COPING BEHAVIOUR IN PIGS

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CONSEQUENCES FOR WELFARE AND PERFORMANCE

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CONSEQUENCES FOR WELFARE AND PERFORMANCE

COPING GEDRAG VAN VARKENS - GEVOLGEN VOOR WELZIJN EN PRESTATIE

(met een samenvatting in het Nederlands)

Proefschrift

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INTRODUCTION

GENERAL STRESS RESPONSE

Stress refers to a state of threatened homeostasis. To counteract disturbances in homeostasis, the body reacts with a stress response that serves adaptation. Psychological factors determine a major part of the stress response. The lower the predictability and controllability of an aversive event, the more stressful it is (Wiepkema, 1990). Moreover, different stressors may evoke their own specific stress responses (Mason, 1971). The central nervous system assesses whether or not a stimulus represents a significant challenge for the animal. If a stimulus is perceived as a threat, the animal can show a multifactorial (behavioural, neurovegetative and neuroendocrine), and relatively non-specific, stress response (Moberg, 1985).

Behavioural aspects of stress

In daily life, behaviour of a vertebrate may be interrupted and disarranged by a great variety of events, such as the absence of food at the expected location or time, or the presence of a rival on an unexpected site. Such events evoke uncertainty and some conflict about what to do next. As a result, the animal may show conflict behaviour, which consists of agonistic behaviour, displacement or interruptive behaviour, redirected behaviour and intentional movements. Normally, these conflict behaviours have a relatively short duration and the animal can relatively quickly solve the problem, e.g. find food, or remove or avoid the rival. Although during such brief responses physiological ‘alarm activities’ can be measured, we assume that they do not greatly reduce welfare. However, when the animal cannot solve the conflict, predictability and controllability of relevant events become low. If the conflict remains present for a longer period of time or if it is severe in nature, symptoms of chronic or severe stress can occur. Then, conflict behaviour can gradually change into disturbed behaviour, such as injurious behaviour (e.g. tail-biting in fattening pigs), automutilation or stereotypies (e.g. chain-biting in tethered sows). In this situation, the animal can no longer cope and welfare is seriously at stake. Although stereotypies are thought to have a stress-reducing effect because of the association with opioid activity in the brain, they do not really remove the cause of stress. Therefore, stereotypies cannot be seen as an effective way of coping, but rather as a signal that environmental conditions for the animals are inadequate (Wiepkema and Koolhaas, 1993).

Physiological aspects of stress

An environmental challenge will induce not only a behavioural but also a physiological response, and both responses to stress are highly integrated. The behavioural and physiological stress responses are initiated and coordinated by the central nervous system (CNS), with two major pathways for the physiological response: the autonomic nervous system and the neuroendocrine system.

The **autonomic nervous system** is divided in the sympathetic and the parasympathetic branch, with both branches innervating almost all organs. A stressor may evoke an almost general activation of the whole sympathetic nervous system, which will cause a wide variety of physiological events. These include an increase in catecholamines: plasma adrenaline from the adrenal medulla and noradrenaline from the sympathetic nerve endings, resulting in an increase in blood glucose levels, heart rate and blood pressure, an elevation of body temperature and changes in the immune system. Cannon (Cannon, 1929) called this the ‘fight-flight’ response because most of these physiological responses prepare the individual for physical activity. Sympathetic action can also be restricted to some parts of the system. For example, psychological stressors usually result in an increase in plasma adrenaline, whereas noradrenaline is associated mainly with physical activity. The parasympathetic branch also reacts to a stressor. For example, in response to the expectation of a stressor, a decrease in heart rate may be observed (Notterman et al., 1952). This bradycardia response is sometimes called the 'orientation attention response', indicating that it reflects an expected stressor (Obrist, 1981).

In the brain, the integration centre for **neuroendocrine** reactions to a stressor is the hypothalamus. These reactions include hormonal systems involved in the regulation of the adrenal cortex (corticotropic releasing hormone (CRH) and adrenocorticotropic hormone (ACTH)), reproduction (gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and prolactin), metabolism (growth hormone and thyrotropic hormone (TSH)) and the regulation of blood-pressure and body fluids (vasopressin and oxytocin). The classical and best-known neuroendocrine pathway that responds to stress is the hypothalamus-pituitary-adrenocortical (HPA) axis (Selye, 1936). In response to a wide variety of stressors such as heat, cold or tissue damage and in response to psychological stressors (uncontrollability, unpredictability), an animal reacts in the same basic pattern: the hypothalamus releases CRH and vasopressin. CRH stimulates the pituitary to release ACTH, which in turn stimulates the adrenal cortex to release glucocorticosteroids, such as cortisol. Vasopressin acts synergistically with CRH, in this respect (Eckert, 1988). Cortisol stimulates mobilization of amino acids from muscle and gluconeogenesis in the liver, to produce elevated blood glucose levels; furthermore, cortisol has an anti-inflammatory effect. Vasopressin or antidiuretic hormone (ADH) promotes water retention in the kidney, concentrating the urine and increasing blood pressure (Eckert, 1988). This vasopressin, originating from the PVN neurons and released from the pituitary, plays a minor role in the HPA response. CRH neurons in the hypothalamus can also produce vasopressin, which influences ACTH secretion in the pituitary. This vasopressin is important in (chronic) stress responses (de Goeij et al., 1992; Janssens et al., 1995).

Function of physiological stress responses

The general function of the changes in peripheral organs such as heart, blood vessels, immune system and gastro-intestinal tract, is preparation of the organism for and the execution of an adequate behavioural and physiological reaction to the stressor. The sympathetic nervous system is involved in the mobilization of energy, the increase in heart rate and blood-pressure and the redistribution of blood flow. This is necessary for muscle activity, allowing the animal to fight or flee. Corticosteroids stimulate gluconeogenesis in the liver, thereby also mobilizing energy.

Stress has a modulating effect on the immune system (Ader et al., 1991). Acute stress stimulates cell-mediated immunity, which can be beneficial if for example an individual is wounded in a fight or during flight (Dhabhar, 1998). Corticosteroids suppress cell-mediated immunity, which is thought to protect the organism against over-activation of the normal defense mechanisms (Munck et al., 1984). However, during chronic stress this suppressing effect of corticosteroid levels can influence health negatively (Dhabhar, 2000).

Several stress-induced hormones affect the CNS, stabilizing hormone levels through negative feedback mechanisms (Huether, 1996). Besides, hormones like adrenaline, ACTH, vasopressin and β-endorphin may affect learning and memory processes, which is important for future behaviour and neuroendocrine stress response to stressors (McGaugh, 1983).

STRESS IN PIG HUSBANDRY AND CONSEQUENCES FOR WELFARE, HEALTH AND PRODUCTION

A pig in the current husbandry systems encounters many stressors, starting with being handled just after birth and ending with transport to and handling in the slaughter house. Several of these stressors are related to the husbandry system(s), whereas others are just an unavoidable part of life. In this thesis, the focus will be on stress factors induced by the management, such as cross-fostering, weaning and mixing of pigs. These factors were chosen because they are important for the welfare of the pig and are typical system-related stress factors that can be reduced or even eliminated by the farmer. Cross-fostering had to be included in our study design, because we needed to create groups based on first backtest results. Handling is not investigated as a separate factor, but it is an essential part of the other investigated procedures. Invasive procedures such as snaring (using a nose sling, e.g. for blood sampling), castration, iron injection and vaccinations, will not be discussed, since in most cases they can not be avoided. Below follows a description of the stressors that have been studied in this thesis.

Handling

In the first few days after birth, most piglets are handled. This happens in order to weigh them, to apply identification marks (tattoos or eartags), to administer iron and for male piglets to be castrated. Animals are usually handled in order to move them to the weaner pens, and later to the fattening units. Finally, pigs are handled when driven onto the lorry for transport to the slaughter house.

Handling by humans is stressful as was shown in several studies. During neonatal handling, cerebral blood flow increased and a higher adrenal activity was found (Stanton et al., 1972; Wootton et al., 1982). In older pigs it was shown that handling increased heart rate and salivary cortisol levels. (Cook et al., 1998; Villé et al., 1993). Handling evokes a behavioural reaction that suggests fear in pigs that are unacquainted with humans. Only after repeated (friendly) handling, these responses disappear (Rushen et al., 2001).

Long-term effects of neonatal handling have been studied in detail in rats and mice. Handled rats and mice showed differences in physiology and behaviour compared to animals that were not handled, ranging from differences in HPA axis activity and behavioural stress responses to differences in body fat distribution and decreased sexual behaviour (e.g. (Beane et al., 2002; d'Amore et al., 1995; Durand et al., 1998; Gomes et al., 1999; Padoin et al., 2001; Sternberg and Ridgway, 2003; Young, 2000).

Also in pigs, several long-term effects of neonatal handling have been reported. Neonatally handled boars showed higher plasma ACTH levels, higher locomotor scores in an open field test and a lower body weight until 7 months of age (Weaver et al., 2000). Handling at a later age has been studied especially with respect to effects of unfriendly handling. Aversive handling can change the pig's behaviour but also production parameters can be negatively influenced (Hemsworth and Barnett, 1991; Hemsworth et al., 1981; Paterson and Pearce, 1992). Maybe it is best to handle pigs as little as possible, but if pigs have to be handled, negative encounters should be avoided, because of animal welfare as well as for economical benefits.

Cross-fostering

Cross-fostering of piglets is common practice in current pig husbandry. If a sow has more piglets than (functional) teats, cross-fostering is necessary for the survival of the piglet surplus. However, cross-fostering is also used to standardise litter size or piglet weight within litters. Because sows accept young piglets shortly after parturition as their own based on association with the nest, it is relatively easy to cross-foster piglets for several days postpartum (Gonyou and Stookey, 1987). Piglets show a behavioural response indicating distress, and also show an autonomous and hormonal response to the handling (and maybe to the moving), which is part of the cross-fostering procedure. This can be interpreted as a stress response (Stanton et al., 1972; Wootton et al., 1982). Negative effects of cross-fostering such as growth retardation, disruption of the teat order and fighting occur when piglets are cross-fostered at >4 days of age (Giroux et al., 2000; Horrell and Bennett, 1981; Milligan et al., 2001; Robert and Martineau, 2001). Therefore, it is assumed that as long as cross-fostering is done < 4 days after birth, no disruption of the sow-piglet bond occurs and no major stress response is evoked. However, long-term effects of the procedure (including handling) cannot be excluded.

Weaning

When domestic pigs are kept in a semi-natural environment, suckling bouts decrease gradually during lactation, and sows finally wean their piglets at 90-120 days of age (Gonyou and Stookey, 1987; Jensen and Recén, 1989). The weaning process of piglets in the current husbandry systems consists of being separated from the mother, replacement of milk by dry feeds and, on many farms, being moved to a new environment. Weaning is an abrupt event and the mean weaning age is 26-28 days (Anonymous, 2003). This early and abrupt weaning causes considerable distress in the piglets, showed by high cortisol levels and a higher level of high vocalisations after weaning. Also belly-nosing, aggression and decreased (solid) feed intake have been observed after weaning, (Ekkel et al., 1995; Fraser, 1978; Gonyou and Stookey, 1987; Mason et al., 2003). The most important reason for weaning this early is the economical benefit for the farmer, since early weaning increases the number of piglets per sow per year. Stress responses and negative effects of weaning might decrease if the weaning process would be more gradual. According to the 'optimal foraging theory', at some point in time it will be more beneficial for the piglets to eat solid food than to suckle and they will decide to stop suckling as an optimal foraging decision. The sow can influence this decision by acting so as to increase the cost:benefit ratio for suckling. She does this by actively terminating most sucklings after about 4 weeks of age, thereby inhibiting prolonged final massage of the udder which would increase milk production (Jensen and Recén, 1989).
In some systems, a more natural weaning age is chosen of 6 weeks or more. This is generally considered to be better for the piglets but may cause stress for sows when they cannot escape the constant attention of the piglets or when they lose too much weight as a result of the prolonged lactation (Anonymous, 2001). Furthermore, weaning in those systems is still abrupt. A solution may be a sow-controlled housing system, with get-away pens where sows can leave their piglets at will (Pajor et al., 1999), or 'interrupted suckling', a daily temporal removal of the whole litter (Matte et al., 1992). In these systems, it seems that weaning stress is reduced and welfare, of sow and piglets, enhanced. Unfortunately these systems require more labour and/or are more costly for the farmer.

Moving, mixing and transport

At weaning, piglets are usually mixed and moved to weaner pens, where they stay until they reach a bodyweight of approximately 25 kilograms. At that time (approximately 10 weeks of age) pigs are usually mixed and moved to the fattening pens. Pigs can be fattened on the same farm, but they can also be transported to a specialised fattening farm. Finally, pigs are transported to the slaughter house. Mixing of unfamiliar pigs usually induces vigorous

fighting, and may be followed by less intensive aggression and social instability in the longer term. Besides injuries, animals may suffer from immunosuppression resulting in health problems, and growth retardation (Arey and Franklin, 1995; de Groot et al., 2001; Ekkel, 1997; Ekkel et al., 1995; Fraser, 1983; Petherick and Blackshaw, 1987; Ruis et al., 2000a; Stookey and Gonyou, 1994). Clearly, welfare in these animals is reduced.
When pigs reach slaughter weight, they have to be driven out of their pens and on to the lorry, in which they are mixed and transported to the slaughterhouse. Sometimes they are mixed in the slaughterhouse as well. During this process the pigs are likely to be subject to rough handling, and often electric goads are used to move pigs (Geverink et al., 1996). Mixing and moving of slaughter pigs can also induce serious welfare problems. During transport, travel sickness is observed, especially on longer and rough journeys (Geverink, 1998), and heart rate is increased (Villé et al., 1993). After transport, cortisol levels are elevated (Becker et al., 1985; Geverink et al., 1998; Parrott et al., 1990). When pigs are mixed in lairage, fighting occurs and skin damage is observed. After mixing, elevated cortisol levels are measured. Meat quality can be impaired, either by bruises due to rough handling, or by a higher post mortem pH caused by stress associated with mixing (Geverink, 1998; Parrott et al., 1990). Transport stress can increase bacterial translocation through the gut wall as well as diminish immunological defense mechanisms (Seidler et al., 2001). In most cases, when pigs are slaughtered directly after transport, this will not have serious consequences, but if pigs are transported again (e.g. breeding sows), this is a risk for health and welfare.
In some systems, piglets are not moved after weaning but stay in their home pen from birth until they reach a bodyweight of 25 kilograms, and are moved only once to the fattening pens. We assume this is a better system than the current system of mixing and moving pigs twice, but it will probably disappear because of high costs and new Dutch law. As from 1-1-2008, regrouping will only be allowed once, within 1 week from weaning. Weaned pigs will then probably be housed in large groups (50-100) until they reach a body weight of 25 kilograms. At that time the groups will be divided in smaller groups (5-15) for the fattening pens, since splitting up is allowed (Anonymous, 1994). The welfare consequences of this system are not yet clear. In a farrow-to-finish or 'Specific Stress Free' (SSF) system pigs stay in the same pen from birth until slaughter. In this housing system positive effects were shown on health, welfare and productivity (Ekkel et al., 1995; Scheepens et al., 1990).

COPING WITH STRESS: INDIVIDUAL DIFFERENCES IN STRESS RESPONSES

Coping is the individual response to a stressor by which harmful effects of the stressor are reduced (Levine and Wiener, 1991). In human psychology, the term 'coping' is used for behaviour shown in situations, which lie outside an individual's competency. If a problem cannot be solved, it must be coped with. In animals, the term is used in general for behaviour shown in difficult (challenging) situations (Erhard and Schouten 2001).

Active and reactive response

Animals differ in the way they react to stressors. Henry and Stevens (Henry and Stevens, 1977) suggested two extreme reaction patterns in response to a stressor. This lead to the idea of 'coping strategies' or 'coping styles' to describe individual differences in coping. In animals, the phenomenon has been described first in rodents (Benus et al., 1987) and later in spiders (Riechert, 1993) and great tits (Verbeek et al., 1994). In the coping style concept, active and reactive (or passive) stress responses are recognized. Coping can be attained by removing the stressor or fleeing from it (active coping) or by adjusting to the stressor and

accept it as it is (reactive coping). Active coping attempts resemble the **fight-flight** reaction (Cannon, 1929), which –in social settings- is characterised behaviourally by territorial control and aggression, and neuroendocrinologically by the release of peripheral catecholamines. The latter indicates a high sympathetic nervous adrenal-medullary activity that prepares the organism to react to a threatening situation. Reactive coping resembles a **conservation-withdrawal** response, characterised behaviourally by immobility and low levels of aggression, and neuroendocrinologically by an increase in adrenocortical activity (Engel and Schmale, 1972; Koolhaas et al., 1999; Selye, 1950). Every individual has both active and reactive coping mechanisms at ones disposal, but a predisposition to one or another coping mechanism might be possible (Henry and Stevens, 1977; Hessing, 1994). This predisposition or coping style can be defined as a coherent set of behavioural and physiological stress responses that is consistent over time and characteristic for an individual. It seems that coping styles have been shaped by evolution, and are general reaction patterns in reaction to everyday challenges in the natural habitat (Huether, 1996; Koolhaas et al., 1999). In a stable environment, an active coping style might beneficial, which is characterised by easily developing routines and a rigid type of behaviour (intrinsically driven behaviour), while animals with a reactive coping style might be better equipped to cope with a changing or unpredictable environment, because they are more flexible and react more adequately to environmental stimuli (cue-bound behaviour) (Koolhaas et al., 1999).

The backtest

In pigs, the backtest (Hessing et al., 1993) has been used to test for the predisposition towards active or reactive coping behaviour. In this test, a piglet is taken out of the farrowing pen and put on its back on a surface (e.g. on a table). The piglet is held in this position and during one minute the number of escape attempts (i.e. bouts of wriggling with at least the hind legs) is counted. The number of escape attempts or 'backtest score' ranges from 0 to approximately 10. The average is about 2 or 3, depending on the age of the piglet. In several studies, only the extremes of the population are used, which can be called HR (high responders) and LR (low responders), leaving the intermediate group out of the analyses, or the whole population is arbitrarily divided in two groups (Bolhuis et al., 2003; Hessing, 1994; Ruis, 2001; Schrama et al., 1997). In this chapter, we will refer to high backtest responses as HR and low backtest responses as LR.

Backtest response as a reflection of coping style

Coping style is a concept, which divides stress responses in rough categories and assumes associations of behavioural responses with physiological responses. The concept is not (yet) perfect, and is constantly being reshaped and fine-tuned. To 'measure' coping style with only one test is still being discussed.
If HR animals in the backtest would be predisposed towards active coping behaviour, we would expect physiological stress responses in these animals that are characterised by elevated heart rate, blood pressure and metabolism, and if LR animals would be predisposed towards reactive coping behaviour, they would favour a response characterised by elevated cortisol levels (Moberg, 1985). It was indeed shown that LR animals have higher basal cortisol levels than HR animals (Hessing et al., 1994a), and some studies also show a higher HPA system reactivity for LR pigs, demonstrated by higher cortisol responses to a novel environment test, to routine weighing at 25 weeks of age, to ACTH administration (Ruis et al., 2000b), and to social isolation (Ruis et al., 2001). Other studies showed the opposite, with increased cortisol levels for the HR animals after an open field test (Hessing et al., 1994a) and

after restraint (Geverink et al., 2002b). However, a time-effect could be involved, with HR animals showing a prolonged cortisol response (Geverink et al., 2002a).
HR gilts vocalised more than LR gilts during isolation and during restraint with a nose sling (Geverink et al., 2002b), excreted more noradrenaline in the urine after 3 weeks of isolation, and seemed to have a prolonged high state of stress, demonstrated by the fact that body temperature remained high during 3 weeks after the start of isolation subsets (Ruis et al., 2001). These results suggest an active coping style.
Studies on heart rate in HR and LR pigs showed that HR animals had a higher mean heart rate, which increased substantially (15%) in reaction to a novel object, while LR animals had a lower mean heart rate which increased only slightly (3%) in reaction to the novel object (Hessing et al., 1994a), while another study showed that in response to feeding, no differences in heart rate were found between HR and LR gilts (Geverink et al., 2002a) while in response to a nose sling, heart rate in HR gilts was lower than in LR gilts (Geverink et al., 2002b).
HR and LR animals may differ in the functional activity of the dopaminergic system, with a higher sensitivity to dopamine for the active individuals. In rats, susceptibility to apomorphine, a dopamine agonist, is used to classify individuals as high (APO-SUS) or low (APO-UNSUS) responders (Cools et al., 1990). APO-SUS rats show higher ACTH and corticosterone levels, and higher amounts of B-cells, resembling the LR pigs, while APO-UNSUS rats show lower ACTH and corticosterone levels and higher amounts of T-cells, resembling the HR pigs (Ellenbroek and Cools, 2002). If rats and pigs are indeed comparable with respect to coping styles, HR pigs are expected to be more sensitive to dopamine and show more stress-induced stereotypies. Indeed, apomorphine induced stereotyped behaviour was shown in HR but not in LR animals (Bolhuis et al., 2000a) and in stall-housing systems, HR animals showed more chain-biting than LR animals (Geverink et al., 2003). This is in contrast with research on stereotypies in tethered sows, where it was shown that the sows that resisted most after first tethering, were the ones with the lowest stereotypy levels later on (Schouten and Wiepkema, 1991). Those sows were not backtested, but another study showed good correlations between backtest response and the behavioural response in a restraint test at 8 weeks of age (Bolhuis and Schouten, 2002).
Thus, coping behaviour in the backtest is associated with, or predictive for, stress responses at a later age. However, what the backtest measures exactly, and which tests should be performed to find correlations, is not yet completely clear.

Some researchers doubt the existence of different coping styles in pigs, and criticised the backtest (Forkman et al., 1995; Jensen, 1995). The criticism is based mainly on the fact that coping behaviour in pigs does not show a bimodal distribution. However, this does not mean that coping styles are non-existent. Although the distribution of reactions to the backtest is unimodal, animals at the ends of the scale may have a preference for one or another strategy. Extreme responders to the backtest differ in levels of aggression at a later age, and have different patterns of stress responses in various situations (Hessing et al., 1994a; Ruis, 2001). Furthermore, the backtest was criticised because of the low intra-test correlations. However, there are certain differences in the way they performed the backtest compared to the afore-mentioned studies. Their backtest scores ranged from 0 to approximately 50, with a mean of 15. Probably, every movement of the piglet was counted, instead of every bout of wriggles.

Backtest response and aggression

If active behaviour in the backtest represents a coping style that favours the fight/flight reaction, it would be expected that HR animals show more aggressive behaviour. Indeed it was shown that HR animals in the backtest were also aggressive in a social confrontation test

(Hessing et al., 1993) and showed more aggression in group-feeding competition tests (Ruis et al., 2000b). Mixing of two HR gilts caused the highest levels of stress, indicated by higher urine catecholamine levels and plasma ACTH levels, compared to mixing of a HR and a LR gilt or two LR gilts. Dominance (d) played an important role in the level of aggression. On the day of mixing, aggression subsided less quickly and increases in body temperature were higher in HR/HR and HR(d)/LR pairs (HR gilt dominant), while in the first week after mixing, feed efficiency was lower and skin damage was higher in these pairs. These results point to much aggressiveness and high stress levels. LR(d)/HR pairs (LR gilt dominant) showed much lower levels of stress. It seems that HR gilts become aggressive when dominant, while dominant, LR gilts do not, and that aggression is suppressed in HR subordinates (Ruis et al., 2002).

Backtest response and environmental challenges

If a reactive coping style is more beneficial in a changing environment and reactive copers are more cue-bound (Koolhaas et al., 1999), and if the backtest reflects (a dimension of) this coping style, than we expect LR animals to show more exploratory behaviour. Furthermore, we would expect the LR animals to show a conservation-withdrawal response to novelty. Indeed, LR animals hesitated longer to leave the home pen or to contact a human in a novel environment test (Ruis et al., 2000b) and they explored more (Ruis et al., 2001) than HR animals.

Backtest response and housing conditions

There is an important influence of housing conditions on the effects of coping behaviour in the backtest. In a study comparing group-housing with stall-housing conditions for sows, relations between backtest response and stereotypies, cortisol response and heart rate were found only in stall-housed gilts but not in group-housed gilts (Geverink et al., 2003). In another study, an interaction between backtest response and housing conditions was found for heart rate after applying a nose sling. Group-housed HR gilts showed a lower heart rate immediately after application of the nose sling than stall-housed HR gilts (Geverink et al., 2002b). In an experiment comparing individual and group housed sows, an interaction between backtest behaviour and housing conditions was found for vasopressin and CRH expression. In individually housed HR gilts, vasopressin in the PVN was increased, while in individually housed LR gilts CRH was upregulated. In group housed gilts, no effects were found (Karman et al., 2002). Finally, an interaction between housing conditions and backtest responses have been found for heart rate (Geverink et al., 2002b).
Evidently, housing conditions are extremely important for the animals, and backtest behaviour may interact with housing conditions.

In conclusion, the backtest response seems to reflect one or more dimensions of the coping style of the animal, but since coping responses may be determined by several dimensions of the personality that can change due to learning and experience consequently the predictive value for behavioural and physiological responses is variable.

INFLUENCES OF STRESS COPING BEHAVIOUR ON HEALTH AND PRODUCTION

In human psychology, some associations between behavioural coping styles and health have been found. For example, avoidance coping was found to be associated with physical illness

(Nowack, 1989) and instrumental mastery-oriented coping (i.e. active problem solving) was found to be related to positive health (Olff et al., 1993). An internal locus of control (i.e. intrinsically driven, believing one is in control of what happens) was found to be associated with more effective coping strategies in contrast to an external locus of control (i.e. reactive to others) (Holahan and Moos, 1987). From these results, it seems that active coping strategies would be healthier than reactive coping strategies. However, if a reactive coping style would be unhealthy, evolution would have eliminated it long ago (Huether, 1996). A problem in studying relations between coping style and health in humans is that cross-sectional studies do not reveal any causal relations. Did the coping style cause the ill health, or did the ill health cause the coping style? Even in longitudinal studies, the coping style might be determined or influenced by previous, unrecorded experiences.

Immune responses

If LR animals show higher cortisol levels and higher cortisol responses to stress, this could suppress cell-mediated immune responses and shift the T_{H1}/T_{H2} balance of the immune system towards T_{H2} (humoral responses). Indeed, several studies showed that HR pigs had a higher in vitro and in vivo cell-mediated immunity, while humoral immunity was highest in the LR pigs (Bolhuis et al., 1999; Hessing et al., 1995; Schrama et al., 1997). In a study where a PHA skin test was performed repeatedly, HR showed more skin swelling than LR 10, 17 and 22 days after weaning. However, 3 days after weaning LR animals showed more skin swelling than HR animals. Before weaning, no differences were found between HR and LR. It seems that weaning stress suppressed cell-mediated immunity in HR but not in LR animals (Hessing et al., 1995). This would suggest an advantage for the LR animals under stressful conditions.

Immune responses and housing conditions

Relations between backtest behaviour and immune responses are sometimes influenced by the housing conditions of the animals in the study. In a study where pigs were housed on bare concrete (‘poor’ housing conditions) or on straw (‘rich’ housing conditions), LR animals on concrete showed a higher cell-mediated immune responsiveness than LR pigs housed on straw, while for HR pigs, opposite effects of rearing conditions were found (Bolhuis et al., 1999). Humoral immune response was influenced by backtest*rearing conditions: Relations between backtest response and humoral immune response were found in pigs housed under ‘poor’ conditions, but not in pigs housed on straw (Bolhuis et al., 2003; Bolhuis et al., 2000b). Housing conditions should therefore always be taken into account, since interactions with backtest responses may occur.

Production parameters

HR animals are expected to be more aggressive (Hessing et al., 1993; Koolhaas et al., 1999). This aggressiveness could be effective in the suckling period, where a teat must be conquered, but also in the rearing and fattening period, where 10 pigs share one feeding trough, although feed is supplied ad lib. In a study comparing HR and LR piglets during the suckling period, the HR animals were indeed predominant at the anterior, presumably most productive teats, and the HR piglets gained more weight in the suckling period (Ruis et al., 2000b). However, HR animals are not automatically the dominant ones when older pigs are mixed. An HR gilt has the same chance of becoming the dominant one of a pair as a LR gilt (Ruis et al., 2002). This is understandable, because dominance is the result of personality and physical traits (Buss, 1988). The pig that combines the right amount of aggression at the right time with enough body weight, strength and fighting skills, will become dominant.

In fattening pigs, we would expect the HR animals to lose more energy with activity. Depending on the feeding system, this would result in a leaner carcass at slaughter (when feeding was ad lib) or a lower daily weight gain (when feeding was restricted). Following this hypothesis, ad lib fed groups with HR animals only are expected to show better production results than groups with LR animals only, and mixed groups (HR/LR) to show intermediate results. However, in a study where pigs were regrouped at 9 weeks of age according to their backtest results, pigs in mixed pens performed better than pigs in pens with HR or LR pigs only. Average daily weight gain was higher, carcass weight and lean meat percentage were higher and carcass classification was better in the mixed pens. The prevalence of pleuritis was lower in the mixed pens, but the prevalence of stomach wall damage was highest in the pens with LR pigs only. Haemorrhages on the heart muscle did not differ between pens (Hessing et al., 1994b). This suggests that mixed groups might be more stable, allowing the best performance results. The stomach lesions in the LR groups might be due to stronger stress responses to mixing and higher cortisol levels in these animals. In another study, the HR pigs showed more haemorrhages in the heart muscle than the LR animals (Hessing et al., 1994a).

A certain coping style can never be only negative for an animal's health or production capacity, because that type of coping style would have been eliminated already in the course of evolution or in the artificial selection process. Each coping style has certain advantages and disadvantages, depending on the environment.

AIM AND OUTLINE OF THE STUDY

Before we started this study, we assumed that the backtest was a reasonably well validated instrument that 'measured' coping style in, according to some, a bimodal distributed population. During the study this view changed drastically. The backtest was not thoroughly validated and we were not sure which aspects of the coping style of an animal are reflected by the backtest response. The distribution of backtest responses in the population is not bimodal, as may have been assumed from Hessing's study (Hessing 1994). Hessing suggested a bimodal distribution of coping styles, but never found a bimodal distribution of backtest responses. Furthermore, in the paragraphs above, many results are mentioned that were published after the start of our experiments. Only some of these results we could take into account during the study. For example, Ruis's thesis was published in 2001, 3 years after the start of our study. It was too late for us to use his results on the interaction between dominance and backtest responses, and we did not record the dominance hierarchy in the groups. Also the studies of Geverink and of Bolhuis on backtest responses were published after we started our experiments.
Several relations have been found between coping behaviour in the backtest and behaviour in other (stressful) situations, physiological and immunological parameters and performance of pigs, and it has been shown that rearing or housing conditions play an important role in determining the final outcome (i.e. coping strategy used and consequences for welfare and production). A high response in the backtest (HR) seems to reflect an active coping style, with stress responses resembling Cannon's fight-flight response, and a low response in the backtest (LR) seems to reflect a reactive coping style resembling Selye's conservation-withdrawal response. The backtest has not been thoroughly validated and standardised, and influences of sow- and piglet parameters as well as some environmental influences should be determined. Several aspects of the relations between coping behaviour and immune parameters after stress have not been investigated, and relations with susceptibility for infectious diseases have not been studied yet. Relations between coping behaviour and production results in fattening pigs

have only been studied in groups consisting of pigs homogenous for backtest response (either HR or LR) or mixed groups (HR/LR) that were formed when the animals were 9 weeks of age, ignoring intermediate animals.

