregnancy or pregnancy rate 28 days post-service.

Factor (versus or change)	Sow level, OR[a] for pregnancy	Group level, beta[b] for pregnancy rate in group
Breed: hybrid (Yorkshire)	2.5[c]*	
Treatment: Stalled (group-housed)	2.3*	+0.1***
Average weaning-to-oestrus interval (+1 day)		+0.06*
Mean parity in group (+1)		+0.05*
Back fat 2 days before weaning (+1mm)	1.3***	+0.04*
Back fat change[d] (+1 mm)	1.8**	+0.09***
Back fat change[d] +1mm in parity 1 (in parity 4)	2.2[e]*	
Back fat change[d] +1 mm in parity 1 (in parity 2)	1.6*	

*p<0.05, **p<0.01, ***p<0.00l
[a]Logistic regression, random factors batch (level 2) and sow (level 1)
[b]Linear regression, random factor: replicate
[c]The ratio of the odds of pregnancy in hybrids to the odds in Yorkshires
[d]From 2 days before weaning to 24 days pregnancy
[e]The ratio of the odds of pregnancy in parity 1 sows with an 1 mm increase in back fat to the odds in parity 4 sows with an 1 mm increase in back fat

Table 9. Factors associated with early disruption of pregnancy in Study III.

Factor (versus)	Sow level, OR[a] for EDP[b]
Breed: hybrid (Yorkshire)	ns
Treatment: group-housed (stalled)	4.6*
WOI[c] 3-4 days (5)	15.4[d]*
WOI[c] 6 days (5)	25.7**
WOI[c] 7-9 days (5)	49.0***
Parity: 5-7 (1)	6.1 (p=0.054)

*p<0.05, **p<0.01, ***p<0.001.
[a]Logistic regression, random factors replicate (level 2) and sow (level 1)
[b]Early disruption of pregnancy
[c]Weaning-to-oestrus interval
[d]The ratio of odds of EDP in sows with WOI 3-4 days to the odds in sows with WOI 5 days

Table 10. Reproductive performance on farms with different housing in the breeding unit. Values are given as average ± SD.

Characteristic	Stall housing	Group housing	Significance
n (farms)	14	14	
Sows per farm	66[a] ± 102	103 ± 88	ns
Piglets/sow/year	20.4 ± 2.8	19.3 ± 3.8	ns
Litters/sow/year	2.08 ± 0.22	2.07 ± 0.28	ns
Piglets born alive	10.9 ± 0.6	10.7 ± 0.3	ns
Piglet death rate birth – weaning	21.0 ± 4.5	24.0 ± 5.2	ns
Non-productive days/litter	35.8 ± 23.9	46.3 ± 33.3	ns
Rebreeding rate	14.8 ± 7.9%	17.3 ± 8.4%	ns
Body condition score in the farrowing unit (0=poor, 3=good)	2.3 ± 1.2	2.7 ± 0.8	ns
Back fat[b] at weaning (mm)	15.0 ± 2.0 (n=8)	15.8 ± 2.7 (n=18)	ns
Difference in back fat between weaned and farrowing sows[b] (mm)	1.0 ± 1.8	2.9 ± 1.6	p=0.007

[a]Non-normal distribution, median 36, range 22-390
[b]On each farm, back fat was measured on different animals during a single herd visit

Table 11. Effects of farm characteristics and A-index subscores on productivity parameters. Only factors with significant effects are included.

Factor (versus or change)	Change in litter size	Change in PSY[a]	Change in LSY[b]
> 50% crossbred sows in herd (> 50% purebreds)		+2.6***	+0.14*
Farrowing pen A-index scores		+0.8***	
floor quality (+10%)		-1.0***	+0.05**
climate (+10%)			
A-index subscores in the breeding unit			
feeding (+10%)		-1.0*	-0.03*
A-index scores in gestation			
feeding (+10%)	+0.2*		
health and stockmanship (+10%)		+1.2***	+0.12**
Feeding subscore in gestation * feeding subscore in the breeding unit[c]		+0.9*	

[a]Piglets/sow/year
[b]Litters/sow/year
[c]Interaction

7.8 ASSOCIATIONS BETWEEN PRODUCTIVITY AND STRESS INDICATORS OR WELFARE (I-IV)

In Studies I and II, no stress indicators (cortisol rhythmicity, behaviour or skin lesions) were associated with ADG. In Study III, a higher (average) BC24 was associated with larger odds for P28. The same was true at a group level for the group average of BC24 and pregnancy rate. An increasing BC24 increased the number of live-born piglets, but only in group-housed sows.