In this thesis, we further validate the backtest, and investigate if backtest results reflect (a dimension of) individual coping styles. Relations between stress coping behaviour in the backtest and immune parameters and disease susceptibility are studied further. Production parameters were investigated in groups of pigs that were formed at 3 days of age on the basis of backtest behaviour, and in fattening pigs that were not regrouped. All pigs, also the ones with intermediate backtest results, were used in the study. Research questions were:
-is the backtest a suitable instrument? (chapters 2, 5)
-what do we measure with the backtest? (chapter 4)
-can we use the backtest to predict behavioural and physiological responses in other stressful situations? (chapter 3)
-can we find relations between backtest response and health and production parameters? (chapters 5, 6, 7)
-could the backtest be of use in practical pig husbandry? (chapters 2-8)

In **chapter 2**, a validation experiment of the backtest is reported. 184 piglets were tested at 3, 10 and 17 days of age in a standardised backtest, and time of testing and test order were recorded. We expected no influence of time of day, since we assume no clear diurnal rhythm in such young piglets, but we expected animals to be influenced by test order, because they could see and hear other piglets during the backtests. Intra-test correlations were calculated.
In **chapter 3**, 315 piglets were backtested at 3, 10 and 17 days of age. At 3 days of age, groups were formed of HR pigs only, or LR pigs only, and mixed pens (MISC) and original litters were used as controls (OR). At 5-7 weeks (after weaning) and at 10-12 weeks (after mixing and moving to the fattening pens), 3 different behavioural group tests were performed in the home pen. In the human approach test (HAT), latency to approach a person was scored; in the novel object test (NOT), latency to approach an unfamiliar red bucket was scored, and in the open door test (ODT), latency to leave the home pen was scored. If the HR backtest response reflects an active coping style, we expected the HR animals to show shorter latencies in contacting the person and the novel object and in leaving the home pen compared to the LR animals, who we expected to show longer latencies.
In **chapter 4**, 814 pigs were backtested and HR, LR, MISC and OR groups were formed as described before. Cortisol responses to weaning and to mixing and moving were assessed, and influences of group composition on backtest behaviour was determined. We expected the LR animals to show higher basal levels of cortisol and stronger cortisol responses to weaning and mixing (reactive coping response) than the HR (active copers) animals.
In **chapter 5**, 566 piglets were backtested at 3, 10 and 17 days of age and influences of several parameters such as gender of the piglet, sow health and sow behaviour on the backtest were determined. 823 piglets were backtested at 10 and 17 days of age and in both datasets, relations with production parameters such as daily weight gain and lean meat percentage at slaughter were determined. We expected some maternal influences on backtest behaviour, since behaviour is the result of genetics and rearing, and we expected a gender difference since in general male animals show more aggressive behaviour than females, and aggression and backtest behaviour might be linked. We expected HR animals to lose more energy due to higher activity levels, which might be compensated by eating more food (ad lib feeding). Therefore differences in daily weight gain or in carcass composition were expected between HR and LR animals.

In **chapter 6**, 812 pigs were backtested at 3, 10 and 17 days of age and production parameters such as daily weight gain, lean meat percentage and backfat were recorded. Differences in daily weight gain or carcass composition were expected, as is explained above.
In **chapter 7**, 173 piglets were backtested at 3, 10 and 17 days of age. Cell-mediated immunity was measured in vivo to PHA at 4 and 10 weeks of age, antibody responses were measured to KLH and ADV at 10-14 weeks of age, and susceptibility to salmonella and influenza (H_1N_1 and H_3N_2) was measured at 6 months of age. Cortisol responses to weaning at 4 weeks of age and to mixing at 10 weeks of age were determined. If LR animals are predisposed towards a reactive coping style, they would show higher cortisol responses, suppressing cell-mediated immune responses and shifting the T_{H1}/T_{H2} balance in the immune system towards T_{H2} (humoral responses). Thus it was expected that HR animals would show better cell-mediated immune responses and that LR animals would show better humoral immune responses.
In **chapter 8**, findings are integrated and discussed.

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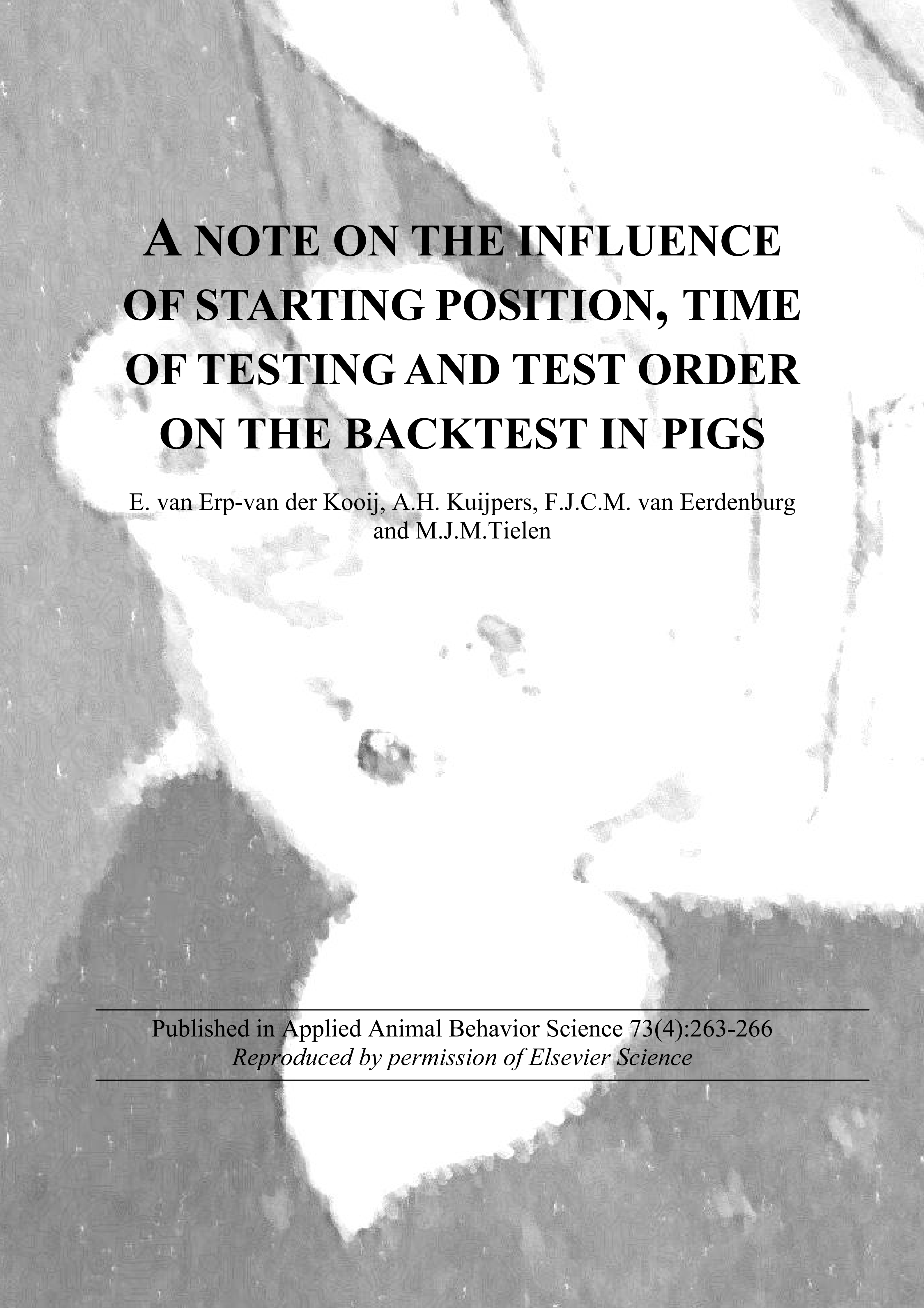

A NOTE ON THE INFLUENCE OF STARTING POSITION, TIME OF TESTING AND TEST ORDER ON THE BACKTEST IN PIGS

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ABSTRACT

The backtest determines the coping behaviour of a piglet in a standardized stress situation, which might be a measure for the coping style of that animal. Backtest results are related to other parameters such as immune responses and production. In this study, the backtest was standardised and it was studied if time of testing or the order in which animals were tested influenced backtest results. 184 piglets from 18 sows were tested at 3, 10 and 17 days of age. Before testing, the sow was disturbed to ensure that all piglets were awake. This standardisation of the starting position before testing did not improve the correlations between backtest results in the successive tests. No relations were found between backtest results and time of testing or test order.

INTRODUCTION

The backtest determines the coping behaviour of a piglet in a standardized stress situation. This coping behaviour might be a measure for the coping style of that animal. Relations have been found between backtest behaviour and immunological and production parameters (Hessing et al., 1995; Hessing et al., 1994; Schrama et al., 1997; van Erp-van der Kooij et al., 2000). Others criticised the backtest (Jensen, 1995).

In former studies (van Erp-van der Kooij et al., 2000), the starting position of the piglets before performing the backtest was not standardised. This could lower the correlation between the successive backtests. The backtest might have provoked a different response in the same pigs when sleeping or suckling before the test on one day, and awake and walking around before the test on another day. In earlier experiments, time of testing was random and the animal that was caught first, was tested first; this animal may react differently to the test than the animal that was caught and tested last. Also the influence of time of testing has not been investigated yet.

MATERIAL AND METHODS

184 piglets bred from 18 NL*GY sows were used. Male piglets were castrated at approximately 5 days of age. Sow and piglets were housed in conventional farrowing pens (1.80*2.40 m), the sow was restricted in a farrowing crate. A backtest according to Hessing (Hessing et al., 1993) was performed at 3, 10 and 17 days of age. In the backtest, the piglet was put on its back and held in this supine position for 1 minute. One hand was placed loosely over the head of the piglet, the other on the hind legs. Each series of wriggles of the piglet was counted as one escape attempt. The total number of escape attempts was called 'backtest score'. Piglets were tested when they were fully awake[1)] and not suckling. If the piglets were sleeping, the experimenter woke them up before testing either by stepping in the pen or by disturbing the sow. If the piglets were suckling, the tests were postponed and testing started just after the last piglet stopped suckling. During the tests, the sow remained in the farrowing crate next to the experimentor. The order in which animals were tested was recorded, and the starting time of each backtest was recorded in 15-minute periods. Times of testing were chosen randomly between 8.30 a.m. and 3.30 p.m. Sow number, litter size and gender were recorded.

[1] 'awake' was defined as 'not lying down'

Statistical analysis

Correlations between the successive backtests were calculated (Pearson correlation test, SPSS, 1999). To study the influence of order in which the piglets were tested (test order) and time of testing, corrected for sow number (random effect), the following model was used:

$$\mathbf{y_{ijklm} = test\ order_i + gender_j + litter\ size_k + time\ of\ testing_l + test\ day_m + \varepsilon_{ijklm}}$$

in which y is the logarythm of the backtest score (backtest results were not normally distributed). The subject was the piglet, and test day was the repeated factor. An unstructured covariance matrix was used (proc mixed, SAS) (SAS_Institute_Inc., 1989). To compare test order of the piglets in the successive backtests, a Spearman correlation test was used (SPSS, 1999).

RESULTS

Backtest results and correlations between successive backtests are presented in Table 1. No relation was found between time of testing or test order and backtest results (Table 2). There was a significant effect of test day (P<0,0005), due to a higher level of escape attempts at day 3, compared to day 10 and 17. A relation was found between successive test orders of the animals on day 10 and day 17 (r=0.18, p<0.05).

Table 1: *Backtest results and correlations between successive backtest scores*

	MEAN NUMBER OF ESCAPE ATTEMPTS	SD	RANGE	N	BACKTEST PAIR	PEARSON CORRELATION	P-VALUE
day 3	3,70	1,80	0-9	184	day 3-day10	0,30	<0,01
day 10	3,08	1,79	0-9	177	day 10-day17	0,40	<0,01
day 17	2,95	1,92	0-9	174	day 3-day17	0,31	<0,01

Table 2: *Relation between backtest results and gender, time of testing, test order and litter size, corrected for test day (repeated) and sow number (random).*

FIXED EFFECT	NDF	F-VALUE	PR > F
gender	1	0.01	0,94
time of testing	1	0.33	0,57
test order	1	0.09	0,76
litter size	1	0.01	0,91
test day	2	12.63	0.0001

DISCUSSION AND CONCLUSIONS

In earlier research (van Erp-van der Kooij et al., 2000), correlations between successive backtest scores (in the same animals) were 0,42 (n=566) and 0,47 (n=823). This is similar to the results of the present study, where r=0,40. Correlations between successive backtests in this study, with only animals tested that were fully awake, were not higher than in earlier experiments where piglets could be sleeping just before testing. The wake up procedure therefore seems unnecessary. Although the piglets were still suckling, it is possible that they already showed a diurnal activity rhythm, based on daylight or on feeding times of the sow (Schrenk and Marx, 1982). However, we found no relation between time of testing and backtest results. The order in which animals were tested had no relation with backtest results. One could imagine that seeing and hearing the testing of littermates would influence backtest results, but we found no indications to support this theory. This implies that the order in which animals are tested and time of day are irrelevant in future analyses. The effect of test day was also found in an earlier study (van Erp-van der Kooij et al., 2000), p.181) and discussed there. There was a relation between the order in which animals were caught at day 10 and day 17 (but not day 3). Certain animals may have been caught sooner due to (subclinical) illness or leg problems, or due to being less frightened of humans. This phenomenon was unrelated to backtest scores. A gender effect was not found, probably due to the small number of animals.

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CAN WE PREDICT BEHAVIOUR IN PIGS? - SEARCHING FOR CONSISTENCY IN BEHAVIOUR OVER TIME AND ACROSS SITUATIONS

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ABSTRACT

Individual differences in animal behaviour could elucidate the differences in stress coping style, which have consequences for production, health and welfare. Therefore, individual behavioural differences in pigs and consistency of responses in different test situations were studied. If differences in behaviour reflect coping characteristics, then behaviour in one situation should predict behavioural reactions in other situations and at other times.

In this study, a backtest was performed on 315 Great Yorkshire * Dutch Landrace piglets at 3, 10 and 17 days of age. On day three, groups of approximately 10 piglets per sow were formed, based on escape behaviour in the first backtest (backtest score): HR (High Resisting, all scores>3), LR (Low Resisting, all scores <3), MISC (Miscellaneous, various scores between 0-10) or OR (Original litters) to determine if group composition would influence coping behaviour. In week 5-7 and/or in week 10-12 a human approach test (HAT), a novel object test (NOT), and an open door test (ODT) were performed with all pigs simultaneously, in the home pen. Pearson correlation coefficients were calculated between the test results and a factor analysis was performed. Furthermore, data were analysed on pen level, and within MISC and OR-pens on animal level, using multivariate linear models.

Significant correlations were found between the backtests and between HAT, NOT and ODT. Backtest results on three ages loaded on the same factor, and HAT, NOT and ODT at one age also loaded at one factor. No differences were found in HAT, NOT and ODT for the different pens (HR, LR, MISC and OR). On animal level, animals with higher backtest scores also had higher HAT scores at 5-7 weeks ($P<0.05$) within the MISC-pens. At 10-12 weeks, no differences were found.

This study suggests that there are consistencies in behaviour of pigs over time and across situations, so coping can be regarded as a trait variable. However, since correlations are well below one, we suggest that other factors such as time (development) and (test) situation may also play an important role in determining an individual's behavioural reaction. The absence of correlations between backtest and the group tests is explained by the theory that these different tests measure different aspects of the coping style.

INTRODUCTION

Over the past two decades, coping has played an important role in human psychology and an increasing role in animal ethology. Coping in humans is viewed as a moderator of the relationship between stress and outcomes such as mood and illness (Schwartz et al., 1999). In animal life, individuals encounter many stressors. Coping with these stressors affects health, welfare and production in the animals. The study of individual differences in adaptive behaviour could elucidate the differences between animals. For production animals, this could either be the first step of selecting animals that are better adapted to their artificial environment, or of selecting a better environment (housing system) for the animals. Welfare of the animal is thereby enhanced (Thodberg et al., 1999).

Change is an essential property of development, yet some element of stability or consistency is necessary for the maintenance of individual distinctiveness (Lyons et al., 1988). Individual responses of animals may be the result of ontogenetic and learning processes, but they are at least partly determined by genetic factors (Benus et al., 1991).

In human behaviour literature, coping is sometimes regarded as a situation-specific variable, while in other studies coping is assessed as a trait-like phenomenon or trait variable (Schwartz et al., 1999). In animals, coping is mostly treated as a trait variable. In this view it is assumed that individuals are relatively consistent in their response to an environmental challenge at

different times (ages) and in different situations (Lawrence et al., 1991; van Erp-van der Kooij et al., 2000). These relatively stable individual characteristics that show some consistency over time and across situations are also referred to as temperament. Temperament represents an individual's basic stance toward continuing changes and challenges in its environment (Lyons et al., 1988).
In certain cases coping behaviour turns out to be situation-specific, which is seen as proof of the non-existence of coping styles (Forkman et al., 1995; Jensen, 1995; Spoolder et al., 1996). However, it is possible that different tests measure different aspects of the coping 'style' of the animal, or different dimensions in the personality, as Gosling et al (Gosling and John, 1999) phrase it. In that case, the coping style (or personality, or temperament) of the animal might be stable, while no correlation would be found between different behavioural tests.

The objective of this study was to investigate individual differences in behavioural responsiveness in pigs. Especially the consistency of behavioural responses in different test situations was explored. If individual differences in behaviour are a reflection of stable coping characteristics, then behaviour in one situation can be used to predict behaviour at other times or in other situations. To determine the importance of cross-fostering and group composition for the outcome of behavioural tests, different groups were created, depending on backtest scores. If the mean outcome of the other behavioural tests would be related to the group composition, the coping style would be a very stable trait and the mixing procedure would not have influenced it. Furthermore, all different tests would have measured the same dimension. If no relation would be found, than there would be several possibilities. 1. Coping might not be a stable trait. This is contradicted by the above-mentioned studies. 2. The mixing procedure might have influenced the coping style, causing a shift in backtest response. This implies that on an individual level, relations between tests could still be found. 3. The different behavioural tests might measure different dimensions of the coping style of the animal. No relations would be found then, either on group or on individual animal level.

MATERIALS AND METHODS

Housing and animals

The study was performed in farrowing and fattening compartments at the farm 'The Tolakker' of the University of Utrecht, The Netherlands. 315 fattening pigs, born from Dutch Landrace * Great Yorkshire sows, were used. Conventional farrowing crates were used. A heating lamp and floor heating were used when necessary. As of the second week, creep feeding was available for the piglets. An all in – all out system was used consequently. Male pigs were castrated at approximately 5 days of age and tails of all pigs were docked, according to standard Dutch procedures. Iron was given to the piglets by injection at 3 days of age. At weaning, around 28 days of age, the sow was removed from the pen and the piglets remained in the same pen. At 9 weeks of age, the fattening period started. At that time, 188 piglets were neither mixed nor moved but stayed in the same pens until slaughter. This is a specific-stress-free housing system (Ekkel et al., 1995; Scheepens et al., 1990). The other pigs were mixed and moved to fattening units, and were not further used in this study. All pens had a partly slatted, concrete floor with floor-heating. Feed and water were supplied *ad libitum*. Pigs were slaughtered at approximately 110 kilograms liveweight (around 6 months of age).

Backtest and mixing procedure

A backtest was performed on all 315 piglets as described by Hessing et al. (Hessing et al., 1993). In this backtest, each piglet was put on its back and restrained in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. Each series of wriggles that the piglet made without a pause was counted as one escape attempt. The total number of escape attempts is called ‘backtest score’. A backtest was performed with every animal at 3, 10 and 17 days of age[1] (Bt3, Bt10, Bt17, respectively).
On day three, different groups of approximately 10 piglets per sow were formed on the basis of the first backtest. These groups were called HR (high resisting), LR (low resisting), MISC (miscellaneous) and OR (original) as controls. In HR-pens only piglets with high backtest scores (>3) were placed, in LR-pens piglets with low scores (<3) were placed, MISC- pens consisted of high, intermediate and low scores (mean backtest score appr. 3) and OR-pens consisted of littermates only, independent of backtest score. The goal of this procedure was to determine whether test results would be influenced by group composition.

Behavioural tests within the group

All piglets were handled in the third and fourth week of the suckling period (just before weaning). The handling procedure consisted of a person sitting in the pen for approximately 10 minutes. The person talked now and then during this time, and moved his or her hands towards the piglets. This procedure was repeated on three or four consecutive days, until all piglets in the pen were considered to be used to humans (they came freely to the person, and did not startle when the person made a sound or moved).
A human approach test (HAT), a novel object test (NOT), and an open door test (ODT) were performed in the home pen with 221 pigs at 5-7 weeks of age (1-3 weeks after weaning) in 6 HR-pens, 10 MISC-pens, 4 LR-pens and 4 OR-pens and with 188 pigs at 10-12 weeks in 5 HR-pens, 8 MISC-pens, 5 LR-pens and 4 OR-pens. 94 animals were tested twice, both at 5-7 weeks and at 10-12 weeks, in 3 HR-pens, 4 MISC-pens, 1 LR-pen and 3 OR-pens.
The three group tests were always performed on the same day and in the same order (HAT-NOT-ODT). In the human approach test (HAT), a person walked into the pen and stood still in the middle. In the novel object test (NOT), a bright red bucket was put in the middle of the pen. It was recorded how quickly animals made snout contact with the person or the bucket. In the open door test (ODT), the door of the pen was opened and it was recorded how fast the pigs left their home pen and walked into the corridor of the compartment (Fig.1). Animals that touched the person or the bucket within 30 seconds received 10 points, within the next 30 seconds 9 points, and so on, until after 5 minutes the test was ended. Animals that left the pen within 30 seconds received 10 points, within the next 30 seconds 9 points, and so on, until after 5 minutes the test was ended. Pigs who did not make snout contact with the person or the bucket, or did not leave the pen within 5 minutes, received 0 points. We chose these tests because they were practical and relatively easy to perform with many animals. The experimental design[2] is in Table 1.

Table 1: *Experimental design*

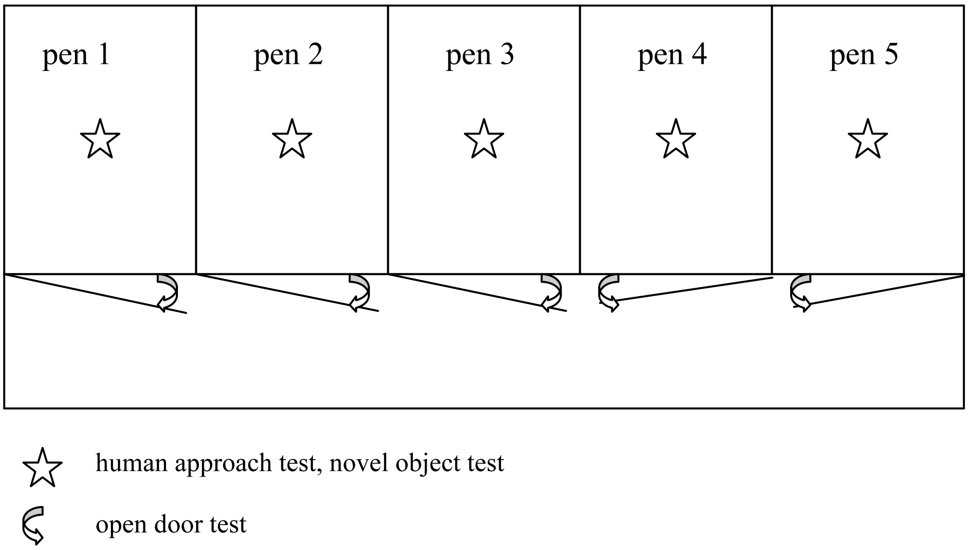
WEEK	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	→

[1] plus or minus 1 day
[2] most animals were subject of only one of the two test series, either at 5-7 weeks or at 10-12 weeks, see also Table 1

	B	Bt Cr	Bt	Bt	W	HAT1, NOT1, ODT1		F	HAT2, NOT2, ODT2			

B=birth; Bt=backtest; Cr=cross-fostering (formation of HR, LR, MISC and OR pens);
W=weaning; HAT=human approach test; NOT=novel object test; ODT=open door test;
F=start of fattening period

Figure 1: *Spatial arrangement of the human approach test (HAT), the novel object test (NOT) and the open door test (ODT).*



Statistical analysis

Pearson correlation coefficients were calculated for all variables (raw data).

A factor analysis was performed on all behavioural data, irrespective of experimental treatment (group composition), with a Harris-Kaiser Oblique rotation method. This was done to search for underlying structures in the variation of the behaviour. With an oblique rotation method, the factors that emerge are correlated. First a factor analysis was performed on all behavioural variables, second two separate analyses were performed for behavioural tests at each test age (5-7 weeks or 10-12 weeks) combined with backtest results, because not all animals were tested at both ages.

On pen level, relations between group (HR, LR, MISC or OR, based on backtest scores) and the results of the group tests (HAT, NOT and ODT) were calculated, corrected for compartment number, by using multivariate linear modelling (PROC GLM in SAS) (SAS_Institute_Inc., 1989).
On animal level, relations between mean backtest scores per animal, calculated as (Bt3+Bt10+Bt17)/3, and the results of the other behavioural tests were calculated within each group, corrected for compartment number and mixing procedure.
Results are considered statistically significant when P-values are below 0.05; P-values between 0.05 and 0.10 are called trends.

RESULTS

Mean backtest results were found of 3,18 ± 1,76 on day 3 (range 0-8), 2,80 ± 1,81 on day 10 (range 0-11) and 2,89 ± 1,92 on day 17 (range 0-11). Correlations between backtests on different test days were 0.27 (between day3 and day10, P<0.0001), 0.31 (between day 3 and day 17, P<0.0001) and 0.48 (between day 10 and day 17, P<0.0001). Mean scores for the group tests at the two sampling ages are in Table 2, and correlations between these tests at both ages are in Table 3.

On pen level, first we compared the pens where piglets were cross-fostered (HR, MISC and LR) with pens where no interventions were done (OR). At 5-7 weeks, no differences in mean results for the group tests were found. At 10-12 weeks, LSMeans of the novel object test and the open door test were higher for the cross-fostered pens than for the original litters (NOT 9.1 vs 7.6, P=0.026; ODT 7.9 vs 6.4, p=0.022).

Table 2: *Mean scores ± sd for the human approach test (HAT), novel object test (NOT), and open door test (ODT).*

	HAT	**NOT**	**ODT**
5-7 WEEKS	5.77 ± 3.98	6.94 ± 3.78	5.84 ± 3.66
10-12 WEEKS	8.35 ± 3.13	8.85 ± 2.71	7.65 ± 2.85

Table 3: *Correlations between behavioural tests: HAT, NOT and ODT. N ranges from 95 to 221. Significant correlations are bold.* **P<0.05,* $^{\#}$*P<0.10*

	HAT1	**NOT1**	**ODT1**	**HAT2**	**NOT2**	**ODT2**
HAT1		**0.44***	**0.24***	0.16	0.12	**0.28***
NOT1			**0.40***	0.17$^{\#}$	**0.26***	0.18$^{\#}$
ODT1				0.14	0.19$^{\#}$	**0.24***
HAT2					**0.35***	**0.18***
NOT2						**0.31***
ODT2						

Scores in the group tests were not affected by group composition. LSMeans are in Table 4. Correlations between backtest results and group tests within the MISC and OR group were also calculated on animal level. This was not done for the HR and LR groups, because in those groups the range of backtest scores on day 3 was too small.
On animal level, there was a significant pen-effect for almost all test results at 5-7 weeks and at 10-12 weeks. At 5-7 weeks, in the MISC group, animals that scored high in the backtest also had high scores for the human approach test (estimate=0.79, F=9.49 (DF=1), P=0.0028). In the original (OR) group, only a trend was found, with animals that scored high in the backtest scoring low in the novel object test (estimate –0.80, F=3.50 (DF=1), P=0.07). At 10-12 weeks, no effect of backtest score was found on any of the other behavioural tests.

Table 4: *Results (lsmeans and SEM) for the human approach test (HAT), novel object test (NOT), and open door test (ODT) per group, corrected for animal compartment. Test results do not differ between groups HR, MISC, LR and OR.*

5-7 WEEKS	**HAT1**	**NOT1**	**ODT1**
HR	6.3	7.9	7.0
MISC	5.8	7.3	5.8
LR	6.6	6.7	5.9
OR	7.3	6.9	4.5
POOLED SEM	1.28	0.81	1.15
F-VALUE (DF=3)	0.24	0.46	0.81
10-12 WEEKS	**HAT2**	**NOT2**	**ODT2**
HR	9.3	8.7	8.5
MISC	7.4	9.2	7.4
LR	8.8	9.4	7.8
OR	8.3	7.7	6.8
POOLED SEM	0.67	0.52	0.63
F-VALUE (DF=3)	1.80	2.01	1.33

Animals that were tested twice (at 5-7 weeks and at 10-12 weeks), scored higher in the open door test at 10-12 weeks than animals that were tested once at 10-12 weeks. For the human approach test, the same trend was found. In the novel object test, no difference was found (Table 5).

Table 5: *Results (lsmeans and SEM) for the behavioural tests at 10-12 weeks (HAT2, NOT2 and ODT2) for animals who experienced the tests for the first time, and for animals who had been tested at 5-7 weeks as well. The model was corrected for animal compartment and pen number.*

	HAT2	**NOT2**	**ODT2**
TESTED ONCE AT 10-12 WEEKS	7.8	8.8	7.0
TESTED TWICE, AT 5-7 AND AT 10-12 WEEKS	8.9	8.9	8.3
POOLED SEM	0.32	0.28	0.28
DIFFERENCE BETWEEN ANIMALS TESTED ONCE AND TESTED TWICE	P=0.011 (F=6.46, DF=1)	P=0.77 (F=0.08, DF=1)	P=0.0013 (F=10.70, DF=1)

Two factor analyses were performed. In the first analysis, all behavioural data were used and three factors were found. Backtest scores loaded on factor 1 and 2, the group tests at 10-12 weeks loaded only on factor 3. Of the tests performed at 5-7 weeks, the open door test loaded on factor 1, the human approach test on factor 2 and the novel object test on factor 3. Secondly, separate factor analyses were performed for the variables Bt3, Bt10, Bt17 and HAT1, NOT1 and ODT1 (at 5-7 weeks) and for the variables Bt3, Bt10, Bt17 and HAT2, NOT2 and ODT2 (at 10-12 weeks). For both ages, two factors were found, with the backtest results loading on factor 1 and the group tests loading on factor 2. The factor loadings of the original variables are in Table 6.

Table 6: *Results from the factor analyses. 6**a**: all variables, n=93; 6**b**, Bt3, Bt10, Bt17 and tests at 5-7 weeks, n=217; 6**c**, Bt3, Bt10 and Bt17 and tests at 10-12 weeks, n=186).*

6a: all variables

Variables	Factor 1	Factor 2	Factor 3
Bt3	0.44	-0.55	0.32
Bt10	0.85	0.40	-0.03
Bt17	0.74	-0.11	0.17
HAT1	0.20	0.85	0.02
NOT1	-0.16	0.37	0.46
ODT1	-0.60	0.20	0.34
HAT2	0.04	-0.12	0.80
NOT2	0.04	-0.02	0.83
ODT2	-0.06	0.10	0.65
Eigenvalue	2.41	1.93	1.16
Variance explained	27%	21%	13%

6b: backtest and tests at 5-7 weeks

Variables	Factor 1	Factor 2
Bt3	0.45	0.39
Bt10	0.83	-0.01
Bt17	0.83	-0.06
HAT1	0.09	0.68
NOT1	0.07	0.82
ODT1	-0.31	0.74
Eigenvalue	1.92	1.62
Variance explained	32%	27%

6c: backtest and tests at 10-12 weeks

Variables	Factor 1	Factor 2
Bt3	0.71	-0.03
Bt10	0.78	-0.06
Bt17	0.80	0.05
HAT2	0.03	0.69
NOT2	-0.10	0.82
ODT2	0.14	0.64
Eigenvalue	1.90	1.46
Variance explained	32%	24%

Discussion and conclusions

Backtests

Mean and range of the backtest results were comparable with earlier studies (van Erp-van der Kooij et al., 2000). Animals reacted consistently in the tests, especially the correlation between day 10 and day 17 was high (r=0.48). In the factor analysis, backtest scores on consecutive days also loaded on the same factor. Correlations between day 3 and day 10 were lower than between day 10 and 17, probably due to the many changes that take place in that period (tail cutting, iron injection, castration of male piglets). Especially the cross-fostering of piglets probably played a role. By changing the social environment of the piglets, possibly the coping style of that piglet and therefore its coping behaviour in the tests may have changed. In future studies, the influence of cross-fostering on backtest results will be investigated elaborately.

Behavioural tests (HAT, NOT and ODT)

Although practical behavioural tests, that had their limitations, were performed under farm conditions, still a consistency in behaviour was found: correlations were found between different tests at the same age, and between the same test at different ages. Also, the different tests (performed at the same age) loaded at the same factor in the second factor analysis (analyzed per test age). Results of the first factor analysis (all variables) were difficult to interpret because most animals were tested at one of both ages and only 94 of the 315 animals were tested twice. In this analysis, only the results at 10-12 weeks loaded on one factor, while at 5-7 weeks the different tests loaded on different factors.
At 10-12 weeks all animals scored high in the HAT, NOT and ODT. We assume that for these animals the tests were not really stressful anymore and therefore the animals did not have to cope. Bolhuis (2001, unpublished results) found that in a restraint test performed twice (week 8 and 20) with gilts, the second test provoked almost no stress response in the animals. In that case, differences in coping style of the animals will not be detected. At 10-12 weeks of age, animals in the cross-fostered pens (HR, MISC and LR) scored higher in the NOT and ODT than in the OR-pens. This suggests that cross-fostering in the first week of age makes the animals in that pen more active or explorative at a later age

Fear and dominance structures

In previous studies, the human approach test (HAT) and the novel object test (NOT) are used to measure fear and are often performed on individual animals (Hemsworth et al., 1994; Hemsworth et al., 1996; Ruis et al., 2001). In practice, it is undesirable when pigs are very frightened of humans, not only because of the consequences for animal well-being, but also from an economic point of view. Hemsworth et al. (Hemsworth et al., 1989; Hemsworth et al., 1981) found that the level of fear of humans was highly associated with the reproductive performance of sows. However, in the present study the tests were performed within the group, in their home pen (the animals were scored individually). Animals within the group in their home pen are less fearful than animals that are tested individually, outside their home pen (Brain, 1990; Epley, 1974; Murphy, 1990). Furthermore, we expect that the element of fear was less important in our tests since the animals were adapted to humans.
An important factor in groups of social living animals is the dominance structure. Since not every animal can come near the human or play with or touch the red bucket at the same time, possibly dominance played an important role. Submissive pigs could be pushed away by dominant penmates, or maybe submissive animals did not dare to come close to some of their penmates and therefore did not come near the human, the bucket or the pen door if the penmates were there. In the NOT, the bucket was sometimes pushed into a corner so that only 2 or 3 animals could play with it and the other pigs could not come near. In the ODT, when a pig was standing in the doorway, sometimes other animals could not pass it. On the other hand, hesitating animals were sometimes pushed through the door by other pigs that were eager to leave the pen. Since we did not score the rank of the individual animals in each group, we cannot correct for dominance structure in the group and we cannot distinguish between dominance and other group processes.

Retesting gave higher scores in the second test (at 10-12 weeks). This suggests that animals got habituated to the tests. In general, animals scored higher in the tests at 10-12 weeks of age than at 5-7 weeks, which suggests that age or development of the animal also influenced test results. Older animals may have experienced more stressful events during their life and maybe were adapted to events happening, while younger animals were still relatively unadapted and easily scared.

Coping as a trait variable

If coping is a trait variable, then consistency should be found in behaviour over time and across situations. This was observed in the present study. High correlations were found between backtests at different ages and animals also reacted consistently in the other behavioural tests: 9 of the 15 possible correlations between HAT, NOT and ODT at two ages were significant, 3 were trends (Table 3). This suggests that coping can (at least partly) be considered as a trait variable.

Consistencies in behaviour were found in some other studies when different tests were performed on the same animals. Lyons et al. (Lyons et al., 1988) found in goats that the latency to approach a human observer (HAT) correlated with the latency to approach a novel object (NOT) and with pituitary-adrenal responsiveness. Stevenson-Hinde et al. (Stevenson-Hinde et al., 1980) found significant correlations between behaviour of Rhesus Monkeys across situations for males, but not for females, and only within each age class (1 year or 2,5 years of age). They concluded that the cross-situational correlations were not straightforward and that 'the search for consistency may take unexpected paths'. Lawrence et al. (Lawrence et al., 1991) found that gilts that responded highly to a series of handling tests (such as latency to leave pen, presenting themselves for testing, walking through a corridor) reacted correspondingly to subsequent tests such as a human approach test, a novel object test or application of a nose sling. Thodberg et al. (Thodberg et al., 1999) found a general reaction pattern across situations in gilts. They performed a food competition test, an open field test, a human test and a social test. Erhard et al. (Erhard and Mendl, 1999) found a relationship between behaviour in a tonic immobility test (comparable with the backtest) and emergence time from a box on the same day, for 3-week old pigs.
These studies provide some evidence for consistency in animal behaviour across time and across situations, but much is still unknown.

Coping as a situation-specific variable

If no correlations would have been found between the behavioural tests over time or across situations, then coping might only be situation-specific and the behaviour of each animal would be random. The fact that we found correlations within animals between different tests, rejects this idea. However, this does not mean that the animals reaction cannot also be influenced by the situation.
Rasmussen (Rasmussen, 1991) suggested that the environment not only determines the coping reaction but changes the coping style of the animal. He found that in conventional pens, pigs explored a novel object more than in an enriched environment. In general, pigs in the conventional pens were more exploratory and aggressive. Olsson et al. (Olsson et al., 1999) found that the reaction to behavioural tests can be influenced by rearing conditions. In their experiment, animals reared in an enriched environment showed longer latencies to approach a novel object. Stolba and Wood-Gush (Stolba and Wood-Gush, 1980) found similar results.
In the present study, all animals were raised in the same environment (i.e. conventional pens). These animals should be more exploratory and aggressive and score high in the NOT. This might explain the relatively high scores we found in the tests.

Relations between backtest scores and HAT, NOT and ODT

No significant differences were found in HAT, NOT or ODT between HR-pens, MISC-pens, LR-pens and OR-pens. There was a large pen effect and a relatively small number of observations on pen level. The lsmeans for NOT and ODT at 5-7 weeks suggest that animals in HR pens have higher test results than animals in LR pens. This would be in concordance with Ruis et al. (Ruis et al., 2001), who found that LR pigs (backtest score at 2-3 days <3) hesitated longer to leave their home pen and to contact a human. However, this is not significant in the present study, which can be explained in several ways. First, there are so many other influencing factors, that possibly this (small) effect is not statistically significant. We are dealing with a group process, dominance may play an important role and previous experiences in the group may have been important for the test reactions. These were uncontrolled factors in the present study. Secondly it is possible that the cross-fostering of piglets changed their coping style. In future studies, we will investigate this phenomenon further. Thirdly, the coping characteristics of a pig might be a composition of several different aspects or personality dimensons. Gosling et al (Gosling and John, 1999) identified five different dimensions in the personality of animals, which are N for Neuroticism (e.g. anxiety, vulnerability to stress); A for Agreeableness (e.g. aggression); E for Extraversion (e.g. activity), O for Openness to experience (e.g. curiosity) and Dominance. It is possible that different tests can measure different dimensions, and the animals characteristics are consistent. This might explain the correlations that we found between the group tests and between the backtests (the same type of test measured the same dimension) and the absence of correlations between the backtest and the group tests. Possibly, the backtests measured activity and (partly) fear, while in the group tests dominance and exploration were measured. The fact that all group tests loaded on the same factor in the factor analysis also suggests that at 10-12 weeks of age these group tests measure the same characteristic of the animal. If the backtest and the human approach test at 5-7 weeks both measure (partly) fear (of humans), then this explains their correlation. In the analysis with all behavioural data the backtest and the human approach test both loaded on factor 2, that may be described as 'fear'. We could call factor 1 with backtest and the open door test at 5-7 weeks 'activity', and an appropriate name for factor 3 with the group tests at 10-12 weeks and the novel object test at 5-7 weeks might be 'exploration' or 'dominance'. At 10-12 weeks and in the novel object test, 'fear' is probably not very important, while 'activity' might only be a distinct dimension in younger animals (Gosling and John, 1999). In older animals, activity might be interwoven with dominance. At 10-12 weeks, pigs are twice as heavy as at 5-7 weeks, and dominance might play a greater role.

Conclusion

There are consistencies in behaviour over time (backtest at day 3, 10 and 17; NOT1-2 and ODT1-2) and across test situations (HAT, NOT and ODT). This suggests that coping is a trait variable. However, when we take the value of the correlations into account ($r=0.2-0.5$, substantially lower than 1), we must conclude that either our test method was fairly imprecise, or that other factors also influenced the test results. This last idea seems the most logical. Probably behaviour is not only a trait variable but also situation-specific. Apart from the coping style (nature) of the animal, we are dealing with developmental and environmental factors in the past (nurture) and in the present.
It is difficult to predict behaviour of individual animals both across time and across test situations (from backtest to HAT, NOT or ODT). Possibly the different test types measured different dimensions of the personality, which would explain the absence of correlations between the tests.

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INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS – INFLUENCES OF GROUP COMPOSITION BUT NO DIFFERENCES IN CORTISOL RESPONSES

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ABSTRACT

To determine the effect of group composition on backtest responses, and to determine the predictive value of the backtest for the physiological stress response to weaning and mixing, 814 pigs were backtested at 3, 10 and 17 days of age. 29% of all pigs were cross-fostered at 3 days according to backtest (Bt) responses and groups were formed of animals with high responses (HR) only, low responses (LR) only, or mixed groups of animals with high, intermediate and low responses (MISC). Original litters (OR, no cross-fostering) were used as controls. Cortisol responses were measured in saliva after weaning at 4 weeks of age and after moving and mixing at 9 weeks of age.
In HR groups, mean Bt responses decreased after cross-fostering while in LR groups, mean Bt scores increased. In both groups, Bt responses of individual animals before and after cross-fostering were not correlated. In MISC and OR groups, all Bt scores were correlated. Weaning and mixing caused a significant rise in cortisol in all animals, while moving or weighing did not. No relations were found between Bt scores and cortisol levels.
We conclude that backtest behaviour can change according to the social environment between 3 and 10 days. This could be intentional, to form a varied group, or it might be caused by a change in HPA function due to social stress. At an older age, this ability is lost and common farm practises such as regrouping, weaning and mixing of piglets at ages >10 days might have a negative effect on the piglets.

INTRODUCTION

Variations in behaviour and physiology are considered important biological individual characteristics to cope with relevant environmental changes that threaten homeostasis (Hessing et al., 1994).
It may be beneficial for the individual animal to behave differently depending on its age, sex or size or on its environment. Behavioural strategies may be depending on what other individuals are doing, or it may benefit animals to possess varying signals to indicate aspects of their identity. Variation may also occur because wide ranges of behaviour are tolerated by the selection process (i.e. evolutionary or an artificial selection), and the exact form of the behaviour is unimportant. Finally, in a varying and unpredictable environment, animals can only guess what behaviour may best be adopted, and a wide range of behaviour will remain in the population (Slater, 1981).
In pigs, individual variation in stress coping behaviour can be measured with the backtest (Hessing et al., 1993; van Erp-van der Kooij et al., 2001). In this test, a piglet is put on its back and restrained in this position for one minute, while the number of escape attempts is recorded (the 'backtest score'). The extremes of the pig population can be defined as active (also referred to as 'pro-active') animals, with high backtest scores, and reactive (also referred to as 'passive') animals, with low backtest scores (for a discussion on terms, see (Koolhaas et al., 1999)). We prefer the terms active and reactive over active and passive, because the latter might be interpreted negatively ('passive' as 'not reacting, not doing anything'). Active animals are the ones that actively try to fight the stressor or try to get away from it, while reactive animals await the end of the stressor in certain test situations; in other situations the reactive animals show the most explorative behaviour. Reactive animals seem to adapt more easily to variable conditions and are more flexible, which is advantageous in changing or unpredictable conditions, while active animals develop routines and seem to anticipate situations, which is advantageous in stable and predictable conditions (Ruis et al., 2001b). Although the backtest has been criticised (Jensen et al., 1995), relations have been

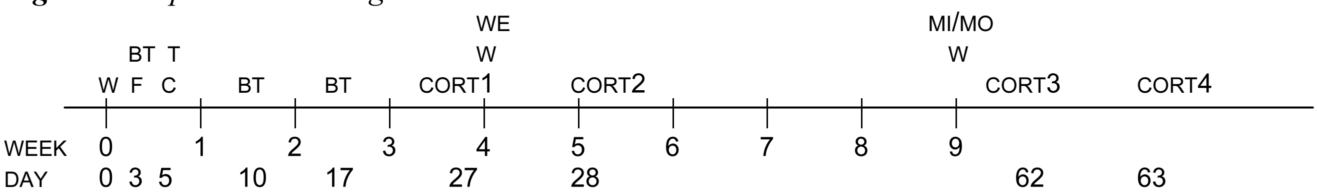
reported between backtest results and other behavioural tests, and physiological and immunological parameters (Hessing et al., 1995; Hessing et al., 1994; Ruis et al., 2000; van Erp-van der Kooij et al., 2000).
Backtest scores range normally from 0 to 9 escape attempts in one minute. Mean backtest results at 10 days of age have risen in the population on the university farm in Utrecht, the Netherlands, from approximately 2 in 1995 to approximately 3 in 1999. Since a positive relation was found between lean meat percentage at slaughter and backtest score, this rise in backtest scores is probably due to the selection on lean meat percentage in commercial pigs (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000).

Because experiences early in life such as cross-fostering and regrouping, which is common in commercial pig husbandry, might have an impact on the coping behaviour of the animals later in life, the first objective of this study was to investigate the backtest response of pigs that were cross-fostered and grouped according to backtest responses at a young age. We assume that backtest responses are relatively stable from 3 to 10 days of age if no major changes in the social environment take place, but if animals are regrouped between the tests, it is unknown what the effect on backtest responses is. Differences might be expected between cross-fostered animals and animals that stayed in the home pen and received new penmates, and between different groups when extreme social environments (with only active or only reactive animals) are created.
In the present study, pigs were weaned at 4 weeks and some were mixed at 9 weeks, to assess the animal's response to those stressors, using salivary cortisol as a tool to assess the physiological stress response of the animals (Barnett and Hutson, 1987; Ekkel et al., 1996).
The second objective of the study was to determine if animals that differed in coping responses to the backtest also differed in cortisol responses to weaning and mixing, because reactive animals might show a higher reactivity of the HPA system (Ruis et al., 2000).

MATERIALS AND METHODS

An outline of the experiment is given in Fig.1.

Figure 1: *Experimental design*



w=weighing
bt=backtest
f=formation of groups HR, MISC, LR and OR[1] by cross-(Ruis et al., 2000)fostering
t=tail docking
c=castration of male piglet
we=weaning
cort=saliva sampling for cortisol
mo=only moving to fattening pens
mi=mixing and moving to fattening pens

[1] HR=high resisting, Bt scores>3; MISC=miscellaneous Bt scores; LR=low resisting, Bt scores <3; OR=original litters, no cross-fostering

Housing and animals

The study was performed at the farm 'De Tolakker' of the University of Utrecht, The Netherlands. 882 piglets, born from 77 NL*GY sows, were used. Sows and piglets were housed in farrowing compartments (N=51 pens, 471 piglets) or multi-purpose compartments (N=40 pens, 411 piglets) and conventional farrowing crates were used. A heating lamp and floor heating were used when necessary. As of the second week, creep feeding was available for the piglets. An all in – all out system was used consistently. At birth, the ears of the piglets were tattooed for identification. 259 piglets were cross-fostered, according to the procedure described in the next paragraph. At approximately 5 days of age male pigs were castrated, tails of all pigs were docked and iron was given to the piglets by injection, according to Dutch standard procedures. At weaning, around 28 days of age, the sow was removed from the pen and the piglets remained in the same pen until 9 weeks of age. After weaning, animals received three plastic eartags, one with the farm number (UBN number, obligatory in the Netherlands) and two others for experimental identification purposes.
At 9 weeks of age, the fattening period started. At that time, 379[2] pigs from the farrowing compartments were moved to fattening units and 290 of these pigs were mixed (see next paragraph). 389[3] piglets in the multi-purpose compartments were neither mixed nor moved but stayed in the same pens until slaughter. This is a specific-stress-free housing system (Ekkel et al., 1995; Scheepens et al., 1990)14). All fattening pens had a partly slatted, concrete floor with floor-heating. Feed and water were supplied *ad libitum*. Pigs were slaughtered at approximately 110 kilograms liveweight (around 6 months of age). This study only covers the period until 9 weeks of age.

Backtest and mixing procedure

A backtest was performed on all piglets (Hessing et al., 1993). In this backtest, each piglet was taken out of the home pen and placed on its back on a table, next to the pen. The animal was restrained manually in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. The pressure was increased slightly when the animal started moving, in order to hold the piglet in the same place: the more an animal moved, the more pressure was needed. When the animal relaxed again, the pressure was decreased again. Each bout of wriggles that the piglet made without a pause was counted as one escape attempt. A pause was defined as a period of time in which the piglet did not move its hind legs, and that lasted at least 1 second. The total number of escape attempts is called the 'backtest score' and ranges from 0 to 11 in this study. A backtest was performed with every animal at 3, 10 and 17 days of age (plus or minus 1 day).
On day three, after performing the first backtest, different groups of approximately 10 piglets per sow were formed on the basis of the first backtest results by cross-fostering of some of the piglets, resulting in 20 HR groups (high resisting), 31 LR groups (low resisting), 18 MISC groups (miscellaneous) and 21 OR groups (original) as controls. In HR-pens only piglets with high backtest scores (>3) were placed, in LR-pens piglets with low scores (<3) were placed, MISC- pens consisted of animals with high, intermediate and low scores and OR-pens consisted of littermates only, independent of backtest score. The cut-off value of 3 was chosen because the mean backtest score on day 3 was 3.26. In the HR pens, 63 out of 192 piglets were cross-fostered (33%), in the MISC pens, 128 out of 304 (42%), and in the LR pens 67

[2] The other 92 pigs either died or were not moved to the fattening pens due to animal density regulations for fattening pigs (min. 0.7 m^2 per pig = max. 10 pigs per pen)
[3] the other 22 pigs either died or were removed from the multi-purpose pens due to animal density regulations for fattening pigs (see footnote [1])

out of 178 (38%). The number of cross-fostered piglets ranged from 1 to 11 in each litter, and cross-fostered piglets usually came from 1 other pen (in 42/64 pens) or 2 other pens (in 16/64 pens). In the remaining 6 pens, cross-fostered piglets came from 3, 4 or 5 other pens. All groups were balanced for gender. In MISC pens, 24% of piglets had high backtest scores (>3), 29% had low scores (<3) and 47% scored exactly 3 in the first backtest. Mean backtest scores per group after the cross-fostering procedure on day 3 are shown in Table 1.

In the first 10 farrowing pens, some behavioural observations were done after cross-fostering. Approximately 30 minuters after cross-fostering, cross-fostered pens were observed for 10 minutes per pen, and aggressive interactions were scored. The following day, during one suckling bout it was checked if all cross-fostered piglets were suckling.

At 9 weeks, all HR, MISC and LR groups from the farrowing compartments were mixed with other HR, MISC or LR groups from the same compartments to create 17 HR, 27 MISC, 17 LR and 18 OR groups in the fattening compartments. Mixing was done only within groups (HR+HR, MISC+MISC and LR+LR). Group sizes sometimes differed before and after mixing, because a) the available space in the fattening pens was limited (a maximum of 10 pigs per pen was allowed) and b) we tried to use the fattening pens as efficiently as possible (e.g. by mixing 20 piglets from 3 farrowing pens into 2 fattening pens, provided that they came from the same group, LR, HR or MISC). Animals from the OR groups and animals in the multi-purpose pens were not mixed.

Table 1: *Mean backtest results (LSMeans) and differences between means at day 3, 10 and 17 in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR). Groups are formed on day 3, on the basis of the first backtest results.*

MEAN BACKTEST SCORE	DAY 3	DAY 10	DAY 17
HR	$4.74^{b} \pm 0.24$	$3.23^{a} \pm 0.31$	$3.40^{a} \pm 0.35$
MISC	$3.07^{a} \pm 0.18$	$2.68^{a} \pm 0.23$	$3.23^{a} \pm 0.26$
LR	$1.52^{c} \pm 0.25$	$1.66^{b} \pm 0.33$	$2.29^{b} \pm 0.37$
OR	$3.53^{a} \pm 0.24$	$2.66^{a} \pm 0.33$	$3.42^{a} \pm 0.36$

Means with a different superscript in the same column differ significantly.

Saliva sampling procedure and radioimmunoassay for cortisol

The saliva sampling and the radioimmunoassay were performed as described previously (Ekkel et al., 1996). Saliva samples were taken from approximately 20% of the animals (2 animals per pen, randomly chosen) on the pre-weaning (cort1) and on the weaning day (cort2), and at 9 weeks of age on the day before weighing (cort3) and after weighing and/or mixing and/or moving (cort4) (Fig. 1). The same animals were used for all the samples. Weaning and weighing started at 9 a.m., saliva sampling started at 11 a.m. (and was finished at the latest at 1 p.m). Because of practical reasons, only 4 samples per animal were taken. The saliva was collected by allowing the pigs to chew on cotton buds (9679396, Hartman, Nijmegen, the Netherlands) until they were thoroughly moistened. Pigs were handled and trained before saliva sampling, therefore fixing was never necessary. The cotton buds were centrifuged for 5 minutes at 400 x g to remove the saliva, which was then stored at –20 °C until analysis.

Concentrations of cortisol in saliva were estimated by a previously validated (Ekkel et al., 1996) direct solid-phase ^{125}I RIA method (Coat-A-Count TKCO; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in general in duplicate samples of 200 µl. For 17% of the samples smaller aliquots were used due to small amounts of collected saliva.

The main cross-reactivities were 11.4, 0.98, 0.94 and 0.26% for 11-deoxycortisol, cortisone, cortisosterone and 11-deoxycortisosterone, respectively, according to the manufacturer. Comparison of added and estimated values when cortisol was added to a pooled saliva sample over the range of the standard curve (0.5-50 ng/ml) produced an average intra-assay coefficient of variation of 7.9% and a recovery of 96.8%. The limit of quantitation was 0.25 ng/ml and the interassay coefficient of variation was 9.5% for the range of 0.5 to 15 ng/ml (n=8).

Statistical analysis

All analyses were performed using the statistical package SAS (Ekkel et al., 1996; SAS_Institute_Inc., 1989).
Pearson correlation coefficients were calculated for individual backtest results on different test days. Confidence intervals around the correlation coefficients were calculated using a Fisher's Z transformation. To compare correlations between day 3 and day 10 for the different groups (HR, LR, MISC and OR), the following model was used (PROC GLM, SAS):
Bt3 = μ + Bt10 + pen(group) + group + Bt10*group
in which Btx is the backtest score at x days of age, pen is the pen number (within group) and group is HR, LR, MISC or OR. If the interaction Bt10*group is significant, this means that the correlation between Bt3 and Bt10 is different in the different groups. The estimates for the betas in the different groups are calculated relative to the OR group (option /solution in SAS); this way we can determine if the correlations in the cross-fostered groups HR, LR and MISC differ from the OR group (original litters).
Log-transformations were used in the analyses for the cortisol rise at weaning (log(cort2/cort1)) and at 9 weeks (log (cort4/cort3)) since cortisol levels were not normally distributed, and for group comparisons the following models were used at weaning (1) and at 9 weeks (2):
(1) log(cort2/cort1)=group
(2) log(cort4/cort3)=log(cort2/cort1) + group + ssf
where group=HR, MISC, LR or OR and ssf=SSF-housing system (no mixing, no moving) or conventional system (moving and/or mixing of pigs at 9 weeks).
To determine the relation between individual backtest result and cortisol rise, the following models were used at weaning (1) and at 9 weeks (2):
(1) log(cort2/cort1)=backtest score
(2) log(cort4/cort3)=log(cort2/cort1) + backtest score + ssf
where backtest score is the mathematical mean of the backtest result on day 10 and day 17 for each individual.
A general linear mixed model (PROC MIXED in SAS) was used, and models were corrected for batch*compartment*pen in the random statement.
Results were considered statistically significant when P-values were below 0.05; P-values between 0.05 and 0.10 are called trends.

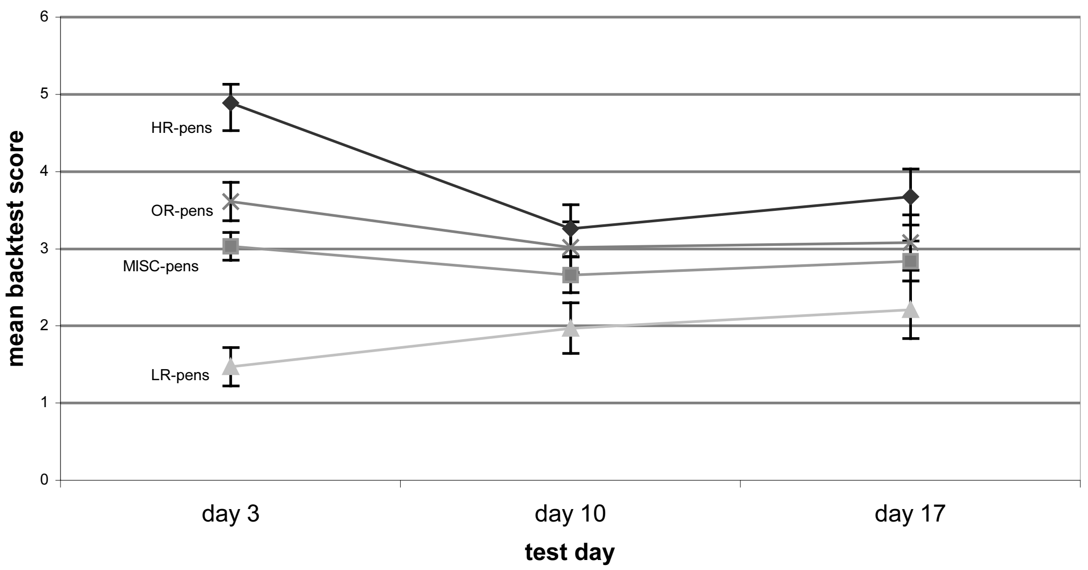
RESULTS

Backtest

Mean backtest results were 3.26 ± 0.06 on day 3 (range 0-9, n=813), 2.76 ± 0.06 on day 10 (range 0-11, n=787) and 2.97 ± 0.07 on day 17 (range 0-11, n=785). Gilts had higher backtest results than barrows on day 3, 10 and 17, but this was significant only on day 10.

By cross-fostering on day 3, we created pens with mean backtest scores on that day of 4.74 in the HR pens, 3.07 in the MISC pens, 1.52 in the LR pens and 3.53 in the OR pens (Table 1). At day 10 and day 17, the differences between the pens were much smaller (Fig.2) and only the mean backtest result of the LR pens was significantly lower than in the other pens. In both the LR pens and in the HR pens, on day 10 almost the full range of backtest results was seen again. This resulted in correlations between backtests on different test days that were dependent on group composition. Correlations between day 3 and 10 and between day 3 and 17 were not significant for the animals in the HR and LR pens. The correlations between day 3 and day 10 for all animals in the LR and HR groups differed from the correlation between day 3 and day 10 in the OR group (P<0.05). The correlation between day 3 and day 10 for all animals in the MISC group did not differ from that in the OR group (P=0.18).

Figure 2: *Mean backtest score for HR, MISC, LR and OR pens. n=186, 294, 170 and 197 pigs for HR, MISC, LR and OR pens, respectively. Cross-fostering to create HR, MISC and LR pens took place just after the first backtest, on day 3.*



Not only the mean backtest scores changed, but also the individual ranking (in backtest results) of the animals changed. Backtest results in typical HR and LR pens are in Figures 3a and 3b.

Figure 3a: *Backtest scores of individual piglets in a representative HR pen, before and after regrouping. Lines represent one or more piglets, numbers are between brackets.*

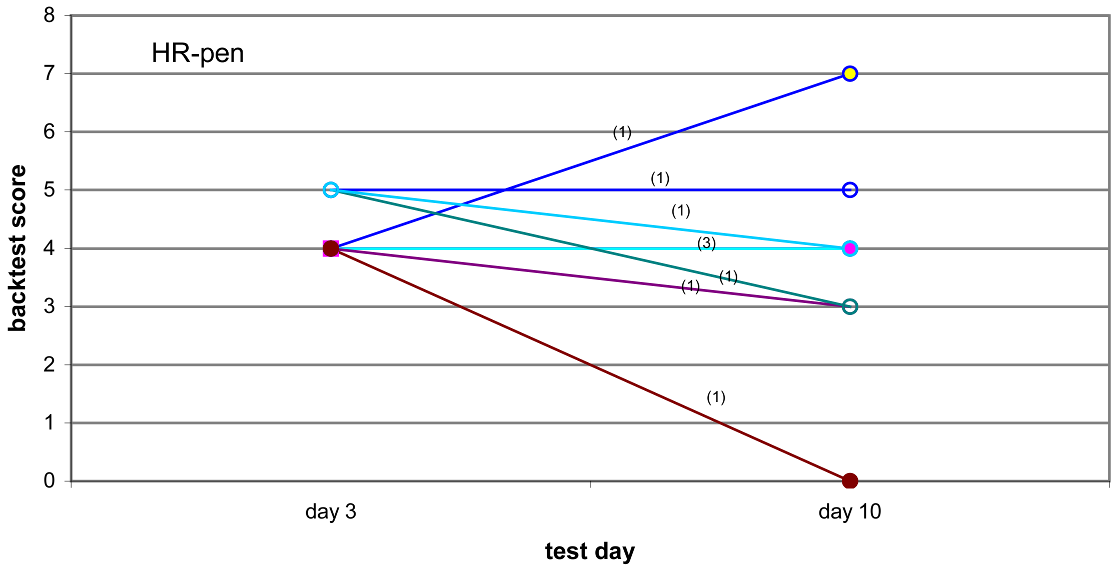
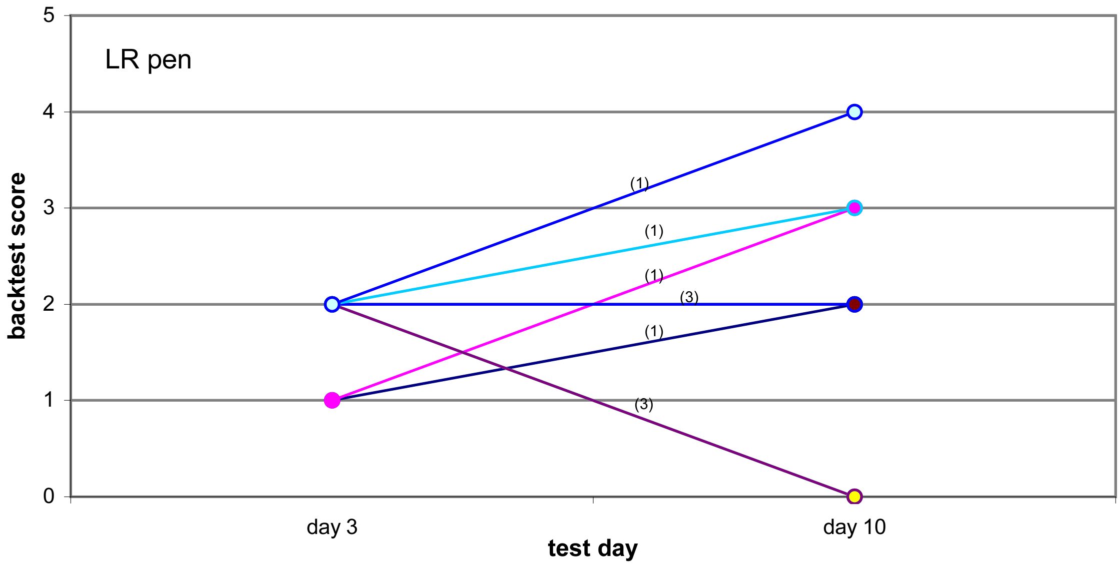


Figure 3b: *Backtest scores of individual piglets in a representative LR pen, before and after regrouping. Lines represent one or more piglets, numbers are between brackets.*



Mean backtest results for all animals in the HR, MISC and OR pens were on an equal level on 10 and 17 days. Backtest results on day 10 and 17 were correlated for all groups (Table 2a) but backtest results on day 3 and day 10 were only correlated for the high resisting animals (Bt scores>3) in the MISC and OR groups. (Table 2b).