In Study IV, WOI was correlated with average back fat at weaning ($r=-0.55$, $p<0.05$) and the SD of back fat at weaning ($r=0.59$, $p<0.05$). The difference in back fat between weaned and farrowing sows was uncorrelated with fertility measures in other than first-litter sows. Certain A-Index subscores were associated with PSY and LSY (see Table 11.). No farm characteristics or A-index subscores were found to predict piglet mortality.

8. Discussion

Environmental effects on behaviour (II), body lesions (II) and stress physiology (I) in growing pigs were mainly as expected. Barren as compared with enriched housing was associated with signs of stress, the most important being the larger tail lesion indices in both nursery (non-significant difference) and fattening stages. These findings agree with a substantial body of literature suggesting that barren housing of pigs causes stress, as measured by behaviour (e.g. Wood-Gush and Beilharz, 1983; Schouten, 1986; Beattie *et al.,* 1995), body lesions (Bøe, 1993; Schouten, 1996) and cortisol secretion (e.g. Barnett *et al.,* 1987; de Jong *et al.,* 2000).

The observed effects of early life experience on later behaviour (II) and cortisol secretion (I) were also supported by the literature. The early environment is known to be an important determinant of later manipulative (Day *et al.,* 2002) and aggressive behaviours in pigs (De Jonge *et al.,* 1996; Olsson *et al.,* 1999), and of stress physiology in a number of species (reviewed by Luecken and Lemery, 2004).

The evidence of stress in barren conditions in Studies I and II was, however, not very strong as compared with results from earlier research. This divergence may be due to differences in experimental treatments. In previous studies (e.g. Wood-Gush and Beilharz, 1983; Beattie *et al.,* 1995; De Jonge *et al.,* 1996), the difference between barren and enriched treatments has been larger than in our study in terms of space allowance, use of bedding and mixing of animals. In the case of Janssens *et al.* (1995), barren-housed subjects were also confined. Previously, substrate has been used in ways known to prevent behavioural signs of stress more efficiently than the bedding style here: the amount has been larger (Day *et al.,* 2002) and the straw has been longer in contrast to our chopped straw (Day *et al.,* 2008).

A few experiments have investigated behavioural effects of straw only, and compared effects of experience and actual housing. They have generally led to a conclusion that housing influences behavioural indicators of stress more than the rearing environment (Schouten, 1986; Fraser *et al.,* 1991; Day *et al.,* 2002; Bolhuis *et al*., 2005, 2006). The present results indicated more effects with early environment than with housing. Again, these previous experiments have been designed with a larger difference between the treatments than in our study. Results by Schouten (1986) and Bolhuis *et al.* (2006), indicating that effects of previous enrichment are more pronounced in barren than enriched fattening pens, suggest that the level of enrichment in the fattening pen may determine to what extent the early environment affects behaviour.

In conclusion, moderate substrate-enrichment was found to decrease signs of stress caused by barren housing. Enrichment status during the first 4 or 9 weeks of age affected signs of chronic stress until 21 weeks of age, when fattening pens were either moderately bedded or barren.

Stall housed sows performed substantially better than sows in groups in Study III, with housing and back fat measures as the most important determinants of fertility. The difference between housing systems was larger than the literature suggests (meta-analysis by McGlone *et al.*, 2004), or results from Study IV would indicate (no significant differences, although most indicators were numerically better on farms with stalls in early pregnancy). The possibility for Study IV farmers to make adjustments to management and feeding in order to optimize performance may be one factor explaining this divergence. Study IV farms with group housing in early pregnancy seemed to feed the animals according to a more abundant strategy than was practiced in Study III, as the Study IV sows increased their back fat 2 mm more than the Study III sows.

Several factors differing between the treatments in Study III may have affected the results: social environment, short-term feed intake, provision of straw and confinement. Unfortunately, the study design does not permit the dissection of the separate and interactive effects of these factors. Nevertheless, they can be discussed to provide background material when designing future experiments.

Literature suggests that social stress may have been an important determinant of fertility in the groups (reviews by Kongsted, 2004, 2005). Although sows in stalls seemed frustrated as they performed far more inactive sitting (Zanella *et al.,* 1996) and possibly also redirected behaviour as defined by Armstrong (1950; manipulation of the trough and drinking nipple), back fat measures indicated no difference in long-term stress between the treatments. The methods of stress assessment in Study III were, however, incomprehensive, and the results thus not very reliable. The behavioural observation was very limited in terms of time, length and sampling method (Martin and Bateson, 1992). The change in back fat during early pregnancy (a time period when most sows need to replenish reserves of body fat) has probably not been used as a stress or welfare measure, although it may be argued to contain such information. The change in back fat is a rough measure of feed intake and health over an extended period of time, seems associated with stressful events in group-housed sows (Kongsted, 2006), and was correlated with the overall A-Index score in Study IV.