Table 2a: *Correlations (with confidence intervals) between backtests in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR).*

	DAY3-DAY10	P-VALUE	DAY3-DAY17	P-VALUE	DAY10-DAY17	P-VALUE
HR	0.08 (-0.06-0.22)	0.26	0.08 (-0.06-0.22)	0.27	0.38 (0.25-0.49)	<0.0001
MISC	0.23 (0.12-0.34)	<0.0001	0.22 (0.11-0.33)	<0.001	0.47 (0.37-0.55)	<0.0001
LR	0.06 (-0.09-0.21)	0.40	-0.07 (-0.22-0.08)	0.36	0.25 (0.11-0.39)	<0.001
OR	0.25 (0.12-0.38)	<0.001	0.36 (0.23-0.47)	<0.0001	0.44 (0.32-0.54)	<0.0001

Table 2b: *Correlations between backtest results for high (HR) or low (LR) resisting animals in groups with high backtest results (>3, HR), with low backtest results (<3, LR), with mixed results (range 0-8, MISC) or original litters (OR).*

BACKTEST RESULTS DAY 3	GROUP	CORRELATION DAY 3-DAY 10	CORRELATION DAY 3-DAY 17	CORRELATION DAY 10-DAY 17	N
LR (0,1 OR 2)	HR	-	-	-	-
	MISC	0.15 (NS)	0.17 (NS)	0.59 (P<0.0001)	80
	LR	0.05 (NS)	-0.05 (NS)	0.29 (P<0.0005)	147
	OR	0.17 (NS)	0.26 (NS)	0.31 (P<0.05)	46
HR (>3)	HR	0.09 (NS)	0.09 (NS)	0.38 (P<0.0001)	166
	MISC	0.40 (P<0.001)	0.22 (P<0.1)	0.45 (P<0.0001)	71
	LR	-	-	-	-
	OR	0.28 (P<0.01)	0.31 (P<0.005)	0.53 (P<0.0001)	91

When, within the groups, cross-fostered piglets were compared with animals that received new penmates, no differences in backtest results were found (Table 2c). No aggressive interactions were seen after cross-fostering, and the following day all cross-fostered piglets were suckling.

Table 2c: *Backtest results on day 3 and 10 and difference (diff) between day 3 and day 10 (Bt day 3-Bt day10) for cross-fostered animals and animals that stayed in the same pen but received new penmates, in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR) or with mixed results on day 3 (range 0-8, MISC). In original litters (OR), no cross-fostering was performed.*

		NOT CROSS-FOSTERED	CROSS-FOSTERED
HR	BT DAY3	4.91±1.08	4.86±0.95
	BT DAY 10	3.24±1.66	3.33±1.87
	DIFF	1.69±1.93	1.46±1.95
MISC	BT DAY3	2.94±1.34	3.18±1.21
	BT DAY 10	2.59±1.65	2.76±1.68
	DIFF	0.35±1.78	0.41±1.98
LR	BT DAY3	1.47±0.70	1.48±0.68
	BT DAY 10	1.92±1.33	2.06±1.50
	DIFF	-0.44±1.41	-0.58±1.67

Cortisol

Cortisol levels in saliva (mean ± SE) were 2.90 ± 0.30 and 4.62 ± 0.26 before and after weaning, and 1.83 ± 0.13 and 2.37 ± 0.20 before and after the start of the fattening period, respectively. The mean difference in cortisol levels before and after weaning was 2.05 ± 0.41 and the mean difference at 9 weeks (before and after the start of the fattening period) was 0.73 ± 0.24. This rise in cortisol levels was significant at weaning, and the animals that were mixed and moved at 9 weeks also showed a significant rise in salivary cortisol at that time. No animals were mixed without moving. Only moving the pigs (no mixing) had no effect on cortisol levels (Table 3).

Table 3: *Cortisol rise in saliva at the start of the fattening period, for animals that are mixed compared to animals that are not mixed and animals that were moved but not mixed compared to animals that were not moved nor mixed.*

	CORTISOL RISE	LOG (CORTISOL)	P-VALUE	N
NO MIXING	0.10 ± 0.14	0.007		73
MIXING	1.48 ± 0.47	0.526	<0.0001	61
NO MOVING	0.14 ± 0.12	0.006		56
MOVING	-0.03 ± 0.50	0.010	NS	17

In the statistical analysis, log transformations were used.

Group effects on cortisol and relations with individual backtest scores

Differences in cortisol levels for the different groups (HR, MISC, LR and OR) before and after weaning, and at the start of the fattening period are presented in Table 4. Relations were found only with housing system (SSF or CONV).
No relations were found between individual backtest results at 3, 10 or 17 days of age and cortisol rise at weaning or at 9 weeks.

Table 4: *Cortisol rise (mean ± SE) after weaning and at 9 weeks in in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR), in different housing systems; SSF=specific stress free (no mixing or moving); CONV=conventional, moving and/or mixing at 9 weeks. Until 9 weeks, housing is identical.*

CORTISOL RISE	HR	MISC	LR	OR
AFTER WEANING	2.31 ± 0.59***	2.42 ± 0.58***	1.29 ± 1.65**	1.84 ± 0.40***
AT 9 WEEKS				
SSF	0.21 ± 0.25	0.23 ± 0.22	0.18 ± 0.20	-0.26 ± 0.26
CONV (MOVING, NO MIXING)				-0.03 ± 0.50
CONV (MOVING AND MIXING)	1.38 ± 0.87[1]	1.20 ± 0.68**	2.20 ± 0.96[1]	

Asterisks indicate if the cortisol rise differs from 0 (analyses on log-transformated data). [1]P<0.10

DISCUSSION AND CONCLUSIONS

Backtest results

The backtest is a behavioural test, which measures the reaction of an individual in a certain stressful situation. Mean and range of the backtest results in this study were comparable with earlier studies, and also gilts had higher backtest results than barrows at day 3, 10 and 17, but this was significant only at day 10 (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000).

So far, it is unknown which specific aspects of the coping style of the animals are measured with this test. According to a recent study (Gosling and John, 1999), five dimensions of the personality can be distinguished in animals. These are organised in the categories Neuroticism (e.g. anxiety), Antagonism (e.g. aggression), Extraversion (e.g. activity), Openness (e.g. curiosity-exploration) and Dominance. It is possible that the backtest measures, for example, a combination of fear of humans, aggression and activity. In order to reveal some of the underlying dimensions of the personality, several extra parameters should be recorded during the backtests such as vocalisations, latency to first escape attempt and defecating behaviour. Because of practical and logistical reasons, this was not done in the present study.

Some authors claim that the backtest is a non-social test (Hessing et al., 1993), but others suggest that being restrained in a supine position during a backtest may represent forced submission, by its resemblance with certain aspects of social fighting (Ruis, 2001). Reactive animals (with low backtest scores) might more readily accept their forced subordinate status and react less fiercely, whereas active animals (with high backtest scores) become frustrated by bodily forced submission and are more eager to resist. In that view, the backtest represents (elements of) a social test. Our results support the idea that the backtest has a social component, since the group composition (=social environment) influenced test results.

Backtest results and group composition

Mean backtest results for all piglets in the HR pens decreased after cross-fostering and correlations before and after cross-fostering were absent, while HR animals in the MISC and OR pens behaved consistently in the successive backtests.
Mean backtest results for all piglets in the LR pens increased after cross-fostering and correlations before and after cross-fostering were absent, but LR animals did not seem to behave consistently in the MISC and OR pens either.
This suggests that in extreme circumstances, the animals can change their behaviour. Furthermore, the behaviour of LR or reactive animals might be more directed by environmental stimuli (extrinsic behavioural control), whereas the behaviour of the active animals might be more routine-like and intrinsically determined, as was suggested in a study with reactive and active rodents (Benus et al., 1991).
Why did the animals' behaviour change? We believe there are two explanations possible. The first explanation is, that the animals changed their behaviour intentionally, because behavioural variation is beneficial for a group. In a study on individual behavioural characteristics in pigs (Hessing, 1994) it is stated that the complementary individual behavioural characteristics of the active and the reactive pigs under stress will result in a better socially integrated group. A socially well integrated group will be more successful in solving their problems, which is beneficial for each individual and for the group as a whole. This is shown in 2 studies, where active (HR) and reactive (LR) pigs were mixed. It was found that the most stable social relationship existed between HR/LR pairs where the LR animal was dominant (Ruis et al., 2002) and that HR/LR groups performed better (Hessing, 1994).
In a study where pigs were categorised as high aggressive (H) or low aggressive (L), it was found that the number of pairs fighting was lowest when H pigs were mixed with L pigs, compared to when only H or only L pigs were mixed. However, the L/L groups integrated faster and the L pigs fought less vigorously than H pigs and therefore it was concluded that it is preferable to mix pigs into groups with low-aggressive pigs only, which has been stated in another paper as well (Erhard et al., 1997; Erhard and Schouten, 2001). If LR pigs are low-

aggressive and HR pigs are high-aggressive, then we might deduce that homogeneous LR or mixed HR/LR groups would be preferred over homogeneous HR groups.
In the above mentioned studies (Hessing, 1994; Ruis et al., 2002) pigs were mixed at 7 and 9 weeks of age. The fact that the results in the different pairs or groups could be related to backtest behaviour before mixing, suggests that mixing did not change backtest behaviour. In the present study, animals were mixed at a much younger age, and we assume that that is why the behaviour of these animals could still change.

A second explanation of the change in backtest results after regrouping, is that in some animals HPA axis functioning and behavioural reactivity might have been affected by the regouping procedure. This is suggested in a study with calves, where regrouped calves showed an increased sensitivity of the adrenal cortex to ACTH (Veissier et al., 2001) and regrouped calves were more agitated during restraint (Boissy et al., 2001). In another study (Weaver et al., 2000) it was shown that neonatal handling permanently affected HPA function in boars. It is concluded fdrom that study that there is a sensitive period during early postnatal development in the pig, during which environmental manipulations can result in permanent changes in HPA function, behaviour and body weight. Differences in body weight were not seen in the present study, but the changes in backtest behaviour might have been a result from changes in HPA function, due to environmental manipulations such as regrouping and handling, that took place in the sensitive period (between 0 and 14 days of age).

Dominance

If the animals changed their behaviour in order to create a more varied and possibly more stable social environment, than dominance hierarchy in the group should be taken into acount. In a study with paired gilts (Ruis et al., 2002), HR gilts that were dominant over LR gilts showed much more aggression than LR gilts that were dominant over HR gilts. LR and HR gilts had almost equal chances of becoming dominant. The authors suggest that LR dominant gilts were able to suppress aggression in the HR subordinates, while HR gilts became aggressive when dominant. Mixing of two HR gilts caused the highest levels of stress. Unfortunately, in the present study we have no information on the dominance hierarchy.

Cross-fostering

No differences were found between cross-fostered animals and animals that received new littermates, within the same group. Differences we found between the groups, were dependent on group composition only. The attachment between sow and piglets is established in the first 3-4 days postpartum (Petersen et al., 1989) so it is possible that cross-fostering at 3 days of age will affect the piglets: they might miss their mother.
No aggression was observed after cross-fostering. We did not expect to see much aggression after cross-fostering, since it was done within a few days after birth (≤ day 4). In a study where piglets were cross-fostered frequently, more fighting occurred in cross-fostered litters compared to control litters when cross-fostering was performed on day 4, 7, 10, 13 or 16 postpartum, but not when cross-fostering was performed on day 1 (Robert and Martineau, 2001). Probably, in our study we cross-fostered early enough not to induce fighting. Under free-range conditions, piglets will join the social group at about 2 weeks postpartum, and in the following period (2-7 weeks pp), mostly friendly interactions are seen. Aggression between residents and intruders is described in older pigs (Ekkel et al., 1995; Erhard and Mendl, 1997; Ruis et al., 2001a).

Cortisol changes

Cortisol was measured in saliva. This non-invasive technique has proven to be reliable and easy to perform. Salivary cortisol is mainly unbound and reflects the biologically active, plasma free fraction (Cook et al., 1996; Ruis et al., 1997). Because a limited number of samples was taken, only major changes in cortisol levels could be found and temporal dynamics of cortisol were not taken into account.

Weaning caused a significant rise in cortisol levels in the piglets of 58%, which we considered important and possibly harmful for the animal (Barnett and Hemsworth, 1990; Mendl, 1991). Since weaning is a known stressor for mammals, this is not surprising. A large variation was found between the animals as was also shown by other authors (Moberg, 1987) and some animals even showed a decrease in cortisol level after weaning. This could be due to some unknown event happening just before or during the first saliva sampling procedure, such as an aggressive encounter with a penmate or a sow vocalising, causing a very high cortisol level at that time. Cortisol levels in pigs may rise quickly in response to an acute stressor (Ruis et al., 2001b).

The procedure at the start of the fattening period (at 9 weeks of age) included weighing of animals and for a number of animals also mixing and moving to the fattening compartments. Animals that were mixed and moved showed a considerable increase in cortisol of 69%, which is also possibly harmful for the animals. It is known that aggression during mixing can increase cortisol in pigs (Parrott et al., 1990) and that mixing of pigs can have a long lasting effect on the physiology and behaviour of pigs (Ekkel et al., 1997; Ekkel et al., 1995). However, cortisol can decrease quickly and be back to basal levels one hour after the stressor (Becker et al., 1985; Geverink et al., 1998; Stricklin et al., 2001). (Weighing and moving without mixing had no effect on cortisol. In the present study, the weighing and moving procedures were new to the animals. Moving meant that a group of pigs was driven through the corridors of the farm to another compartment. Weighing at birth and at weaning was performed with a small balance on which animals were lifted by the researcher, while at 9 weeks pigs were driven on a large weighing device. We therefore expect these treatments to have been stressful for the animals. However, these procedures might have caused a short-lived increase of cortisol, and at the time of measuring (appr. 2 hours later) the effect may already have disappeared.

Cortisol changes and backtest results

Several studies indicate that some differences in cortisol response to stressors in pigs, depend on coping style (Haemisch, 1990; Hessing et al., 1994; Ruis et al., 2001b; Ruis et al., 2000) while others do not (Geers, 1995).

In the present study, no differences were found in mean cortisol rise after weaning or at 9 weeks between the groups. This is not surprising, since at 10 days of age mean backtest results in all groups were approximately at the same level, and the whole range of backtest results was found again in the originally different groups. However, relations between individual backtest scores and cortisol rise were not found either. This means that coping style, as measured with the backtest, could not predict the individual stress responses to weaning or to mixing at 9 weeks of age for these pigs. If a relation with backtest response at 3 days would have been found, we would have concluded that animals had kept their original coping strategy (before cross-fostering) but a phenotypical change in test results after regrouping had occurred, while a relation with backtest responses at day 10 or 17 would have suggested that animals had changed their coping behaviour permanently.

It is possible that the time between the start of the stressor and the cortisol sampling was too long in our study. The animals that differed in coping style, may also have had a different cortisol curve, meaning that the time and the level of the cortisol peak has differed, in accordance with the results of a study with gilts differing in coping response to the backtest (6). It was found that LR gilts (<3 escape attempts in the backtest on day 3) showed higher cortisol responses than HR gilts (>4 escape attempts) at 15 and 30 minutes after the start of an isolation period, but subsequent levels at 45, 90, 180 and 300 minutes did not differ between HR and LR animals. With the single saliva sample approximately 2 hours after the start of the stressor in the present study, this could not be determined. In another study by the same author (21), it was found that not only backtest behaviour at 3 days, but also dominance and the interaction between dominance and backtest behaviour influenced the stress response to mixing at 7 weeks. In future studies on mixing of pigs, dominance should therefore be taken into account as an important factor which might explain differences in cortisol responses after mixing, especially when group stability is discussed.

CONCLUSION

The backtest measures some aspects of the coping style, but which ones exactly is still unknown. The coping response in the backtest is a relatively consistent characteristic of the animal in a stable social environment. When piglets with similar backtest responses (HR or LR) are put together at a young age, backtest responses of the animals change and the group becomes heterogeneous again, containing active (HR) and reactive (LR) individuals. This change could be intentional, because a varied group might be more beneficial for the animals, or might be caused by a change in HPA function due to stress.

Salivary cortisol was increased after weaning and also after mixing of unfamiliar pigs at 9 weeks, but we found no relations with backtest scores in this study.

It is important that people who work with pigs realise that common farm practises such as regrouping of piglets and mixing of older pigs have such an influence on the behaviour and the physiology of the animals. Although cross-fostering has less (visible) effects than regrouping at a laer age, the procedure can change the animals behaviour. We therefore advise strongly not to mix pigs unless it is absolutely necessary, and if mixing is unavoidable, to do it at a very young age.

Hopefully, if farmers (and consumers) see our production animals as individuals again, each with their own personality, they will treat them better (or demand that they be treated better), and spare them some of the (unnecessary?) stressors of everyday life on the commercial pig farm.

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INDIVIDUAL BEHAVIOURAL CHARACTERISTICS IN PIGS AND THEIR IMPACT ON PRODUCTION

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ABSTRACT

Two studies have been carried out in pigs, to determine the relation between escape behaviour and production parameters and between escape behaviour and other factors. In the first, 823 piglets were tested with the backtest at 10 and 17 days of age. Production parameters such as average daily weight gain and lean meat percentage were recorded. In the second, the backtest was performed on 566 piglets at 3, 10 and 17 days of age. Escape behaviour in the backtest (backtest score) of the mother was known for 364 piglets. Parameters concerning the health of sow and piglets were recorded, as well as the sow's reaction on piglet removal for testing. Relations between production parameters and backtest scores of the animals were calculated, as well as the influence of birth weight, gender (all males were castrated), parents and health parameters on backtest scores.

Backtest scores were fairly consistent over successive tests. Males had lower backtest scores than females, and piglets from sows with low backtest scores had low scores themselves. Finally, a higher backtest score gave a higher lean meat percentage and a better carcass grading at slaughter; no relation with daily weight gain was found.

It is concluded that there are individual differences between the ways in which pigs cope with a stressful situation, as is measured with the backtest, and that this coping behaviour is consistent. A positive relation exists between backtest scores and lean meat percentage, and a heritability of backtest scores is assumed.

INTRODUCTION

The way vertebrates react in a stressful situation varies and largely determines their adaptive capacity. Animals will react to a stressor with a series of behavioural, physiological and neuroendocrinological activities regulated by the brain. However, not every animal of the same species will react to a stressor in the same way (Benus et al., 1987).

In mice and rats, evidence has been found for two different ways of dealing with a stressor. The theory is, that each individual has its own coping style, which can be either active or passive. To determine this coping style, animals are tested for behavioural traits like aggression or nest building behaviour, or reactions in an open field test or during tonic immobility are determined. The distribution of these traits and the results in several tests are bimodal and consistent over time (Benus et al., 1991; McGraw and Klemm, 1973; Sluyter et al., 1995). Most of these studies are done with SAL and LAL mice, two genetically selected lines of wild house mice. Comparable results are found in poultry (Beuving et al., 1989; Jones and Satterlee, 1996).

In humans, several different coping approaches are recognized. In the first theory two coping styles were recognized, resulting in type A and type B humans (Glass, 1977). Later, some authors defined up to twelve different coping strategies, others only three or four (Pines and Kafry, 1982; Simoni and Paterson, 1997).

In primates, individual differences in behaviour have been found but two or more distinct coping styles have not been established. In experiments with rhesus monkeys, results of behavioural tests are fairly consistent when the same test is done twice at the same age, but they are not consistent over time and for different situations (Stevenson-Hinde et al., 1980).

In pigs, different theories about coping styles exist. Hessing and co-workers (Hessing et al., 1994a; Hessing et al., 1993) suggest that there are two different coping styles in pigs, that can be determined in piglets by performing a backtest. In this test, the piglet is put on its back and held by the experimentor, and the number of escape attempts is counted during one minute. Based on two tests, piglets can be divided into the category of High Resisters (HR), with more

than two escape attempts in each test and Low Resisters (LR), with less than two escape attempts in each test. The animals that did not fit in one of those groups, namely those with exactly two escape attempts in both tests or with varying test results, were called Doubtful (D). HR and LR pigs displayed consistent differences in behavioural, physiological and endocrine responses to stress situations. These responses were associated with different stress pathologies. Moreover, differences in cell-mediated and humoral immunity were found between HR and LR pigs (Hessing et al., 1995; Schrama et al., 1997). Hessing (Hessing et al., 1994b) also found a relation with production: animals in mixed pens (with HR and LR pigs) performed better than pigs in pens with only HR or only LR pigs.
Lawrence and co-workers (Lawrence et al., 1991) have found that female pigs responded consistently to a series of non-social tests, but in social tests, not all responses were consistent. Erhard and Mendl (Erhard and Mendl, 1997, 1999) used a tonic immobility test (TI) to determine behavioural strategies in pigs. They suggest that TI reflects an active or passive strategy. Controversial to these studies are the results of Jensen, Forkman and co-workers, stating that there are no coping styles in pigs. They found no relations between the backtest (modified after Hessing) and other behavioural tests and the intra-test consistency was low. Using factor analysis, they did find correlations between several behavioural traits, excluding the backtest. Three personality factors are suggested by these experiments: aggression, sociability and exploration (Forkman et al., 1995; Jensen, 1995; Jensen et al., 1995a; Jensen et al., 1995b).
The objectives of our studies are threefold. Firstly, we investigated if individual characteristics of pigs, measured with the backtest, are consistent. Secondly, we determined if these individual characteristics were related to sow, boar or gender. Thirdly, we studied the relationship between these individual characteristics and the performance of the pigs. If backtest results in pigs are related to performance, this would mean that a selection of the best performers can be made at an early age. Also, if backtest results are heritable and related to performance, a selection in sows can be made according to their own backtest results.

MATERIALS AND METHODS

Housing and animals

The studies were performed in farrowing and fattening units at the farm 'The Tolakker' of the University of Utrecht, The Netherlands. In two studies, 823 and 566 fattening pigs, born from Dutch Landrace * Great Yorkshire sows, were used. The piglets were born in conventional farrowing pens. A heating lamp and floor heating were used if necessary. An all in – all out system was used consequently.
Male pigs were castrated at approximately 5 days of age and tails of all pigs were docked, according to standard Dutch procedures. Iron was given to the piglets by injection at 3 days of age. At weaning, around 28 days of age, the sow was removed from the pen and the piglets remained in the same pen until 9 weeks of age. At that time, the fattening period started. All fattening pens had a partly slatted, concrete floor with floor-heating. Feed and water were supplied *ad libitum*. Pigs were slaughtered at approximately 110 kilogrammes liveweight (around 6 months of age).

Backtest

A backtest was performed as described by (Hessing et al., 1993). In this backtest each piglet was put on its back and restrained in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. Each series of

wriggles that the piglet made without a pause was counted as one escape attempt. The total number of escape attempts is called ‘backtest score’.
In the first of our studies, all piglets were tested twice during the suckling period, at 10 and 17 days of age. In the second study, piglets were tested at 3, 10 and 17 days of age.

Data

In the first study, 823 pigs were followed from birth until slaughter. The following performance parameters were recorded for each pig: weight at birth, at weaning, at approximately 9 weeks and at slaughter, lean meat percentage at slaughter and carcass grading. Lean meat was calculated using muscle- and backfat thickness, measured on carcasses with the Henessy Grading Probe (HGP) (Engel and Walstra, 1993). Live weight just before slaughter was calculated as slaughtered weight * ((83-slaughtered weight)*0.0025) + 1,3). Gender, sow and boar identity were recorded. Carcass grading (meatiness) in the slaughterhouse was recorded using the SEUROP-system. With this system, carcasses are graded according to the muscularity of the slaughtered pig: S=Superior, E=Excellent, U=Very good, R=Good, O=Moderate and P=Poor.
In the second study, data from 566 piglets were collected until weaning. Weight at birth and at weaning were recorded, as well as gender and health of the piglet. Sow identity, sow reaction to piglet removal and medical treatments of the sow were recorded. Sows were backtested before the start of the study, at the age of 10 and 17 days. Sows were divided into three backtest score groups, based on the outcome of two backtests, according to Hessing (Hessing et al., 1993)

* An animal was classified Low Resisting (LR) when the total number of escape attempts was less than 4, with a maximum of 2 attempts per test (for example, 0+0 or 1+2)
* An animal was classified High Resisting (HR) when the total number of escape attempts was more than 4, with a minimum of 2 attempts per test (for example, 2+3 or 4+4)
* The other animals were classified Intermediate (I) (for example, 0+3 or 2+2)

The reaction of the sow to piglet removal was scored as 'quiet' or 'aggressive'. A sow was 'aggressive' when she reacted hostile (e.g. making noise, jumping up) when one of her piglets was taken out of the pen for testing. A sow that did not react was classified as ‘quiet’.

Statistical methods

Consistency of backtest scores

The Pearson correlation coefficient is used to determine the consistency of backtest scores in the successive tests.

Backtest scores and related factors

Relations between factors such as birth weight, gender, boar and sow, and backtest scores were calculated for both studies. Also the effect of test day was calculated.

First study

Preliminary analysis showed no effect of the different pens, so this variable is not used in the models.
To determine the effect of test day (10 or 17 days of age) on backtest score per test (y), the following model was used:

$$\mathbf{y_{ij} = \mu + piglet\ number_i + test\ day_j + \varepsilon_{ij}}$$

Since there was no effect of test day in the first study, the rest of the models were not corrected for test day and the mean number of escape attempts in the two tests was calculated for each animal.

To determine the effect of AI or natural service and the effect of boar number on backtest scores, the mean backtest score per litter was calculated (y). This means that first the mean score per animal was calculated (from the results of the two tests) and these figures were used to calculate the mean score per litter. The following models were used:

$\mathbf{y_i = \mu + AI_i + \varepsilon_i}$

$\mathbf{y_i = \mu + boar\ number_i + \varepsilon_i}$

To determine the effect of gender, mean backtest score per animal (from two tests) (y) was calculated and the following model was used:

$\mathbf{y_{ij} = \mu + litter_i + gender_j + \varepsilon_{ij}}$

Second study

To determine the effect of litter, gender and test day (3, 10 or 17 days of age) on backtest score per test (y) , the following model was used:

$\mathbf{y_{ijkl} = \mu + litter_i + gender_j + \varepsilon_{1:ijk} + test\ day_l + \varepsilon_{2:ijkl}}$

in which 'litter' is the combined effect of sow and boar (each sow is used only once), ε_1 is the errorterm of litter and gender nested within piglet number and ε_2 is the errorterm of the total model.

The relation between sow reaction, sow health and sow backtest scores were calculated using logistic regression. Since sow reaction and sow health were related, we used only sow reaction and sow backtest scores in the final models.
To determine the effect of sow reaction and sow backtest score on backtest score of the piglets, mean backtest score per litter was calculated. This means that first the mean backtest score per animal was calculated (from the results of the three tests), and these figures were used to calculate the mean score per litter. The following model was used:

$\mathbf{y_{ij} = \mu + sow\ reaction_i + sow\ backtest\ score_j + \varepsilon_{ij}}$

Relations between backtest scores and production parameters

For the *first study*, relations between production parameters (lean meat percentage, carcass grading and daily weight gain) and backtest scores were calculated. Since no day-effect was found, the mean number of escape attempts (mean of two tests) per animal is used. To determine the effect of mean backtest score on daily weight gain (y), the following model was used:

$\mathbf{y_{ijkl} = \mu + litter_i + weight_j + gender_k + backtest\ score_l + \varepsilon_{ijkl}}$

in which 'y' is daily weight gain in the suckling, growing or fattening period and 'weight' is birth weight, weight at weining or weight at the start of the fattening period.

To determine the effect of mean backtest score on meat percentage and carcass grading (y), the following model was used:

$\mathbf{y_{ijkl} = \mu + litter_i + weight_j + gender_k + backtest\ score_l + \varepsilon_{ijkl}}$

in which 'y' is lean meat percentage or carcass grading and 'weight' is birth weight, weight at weaning or weight at the start of the fattening period.

General

For all models the Shapiro-Wilk statistic (W) was calculated as a statistical test for normality and the skewness and kurtosis of the rest value of the models were determined. All calculations were performed using the statistical package SAS (SAS_Institute_Inc., 1989). P-values<0,05 were considered to be significant.

RESULTS

General

The rest values of all models had a normal distribution, so no transformation of y-values were necessary. Skewness and kurtosis was between -2 and 2 and the Shapiro-Wilk statistic was not significant.

Backtest

In the first study, a backtest was performed on day 10 for 832 piglets and on day 17 for 825 piglets. The average number of escape attempts on day 10 was 1.89 (range 0-7), on day 17 it was 2.01 (range 0-8). In the second study, the backtest was done three times with 566 piglets. The average number of escape attempts was 3.20 (range 0-12) on day 3, 2.57 (range 0-8) on day 10 and 2.60 (range 0-10) on day 17. The number of escape attempts for each test had a unimodal distribution (Fig. 1a, 1b).

In the first study, the correlation between the successive backtest results (day 10–day 17) was 0.42 . In the second study, the correlations were 0.41 (day 3–day 10), 0.39 (day 3–day 17) and 0.47 (day 10–day 17). All P-values were 0.0001.

A day-effect was found in the second study for backtest scores between the test on day 3 and the test on day 10 or 17 (P<0.0005). This was caused by the high test scores in the first test, compared to the other two tests. The mean level of escape attempts was higher on day 3 than on day 10 or 17. In both studies, no day-effect was found between the tests on day 10 and 17 (P=0.49) (Fig. 2, 3).