According to a list of recommendations given in a review by Spoolder *et al.* (2009), the lack of visual barriers and the group size in Study III may have impaired fertility in the groups. Both factors are possible sources of social stress. The moderate group size (20 sows) may have caused high levels of aggressive behaviour. An optimal group in terms of sow welfare consists of no more than 3-7 individuals (Bracke *et al.*, 2002), on the other hand, in very large groups sows change their social strategy from aggressivity to avoidance (Turner and Edwards, 2000). The high incidence of EDP in the groups, most of them between days 20 and 24, indicates that some factors (that may have been a decreased feed intake or social stress) took place in the groups around the end of the 3rd week of pregnancy (Geisert and Yelich, 1997).

The role of WOI as an intervener, mediating the effects of housing on the odds of pregnancy on day 28, provides further evidence for involvement of social stress in Study III. The social environment is known to affect WOI (Dial *et al.,* 1992). Although acute stress is known to stimulate oestrus (du Mesnil du Buisson and Signoret, 1962), low social status may shorten oestrus and disturb the standing reflex (Pedersen *et al.,* 1993). Post-mixing fighting in the groups may have affected WOI, as it is a well-known source of stress (Mendl *et al.,* 1992; Pedersen *et al.,* 1993).

The change in back fat during early pregnancy was an important determinant of fertility in Study III, indicating an involvement of stress unassociated with housing. Behavioural stress indicators were, on the other hand, not associated with fertility. The result is not surprising, as stress effects on fertility are known to be extremely variable between individuals (Turner *et al.,* 2005).

Overall welfare scores in Study IV were not associated with fertility. A measure of total distress levels may have been expected to correlate with fertility, but this result is not surprising if we recall that the association between welfare and distress in not continuous (Veissier and Boissy, 2007).

In contrast to overall welfare scores, certain A-Index subscales correlated with reproductive performance in Study IV. A subscale could be referred to as a subcategory of welfare, such as health-related or social. LSY and PSY were enhanced especially by high A-Index subscores for floor quality, health and stockmanship. Floor type and condition are associated with slipping, lameness, decubital ulcers in the shoulder region, injuries on feet and teats (Boyle *et al.,* 2000; Bonde *et al.,* 2004) and even culling rate (D'Allaire *et al.,* 1989). Hence, it would seem logical that good-quality footing would affect farm-level fertility measures through a decreased number of leg (and other types of) pathologies. Leg problems often cause considerable pain and affect the animal for extended periods of time.

The possibility of leg health as one factor mediating fertility effects of housing in Study III cannot be excluded, although the difference in the leg-related culling rate was non-significant (3.7% in group housing vs. 3.0% in stalls, $p>0.1$). The small difference is not surprising since the exposure time was only one month. In Study IV, the animals were kept on the same floors continuously, over time causing accumulation of injuries on inappropriate surfaces and thereby probably more evident effects on reproduction.

Applicability of the models built in Study IV for data from Study III was fairly good. The calculated difference between accommodations in LSY was 1.8 times larger than predicted based on A-index scores, and PSY was about 1/3 of the predicted difference.

When comparing results or drawing conclusions from Studies III and IV, several factors have to be taken into account. The very short exposure time to the different environments for the animals in Study III (4 weeks) is an important deviation from the commercial conditions in Study IV, where the sows return to the breeding unit every four to six months. Health and productivity effects of the accommodation will of course become more pronounced over time. If Study III sows would have been followed over several parities, differences in A-Index scores for health would probably have emerged between the treatments, changing the applicability of the model from Study IV.

Another important deviation between Studies III and IV was exclusion of sows not coming into or returning to oestrus, or culled in Study III. The non-productive days such animals cause will impair productivity at farm level, and are one factor explaining the differences between predicted and observed performance when using the model from Study IV for Study III data. Likewise, removal of the sick and culled animals in Studies I and II affected applicability of the results for commercial conditions, especially attempts to make economic calculations.

In Study IV, farms were managed conventionally without intervention. Although an observational approach is useful for monitoring the actual situation on the field and making hypotheses for future experimental confirmation, causal relationships between collected parameters cannot be established, nor can all factors (e.g. culling policy) affecting the observed results be taken into account.

In conclusion, factors impairing fertility in groups as compared to sows in Study III could not be ensured. Stress was probably involved in short periods of time around oestrus and/ or the end of the third week of pregnancy. In Study IV group and stall housed sows seemed to be managed differently in order to perform at the same level. High quality floors and stockmanship enhances productivity in sows.