Figure 1a: *Percentage of animals for each backtest score (first study)*

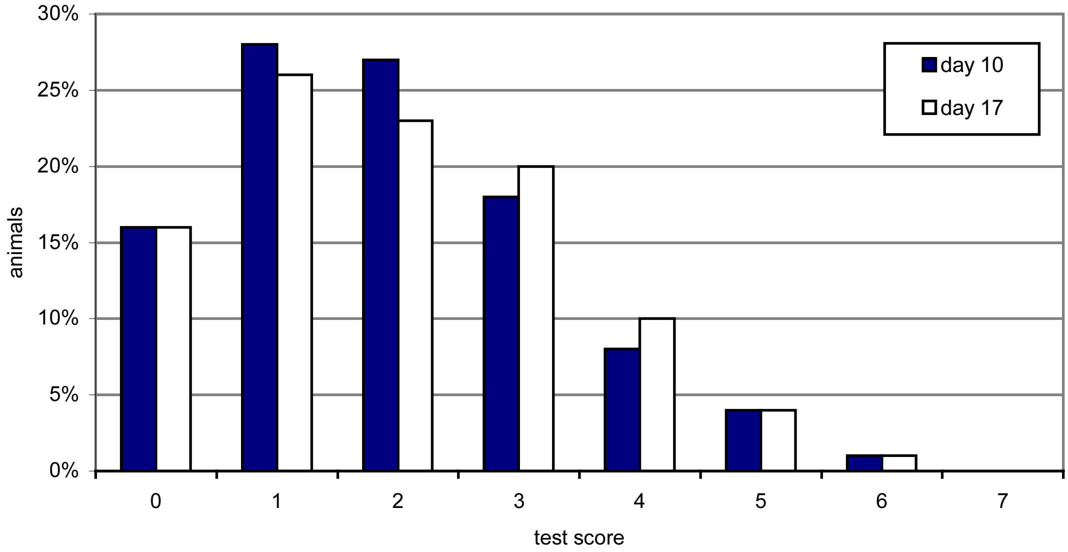
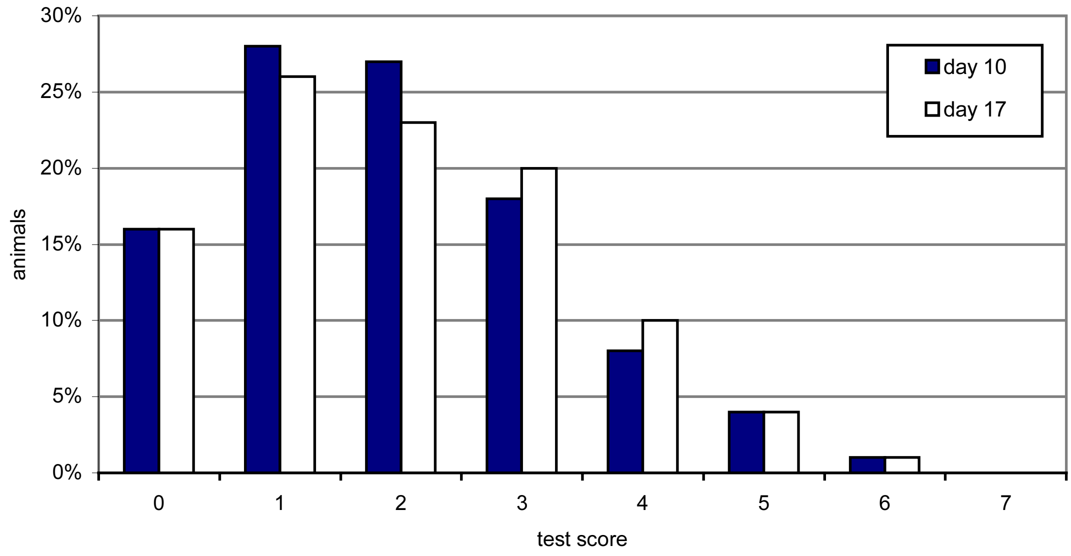

30%
25%
20%
15%
10%
5%
0%
animals
0
1
2
3
4
5
6
7
test score
day 10
day 17

***Figure 1b:** Percentage of animals for each backtest score (second study)*



Parameters related to the backtest scores

In both studies, female piglets showed a higher number of escape attempts than castrated males (Fig. 2, 3). No interactions between variables (such as day*gender) were found.
In the first study, relations were found between backtest scores and litter (P<0.001), gender (P<0.0005) and AI (P<0.05) (Fig. 2). Piglets born from natural serving boars had higher backtest scores than piglets born from AI. No relations were found with weight, boar number or pen number.
In the second study, the backtest score per piglet was related to litter (P<0.01), sow backtest type (P<0.01) and gender (P<0.01). Sows with lower backtest scores when they were piglets, had offspring with lower backtest scores. A trend was found between backtest score of the piglet and sow reaction (P<0.10) (Fig. 3). No relation was found with illness of the piglets. When the data were analyzed per test day, a small gender effect was observed at 3 days of age, which was not significant. At 10 days the difference was larger but was still not significant, while at 17 days the difference was significant.

Figure 2: *Backtest scores and related parameters (first study): effect of test day, AI and gender.*

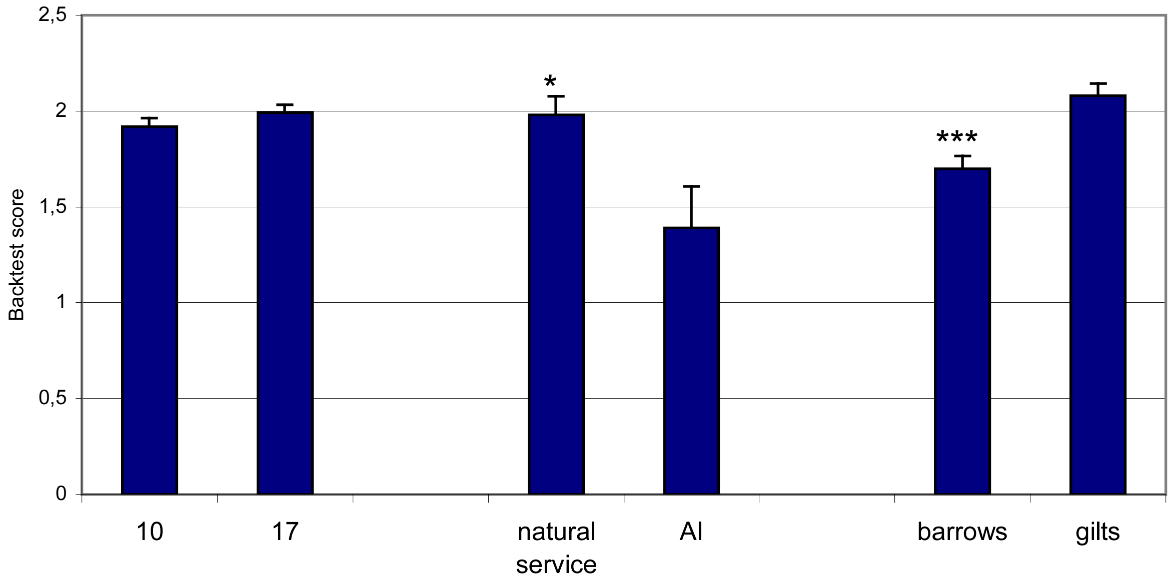
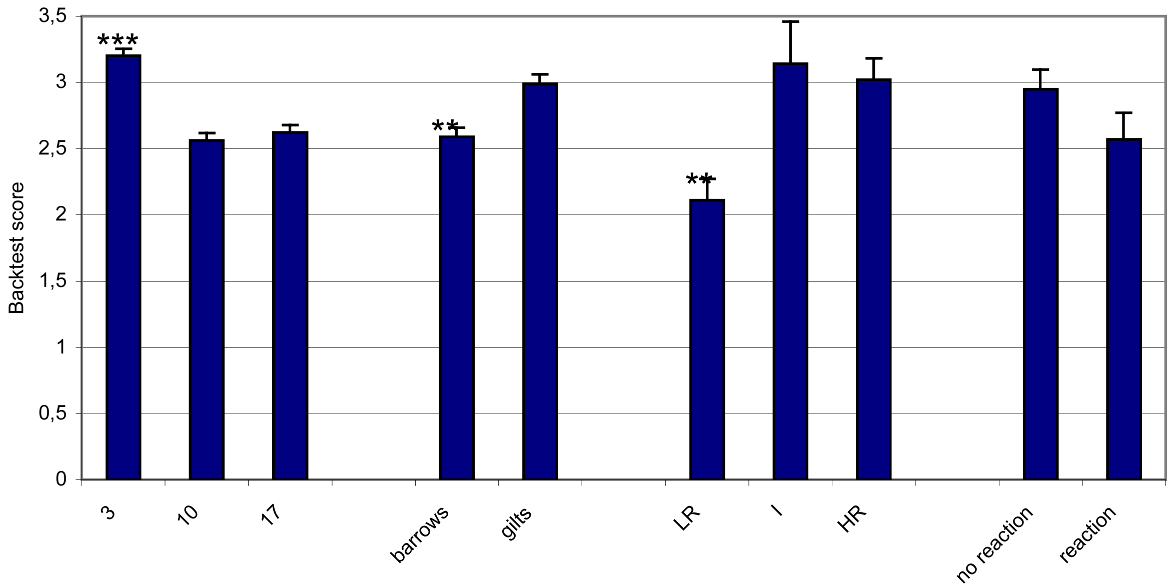


Figure 3: *Backtest scores and related parameters (second study): effect of test day, gender, sow backtest type and sow reaction.*



Performance

Due to selling of fattening pigs at 10 weeks of age or missing data, performance data were collected from 747 of the 823 animals in the first study. The results are shown in Table 1. The correlation between lean meat percentage and carcass grading was high (r=0.91;P<0.01).

Table 1: *Production performance of pigs in the first study (n=747) end estimates of backtest scores on performance parameters*

	MEAN	SEM	N	ESTIMATE OF BACKTEST	P-VALUE
WEIGHT (KG)					
AT BIRTH	1.474	0.312	747	-0.19	0.25
AT WEANING	8.6	1.76	747	-0.06	0.05
AT 9 WEEKS	24.3	4.442	746	-0.02	0.08
AT SLAUGHTER	113.1	6.565	747	0.008	0.27
DAILY WEIGHT GAIN (KG/DAY)					
SUCKLING PERIOD	0.265	0.056	747	-0.002	0.14
WEANING PERIOD	0.442	0.098	746	-0.002	0.47
FATTENING PERIOD	0.804	0.088	746	0.001	0.63
LEAN MEAT%	54.03	3.42	740	0.33	<0.01

Relations between backtest scores and production parameters

In the first study, performance data were gathered. Since no day-effect was found in both studies between the backtests on 10 and 17 days, in the following calculations the mean backtest score for these two ages per piglet was used.

No relations were found between daily weight gain in the suckling, weaning or fattening period and backtest scores, or between backtest scores and weight (Table 1).

Backtest score and lean meat percentage at the slaughterhouse were related positively ($P<0.01$) just as backtest scores and carcass grading at the slaughterhouse ($P<0.05$), corrected for gender, sow identity, AI and weight. Piglets with higher backtest scores had a higher carcass grading and a higher lean meat percentage at slaughter (Fig. 4, 5).

Figure 4: *Relation between backtest score and carcass grading.*

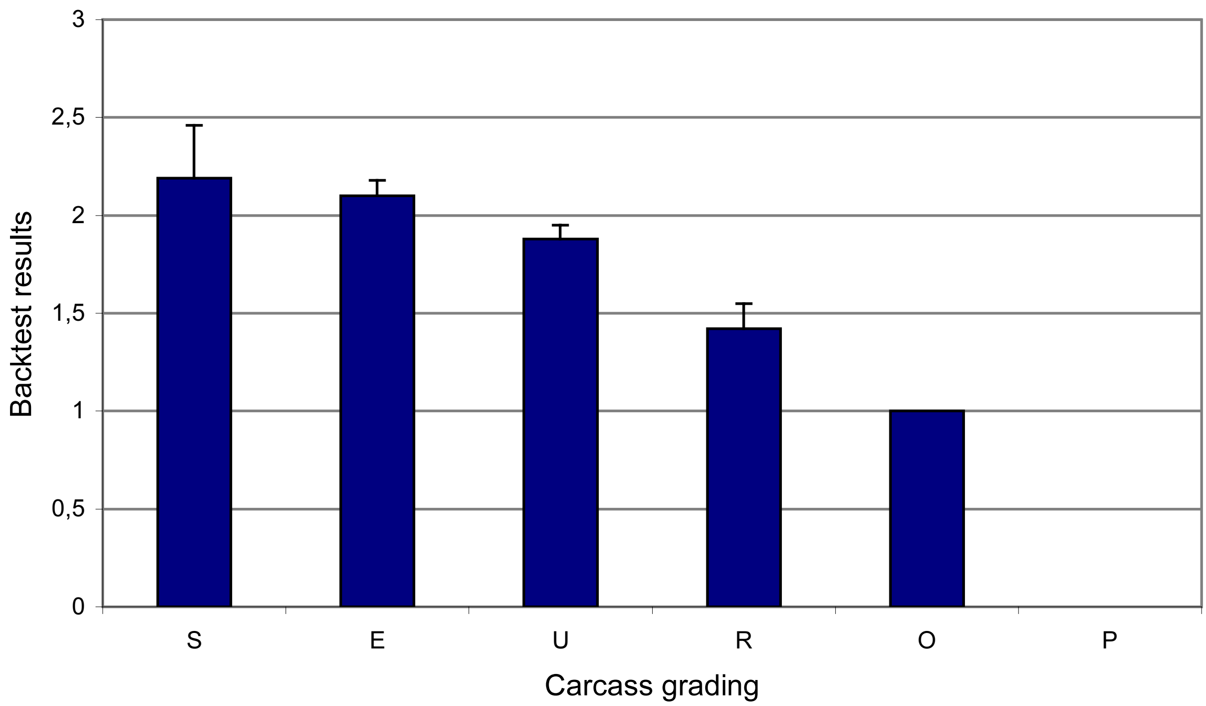
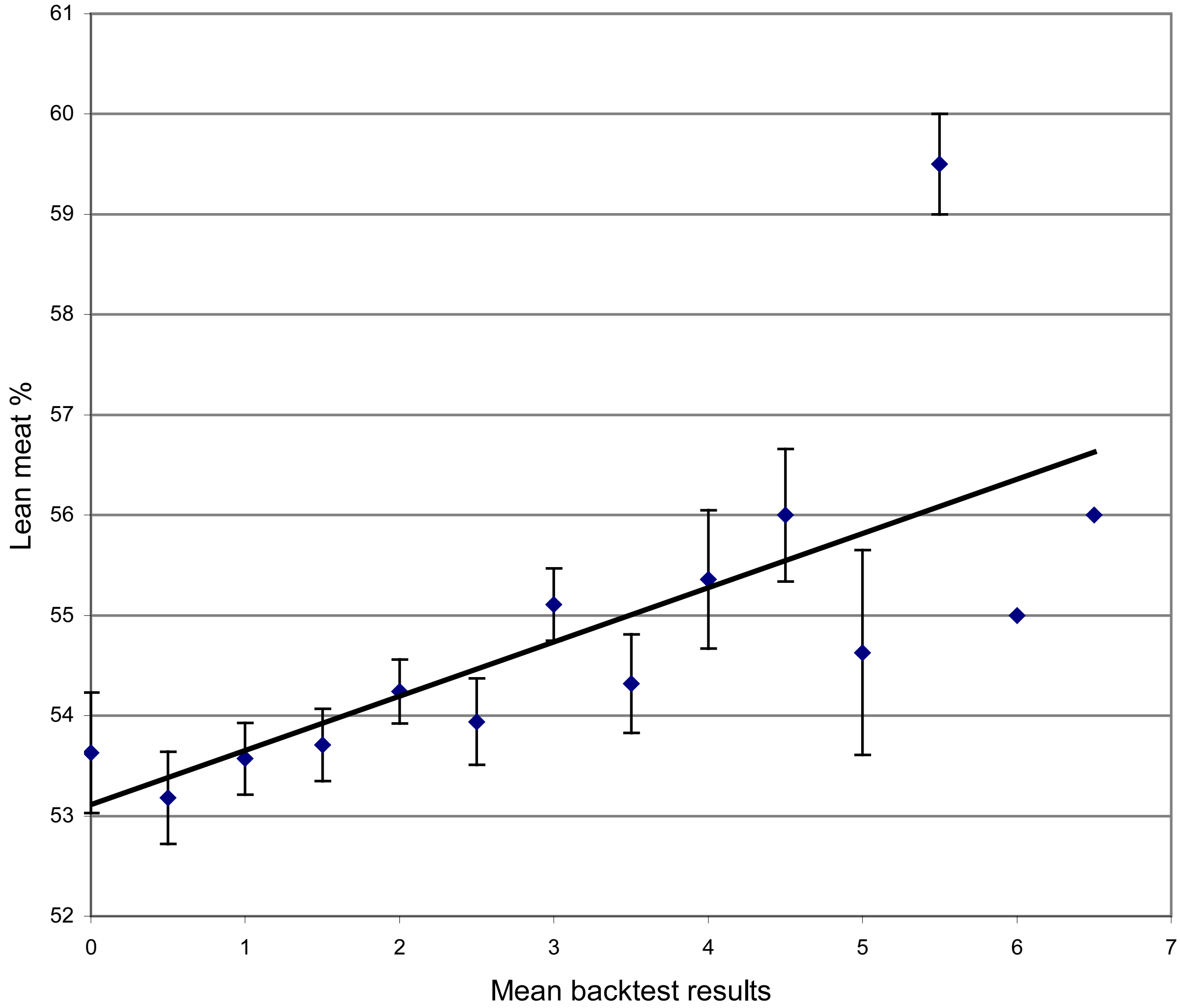


Figure 5: *Relation between backtest score and lean met percentage.*



Discussion

Backtest

The distribution of backtest scores and the number of escape attempts (range 0 to 8) were comparable to Hessing's results (Hessing et al., 1993). In his experiments, pigs were divided into active and passive animals according to two backtests, but pigs with intermediate results were disregarded. This can give the impression that there are only two different types of pigs, active and passive, which is not the case. The distribution is unimodal, as can be seen in Fig. 1a and 1b. In our report, backtest scores of all animals were used and animals were not divided into categories, with an exception for the sow backtest scores since the original backtest scores for those animals were not available.

Jensen (Jensen, 1995) stated that when the distribution of backtest types does not deviate from a normal distribution, there is no evidence for distinct individual types of piglets. In some lines of rodents, the distribution of coping behaviour (measured with several behavioural tests such as open field tests) is bimodal, and therefore it is stated that there are two different coping strategies. However, in rodents a diversifying selection has taken place in almost every

population, either natural (wild populations) or artificial (in the laboratory populations, e.g. SAL and LAL mice). By selecting for aggression or nest-building behaviour, a bimodal distribution of (test) reactions developed. Selection for certain reactions might also have happened in wild populations, because different strategies (active or passive) may have been beneficial (Benus et al., 1991; Sluyter et al., 1995) In contrast to the rodent experiments, no direct artificial selection for behaviour has been performed in the pig population used. Indirectly, selection for a certain coping strategy might take place in domestic pigs if this strategy would somehow be correlated to high production or low disease incidence or mortality. Therefore, a bimodal distribution was not expected but a tendency for change in overall coping strategy would be possible. For example, if an active coping strategy would be more beneficial than a passive one, coping strategies of all animals would change over the years. If the backtest measures an aspect of this coping style, than higher backtest scores would have been found in the population. If selection for backtest types in pigs were possible, a bimodal distribution of backtest types might be created by active selection.
At 3 days of age, the animals resisted more in the backtest than on day 10 and 17. The higher scores at 3 days could well be caused by the relative naïveté of the piglets. At this early age, the animals are not used to seeing or hearing people, let alone to being handled by them. Between 3 and 10 days, many things happen to a piglet, such as handling, tail docking, an iron injection and, for male piglets, castration. In contrast, no major events happen between 10 and 17 days. It is possible that the (negative) experiences in the first week change the piglets reaction in the backtest. This is in line with findings of Erhard and co-workers (Erhard and Mendl, 1999), who found that handling of piglets before performing a tonic immobility (TI) test influenced test results. Piglets that he tested three times, showed an increasing susceptibility for TI. Furthermore, in the first days of life, piglets must fight in order to gain milk. In these few days, the teat order is established (Gonyou and Stookey, 1987). This might also explain the higher scores for all animals on day 3, while the piglets are fighting for their place at the udder and not on day 10 or 17, when the teat order has been established. This theory is in line with findings of Hessing and co-workers (Hessing et al., 1993), who found a positive relation between aggressive behaviour and backtest scores in piglets.

Relation between backtest scores and sow, AI and gender

Backtest scores of the piglets was related to the backtest type of the mother. This suggests a heritability of backtest scores. This is in line with findings in mice, where a heritability of several behavioural traits is found (Benus et al., 1991; Sluyter et al., 1995). Also, a litter effect was found, which might point to a maternal as well as a genetical effect. No relation with boar identity was found, probably due to the distribution of the data: for 20% of the sows AI was used, some natural serving boars were used for several sows while others were used for one sow only.
When AI and natural service were compared, a difference in backtest scores of the piglets was found. This could be caused by a difference in handling of the sow (AI or natural service), by a difference in sperm treatment (sperm is older and diluted) or by a difference in the two boar populations. From our point of view, the latter is the most probable cause for this difference. The natural serving boars used in the study are owned by and raised on the experimental farm, in their own breeding program, while the AI boars are owned by and raised on the AI-station. It seems that the boars of the AI-station produce offspring with lower backtest scores than the boars of the experimental farm.

There was a weak, but non-significant, relation between sow reaction and the piglets' test scores. Mothers who reacted fiercely when piglets were removed, had piglets that resisted less

in the backtests. Probably this ‘aggression’ of the sow is actually good motherly behaviour. Sows who defend their piglets, might have piglets that feel they do not have to deal with a stressor themselves (their mother will do that for them) and those piglets might therefore be less resistant. They use a passive coping strategy in the backtests. Piglets with unattentive mothers might feel ‘left alone’ and therefore try to fight the stressor, thus using an active coping strategy in the backtests. The reaction of the sow may also have influenced the backtest. The piglets are tested next to the farrowing pen so they can hear the sow but not see her. If this would influence the test, then in future studies the tests should be performed away from the sow. These relations suggest an effect of the environment (e.g. the sow) on backtest scores.
To distinguish between genetical influences and environmental effects, cross-foster studies should be performed.
No relation was found between backtest scores of the sow and reaction of the sow on piglet removal, but ill or medically treated sows reacted less when piglets were removed for testing. This means that the sows motherly behaviour is mainly influenced by her health state.

In both studies, backtest scores of piglets were related to gender. Castrated males resisted less than females. The gender effect became more apparent in the successive tests. However, the expected gender*day effect was not found.
In our study, piglets were not handled before the first test at day 3 , which showed no gender effect. After handling the piglets in the first week, the gender effect emerged in the second and third test. This is in line with findings by Erhard and co-workers (Erhard and Mendl, 1999) who reported that after handling, female piglets were more active in a tonic immobility test than males but found no gender effect in piglets that were not handled before the tests. Between day 3 and day 10, male piglets were castrated. If this castration caused the gender difference, is not clear. The gender difference is also found before castration at day 3, even though not significant. It is possible that castration and/or handling of piglets between day 3 and day 17 makes the gender differences more evident or that these differences develop during the first weeks of life. Another possibility is, that the effect of castration becomes more evident with time, due to the decreasing level of hormones (i.e. testosterone).

Relation of backtest scores with production parameters

The first study revealed that mean backtest scores were related to lean meat percentage and carcass grading of the slaughtered pigs. Animals that had high scores in the backtest had a higher lean meat percentage and a better carcass quality than pigs with lower scores. Lean meat percentage and carcass quality are highly related.
Feeding was *ad libitum* but only one animal could eat at the same time. If the active animals could get to the feeding place more often, then a higher daily weight gain would be expected in the HR animals, which our data did not show. Active animals (i.e. with high backtest scores) possibly used more energy in walking or fighting, and therefore became leaner. According to Hessing et al. (Hessing et al., 1993), a high backtest score is related to aggression in pigs. The relation between aggression and production in pigs is described by Hartsock et al. (Hartsock and Graves, 1976), who found that more fighting succes during the suckling period resulted in a higher protein percentage at slaughter. This might explain the relation between backtest type and lean meat percentage and carcass quality in the present study.

Consequences of selection

When backtest scores in pigs are related to lean meat percentage, this means that a selection of the best performers can be made at an early age by use of the backtest.

If backtest scores are heritable, an even earlier selection can be performed. However, selection for high backtest scores could lead to uniform pens with high resisting animals, which, according to Hessing et al. (Hessing et al., 1994b), results in less average daily weight gain.

However, in the practice of pig production, selection for lean meat percentage does take place directly, which might lead to higher backtest scores in the population. This hypothesis is supported by the fact that we found higher backtest scores in the second study, which took place about two years after the first one.

If, as according to Hessing et al. (Hessing et al., 1993), backtest scores and aggression are related, we are indirectly selecting for aggression in the population.

CONCLUSIONS

Although there was a consistency in the successive backtests, two different behavioural strategies in pigs could not be distinguished. In these studies backtest scores reflect a possibly heritable behavioural trait which might be influenced by the environment (e.g. the sow) and by previous experiences (e.g. handling). Relations exist between backtest scores and some production parameters.

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COPING CHARACTERISTICS AND PERFORMANCE IN FATTENING PIGS

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ABSTRACT

Pigs vary in their individual behaviour in response to stress, and this coping behaviour can be measured with a backtest. In this test, a piglet is put on its back and escape attempts are counted during one minute. Backtest results are linked to individual performance, and regrouping at 9 weeks of age on the basis of coping characteristics can influence production parameters . In the present study, piglets were cross-fostered at 3 days of age on the basis of backtest results, and pens with only active piglets (HR), with only reactive piglets (LR) and mixed pens were formed next to original litters. At 9 weeks animals were mixed and moved. The relation between backtest results and production performance was investigated. Cross-fostering induced a change in backtest results, resulting in approximately the same mean backtest results in each group and consequently on pen level no relations were found between initial coping behaviour and performance. Individual backtest results were linked to production parameters: active animals tended to grow faster and have a leaner carcass. If pig breeding companies would use the backtest to select the most active animals that are potentially the best performers, aggressive behaviour should also be considered (e.g. as part of the breeding goal), because of the link between activity and aggressive behaviour.

INTRODUCTION

Pigs vary consistently in individual coping behaviour. On one side of the scale, animals actively try to avoid a stressor, while at the other end animals tend to wait until the stressor disappears. The extremes of the population can be described in terms of active (HR) and reactive (LR). These extremes do not represent distinct categories of pigs, but different ways of coping are based on a differential and consistent use of various adaptation mechanisms (Koolhaas et al., 1999; Ruis et al., 2002). In pigs, coping behaviour can be measured with a backtest. In this test, a piglet is put on its back and restrained in this position for one minute while the number of escape attempts is counted (Hessing et al., 1993). Coping characteristics of pigs are linked to individual performance, such as lean meat percentage at slaughter (van Erp-van der Kooij et al., 2000). Regrouping at 9 weeks, based on coping characteristics, can influence production parameters such as daily weight gain and lean meat percentage (group means). When mixed pens were formed with active and reactive pigs (HR/LR), production results were better then in pens with HR or LR animals only (Hessing et al., 1994). Cross-fostering and mixing of animals is common practice in pig husbandry. Important factors for the animals in the processes of cross-fostering and mixing are age, dominance and individual behavioural characteristics. Cross-fostering and mixing can be ‘major life events’ for the pigs and may have profound negative effects for animal welfare as well as for production performance.

The present study investigated if coping characteristics in pigs, as determined with the backtest, could predict the individual performance of these pigs when they are cross-fostered at 3 days of age and regrouped at 9 weeks. If relations are found, the backtest can be used as a tool to accomplish optimal production results, and animal welfare might be enhanced.

MATERIALS AND METHODS

Housing and animals

The study was performed with 812 fattening pigs at the farm 'The Tolakker' of the University of Utrecht, The Netherlands. All genetic pig lines that were present on the farm, were used in the experiments: Dutch Landrace (DL), Great York (GY), F1 and F2 piglets.
Sows with piglets were housed in conventional farrowing pens (conventional housing system) or in multi-purpose pens (farrow to finish system, see next paragraph). Conventional farrowing crates were used, with a heating lamp and floor heating for the first 14 days *post partum*. The farrowing pen was 180*240 cm, with a partly slatted floor and a diagonally placed sow crate. The multi-purpose pens were 200*250 cm when used for sows and piglets and had a movable front barrier. These pens also had a partly slatted floor, and a diagonally placed sow crate that was removed at weaning. As of the second week, creep feeding was available for the piglets. At birth, piglets received an identification tattoo. At approximately 5 days of age an iron injection was given, tails were docked and male piglets were castrated. At weaning (28 days of age), the sow was removed from the pen and the piglets remained in the same pen. At 9 weeks of age, animals in the multi-purpose pens stayed in the same pen, which was then enlarged to 200*350 cm, while animals from the farrowing pens were moved to the fattening compartments. The fattening pens were 200*350 cm, with a partly slatted, concrete floor and floor-heating. Group size was approximately 10 pigs per pen. Each pen contained a nipple and a food trough, accessible for one pig. Feed (commercial pelleted dried diets) and water were supplied *ad libitum*. Pigs were slaughtered at approximately 110 kilograms liveweight (6 months of age).

Backtest and mixing procedure

A backtest was performed on all 812 piglets as described by Hessing et al. (Hessing et al., 1993), in the same way as in previous studies (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000). In this backtest, each piglet was put on its back and restrained in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. Each series of wriggles that the piglet made without a pause was counted as one escape attempt. The total number of escape attempts is called ‘backtest score’. A backtest was performed with every animal at 3, 10 and 17 days of age (±1 day).
On day three, cross-fostering was used to form groups of approximately 10 piglets per sow, on the basis of the first backtest. In HR pens only High Resisting piglets (with high backtest scores, >3) were placed, in LR pens only Low Resisting piglets (with low scores, <3) were placed, MISC (Miscellaneous) pens consisted of piglets with high, intermediate and low scores (mean backtest score appr. 3) and OR (Original) pens consisted of the original littermates only, independent of backtest score. The range of the group size was 6-13, with the extremes caused mostly by the original litters. At 9 weeks of age, 323 pigs from the farrowing compartments were moved to fattening units (conventional system, CONV) and of these animals the HR groups, MISC groups and LR groups were mixed within the group (HR+HR, MISC+MISC and LR+LR). 384 piglets in the multi-purpose compartments were neither mixed nor moved but stayed in the same pen until slaughter. This farrow-to-finish system is also called the specific-stress-free (SSF) housing system (Ekkel et al., 1996; Scheepens et al., 1990). The rest of the piglets either died before 9 weeks of age, or were sold at 9 weeks of age due to animal density restrictions in the Netherlands (0.7 m^2/pig)[(2)]. Pigs were chosen randomly to be removed at 9 weeks.

Measurements and procedures

Pigs were individually weighed at birth, after weaning and at 9 weeks of age. At weaning, animals received plastic eartags for identification purposes. Live weight at slaughter (LWS) was estimated from cold slaughter weight using the following formula from SIVA (SIVA, 1991):

LWS = (1.3 + ((83-SW)*0.0025))*SW

where SW=slaughter weight (kilograms).

Daily weight gain (DWG) was calculated from birth to weaning (suckling period), from weaning to 9 weeks (nursing period) and from 9 weeks to slaughter (fattening period). DWG for the fattening period was calculated as follows:

DWG = (LWS – W2)/LFP + (23-W1)*0.003

where W1=weight at weaning, W2=weight at 9 weeks and FP=length of the fattening period. Lean meat percentage at slaughter (%LM) was calculated using muscle- and backfat thickness, measured on carcasses with the Henessy Grading Probe (HGP) (Engel and Walstra, 1993).

Statistical methods

The statistical package SAS was used for all the calculations (SAS_Institute_Inc., 1989). To determine differences between mean backtest scores per group, the following linear mixed model was used (PROC MIXED), with farrowing pen number (unique) as random factor:

(a) Bt day 3 = μ **+ gender + W(b) + breed + group +** ε

(b) Bt day 10 = μ **+ Bt day 3 + gender + W(b) + breed + group +** ε

(c) Bt day 17 = μ **+ Bt day 3 + Bt day 10 + gender + W(b) + breed + group +** ε

in which Bt = the individual backtest score on day 3, 10 or 17, gender = the gender of the pig (barrow or gilt), W is the weight at birth (b), breed = the breed of the piglet (Dutch Landrace, Great York or a combination of these breeds) and group is HR (>3 escape attempts on day 3), LR (<3 escape attempts on day 3), MISC (mixed pens with high and low backtest scores) and OR (original litters).

To determine the relation between production parameters and backtest scores, the following linear mixed models were used (PROC MIXED), with the (unique) pen number as random factor:

(1) DWG(S) = μ **+ gender + W(b) + breed + Bt + crossf +** ε

(2) DWG(W) = μ **+ DWG(S) + gender + W(w) + breed + Bt + crossf +** ε

(3) DWG(F) = μ **+ DWG(S) + DWG(W) + backfat + muscle + houssys + gender + W(9w) + breed + Bt + crossf +** ε

(4) LM% = μ **+ W(9w) + W(s) + houssys + gender + breed + Bt + crossf +** ε

(5) BACKFAT = μ **+ W(9w) + W(s) + houssys + gender + breed + Bt + crossf +** ε

(6) MUSCLE = μ **+ W(9w) + W(s) + houssys + gender + breed + Bt + crossf +** ε

in which DWG = daily weight gain in the suckling (S), weaner (W) or fattening (F) period, W is the weight at birth (b), at weaning (w), at 9 weeks (9w) or at slaughter (s), crossf = cross-fostering of the piglet (yes/no), backfat and muscle are the backfat and muscle thickness in the carcass, and houssys is the housing system of the pigs (CONV=conventional, moving and mixing at 9 weeks; SSF= specific stress free, a farrow-to-finish production system).

RESULTS

All calculations were performed on individual pig level, unless mentioned otherwise.
Mean backtest scores were 3.26 ± 0.06 on day 3 (range 0-9, n=813), 2.76 ± 0.06 on day 10 (range 0-11, n=787) and 2.97 ± 0.07 on day 17 (range 0-11, n=785). No breed or gender differences in backtest results were found. At day 3, part of the piglets were cross-fostered, resulting in HR pens with the highest mean backtest scores per pen and LR pens with the lowest mean scores.

At day 10 and 17, backtest results in HR and LR pens had changed. In HR pens, the mean backtest score had decreased to the level of the OR and MISC pens, while in the LR pens the level had increased but was still below the level of the other pens (Table 1). In HR and LR pens, backtest scores between day 3 and day 10 were not correlated, in contrast to the results in MISC and OR pens. Correlations between backtest results on days 10 and 17 were significant in all groups.

Table 1: *Mean backtest (Bt) results (Least Squares Means ± SEM) on day 3, 10 and 17 in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR).*

	HR	MISC	LR	OR
BT DAY 3	5.16 ± 0.13^{a}	3.29 ± 0.13^{b}	1.70 ± 0.15^{c}	3.82 ± 0.14^{d}
BT DAY 10	2.95 ± 0.15^{a}	2.76 ± 0.10^{a}	2.37 ± 0.16^{b}	2.94 ± 0.13^{a}
BT DAY 17	3.18 ± 0.15^{a}	2.94 ± 0.10^{a}	2.84 ± 0.15^{a}	3.06 ± 0.13^{a}
N	167	283	155	178

Values differ significantly within rows if superscripts are different

The average daily weight gain in the fattening period was 874.2 ± 93.4 grams (n=669), with a %LM of 55.3 ± 2.74 (n=659)[1]. Mortality from 3 days to weaning was 4.7%, from weaning to 9 weeks 3.9% and in the fattening period it was 2.3%. Piglets that died before the first backtest were for obvious reasons excluded from the study.
The backfat and muscle thickness in the carcass depended on the breeding line used (Table 2).

Table 2: *Influences of genetic pig line on production parameters (Least Squares Means ± SEM); GY=Great York (sire line), DL=Dutch Landrace, F1=DL*GY_z (sow line), F2=F1*GY_s.*

	GY_s (N=61)	DL (N=10)	F1 (N=67)	F2 (N=418)
DWG 9 WEEKS-SLAUGHTER	$848.5\pm12.5^{**}$	$845.9\pm41.9^{**}$	$904.6\pm12.2^{**}$	$874.3\pm5.7^{**}$
LEAN MEAT%	$56.2\pm0.3^{***}$	$55.9\pm0.8^{***}$	$55.2\pm0.1^{***}$	$54.5\pm0.3^{***}$
BACKFAT THICKNESS	$16.9\pm0.4^{*}$	$17.2\pm1.1^{*}$	$18.4\pm0.4^{*}$	$17.7\pm0.2^{*}$
MUSCLE THICKNESS	$58.4\pm0.8^{***}$	$61.9\pm2.0^{***}$	$54.1\pm0.8^{***}$	$56.3\pm0.3^{***}$

[1] data on %LM was missing for 10 animals

These parameters were positively related to slaughter weight, and +1 mm backfat equalled –0.75% lean meat and +1 millimeter muscle equalled +0.14% lean meat. Gilts had a lower DWG in the fattening period, a higher %LM and lower amount of backfat than the castrated males (Table 3). DWG in the suckling period and in the fattening period were positively related (estimate 0.30, P<0.01). DWG in the suckling and in the nursing period were negatively related (estimate –0.81, P<0.0001) (Table 4).

Table 3: *Least Squares Means (LSMeans ± SEM) of production parameters for barrows and gilts*

	GILTS	BARROWS	P-VALUE
DWG (GRAMMES)			
SUCKLING PERIOD (BIRTH-WEANING)	251.7±7.4	248.8±7.6	<0.0001
WEANER PERIOD (WEANING-9 WEEKS)	422.9±15.9	440.3±16.3	<0.05
FATTENING PERIOD (9 WEEKS-SLAUGHTER)	850.1±12.2	892.8±12.5	<0.0001
LEAN MEAT%	55.9±0.3	53.8±0.3	<0.0001
BACKFAT THICKNESS	16.9±0.4	19.4±0.4	<0.0001
MUSCLE THICKNESS	56.0±0.7	55.0±0.7	<0.0001

***Table 4:** Solution for fixed effects of weight and backtest scores (Bt) at 3, 10 and 17 days (estimates).*

	WEIGHT	DWG SUCKLING PERIOD	DWG WEANER PERIOD	BT DAY 3	BT DAY 10	BT DAY 17	MEAN (BT DAY10 +DAY17)
DWG (GRAMMES)							
SUCKLING PERIOD	+0.08^{***a}			+0.19	-3.42**	+0.77	-2.31^{1}
WEANER PERIOD	+57.2^{***b}	-1.31***		-0.13	-2.75	+0.93	-1.62
FATTENING PERIOD	+4.44^{***c}	+0.30**	+0.04	-1.35	+0.38	+3.93*	+3.92^{1}
LEAN MEAT%	-0.08^{***d}			+0.00	+0.11^{1}	-0.01	+0.03
BACKFAT THICKNESS	+0.17^{***d}			+0.00	-0.16^{1}	+0.00	-0.02
MUSCLE THICKNESS	+0.26^{***d}			-0.31*	+0.17	-0.12	+0.16

^{1}P<0.10; ^{a}birth weight; ^{b}weaning weight; ^{c}weight at 9 weeks; ^{d}slaughter weight

Cross-fostering had no effect on daily weight gain or on carcass composition. Animals that were mixed and moved at 9 weeks showed a lower amount of muscle in the carcass than animals that stayed in the same pen (SSF-system). No differences in daily weight gain, backfat or %LM were found between these animals and the ones from the SSF-system (no mixing or moving) (Table 5).

***Table 5:** Effect of mixing and moving on production performance: the SSF-system (farrow-to-finish) versus a conventional system (mixing and moving at 9 weeks). DWG(f) = daily weight gain in the fattening period (25-110 kilogrammes).*

LS Means	conventional system	SSF system	P-value
DWG (f)	869.1 ± 12.0	865.9 ± 11.2	0.16
Lean meat%	54.9 ± 0.30	54.7 ± 0.28	0.40
Backfat thickness	18.0 ± 0.41	18.4 ± 0.36	0.30
Muscle thickness	54.5 ± 0.77	56.1 ± 0.66	<0.005

No differences in production results were found on pen level between the groups (HR, LR, MISC and OR). On individual level, muscle content of the carcass was negatively related to the backtest score at day 3. DWG in the suckling period was positively related to the backtest score at day 10, and in the fattening period DWG was positively related to the backtest score at day 17. Backtest score at day 10 tended to be positively related to %LM and negatively related to mm backfat. (Table 4).

DISCUSSION

Production parameters

Most relations found between production parameters and with gender were to be expected. Barrows grow faster than gilts. When pigs become heavier, the backfat and muscle content of the body will also increase, but the %LM will be lower. Fast growing animals are sometimes inclined to put on more fat than normal growing animals, especially barrows. However, in the present study this relation was not observed, although daily weight gain in the fattening period was high (874 grammes/day) (Bittante, 1983; Bruwe et al., 1991).
The lower %LM but higher muscle content of the carcass in the SSF-system as compared to the conventional system is a contradiction. In contrast to findings of Ekkel et al. (Ekkel et al., 1996) differences in DWG were absent in the present study. We can therefore not conclude that animals in the SSF-system produced better than in the conventional system where piglets were moved and mixed one time at approximately 9 weeks of age.

DWG in the suckling period was positively related to DWG in the fattening period, but negatively related to DWG in the weaner period. Furthermore, DWG in the weaner period was not related to DWG in the fattening period. This might be an effect of compensatory growth (Lovatto et al., 2000): piglets that had a low-producing teat, will make up for the growth losses by growing extra fast in the nursing period. Another possible explanation might be that piglets who had easy access to the teat, which would be the animals with the highest DWG in the suckling period, adapted slower to the solid pig feed and the post-weaning situation than the animals who received less milk from the sow. See also the next paragraph.
No relations were found between cross-fostering and production performance. Horrell found an effect of cross-fostering on production performance in 1-week old pigs (Horrell and Bennett, 1981), while in our study piglets were cross-fostered at a maximum age of 4 days. According to Petersen et al. (Petersen et al., 1989), the attachment between sow and piglets is established between day 2 and day 4 post-partum. Probably up to 4 days of age cross-fostering is possible, while at a later age problems will occur.

Backtest results and production parameters

Mean and range of backtest results were comparable to earlier studies (van Erp-van der Kooij et al., 2001).
On pen level, no differences in production were observed. This is not surprising, since at 10 days of age no differences in mean coping responses per pen were found anymore although they differed originally, based on the regrouping at 3 days of age. Therefore, it is not possible to fully compare the present study with Hessing's results (Hessing et al., 1994). He reported differences in production results depending on group composition. Piglets of 9 weeks of age were allocated to different groups, depending on backtest scores. Animals in his mixed pens (with both high and low backtest scores) produced better than pigs in the HR (only high backtest scores) or LR (only low backtest scores) pens. The animals in this experiment apparently did not change their coping style. It seems, as if between 3 and 10 days the coping behaviour of the piglets is still flexible and animals can change their behaviour according to the social environment, while at 9 weeks the coping characteristics of the individual animals are much more stable and mixing will have a higher impact on the animal.

On individual animal level, relations were found between backtest and production. Animals with high backtest scores had less mm muscle in the carcass, a lower DWG in the suckling period, and a higher DWG from 9 weeks until slaughter. The lower DWG in the suckling period is in contrast with results from Ruis (Ruis et al., 2000), who found that HR pigs (backtest result >4 at day 3) were heavier than LR pigs (backtest result <3) at 4 weeks of age. Furthermore, the HR pigs tended to have a higher %LM and less mm backfat in the carcass at slaughter. The leaner carcass is in accordance with an earlier study, in which a positive relation was found between backtest results and %LM at slaughter, but not DWG (van Erp-van der Kooij et al., 2000).
The fact that in the present study most relations are not statistically significant may be due to the changing of backtest scores in the cross-fostered groups and to the smaller number of animals per group compared to earlier studies. However, all results point to a tendency of HR animals to grow faster and have a leaner carcass. This would also mean that due to selection on %LM in the carcass, which has been done over the past decades, selection has also been performed on active behaviour. Since activity and aggression are linked, pigs might become more active and thus also more aggressive over the years (Hessing et al., 1993; van Erp-van der Kooij et al., 2000).
Animals with high backtest responses had a low DWG in the suckling period and a high DWG in the fattening period. These animals could be the ones who spend the most time in

active, exploratory and/or aggressive behaviours during the suckling period, thus losing energy and losing valuable time for drinking milk, resulting in a low DWG in the suckling period. After weaning, the lack of consistency in growth rate might be explained with the theory of optimal foraging (Jensen and Recén, 1989). This theory states that weaning is caused by the piglets, because it is at some point more beneficial to eat solid food than to suckle. HR piglets might be less sensitive to environmental stimuli such as the presence of solid food in the farrowing pen, consequently eat less solid food during the suckling period, and have more problems due to the weaning process. Later they would catch up, which may lead to a higher DWG in the fattening period. In the fattening period, the aggressive behaviour of the active pigs may be advantageous, because only one feeding place was available per pen of 10 pigs and although food is provided *ad libitum*, aggressive or dominant pigs might get more food than the more timid penmates. However, Ruis et al (Ruis et al., 2002) found that in mixed HR/LR pairs of gilts there was an almost equal number of LR and HR dominants (11:12).
Eating can occur continuously in the fattening pen, in contrast to the farrowing pen, where time per suckling bout is limited and fighting can result in a partially missed bout.
Another explanation might be that animals with high backtest scores have a higher activity level, requiring more energy. With *ad lib* feeding (in the fattening period) this can lead to a leaner carcass for more active pigs, with the same growth rate. When the food source is limited (in the suckling period), this might lead to less daily weight gain for the more active piglets. In future research, it would be interesting to record individual feed intake and feed efficiency in pigs with known backtest results.

Practical implications

From the results of the present study, one might conclude that farmers should backtest their piglets and use activity as a selection criterion, as the more active pigs will probably become the best performers. However, if piglets are regrouped at an early age according to coping style, the behaviour might change and the coping response of some of the active piglets might decrease. Furthermore, it has been reported that mixing at a later age (e.g. 9 weeks) according to backtest responses will result in worse production results due to unstable groups. Pig breeding companies might use the backtest to select the most active piglets, because they might also be the best producers. The risk of this strategy, however, is that this selection process will also favour the most aggressive pigs. This will cause problems when pigs are mixed at 4-5 weeks of age, or maybe even throughout the pig's life, since they are kept in groups. On the other hand, pig breeders should realise that they are not only breeding for performance, but that behaviour should also be involved in the selection process. If pigs are becoming too aggressive, maybe behavioural traits should be in the breeding goal. The backtest might be useful for that purpose. The animals with extremely high backtest scores could then be eliminated from the breeding process, or only used in a farrow-to-finish production system where animals are not mixed.
Cross-fostering at a young age (<5 days) on the basis of coping characteristics had no negative consequences for production of the pigs. It seems that young piglets have no problems adapting to a new social environment. Under freerange condictions, piglets join the social group at week 2 post-partum. Interaction around that time are peaceful and no vigorous fighting is seen (Petersen et al., 1989). This means that if one wants to reallocate pigs, cross-fostering at 3 days of age or mixing around 2 weeks of age is a much better method than mixing at a later age.

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SOCIAL STRESS, COPING BEHAVIOUR AND IMMUNITY IN PIGS

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Submitted

ABSTRACT

From 173 piglets coping behaviour in the backtest was recorded at 3, 10 and 17 days of age. Results from the backtest range from active (many escape attempts) to reactive (low number of escape attempts). Based on earlier studies, classification of coping behaviour in the backtest might result in different immune responsiveness and thus disease resistance. Active animals might be prone to T_{H1} responses enhancing PHA test results, while reactive animals might be prone to T_{H2} responses, enhancing humoral immune responses. We measured cell-mediated immune reactivity in vivo to PHA, and antibody responses to KLH and ADV. A PHA skin test was performed after weaning at 4 weeks of age, and after mixing at 9 weeks of age. Maternal antibody levels to ADV were determined in suckling pigs, and antibody responses to ADV vaccination and to KLH immunization were determined at 10-14 weeks of age. In addition, the relation between coping behaviour and susceptibility to salmonella was determined at 6 months of age. Salivary cortisol levels were measured after weaning at 4 weeks of age and after mixing at 9 weeks of age.

We found no relation between antibody responses to KLH and ADV and coping behaviour, and no relation between coping behaviour and PHA responsiveness. However, social stress enhanced PHA responsiveness. Susceptibility to Salmonella was related to mixing and high cortisol levels, and active coping behaviour was associated with a lower risk of infection with Salmonella.

We conclude that effects of coping behaviour in the backtest on immune responsiveness probably depend on the environment in which the immune responses are determined.

INTRODUCTION

To maintain homeostasis, vertebrates react to changes in the internal and external milieu with a chain of behavioural, physiological, neuroendocrine and immunological responses (Ader et al., 1991). Variation in reactions between individual animals was shown (Dantzer and Mormede, 1983). However, insight in the mechanisms underlying these individual differences is limited. In pigs, individual differences in coping with stress was measured with a backtest (Hessing et al., 1993; van Erp-van der Kooij et al., 2000). In this test, a young piglet (<4 weeks of age) is put on its back and held in this supine position for 1 minute, while the number of escape attempts (bout of wriggles) is counted. The reactions range from active responses to avoid a stressor to a more passive ‘waiting until the stressor ends’ or ‘trying to live with it’ (reactive response).

Hessing (Hessing et al., 1995) demonstrated a relation between immune reactivity of pigs and their backtest response. They found that in active pigs (high backtest scores) cellular immune responses were higher than in reactive pigs (low backtest scores), which was reduced, however, after stress. On the other hand, humoral immunity was highest in the reactive pigs, as confirmed by others (Bolhuis et al., 2000; Schrama et al., 1997).

Cortisol and other neuroendocrine components of the stress response affect the immune system. In pigs, an increase in cortisol concentration was shown to suppress cell-mediated immune events, e.g. proliferation of lymphocytes to mitogens, natural killer cell activity and neutrophilic chemotaxis (de Groot et al., 2001; Westley and Kelley, 1984). PHA-induced skin swelling was suppressed after weaning (Hessing et al., 1995) and after mixing of unfamiliar pigs (Ekkel et al., 1995a; Moore et al., 1994). However, acute stress can also enhance cell-mediated immune responses (Dhabhar, 2002). Weaning and mixing can be considered as both acute and chronic stressors, because of short and long term effects (Ekkel, 1997; Ekkel et al., 1995b; Jensen and Recén, 1989; Mason et al., 2003; Scheepens et al., 1990).

Measurement of one specific immune parameter at one specific time-point after stress is not representative of the functional immune status of the animal. For example, anti-viral immune responses are restored to control levels soon after stress-induced suppression. Therefore, effects of stress on the immune system should be measured at several moments after stress. Several immune parameters should be taken into account, to extrapolate the results towards a functional immune status of the animal (de Groot et al., 2001; de Groot et al., 1999) and susceptibility for infection. To determine relations between coping behaviour in the backtest, stress responses, and immunological responses in individual pigs, in the present study different immune parameters were studied in slaughter pigs which differed in their backtest responses, and were housed in different housing conditions: the PHA skin test (in vivo cell-mediated response), specific antibody responses to KLH and Aujeszky's Disease Virus (ADV) after vaccination at several time points, and antibodies against naturally occurring Salmonella and Porcine Influenza virus. Cortisol responses after weaning at 4 weeks, and after mixing at 9 weeks of age were measured in saliva, to assess individual physiological reactions to these stressors.

MATERIALS AND METHODS

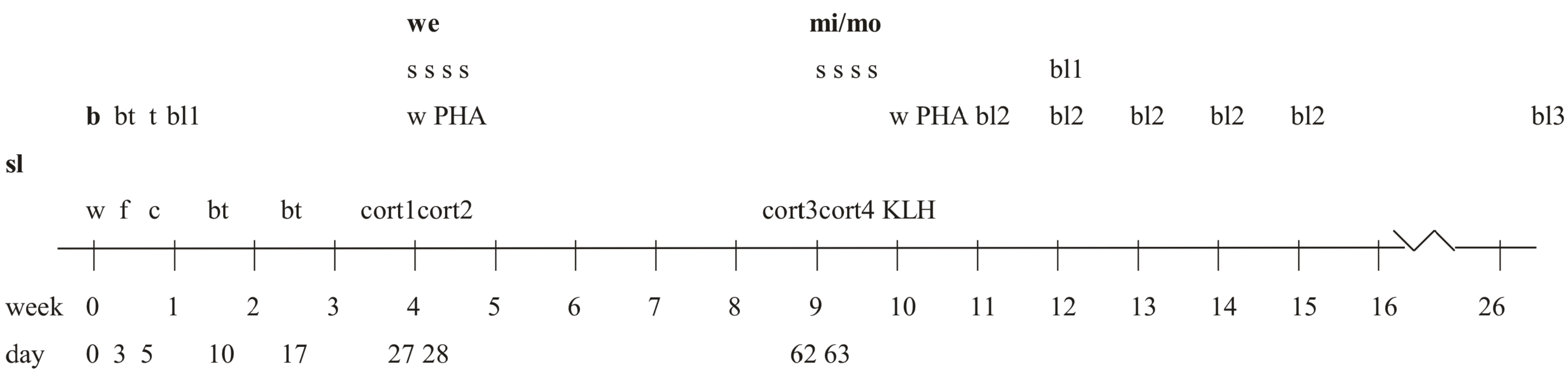
An overview of the experiment is given in Figure 1.

Housing and animals

The study was performed with 173 fattening pigs from birth until slaughter. They were born from Dutch Landrace (DL) * Great York (GY) sows housed with 710 other piglets (2 test pigs + 8 other pigs per pen) in farrowing and fattening units at the experimental farm of the University of Utrecht, The Netherlands. Gender and pig line of each piglet were recorded. Conventional farrowing crates were used, with a heating lamp and floor heating for the first 14 days post partum. From the second week, creep feeding was available for the piglets. Piglets were identified with an ear tattoo. At 5 days of age an iron injection was given, tails were docked and male piglets were castrated. At weaning at 28 days of age, the sow was removed from the pen but the piglets remained in the same pen, and received plastic ear tags for identification purposes. Pigs were weighed at birth, after weaning, and at 9 weeks of age. At 9 weeks of age, animals either stayed in the same pen, or were moved to the fattening compartments, with a partly slatted, concrete floor with floor-heating. Feed and water were supplied *ad lib.* Pigs were slaughtered at approximately 6 months of age, and approximately 110 kilograms live weight.

Backtest and mixing procedures

A backtest was performed on 3, 10 and 17 days of age (plus or minus 1 day) on all piglets (test pigs and littermates) as described; (Hessing et al., 1993); (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000; van Erp-van der Kooij et al., 2002). In brief, each piglet was put on its back and restrained in this supine position for one minute. One hand was placed loosely over the head of the pig, the other was placed loosely on the hind legs. Each series of wriggles that the piglet made without a pause was counted as one escape attempt. The total number of escape attempts was called 'backtest score'.
On 3 days of age, cross-fostering of some of the piglets was performed on the basis of the first backtest, in order to create pens (HR) with only High Resisting piglets (backtest scores >3), pens (LR) with only Low Resisting piglets (backtest scores <3), pens (MISC) with piglets with active, intermediate and reactive piglets (miscellaneous backtest scores) and pens (OR) with original littermates only, independent of backtest score.



Routine and experimental procedures

b=birth

bt=backtest

f=formation of groups HR, MISC, LR and OR

t=tail docking

c=castration of male piglet

w=weighing

we=weaning

cort=saliva sampling for cortisol

mi=mixing and moving to fattening pens

mo=only moving to fattening pens

PHA=intradermal injection with PHA

KLH=immunization with KLH

s=measuring skin thickness

sl=slaughter

bl1=blood sample for ADV specific Ig titer

bl2=blood sample for KLH specific Ig titer

bl3=blood sample for salmonella/swine influenza specific Ig titer

Test pigs were distributed evenly over the pens. At 10 days of age, due to changing of individual backtest scores of some piglets, mean backtest scores in all pens were at the same level and therefore all pens were considered miscellaneous pens (van Erp-van der Kooij et al., 2003).

At 9 weeks of age, 471 pigs (including 98 test pigs) were moved from the farrowing compartments to the fattening units, and 74 of these animals were mixed. 412 pigs (including 77 test pigs) in the multi-purpose compartments were neither mixed nor moved, but stayed in the same pen until slaughter. This is called the specific-stress-free (SSF) housing system (Ekkel et al., 1995b; Scheepens et al., 1990).

Immune parameters

Phytohaemagglutinin (PHA) skin test

PHA skin tests were performed after weaning at 4 weeks of age with 173 test pigs, and at the start of the fattening period at 9 weeks of age with 114 test pigs. Two circles with a diameter of approximately 2 cm were drawn at the right and left sides of the ventral groins of the pig. In the center of each circle, 2.5 mg/ml PHA (phytohaemagglutinin; Sigma, L8754) dissolved in 0.1 ml Eagle's Minimal Essential Medium (MEM, Flow Laboratories, Irvine, UK) was injected intradermally. The skin reaction was assessed by measuring skinfold thickness with a cutimeter (Aesculaap, VA 110), prior to injection with PHA as described earlier, and at 18h, 24h, 42h and 48h after injection (Ekkel et al., 1995a; Moore et al., 1994).

Serological tests-general

Blood samples were taken from the jugular vein from all test pigs once in the suckling period, 5 times in the fattening period (10-14 weeks of age) and once in the last month before slaughter.

Antibody responses to Keyhole Limpet Haemocyanin (KLH)

At 4 days after the start of the fattening period (9-10 weeks of age), 119 test pigs were immunized intramuscularly with 1 mg KLH (Keyhole Limpet Haemocyanin, Calbiochem-Novabiochem Co., La Jolla, CA. USA no. 374811). In 1 ml Phosphate Buffered Saline (PBS) and Specol (ID-DLO, Lelystad, the Netherlands) 1:1 v/v. The adjuvant gives a slow release and therefore a relatively long exposure to KLH, which increases the chance of an effective immune response in all individuals.

Blood samples were taken just prior to immunization (d0) and on day 7, 14, 21 and 28 after immunization from the anterior *vena cava*. After clotting and centrifugation at 2000 rpm for 10 min., serum was stored at minus 18° C until use. Titers of total antibodies binding KLH were determined by ELISA using routine procedures. In brief, 96 well flat bottomed ELISA microtiter plates (Greiner) were coated with µg/ml KLH in carbonate buffer (pH 9.6)
Serial two-step dilutions starting at 1:10 in 0.05% Tween-20 and 1% Normal Horse Serum in PBS. The plates were incubated for one hour at room temperature and subsequently after washing incubated with 1:8000 diluted Rabbit Anti Swine IgG H+L/PO (Cappel) for one hour at room temperature. Binding of antibodies to KLH was induced with 0.133 ml Tetra Methyl Benzidine (TMB) as a substitute.
Colouring was stopped by adding 50 µl 2.5 M sulphuric acid. Extinctions were measured at 450 nm with a Labsystems Multiscan MS. Titers were calculated by computer by comparison

with two standard positive serum samples. Titers were expressed as the ^{2}log values of the highest dilution giving a positive reaction.

Antibody responses to Aujeszky's Disease Virus (ADV) vaccination

Antibodies binding ADV were determined 3 times in all test piglets, once in the suckling period (n=216), once 2-3 weeks after vaccination with ADV vaccine (Suvaxyn Aujeszky NIA3-783, Solvay Duphar) in the fattening period (n=128), and once before slaughter (n=124). The number of animals with complete records in the fattening period is lower than in the suckling period, due to missing values for cortisol at 9 weeks and because not all piglets were fattened (due to density regulations for fattening pens). One batch of fattening pigs was vaccinated with a different vaccine (n=9 test animals), and these results were not used in the analysis. All sows were vaccinated three times per year in April, July and November. Postpartum antibody titers differed between sows in blood as well as in colostrum. Therefore, blood samples of the sows were taken and these values were used in the analysis of piglet's antibody levels. Samples of whole blood were send to the Animal Health Service (Boxtel, the Netherlands) where serum was obtained and stored at –20 °C until assayed. Sera were tested for neutralizing antibody against the NIA-3 strain using porcine kidney cells (PK15) and a serum/virus incubation period of 24 h at 37 °C (Bitsch and Eskildsen, 1976; Kimman et al., 1992). Titers were expressed as the reciprocal of the of the highest serum dilution inhibiting the viral cytopathological effect (CPE) in 50% of the cell cultures.

Antibodies against Salmonella and Porcine Influenza virus

Blood samples were obtained from fattening pigs 1-2 weeks before slaughter. Antibodies against Salmonella were determined at the Animal Health Service (Boxtel, the Netherlands) by indirect ELISA. This ELISA uses lipopolysaccharides (LPS) isolated from *S. typhimurium* and *S. livingstone* strains representing the O-antigens 1, 4, 5, 6, 7 and 12 of *Salmonella,* and will detect antibodies from approximately 90% of all *S. enterica* serovars present in Dutch finishing pigs. The results are presented as percentage of optical density of the sample, relative to the optical density of positive reference samples (OD%). OD% >10 was used as the cut-off value for positive samples (van der Heijden et al., 1998; van der Wolf et al., 2001). First, a random check of 40 samples was investigated to determine if Salmonella antibodies were present on the farm. In this way, the probability of diagnosing at least one animal as (truly) positive is 98,67% when the estimated prevalence is 10%. Positive samples were found, and subsequently 59 samples were analysed, adding up to a total of 99 tested samples. Antibodies against Porcine influenza H1N1 and H3N2 were determined using a haemagglutination inhibition test for antibodies against H1 and H3 (Loeffen et al., 1999). Antibody titers ≥ 18 were considered to be seropositive (Elbers et al., 1990).

Cortisol measurements

Saliva sampling and radioimmunoassay were performed as described (Ekkel et al., 1996). Saliva samples were taken from the test animals on the pre-weaning (cort1) and on the weaning day (cort2), and at 9 weeks of age on the day before weighing (cort3) and just after weighing and/or mixing and/or moving (cort4). Weaning and weighing started at 9 a.m., saliva sampling started at 11 a.m. in each compartment. The saliva was collected by allowing the pigs to chew on cotton buds (9679396, Hartman, Nijmegen, the Netherlands) until they were thoroughly moistened. Pigs were handled and trained before saliva sampling, therefore

fixing was never necessary. The cotton buds were centrifuged for 5 minutes at 400 x g to remove the saliva, which was then stored at –20 °C until analysis.
Concentrations of cortisol in saliva were estimated by a previously validated direct solid-phase ^{125}I RIA method (Coat-A-Count TKCO; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in general in duplicates of 200 µl. For 17% of the samples smaller aliquots were used due to small amounts of collected saliva.
The main cross-reactivities were 11.4, 0.98, 0.94 and 0.26% for 11-deoxycortisol, cortisone, cortisosterone and 11-deoxycortisosterone, respectively, according to the manufacturer. Comparison of added and estimated values when cortisol was added to a pooled saliva sample over the range of the standard curve (0.5-50 ng/ml) produced an average intra-assay coefficient of variation of 7.9% and a recovery of 96.8%. The limit of quantitation was 0.25 ng/ml and the interassay coefficient of variation was 9.5% for the range of 0.5 to 15 ng/ml (n=8).

Statistical analyses

To determine correlations between the PHA skin swelling responses and KLH antibody titers, the Area Under the Curve (AUC) was calculated per animal for each test, and Pearson correlation coefficients were calculated.
To determine relations between the backtest scores, immune parameters, and salivary cortisol levels, the following models were used.
For the PHA skin test and the KLH antibody titers, the following repeated measurements models were used (PROC MIXED, SAS):

PHA (w) = μ + cort1 + cort2 + gender + bt3 + bt10 + bt17 + line + time + ε

PHA (w) = μ + cort1 + cort2 + gender + type3 + type1017 + line + time + ε

PHA (f) = μ + cort1 + cort2 + cort3 + cort4 + gender + bt3 + bt10 + bt17 + line + mixing + time + ε

PHA (f) = μ + cort1 + cort2 + cort3 + cort4 + gender + type3 + type1017 + line + mixing + time + ε

in which PHA = repeatedly measured skin thickness in the PHA skin test after weaning (w) or at the start of the fattening period at 9 weeks (f), cort is the logarithm of the salivary cortisol level before (1) and after (2) weaning, and before (3) and after (4) the start of the fattening period (9 weeks of age). Bt is the backtest score on day 3, day 10 and day 17, type3 is a classification based on backtest scores of day 3 (Bt d3≥3.2=HR, Bt d3<3.2=LR) and type1017 is based on backtest scores of day 10 and day 17 ((Bt d10+Bt d17)/2≥2.8=HR, (Bt d10+Bt d17)/2<2.8=LR). Gender is gender of the piglet and line is genetic pig line (GY, DL, F1 or F2). Time is the time of the skin measurement (18, 24, 42 and 48 hr after PHA injection).
In both PHA(w) models, sow number (=mother of the piglet) was added as a random factor, while in the PHA(f) models, unique pen number was added as a random factor. The PHA models were also corrected for weight of the animals, but in the PHA(f) models, this meant that correction for pen number was not possible in the same model.

To determine the relation between KLH specific antibody titers after the start of the fattening period, the following model was used:

KLH = μ + cort3 + cort4 + gender + bt3 + bt10 + bt17 + mixing + weight (9w) + DAF + day + ε

in which KLH is the repeatedly measured antibody titer against KLH, DAF is the time (5, 8 or 12 days) between start of the fattening period and immunization, and day is the number of days (0, 7, 14, 21 or 28) between immunization and blood sampling The model was corrected for (unique) pen number as a random effect.