In Studies I and II, no evidence was found for associations between stress measures and growth. In preliminary analyses, the presence of a rhythm at N9 clearly increased nursery ADG, but the effect was confounded by enrichment status. The mechanisms mediating enrichment effects on growth are probably complex and interrelated (e.g. better health through enhanced hygiene at least in comparison with solid floors, thermal comfort and decreased stress levels). Thus, absence of associations of the chosen stress indicators and ADG cannot be taken as evidence of no involvement of stress.

Any practical recommendations for commercial conditions based on this research should be applied with caution. The Study IV farms were collected by nonprobability sampling, possibly biasing the results. Studies I-III were conducted on one single farm (Studies I-II on one farm, Study III on another), thus the results cannot be generalized. Removal of badly performing animals makes economic calculations difficult. Nevertheless, the outcome from Study II indicates, that in order to decrease agonistic behaviours in barren or moderately bedded fattening pens (e.g. most commercial environments), farrowing pens should be littered.

The results obtained do also provide useful indications for follow-up studies. The causes for the observed deviations in cortisol secretion (stress and/ or delayed maturation) in Study I should be established using a larger number of sampling days and subjects. The biological significance of the deviations should be investigated by, for instance, behavioural observations. Results from Study III did not clarify why the difference in performance was so big. The results merely left questions regarding the underlying mechanisms, especially the role of WOI. Clearly, there are factors associated with group housing in early pregnancy that need to be clarified in order to tackle their effects on reproduction.

9. Conclusions

Behavioural and physiological signs of chronic stress (tail biting lesions, decreased exploration, blunted cortisol rhythm) in barren-housed growing pigs are prevented by moderate substrate-enrichment.

Moderate provision of bedding in early life (0-4 or 0-9 weeks of age) may prevent or decrease signs of chronic stress in barren pens (blunted cortisol rhythm, performance of agonistic behaviours) at least until an age of 21 weeks.

A moderate, in contrast to an abundant supply of substrate in fattening pens does not diminish signs of stress caused by barren rearing conditions.

Measures of stress in growing pigs (cortisol rhythmicity, body lesions and behaviour) do not seem to correlate with daily gain or feed conversion ratio.

Bedding of nursery pens may decrease post-weaning diarrhoea and increase daily gain.

Group as compared with stall housing post-weaning may increase early embryonic death and decrease pregnancy rate in sows substantially, although behaviour indicates presence of chronic stress in stalls.

Back fat gain in early pregnancy is associated with good fertility.

Farm-level fertility is not associated with an environment-based animal welfare score, but it may be enhanced by high-quality floors and good stockmanship.

10. Acknowledgements

This work was part of the project Welfare and Production in the Pig. All financial supporters, the Finnish Ministry of Agriculture, Raisio Feed Ltd., Snellman Ltd., Atria Ltd., the University of Helsinki and the Mercedes Zachariassen Fund are gratefully acknowledged.

The people mentioned here are a representative sample of the most important contributors to this thesis. Juha Virolainen and Olli Peltoniemi first introduced me to the fancy Pig Research Group at Saari. Olli also arranged for me to work with Veikko Tuovinen, who gave me a comprehensive introduction to bold thinking and provided me with quite a playground to try my wings in the field of pig health care.

The supervisor squad Anna Valros, Mari Heinonen and Olli Peltoniemi deserves special thanks for all the support, help and excellent guidance through all stages of this process.

This manuscript was greatly improved by valuable comments by my official reviewers, Cheryl Ashworth and Hans Spoolder. I am indebted to them for their thorough evaluation and insightful criticisms. Bo Algers is thanked for agreeing to serve as my opponent.

Co-author Outi Hälli is acknowledged for her gift of producing incisive comments that really make a difference. Our project at MTT Animal Production Research in Hyvinkää was successfully managed by co-author Hilkka Siljander-Rasi and organized by co-author Maija Karhapää. However, the experiment would not have been finished without Tapio Helenius, who engineered the whole camera show and kept up a joyful atmosphere.

In Katri Rantsi, we found a farmer able to carry out an experiment all by herself, undeterred by the less glamorous reality of data collection, all of this on her own farm – yes, she really does exist!

At the Saari lab, Marja-Liisa is thanked for the time she put in teaching a beginner to manage on her own. Furthermore, there is a bunch of students deserving heaps of gratitude for helping out with practicalities during hours that should not be revealed anywhere.

Tuomas Herva is acknowledged for being a highly valued companion capable of intriguing scientific debating at any hour. He is also thanked for our daughter Linnea, the most wonderful creature on earth, an already experienced but absolutely terrible co-author.

Finally, I want to thank my parents for giving me an opportunity to go all this way. I hope I have made them proud.

Camilla Munsterhjelm

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