Relations between ADV specific antibody titers and other parameters were investigated using the following models (PROC MIXED, SAS):

ADV(p) = μ + ADV(s) + gender + bt + weight (b) + weight (w) + age + ε

ADV (f) = μ + ADV (p) + cort(f) + gender + bt d3 + bt d10 + bt d17 + weight (9w) + mixing + DAV + ε

ADV (s) = μ + ADV(f) + cort(f) + gender + bt d3 + bt d10 + bt d17 + weight (9w) + weight(s) + mixing + ε

in which ADV is the 2log of the IgG titer against ADV of piglet (p), sow (s), fattening pig after vaccination (f) or at the end of the fattening period (s), weight is weight at birth (b), at weaning (w), at 9 weeks (9w) or at slaughter (s), age is the piglet's age when bled, and DAV is the number of days (14, 17 or 19) between vaccination and sampling. Models were corrected for (unique) pen number.

For Salmonella, a logistic regression analysis was performed with salmonella status as a binomial outcome variable (cut-off value for positive sample OD%>10). The following model was used:

Salm = μ + cort(f) + gender + bt + mixing + weight (9w) + ε

in which Salm is positive for Salmonella (0/1) and W(9w) is the starting weight at 9 weeks. This analysis was only performed in units with at least one positive sample for the relevant antibody.

For all outcome variables, an extra model was tested with group (HR, MISC, LR or OR) as a class variable in the model. This parameter was significant only for PHA skin test results at 9 weeks of age. In this statistical model, correction for pen number as a random factor was not possible. Results are reported in the paragraph regarding PHA.
For all models, relevant 2-way interactions were tested. Residuals of the models were tested for normality by examining the normal probability plot and by plotting the residuals against the predicted values. All analyses were performed using the statistical analysis program SAS (SAS_Institute_Inc., 1989).

RESULTS

Backtest and cortisol levels

Results of backtest typing in four different pens are shown in Table 1. Range and mean backtest results were comparable with earlier studies (van Erp-van der Kooij et al., 2001; van Erp-van der Kooij et al., 2000; van Erp-van der Kooij et al., 2002).
Cortisol results were reported earlier (van Erp-van der Kooij et al., 2003). Weaning and mixing caused a rise in cortisol levels of 58% and 69%, respectively. Cortisol levels at 9 weeks were related to cortisol levels after weaning (estimate 0.13, P<0.01).

Table 1: *Mean backtest (Bt) results (Least Squares Means ± SEM) on day 3, 10 and 17 in pens with high backtest results on day 3 (>3, HR), with low backtest results on day 3 (<3, LR), with mixed results on day 3 (range 0-8, MISC) or original litters (OR).*

	HR	MISC	LR	OR
BT DAY 3	4.77 ± 0.20^{a}	3.05 ± 0.16^{b}	1.45 ± 0.22^{c}	3.42 ± 0.13^{b}
BT DAY 10	3.29 ± 0.27^{a}	2.61 ± 0.22^{a}	1.51 ± 0.29^{b}	2.94 ± 0.18^{a}
BT DAY 17	3.71 ± 0.29^{a}	3.15 ± 0.23^{a}	2.21 ± 0.31^{b}	3.22 ± 0.18^{a}
N	38	60	33	90

Values differ significantly within rows if superscripts are different.

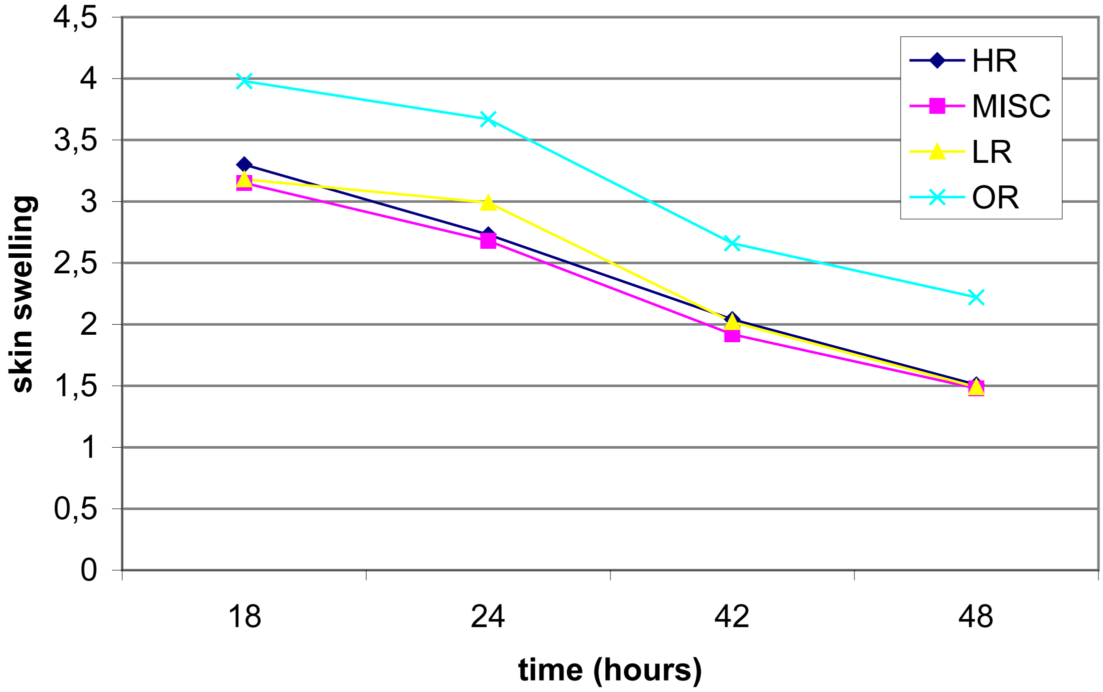
Relations between immune parameters, salivary cortisol and individual backtest scores

PHA skin test

The PHA skin tests at 4 weeks and at 9 weeks were positively correlated (r=0.39, P<0.01, based on AUC).
A positive relation was found between backtest results at day 10 and skin swelling in the PHA test after weaning (estimate 0.09, P<0.05). No relations were found between skin swelling in the PHA test after weaning and cortisol levels at that time, nor with weight at weaning, gender or line of the piglet.

Results of skin swelling due to PHA at 9 weeks of age are shown in figures 2, 3 and 4. At 9 weeks, animals from the OR group showed more skin swelling than animals in the HR, MISC or LR groups (Figure 2) (P<0.01), in the statistical model that was corrected for mixing. When only unmixed animals were included in the analysis, the same trend was found (P<0.10).

Figure 2: *Skin reaction to PHA at 9 weeks of age in cross-fostered groups with high resisting pigs only (HR), low resisting pigs only (LR) and mixed groups (MISC), and original litters (OR, no cross-fostering).*



At 9 weeks, mixed animals showed more skin swelling in the PHA test than unmixed animals ($P<0.05$) (Fig. 3). An interaction was found between mixing and backtest classification based on bt d3 (Fig. 4). At 18 and 24 hours after PHA injection, unmixed HR animals tended to have less skin swelling than unmixed LR animals ($P<0.10$), while mixed HR and LR animals showed no differences. At 42 and 48 hours post-injection, no differences between HR and LR animals were found within the mixed or the unmixed groups. No relations were found between skin swelling and cortisol levels, gender or genetic pig line.
A positive relation was found between weight at 9 weeks and skin swelling: 1 kilogram extra weight was associated with 0.03 mm extra skin swelling ($P<0.01$).

Figure 3: *Skin reaction to PHA at 9 weeks of age in mixed and unmixed animals.*

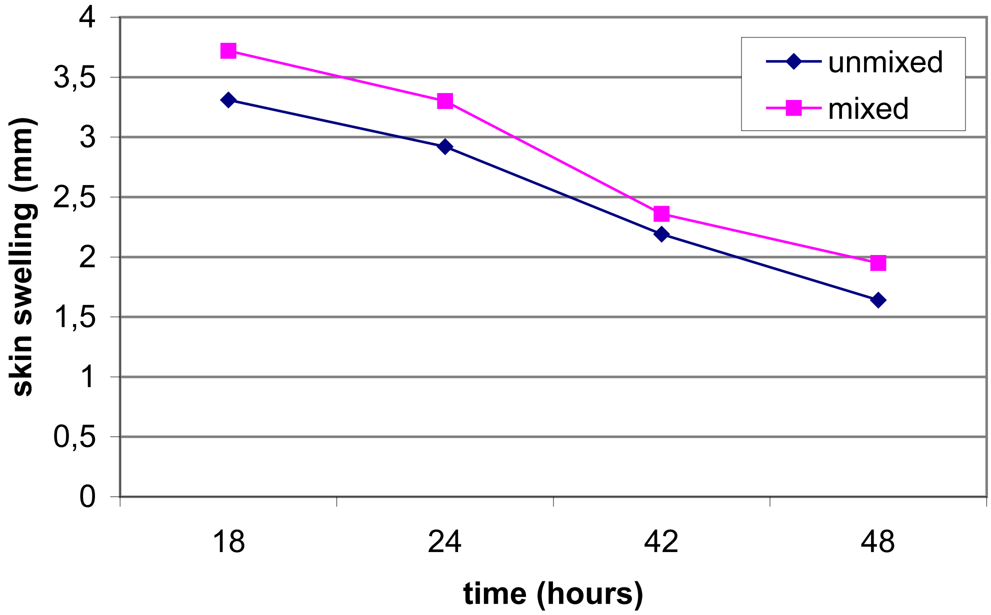
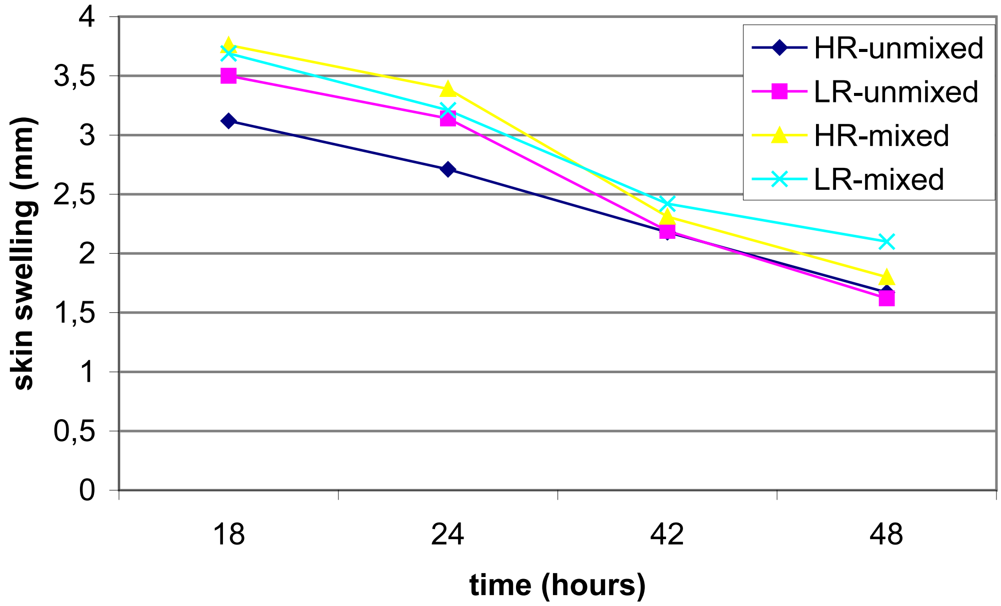


Figure 4: *Skin reaction to PHA at 9 weeks of age in mixed and unmixed animals with high (HR) and low (LR) backtest results at day 3.*



Antibody titer to KLH

Mixed animals showed higher titers than unmixed animals (P<0.01) (Fig. 5) and pigs that were immunized with KLH at a later moment after the start of the fattening period showed lower KLH-specific Ig titers (Fig. 6). No relations were found with backtest results, weight at 9 weeks, pig line or gender.

Figure 5: *Antibody titers to KLH for mixed and unmixed animals at 9 weeks of age.*

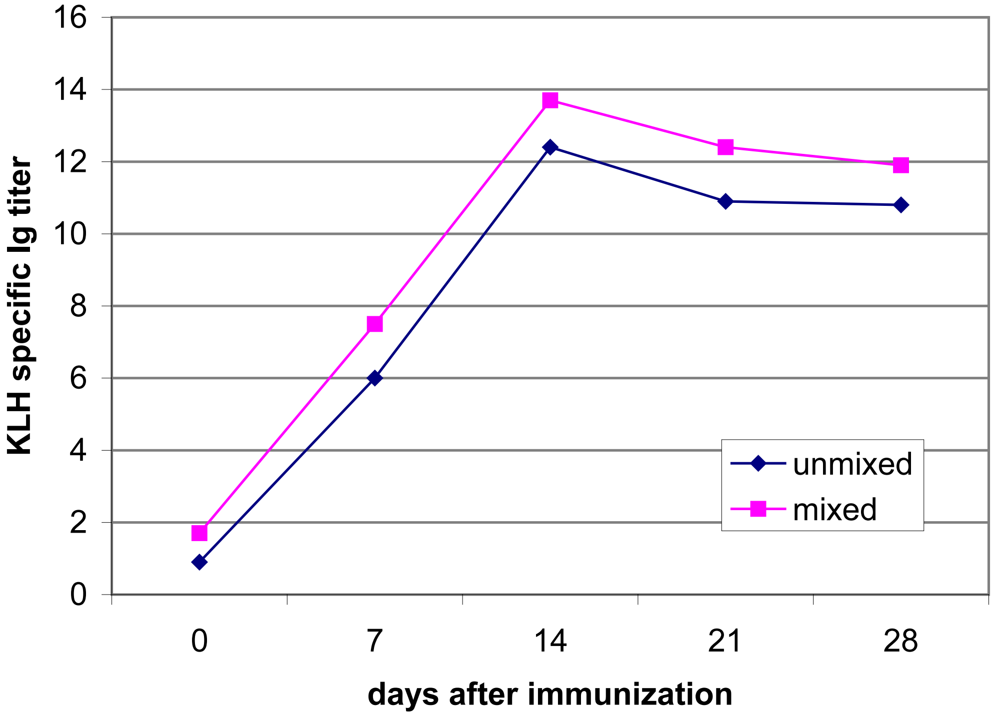
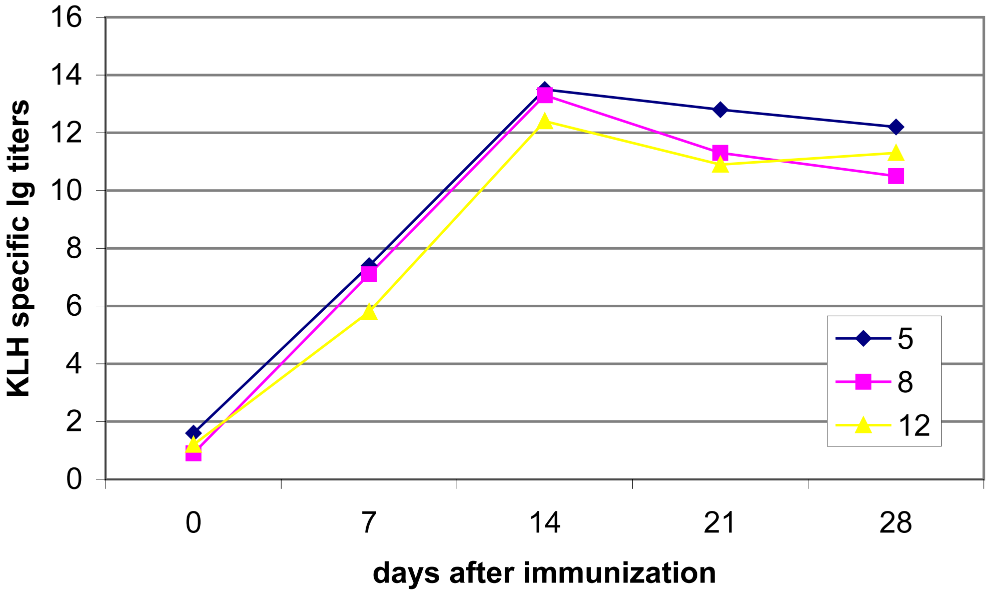


Figure 6: *Antibody titers to KLH for pigs that were immunized at different timepoints after the stressors at 9 weeks of age (weighing, mixing, moving)*



PHA skin test and KLH antibody titer

The amount of swelling in the PHA skin test at 4 weeks, and the antibody titers to KLH were negatively correlated ($r=-0.27$, $P<0.01$, based on AUC).

Antibody titers to ADV

A positive relation was found between ADV specific antibody titers of the piglets and their mothers (estimate 0.58, $p<0.0001$) and between piglet titers and birth weight (estimate 0.67, $p<0.01$), but a negative relation was found between ADV specific antibody titers of piglets and their weight at weaning (estimate –0.11, $p<0.01$). Piglets that were sampled at a later age, showed lower titers (estimate –0.1, $p<0.001$). Castrated male piglets tended to have higher antibody titers than females (lsmeans 2log(titer) 9.60 ± 0.12 vs. 9.43 ± 0.11, $p<0.10$). No relations were found with backtest results.

A positive relation was found between ADV specific antibody titers of the fattening pigs after vaccination and of the piglets in the suckling period (estimate 0.37, $p<0.001$). A longer time between vaccination and sampling caused higher antibody titers in the pigs (estimate 0.37, $p<0.01$). No relations were found with cortisol, gender, mixing or backtest results.

A positive relation was found between ADV specific antibody titers of the fattening pigs after vaccination and before slaughter (estimate 0.48, $p<0.001$). Animals with higher titers had a lower weight at slaughter (estimate –0.05, $p<0.05$). No relations were found with cortisol, gender, mixing or backtest results.

Antibodies binding Salmonella

Antibodies binding Salmonella LPS were found in 22 out of 99 tested animals (22%). The Salmonella-positive animals were housed in 4 different fattening units, and came from different farrowing compartments. No relation between farrowing compartment and infection was found. It was therefore assumed that the animals were infected in the fattening units. About 4 months later, after finishing this study, several other animals that were housed in these fattening units showed clinical signs of Salmonella (diarrhoea). Bacteriological surveys revealed Salmonellas from the B-group.
Logistic regression suggested that a higher cortisol level at 9 weeks gave more risk of infection ($P<0.10$, $OR=2.87$). A higher backtest score at day 17 was a preventive factor for infection ($OR=0.50$, $P<0.05$), just as a higher weight at 9 weeks ($OR=0.80$, $P<0.05$). Mixed animals also showed a higher risk of getting infected with salmonella, though not significantly ($OR=3.91$, $P<0.10$).

Swine influenza

Antibodies against Influenza H1N1 and H3N2 were found in respectively 2 and 6 out of 97 samples. No statistical analyses were performed with these results.

DISCUSSION AND CONCLUSIONS

Stress and immune status

Stress may lead to suppression of immune functions. Besides direct sympathetic innervation of most lymphoid tissues, the nervous system directly or indirectly controls the output of various hormones such as corticosteroids, growth hormone, thyroxin and adrenaline.

Corticosteroids inhibit T_{H1} cytokine production while sparing T_{H2} responses (Cooke, 1998). This means that effects of enhanced levels of cortisol due to stress are expected mostly on cell-mediated responses, and little or no effects are expected on humoral immune responses.

The T_{H1}-T_{H2} paradigm states that cell-mediated and humoral immunity are negatively related, due to cytokines secreted by the two subsets. For example, Interferon gamma (IFNγ), a signalling peptide secreted by T_{H1}-cells and important in antiviral resistance, inhibits the responsiveness of T_{H2} cells, while Il-10, a signalling peptide produced by T_{H2} cells, downregulates B7 and Il-12 expression by antigen presenting cells, which in turn inhibits T_{H1} activation.

Effects of stress on immune responses may depend on four different levels: 1. the sensibility of an individual for a certain stressor may vary, 2. responses to this stressor may vary, resulting in different levels of the various stress hormones such as cortisol, 3. the sensitivity of the cell-mediated immune response for cortisol and of the humoral immune response for other stress-related hormones may differ between animals and 4. the (undisturbed) level of the immune response may differ between individuals. This might explain the fact that we found only low relations between coping behaviour, cortisol levels and immune parameters.

Discussion

Relation between KLH antibody titer and PHA skin swelling responses

A negative relation between the reaction to PHA (cell-mediated immunity) and to KLH (humoral immunity) was observed in the present study. This inverse relationship can be explained by the cross-regulation of T_{H1} and T_{H2} cells as mentioned above. In other studies, classification of piglets based on coping styles resulted in differences in cellular and humoral immune responses (Hessing et al., 1995)

KLH antibody titer

Previously, higher humoral immune responses were found in low reactive piglets (Hessing et al., 1995) In the present study, no relations were found between backtest results and KLH-specific antibody titers. This is in line with a study in gilts (Geverink, 2002). Another study showed an interaction between housing conditions and backtest results on immune responses to KLH, but also no effect of backtest results alone (Bolhuis et al., 2003). It must also be mentioned, that in our study all animals were used, while in the afore-mentioned studies only animals with high (≥3) or low (0,1) backtest results were used. Comparing the extremes might give better results in the analyses than using intermediates as well.
Mixing at 9 weeks was associated with higher responses to KLH. The stress, associated with mixing at the time of immunization, may have suppressed cell-mediated but not humoral immune responses. Because of the cross-regulation of the immune system, this might have resulted in higher responses to KLH (Cooke, 1998).
If immunization happened longer after the 9 weeks' stressors (which were moving, weighing and for some animals mixing), KLH specific Ig titers were lower. This suggests that shortly after the stressor, the humoral immune response might be enhanced. In a study with mice, it was found that mice that were challenged on the 7th and 14th day of a daily social confrontation period showed an increase in primary immune response to SRBC, compared to mice that were challenged on the 1st and 7th day. Thus, the timing of stress exposure seems to have an important effect on the humoral immune response (Gasparotto et al., 2002).

PHA skin test

Delayed-type hypersensitivity (DTH) reactions such as in PHA skin swelling represent cell-mediated (T_{H1}) immune responses that exert important immunoprotective (resistance to viruses, bacteria and fungi), or immunopathological (allergic or autoimmune) responses (Dhabhar and McEwen, 1997)). It has been shown that chronic stress can suppress the DTH response (Dhabhar, 2002). The positive relation between PHA skin swelling at 4 and at 9 weeks of age suggests that this response is animal-specific.
Animals in the HR, MISC and LR groups showed less skin swelling than animals in the undisturbed OR group. Possibly in these groups the overall stress level was higher due to cross-fostering and social stress, and consequently cell-mediated immunity was suppressed.
A positive relation was found between backtest results at day 10 of age and skin reaction to PHA after weaning. This is in accordance with other studies, showing that active animals have an enhanced cell-mediated immune response, either measured with a PHA skin test (Hessing et al., 1995) or measured with in vitro KLH-specific lymphocyte proliferation (Bolhuis et al., 2000).
At 9 weeks, mixed animals showed more skin swelling than unmixed pigs. This is in contrast with a study on the effects of regrouping of pigs, where the reaction to a PHA-skin test 8 hours post-regrouping was reduced in mixed pigs, but no difference was found between treatments at 24 hours after regrouping (Moore et al., 1994). Other studies show that acute stress experienced before primary or secondary antigen exposure induces a significant enhancement of skin DTH (Dhabhar, 2002). It is suggested that during acute stress, endogenous stress hormones enhance skin immunity by increasing leukocyte movement and cytokine gene expression at the site of the antigen entry (Dhabhar, 2002) .
In the unmixed group, active animals tended to have less skin swelling than reactive animals. Studies on coping characteristics and physiology in pigs showed that reactive (LR) animals mounted higher cortisol responses to routine weighing and to ACTH challenge than active (HR) animals (Ruis et al., 2000). In another study however, where HR and LR gilts did not differ in cortisol increase due to restraint by a nose sling, the HR gilts showed a prolonged cortisol response compared to LR gilts. This might explain the absence of relations between backtest results and PHA skin swelling results in the mixed group. Unmixed LR pigs showed enhanced skin swelling. In unmixed animals, routine weighing may have caused a short-term stress response, especially for the LR animals. LR animals have been reported to show higher baseline cortisol concentrations (measured hourly during 24 h) (Geverink et al., 2003). Possibly the overall stress levels in these animals are higher, enhancing skin reaction to PHA.

ADV

ADV specific antibody titers of piglets were highly correlated with ADV specific antibody titer of the sow (the own mother), decreasing with age.
Heavier piglets at birth had higher antibody titers to ADV, possibly because of a higher colostrum intake. These heavier piglets also gained more weight in the suckling period. Weaning weight was negatively related to Ig titers, which can be explained by the diluting effect of increased body weight. Castrated males showed higher ADV specific titers, possibly also because of a higher colostrum intake.

We found no relation between coping behaviour and ADV specific antibody titers after vaccination (at. 10 weeks of age), but ADV specific antibody titers after vaccination were related to titers of the piglets in the suckling period.

Salmonella

Antibodies against Salmonella were found using an aspecific ELISA against LPS from the serogroups B, C and D.
Stress factors such as high animal densities, transport or other diseases are assumed to increase shedding by carriers as well as susceptibility of exposed pigs to Salmonella due to increase of gut permeability (Schwartz, 1999), or because of a decrease in immune response, due to catecholamines and glucocorticoids (Groot et al., 2000; Seidler et al., 2001). From the present study, both mixing and cortisol levels at 9 weeks were identified as risk factors for infection, suggesting a higher chance of gut translocation of salmonellae due to stress.

Resistance to Salmonella rests primarily on cell-mediated immunity (Schwartz, 1999).
If HR animals have enhanced cell-mediated immunity, they are expected to be less susceptible to salmonellae. This may be exemplified by lower antibody titers to Salmonella, either because the pathogen was effectively eliminated in an early stage, or because it takes more time for these animals to become infected. At slaughter, antibody titers to Salmonella were not yet high. Active coping behaviour at day 17 of age was identified as a preventive factor for infection with salmonellae. Though it is unknown whether antibody titers to LPS reflect resistance or infection, our results suggest a lower susceptibility for Salmonella in the active pigs.

Conclusions

From the present study we conclude that social stress due to mixing had a direct, positive effect on the skin swelling responses, and an indirect positive effect on the KLH specific antibody titers. A negative effect of chronic (social) stress was observed in the cross-fostered and mixed pens, where skin swelling to PHA was suppressed, compared to the original (undisturbed) litters.
Mixing increased the risk for salmonella infection, and higher backtest results related with enhanced skin swelling in the PHA test at weaning was related with a lower risk for salmonella infection. In contrast to previous studies (Hessing et al., 1995), we found relatively stronger effects of (social) stress on immune responsiveness as compared to coping behaviour, which is in accordance with studies showing an important effect of the environment in which coping behaviours are functioning (Bolhuis et al., 2003).
We conclude that not only the stressors but also the way the individual animal copes with these stressors have an important effect on the immune function and health of that animal.

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DISCUSSION

In this thesis, differences in the way individual pigs cope with management-related stressors are investigated. Attention was focussed on direct and indirect effects of cross-fostering, weaning, mixing and moving of pigs: stress-factors that are related to the common husbandry systems, and that are important for the pigs' welfare. The backtest response was used as an instrument, assuming it reflects (a dimension of) the coping 'style' of the individual animal. Backtest responses may predict responses to stress in other situations, and relations between backtest response and health and production parameters were studied and discussed.

CONSEQUENCES OF STRESS IN PIG HUSBANDRY FOR WELFARE, HEALTH AND PRODUCTION

Cross-fostering

In practice, cross-fostering is often a necessary procedure, saving piglets that otherwise would have died. According to earlier studies, cross-fostering before day 4 does not induce much fighting and sows will accept any piglet which is in the proximity of her nest site (Gonyou and Stookey, 1987; Robert and Martineau, 2001). In our study, cross-fostering was always done within 4 days postpartum and indeed no fighting or rejection by the sow was observed. Therefore, initially one might conclude that cross-fostering is only beneficial for piglet welfare. However, the handling involved in the cross-fostering process might influence the animals negatively. It has been shown in other studies that neonatal handling can influence behaviour and stress responses at a later age (see chapter 1). Repeated (friendly) handling of pigs will reduce fear of humans (Rushen et al., 2001), but at 3 days of age piglets are not used to humans and handling will evoke a (short-term) stress response (Stanton et al., 1972; Wootton et al., 1982).

In our studies, apart from the acute reaction to handling (some struggling and vocalising), we observed no behavioural responses of piglets to the cross-fostering process that indicated stress, such as agonistic or avoidance behaviour, escape behaviour from the new pen or increased defaecating/urinating (chapter 4). Only some of the piglets that were fostered into a pen with a differently positioned sow crate needed some time to find their way in the new pen, and those piglets vocalised more on the cross-fostering day than piglets that were not cross-fostered. The next day however, we were not able to distinguish between infostered piglets and piglets from the original litter on the basis of behaviour. However, no physiological parameters were recorded at that time. Weaver et al (2000), found higher locomotion in an open field test for neonatally handled boars and suggested that handling influenced HPA-axis functioning. In our study, cross-fostered animals showed shorter latencies in the novel object test (NOT) and the open door test (ODT) at 10-12 weeks of age (chapter 3), but cross-fostered and resident piglets did not differ in their cortisol responses to weaning at 4 weeks or mixing at 9 weeks of age. This suggests that cross-fostering in the first week of age makes the animals more active or explorative at a later age, but we could not confirm any change in the HPA axis functioning. At 9 weeks, cell-mediated immunity was suppressed in the cross-fostered pens in comparison with the original litters. No differences in cortisol levels were found, but this might be attributed to our measuring protocol: salivary cortisol was determined only once at approximately 2 hours after the stressor. Although no aggressive interactions were seen the first few days after cross-fostering, possibly in the long run, a higher level of (social) stress in these groups occurred, suppressing cell-mediated

immunity (Dhabhar, 2000). All piglets were handled after birth, for weighing and for applying an identification tattoo, and at 3 days of age for the backtest. The differences between the effects of the different amounts of handling for cross-fostered and resident piglets may have been absent or just too small to detect.
Cross-fostering had no effect on daily weight gain in the suckling, rearing or fattening period or on carcass composition in fattening pigs (chapter 6). It has to be concluded that although no immediate effects of cross-fostering were observed, and no direct effect of cross-fostering on welfare of the piglets was found, some negative long-term effects occurred, and maybe the impact of the procedure is underestimated.

Weaning, mixing and moving

Responses of the piglets to weaning at 4 weeks and to mixing at 9 weeks were evident (chapter 4), with an increase in salivary cortisol of 58% after weaning and 69% after mixing. The relevance of these increases is indicated in studies on the validity of physiological and behavioural measures of animal welfare. It was concluded that high levels of cortisol (>40% increase) are significant and possibly harmful for the animals (Barnett and Hemsworth, 1990). However, lower or absent rises of cortisol levels do not per se indicate that no stress is involved (Mendl, 1991). Mixing causes acute responses in pigs but also long-term effects (Ekkel et al., 1997). In terms of welfare, the long-term effects are probably worse than the acute stress responses. When unacquainted pigs meet, they will fight because of the possible advantages of becoming dominant; however, in doing so they pay a price, with no guarantee of receiving positive returns (e.g. access to more food, or a better lying space). Fighting offers a direct health risk for the animal, because the animal might get hurt, and if the animal loses the fight, a stress response will follow. Social defeat in pigs leads to acute endocrine changes indicating stress, but also some long-term effects have been reported (Ruis et al., 2001).
Moving of pigs from the farrowing to the fattening pen (while keeping the group intact), caused no increase in salivary cortisol levels (chapter 4), measured 2 hours later. However, any short-term effects on cortisol levels may have been missed. Cortisol can decrease quickly and be back to basal levels 1 h after the stressor (Becker et al., 1985; Geverink et al., 1998). Such a short-term or acute stress response should be considered as an adaptive mechanism, with potentially beneficial effects, while the long-term or chronic response can result in immune suppression and detrimental effects on health (Barnett and Hutson, 1987).

Mixing of pigs at 9 weeks and high cortisol levels due to mixing or other stressors, increased the risk of infection with Salmonella (chapter 7). This suggests that stress increases the chance of gut translocation of salmonellae, as was reported before (Seidler et al., 2001). Mixing enhanced humoral and cell mediated immune responses shortly after the stressor. The enhanced skin swelling could be an effect of acute stress (Blecha et al., 1982). Endogenous stress hormones such as corticosterone or adrenaline enhance skin immunity by increasing leukocyte movement and cytokine gene expression at the site of the antigen entry (Dhabhar, 1998). The enhanced humoral immunity can be a more long-term effect of a stress-induced shift in T-cell subsets towards T_{H1} (Cooke, 1998). This shift might be an effect of increased glucocorticoid levels (Agarwal and Marshall, 2001; Daynes and Araneo, 1989). The PHA skin test was performed directly after mixing, while the KLH immunization was performed 5 days after mixing, and antibodies were determined 1-4 weeks later.
In our study, weaning and mixing of pigs induced physiologically measurable stress responses (chapter 4). At an older age, negative effects on health were found for mixed pigs, reducing welfare (chapter 7). No short- or long-term effects of moving of pigs were found (chapter 4).

These results suggest that moving of pigs is not influencing welfare, as long as penmates are kept together.

BACKTEST RESULTS

Backtest standardisation and validation

All backtests in this study have been performed according to a protocol that was used by other researchers as well (Bolhuis et al., 2003a; Hessing, 1994). In this protocol, the exact position of the piglet and the researcher's hands in the backtest, as well as the scoring method is described, and 'escape attempt' is defined. If piglets show an activity pattern during the day, this might influence backtest responses. In a study on activity rhythm in suckling pigs (>15 days of age), piglets were active mostly between 6.00-8.00 hours and between 14.00-16.00 hours (Schrenk and Marx, 1982). It is unclear when this activity rhythm already exists at the time of the first two backtests (day 3 and day 10). However, our study did not show any relations between time of day and backtest results. Seeing and hearing the testing of littermates did not influence test results, since we found no relation between order in which piglets were tested and backtest results (chapter 2).
A remaining problem in standardisation of the backtest is the 'state' of the animal at the time of testing. An animal might be hungry, sleepy, or it may previously have lost a fight. The experimental protocol did not allow correcting for these different states, which may well influence backtest results. An attempt has been made to standardise the state of the animal before the backtest by a wake-up procedure to ensure that all piglets were awake and on their feet prior to the backtest (chapter 2), but this did not increase correlations between successive backtests.

It is important at what age piglets are backtested (chapter 5). Piglets resisted more in the backtest in the first week than in the subsequent backtests, possibly because of the overall higher activity level of these young piglets, that are still actively fighting for a teat (Gonyou and Stookey, 1987). Having experienced a previous backtest (at day 3) or not, did not influence backtest responses at day 10 and 17, so it is assumed that there is hardly any habituation to the test.
Backtest scores of the piglets were related to backtest scores of the sow, and a litter effect was found (chapter 5). This points to a maternal and/or a genetical effect. Heritability of behavioural traits has been shown in several species (Benus et al., 1991; Boecking et al., 2000; Kenttamies et al., 2002; Kilgour et al., 1984; Kjaer et al., 2001; Ruefenacht et al., 2002; Sluyter et al., 1995; van der Steen et al., 1988; Wilsson and Sundgren, 1998).
In the last few years, backtest scores in pigs from our breeding population have increased. This could be due to the association with lean meat percentage, because selection for that trait favours HR piglets (chapter 5). When at a young age (< 4 days) only HR or only LR piglets are put in a pen, a redistribution of backtest responses occurs (chapter 4). Possibly these (extreme) changes in the social environment interact with or even overrule the genetic predisposition for a certain type of coping behaviour. This is discussed further in paragraph 8.2.3..

Females resisted more in the backtest than (castrated) males, and this gender difference became more apparent in the successive backtests (chapter 5). This was also found in a tonic immobility test, which resembles the backtest (Erhard et al., 1998), where handled females were more active than handled males; however, pigs that were not handled, showed no gender difference in test results. In a study using a modified backtest, no gender differences were found for number of escape attempts but female piglets vocalised more than male piglets (Forkman et al., 1995).
Gender differences in coping strategies have also been found for humans, but comparison with animal studies is difficult. Coping styles that were studied involved feelings and thoughts, which we cannot record for animals (Gadzella et al., 1991).

Coping style and personality

In human psychology, personality and stress-coping are mostly considered different fields of study (McCrae, 1992). In humans, five personality traits are distinguished, the human Five-Factor Model (FFM) (Gosling and John, 1990; John, 1990): N=Neuroticism, anxiety,E=Extraversion, assertiveness, activity, dominance,
O=openness, curiosity, A=agreeableness, aggression and C=conscientiousness, dutifulness, order. Genetic influences on these ‘Big Five’ are estimated 35-60% (Jang et al., 1996; Lensvelt-Mulders and Hettema, 2001). Coping styles are for example ‘informational’ (actively seeking information), ‘normative’ (reactive to others) and ‘diffuse/avoidant’ (situation-specific reactions) (Anshel and Kaissidis, 1997; Berzonsky, 1992).
Influences of personality traits on coping strategies have been determined in several studies. One study shows for example that active coping behaviour is linked with certain personality traits such as an ‘easygoing personality style’ and an ‘internal locus of control’ (i.e. intrinsically driven) (Holahan and Moos, 1987), while another states that personality traits can influence coping behaviour in addition to the influence of disposition (=coping style), and that traits like neuroticism and locus of control might be stable determinants of coping responses (Terry, 1994).
When comparing studies on coping style and personality for humans and animals, many parallels are found although terminology sometimes differs. In animal studies, sometimes the terms ‘personality’ and ‘coping style’ are mixed up, and words like ‘temperament’ and ‘personality types’ are used (Lyons et al., 1988; Ruis et al., 2000). In a study with mice, aggression is used as a measure for coping style, while in human studies, aggression is a personality trait that might be associated with coping style. In one study it is stated that animals might have different personality types within populations, and each type can be classified as having an active or reactive coping style (Benus et al., 1991). Personality studies in animals show that some, if not all, of the same personality dimensions are found in many different animal species, including pigs (Creighton, 2003; Forkman et al., 1995; Gosling and John, 1990).
Coping styles that are distinguished in animals resemble the coping styles that have been identified in humans. For example, the human ‘problem-focused strategies’ and ‘emotion focused strategies’ (including avoidance) (Terry, 1994) resemble active and reactive coping styles in animals, respectively. Probably in animals, like in humans, the interaction between the different personality traits determines the coping style of an individual (Holahan and Moos, 1987). Experience can influence personality traits, but also coping style. Every trait determines an aspect of the complex response in interaction with the environment. In the end, there are situational and stable factors which determine coping: the stable factor is the

disposition that influences coping response, or the coping style (Terry, 1994), which is determined by aspects of the personality.

Backtest as a measure for coping style

In the backtest, behaviour in a certain situation is recorded on a one-dimensional scale (0-10). Behaviour is influenced by several personality traits such as exploration, curiosity and anxiety, and possibly on a different level by the coping style of the animal. Consequently, what we measure with the backtest is the result of several personality traits, and the situational appraisal. If the backtest response reflects a social response (e.g. submission) (Ruis, 2001), than correlations would be expected in a social context. If the backtest measures fear, than we would expect correlations with behaviour in other fearful situations. Whatever correlations are found, they only reflect a part of all factors that play a role in stress coping.

Intra- and inter-test correlations

A test that measures the state of an animal, should be consistent within that state, e.g. an animal has to show fear-related behaviour every time it is afraid (and not every time it is in the same test situation). When a test is used as an indicator of a trait, it should be consistent across time. Therefore, it may be more appropriate to show a consistency between different test situations in the same context than to show the same test results in the same test (Erhard and Schouten, 2001; Romeyer and Bouissou, 1992). The repeatability of behavioural tests depends on the extent to which the animals adapt their behaviour according to the experience they had in the test situation (Erhard and Mendl, 1999). For example, through habituation an animal may cease to find a situation threatening and therefore show less fear-related behaviour in the successive tests. This is important information for the application and interpretation of a test (Erhard and Schouten, 2001).

In most backtest studies, correlations between successive backtests are not mentioned (Bolhuis et al., 2000; Geverink et al., 2002; Hessing et al., 1993; Schrama et al., 1997). Others found no correlations or a low consistency of backtest results over time (Forkman et al., 1995; Ruis et al., 2000). In original litters and mixed pens (HR/LR) intra-test correlations of about 0.4 were found. When at a young age (< 4 days) only HR or only LR piglets are put in a pen, a redistribution of backtest responses occurs (chapter 4) and correlations between backtests before and after cross-fostering are absent. Two explanations are possible for the behavioural change in the extreme groups. First, animals may have changed their behaviour intentionally, because behavioural variation is beneficial for a group. The mechanisms for this change are still unclear. It has been shown by others that mixed HR/LR groups show less aggression and better performance results (Hessing et al., 1994; Ruis et al., 2002). The fact that, in our study, the regrouping procedure only changed backtest responses in the HR and LR pens, and not in the mixed pens, in which animals were also handled and regrouped, favours this first explanation.

Second, it is possible that the regrouping procedure changed HPA axis functioning and behavioural reactivity in some animals. It has been shown in studies on calves that regrouping can increase sensitivity of the adrenal cortex to ACTH and that regrouped calves were more agitated during restraint (Boissy et al., 2001; Veissier et al., 2001), and in boars it has been shown that neonatal handling can change HPA function and behaviour (Weaver et al., 2000). Experiments where rats were bred selectively for apomorphin (leading to APO-SUS, with high gnawing scores in response to apomorphine, and APO-UNSUS, with low gnawing scores in response to apomorphin) have shown that cross-breeding, cross-fostering and

maternal deprivation can change apomorphin-susceptibility. These results show a contribution of both genetic and early environmental factors to the susceptibility of apomorphin (Ellenbroek et al., 2000), and suggest an environmentally induced change of a trait. This favours the second explanation. Possibly both mechanisms play a role.
We conclude that backtest behaviour is a relatively consistent characteristic in a stable social environment, but, at a young age, animals can still adapt if extreme environmental changes occur.

Distribution of backtest responses

The distribution of backtest results is unimodal, as Ruis already showed (Ruis et al., 2000), and not bimodal as Hessing (Hessing et al., 1993) suggested. This does not necessarily mean that coping styles in pigs do not exist, as Jensen (Jensen, 1995) suggested, but we argue that the coping style of a pig can be anywhere on a scale from active to reactive. Most pigs have a backtest response that lies somewhere in the middle of the scale, with a backtest score of approximately 2 escape attempts in 1 minute. Animals towards the ends of the scale may have a prevalence for a more active or a more reactive coping strategy (Ruis, 2001). The difference between HR and LR or between active and reactive responses is gradual, and is determined by emotion (appraisal) and constitution (personality). The (social) environment also influences coping behaviour, as was discussed above.

Predictive value of the backtest

If the backtest response reflects coping style, or a part of the genetic background of coping behaviour, it might predict the way animals cope in other situations. However, not only genetic constitution but also social and non-social environmental factors influence brain development and therefore behaviour. At a later age, behaviour is the result of prenatal and postnatal interacting factors. The backtest is performed very early in life, to avoid as many environmental influences as possible.

If the backtest reflects a more active or more reactive coping style, LR pigs were expected to show higher cortisol responses to weaning and mixing. However, we found no correlations between coping behaviour in the backtest and cortisol levels 2 hours after stress (chapter 4). Differences in cortisol responses between HR and LR animals to these stressors were absent or at least short-lived. Furthermore, we found that the backtest response only has a limited predictive value for behaviour in other (test) situations and most inter-test correlations were absent. In our study, HR animals scored high in a human approach test, but no relations were found with latency to approach a novel object test or latency to leave the home pen. This is in accordance with one other study (Forkman et al., 1995), but other authors did find correlations between backtest responses and other behavioural tests (Bolhuis and Schouten, 2002; Ruis et al., 2000). From a lack of inter-test consistencies, it is sometimes stated that coping behaviour is situation-specific, thereby rejecting the theory that coping is a trait variable. In reality, coping behaviour is probably the outcome of an innate preference for a certain coping behaviour (trait) combined with situational factors. Possibly experience can influence this preference directly or indirectly (via one or more personality dimensions). When the backtest is used as a predictor for behaviour in a group test, dominance structures might be important as well as individual determinants of coping. Besides that, situational factors always play a role in determining coping behaviour (Holahan and Moos, 1987; Terry, 1994).

When using the backtest response as a predictor for behavioural responses in other tests performed in the group, **dominance** may play an important role. Dominance is the result of personality and physical traits (Buss, 1988). Environmental influences have also been described (Verbeek et al., 1996). There is probably a direct effect of dominance status of the animal on the outcome of a behavioural group test. For example, a dominant animal can block the way to the person or the novel object in a human approach test or novel object test. Furthermore, an interaction between dominance and backtest response has been reported. In a study with HR and LR gilts, HR gilts showed more aggressive behaviour than LR gilts, and in mixed HR/LR pairs the aggression levels were much higher when the HR gilt was dominant (Ruis et al., 2002). In a study where 3 HR and 3 LR pigs were mixed per pen, fighting efforts (frequency, duration and latency) were highly correlated with the achieved social rank in LR but not in HR pigs. This suggests that the LR pigs have a more flexible, adaptive coping style in social confrontations and that the LR pigs can measure out their aggressive behaviour according to the circumstances, while the HR pigs are aggressive, irrespective of the fighting success (Bolhuis et al., 2003b). Since HR and LR pigs have an equal chance of becoming the dominant pig of a pair (Bolhuis et al., 2003b; Ruis et al., 2002), aggression level in a group can not be predicted from backtest results only, without knowledge on the dominance hierarchy in the group.

In human psychology, different models are used to describe the interaction between personal (coping style) factors and **situational factors** that predict an individual's selection of coping strategies in reaction to stressful events. Supporters of the *trait model* argue that dispositional factors tend to explain an individual's stable and consistent use of coping response. The *situational-mediating model* assumes that appraisal shapes the effects of personal factors on coping, so that situational characteristics form a primary predictor of coping strategies. This means that only the situation determines the individual's reaction. The *interactive model* suggests that coping is a result of the interactions between personal dispositions and situational appraisals (Kaissidis-Rodafinos et al., 1997). The differences we observe in test outcome across situations for pigs point to an interactive model. We therefore assume that coping behaviour in pigs is determined both by coping style and the situation the pig is in (chapter 3). In different situations, different coping behaviour is needed and most animals are flexible enough to adapt their behaviour to different situations. Coping behaviour should be partly situation-dependent. Younger pigs can change their coping behaviour in an extreme social environment (chapter 4). If coping behaviour was solely determined by an inflexible coping style, no change, no learning would be possible and this would not benefit an animals' survival. Change is an essential property of development, yet some constancy or stability is necessary for the maintenance of individual distinctiveness (Lyons et al., 1988). Thus, coping behaviour will (and should) differ across situations, and inter-test correlations in behavioural tests will never be 1.0. However, if a test method was developed that measured intention instead of resulting behaviour, and in two tests, the same underlying dimensions were measured (the 'trait' part of the behaviour), correlations should be higher than zero (depending on the proportion trait and situation-specificity).

In conclusion, the backtest can be used as a predictor for other behavioural or physiological responses, but the above-mentioned factors should be taken into account. During the backtest, additional parameters can be recorded such as vocalisations and latency to first resistance. The backtest might be useful as part of a series of tests to determine the coping style in the pig. Possibly the predictive value of the backtest will increase, when more parameters are recorded and taken into account.

EFFECTS OF COPING BEHAVIOUR ON HEALTH AND PRODUCTION

Coping behaviour and health

Several authors reported relations between backtest behaviour and immune responses (Bolhuis et al., 2003a; Hessing et al., 1995; Schrama et al., 1997). In our study, we found some relations between backtest responses and cell-mediated immune responses, but no relations with humoral immune responses. An association between backtest behaviour and Salmonella infection was observed: HR animals had a lower risk of infection with Salmonella (chapter 7).

Relatively stronger effects of social stress on immune responsiveness, as compared to the effects of coping behaviour were found. This is in accordance with studies showing an important effect of the environment on immune responses (Bolhuis et al., 2003a; Geverink et al., 2003). Possibly the situation-specificity of these parameters is high, while the trait we measured (a dimension of the coping style) is only a minor determinant of these immune responses.

Coping behaviour and production

HR animals showed a higher lean meat percentage and a better carcass quality at slaughter (chapters 5 and 6). HR animals had a higher daily weight gain in the fattening period than LR animals, but in the suckling period the HR animals showed a lower daily weight gain, in contrast to findings of Ruis (Ruis et al., 2002). It is possible that HR animals spent more time fighting or in general with active behaviour. During the suckling period, this might lead to missed suckling bouts and lost energy, leading to lower daily weight gain. In the fattening period, with ad lib feeding, the HR animals can compensate the energy loss with extra food, and the result is a leaner pig at slaughter. It is also possible that the metabolism of an HR animal differs from an LR animal, leading to differences in carcass composition.

Since backtest response and carcass composition in pigs are associated, selection for lean meat in pigs is also indirect selection for active behaviour. This might be an undesirable side effect of selection for production parameters. More activity in a pen with slaughter pigs will lead to more social confrontations, and especially when this activity is associated with aggression, more fights will occur. This could lead to a higher feed conversion, which leads to less benefits for the farmer. In groups of sows, this activity and aggression could have a negative effect on piglet production and lead to a higher replacement rate for sows, which also leads to higher costs for the farmer.

SUMMARY OF RESULTS AND DISCUSSION

From the management-related stressors we focused on, weaning and mixing of pigs induced physiologically measurable stress responses, while moving of pigs did not. Cross-fostering and mixing of pigs resulted in some long-term negative effects on health. Cross-fostered animals showed lower cell-mediated immune responses at 9 weeks of age than piglets that were not cross-fostered, and showed more explorative and active behaviour at 10-12 weeks of age. Mixing enhanced both humoral and cell-mediated immune responses shortly after the stressor, but mixed animals were more susceptible for infection with Salmonella later on. Individual differences in the ways animals cope with these (or other) stressors can be described with the coping style theory, which states that every animal has an innate preference for a certain behavioural and physiological coping strategy. Coping strategies can range from active (fight/flight response) to reactive (conservation-withdrawal response). Coping styles are determined by (aspects of) the personality, and the coping style and the situation determine the coping behaviour. In this study, the backtest was used as an instrument to measure (a dimension of) the coping style of the pigs. The backtest is relatively consistent in a stable environment, but our cross-fostering study showed that backtest response in young piglets can change in an extreme social environment (uniform HR or LR pens). Some associations were found between backtest response and immune parameters, with HR animals showing a better cell-mediated immune response after weaning and a lower risk of infection with Salmonella. HR animals also showed better performance results, with a higher daily weight gain in the fattening period, and a higher lean meat percentage and better carcass classification at slaughter. In the current husbandry systems, selection for production parameters will therefore probably favour HR animals. Other studies showed that HR animals display the most aggression.

PRACTICAL RECOMMENDATIONS FOR PIG HUSBANDRY

Cross-fostering probably does not cause much stress when it occurs in the first few days postpartum. On most farms it is used as a necessary procedure that saves piglets, and therefore in general it enhances welfare. However, some effects of cross-fostering (<4 days) on behaviour and cell-mediated immune response at a later age were observed, which might be considered negative for animal welfare.
Weaning and mixing of pigs are stressful events in a pig's life, as was shown by the high cortisol responses and, for mixing, the long-term effects on immune responses and disease susceptibility. Weaning of piglets is unavoidable but should be more gradual. However, a comparison between different weaning methods was not part of this study. Mixing of pigs should be avoided when possible. Long-term negative effects of mixing on immune responses and susceptibility to Salmonellae were found. Weaning and mixing in the current husbandry systems are factors that decrease welfare in the pig, irrespective of the coping strategy that is employed.

HR animals were the best performers (lean meat percentage, carcass classification) in mixed groups of pigs, but pigs should not be selected on the basis of backtest behaviour in order to make uniform pens with HR animals only. In young piglets, this selection would be useless, because of the flexibility of coping behaviour at this age, while in older pigs, this selection would probably lead to worse performance results and problems with aggression (Hessing et al., 1994). Since a link with aggression has been found, the backtest might be used to exclude the extremely high responders in the selection process. In that way, the negative side effect of selection on lean meat percentage, namely selection on HR and possibly aggressive behaviour, is reduced. However, a more direct test might be developed for that purpose, which measures aggression in young pigs in a quick and practical way, and which correlates with aggression at a later age.

No indications were found that either one coping style, active or reactive, is more beneficial for the animal (or the farmer) than the other. HR animals have the best carcass quality at slaughter (lean meat percentage, muscle content), but also display more aggressive behaviour. HR animals have a smaller chance to become infected with salmonellae, but they might show a lower humoral immune response once they are infected. Of course, if one of the coping styles were really detrimental for an animal, it would be eliminated from the population. Evolution would already have removed ‘unhealthy’ coping styles from the population, and coping styles that would be highly associated with low production parameters would have been eliminated by artificial selection. However, it is possible that in different (housing) conditions, different coping styles would be favoured.

In the current pig husbandry systems, HR animals might have the benefit of their aggressive and routine-like behaviour, because the environment is stable, and selection for lean meat percentage will also favour the HR animals. A changing environment like a biological housing system, providing various stimuli, might be favourable for LR animals, while selection for non-aggressive individuals will also favour the LR animals. This does not imply that the range of behavioural coping styles in a group will (or should) disappear, only that the mean of the unimodal distribution of coping styles will move up or down the scale when (indirect) selection takes place.

Analogous to results found by others, we conclude that in a group, different animals are needed with varying coping styles, ranging from HR to LR in the backtest. Uniform pens should be avoided, since they are not enhancing welfare, and extremely high responders in the backtest may have to be excluded in the selection process.

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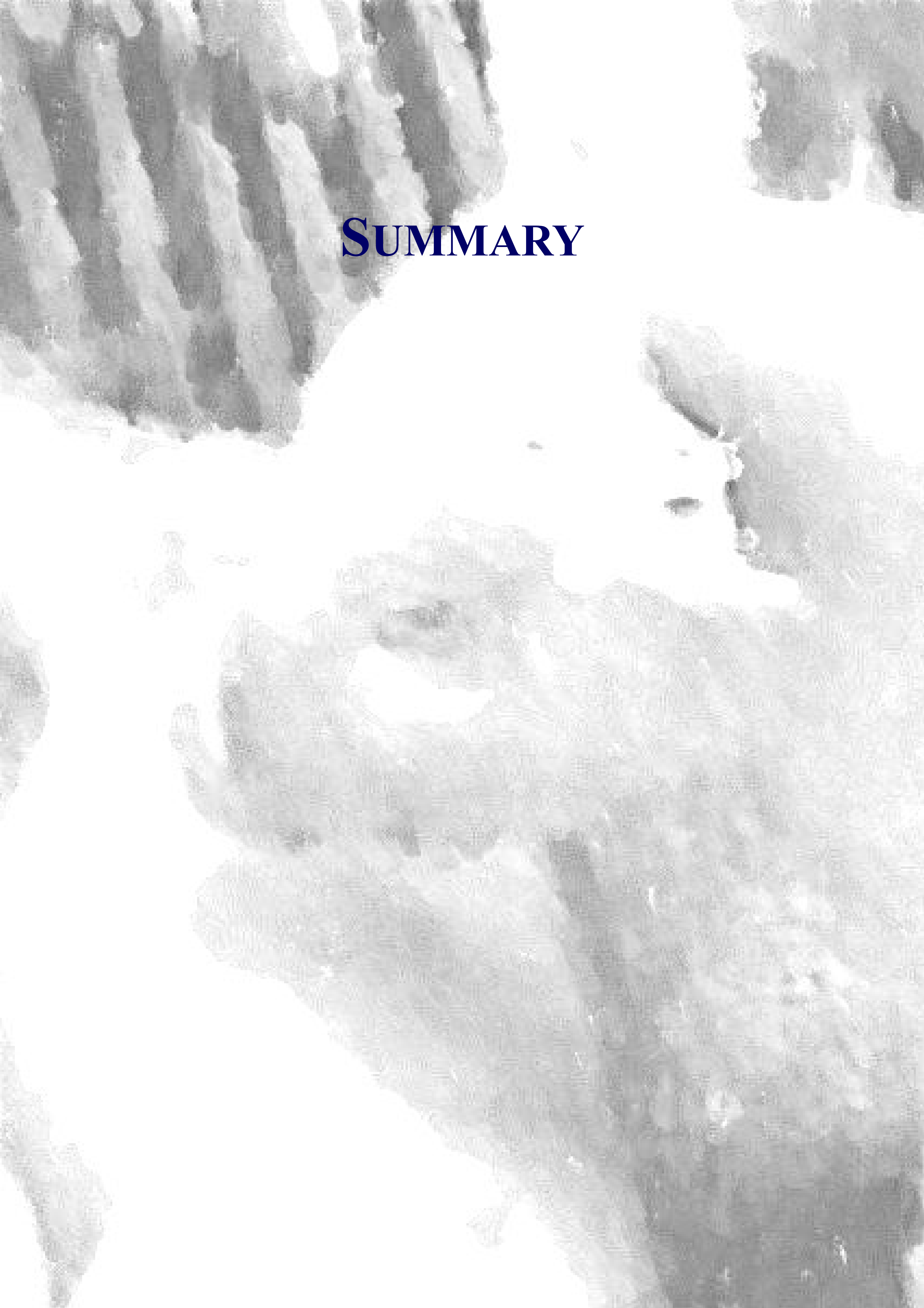
SUMMARY



SUMMARY

In the current husbandry systems, pigs encounter many stressors. Shortly after birth piglets are handled, some are cross-fostered, and at 4 weeks piglets are weaned. Most pigs are mixed and moved to other units at 4 weeks and again at 9 weeks of age. At 6 months pigs are mixed and transported to the slaughterhouse. In this study we have investigated the ways animals cope with these stressors, and studied individual differences in coping behaviour. Practical consequences for pig husbandry are discussed.

Our study results confirmed that weaning and mixing of pigs induced acute, physiologically measurable stress responses, while moving of pigs did not. Cross-fostering and mixing of pigs resulted in some long-term negative effects on health. Cross-fostered animals showed lower cell-mediated immune responses at 9 weeks of age than piglets that were not cross-fostered, and showed more explorative and active behaviour at 10-12 weeks of age. Mixing enhanced both humoral and cell-mediated immune responses shortly after the stressor, but mixed animals were more susceptible for infection with Salmonella later on.

Animals differ in the way they cope with stressors. An active coping strategy is characterized by an autonomous response, with higher heart rate, blood pressure and blood glucose levels, necessary for a fight or flight response, while a reactive (or ‘passive’) coping strategy is associated with conservation and withdrawal and characterized by a HPA response with elevated cortisol levels. The coping style is the preference of each individual for a certain coping strategy. Coping styles are determined by (aspects of) the personality, and the coping style and the situation determine the coping behaviour.

In pigs, the backtest can be used as an instrument to measure (a dimension of) the coping style of the pigs at a young age. In this test, a piglet is put on its back and during 1 minute the number of escape attempts is recorded (mean 2-3, range 0-10). Active copers respond with many escape attempts and can be called HR (high resisting), while reactive copers resist less and can be called LR (low resisting). We performed backtests at 3, 10 and 17 days of age. In a validation experiment (chapter 2) we found that time of day and test order did not influence backtest responses. Correlations between successive backtests were about 0.4, and were not increased by further standardization of the backtest (with respect to the ‘state’ of the animal, by inducing all piglets to stand or walk before testing). The backtest is relatively consistent in a stable environment, but our cross-fostering study (chapter 4) showed that backtest response in young piglets could change in an extreme social environment (uniform HR or LR pens). We found no associations between backtest response and salivary cortisol rise after weaning or after mixing, but this could be due to our protocol. We took saliva samples 2 hours after the stressor, transient, short lasting, differences between HR and LR animals could have been missed in this way. Backtest responses were predictive for a human approach test at 5-7 weeks of age (chapter 3), but no relations were found between backtest response and results of a novel object test or an open door test, also no relations were found with these tests at 10-12 weeks of age. Possibly, in these tests other aspects of the coping style are measured, or aspects that are determined by different combinations of personality traits.

Some associations were found between backtest response and immune parameters (chapter 7), with HR animals showing a better cell-mediated immune response after weaning and a lower risk of infection with Salmonella. HR animals also showed better performance results (chapters 5 and 6), with a higher daily weight gain in the fattening period, and a higher lean meat percentage and better carcass classification at slaughter.

It cannot be concluded that one coping style is “better” than the other from the results of this study. HR animals showed better performance results, but only in mixed HR/LR groups. HR

animals showed better cell-mediated immunity and lower susceptibility for infection with Salmonella, but once infected they might show a lower humoral immune response. Because of the association with lean meat percentage and daily weight gain, selection for production parameters will probably favour HR animals in the current husbandry systems. The organic farming systems, which offer more stimuli, like other sows in the group, straw bedding and an outdoor area, probably favour the LR sows, because they may be better equipped to cope with a less predictable environment, as other studies showed.

SAMENVATTING

Samenvatting

In de huidige varkenshouderijsystemen komt een varken in aanraking met vele management-gerelateerde stressoren. Kort na de geboorte worden biggen opgepakt, sommige biggen worden overgelegd naar een andere zeug en op 4 weken leeftijd worden de biggen gespeend. De meeste dieren worden op dat moment ook gemengd en verplaatst naar de gespeende biggenafdeling. Op 9 weken leeftijd worden de dieren opnieuw gemengd en verplaatst naar de vleesvarkenafdeling. Wanneer de dieren 110 kilo wegen (ca. 6 maanden leeftijd) worden ze naar het slachthuis vervoerd, waarbij ze vaak weer gemengd worden. In dit onderzoek hebben wij gekeken naar de manier waarop dieren omgaan met deze stressoren (‘coping’), en naar individuele verschillen tussen dieren in hun omgang met stress. Mogelijk zijn bepaalde manieren van omgaan met stress effectiever dan andere. Ook bespreken we de gevolgen voor de praktijk.

Onze onderzoeksresultaten bevestigen dat spenen en mengen van dieren fysiologisch meetbare stress-responsen veroorzaakt op de korte termijn (verhoogde waardes van speeksel-cortisol). Er werden geen effecten gemeten van het uitsluitend verplaatsen van dieren (hoofdstuk 4). Het overleggen van biggen en het mengen van varkens veroorzaakten negatieve effecten op de gezondheid op langere termijn. Cellulaire immuunresponsen werden gemeten op 4 en 9 weken leeftijd door middel van een huidtest, waarbij intradermaal Phytohaemagglutinine (PHA) werd ingespoten en de resulterende huidzwelling werd gemeten. Humorale immuunresponsen werden gemeten door dieren te immuniseren met Keyhole Limpet Haemocyanine (KLH) en te vaccineren met Aujeszky vaccin, waarna Ig titers in bloed werden bepaald. In de hokken waarin was overgelegd vertoonden de dieren een lagere cellulaire immuunrespons dan in de hokken met originele tomen (niet overgelegd) (hoofdstuk 7). Verder scoorden de dieren in de hokken waarin was overgelegd hoger in de ‘novel object test’ en de ‘open door test’ op 10-12 weken leeftijd (hoofdstuk 3). In deze testen werd gemeten hoe snel elk dier binnen de groep naar een emmer (novel object) of uit het hok (open door) liep wanneer de hokafscheiding werd opengezet. Het mengen van dieren op 9 weken leeftijd stimuleerde de cellulaire en humorale immuunresponsen kort na de stressor, maar gemengde dieren waren gevoeliger voor een Salmonella-infectie (hoofdstuk 7).

Dieren verschillen in hun omgang met stressoren. Een actieve coping strategie wordt gekarakteriseerd door een respons van het autonome zenuwstelsel, dat een hogere hartslag en bloeddruk en een hogere glucosespiegel in het bloed veroorzaakt. Dit wordt ook wel de ‘fight/flight’ (vechten of vluchten) respons genoemd. Een reactieve coping strategie wordt gekenmerkt door een respons van de hypothalamus-hypofyse-bijnieras, met verhoogde gehaltes van cortisol in het bloed. Dit wordt ook wel de ‘conservation-withdrawal’ (behouden-terugtrekken) respons genoemd. Elk mens of dier heeft beide systemen tot zijn beschikking, maar kan een aangeboren/aangeleerde voorkeur hebben voor een bepaalde strategie en geneigd zijn om meestal op die manier te reageren. Deze voorkeur noemen we de coping stijl. Coping stijlen worden bepaald door (aspecten van) de persoonlijkheid. De coping stijl en de situatie bepalen gezamenlijk welke coping strategie gebruikt zal worden.

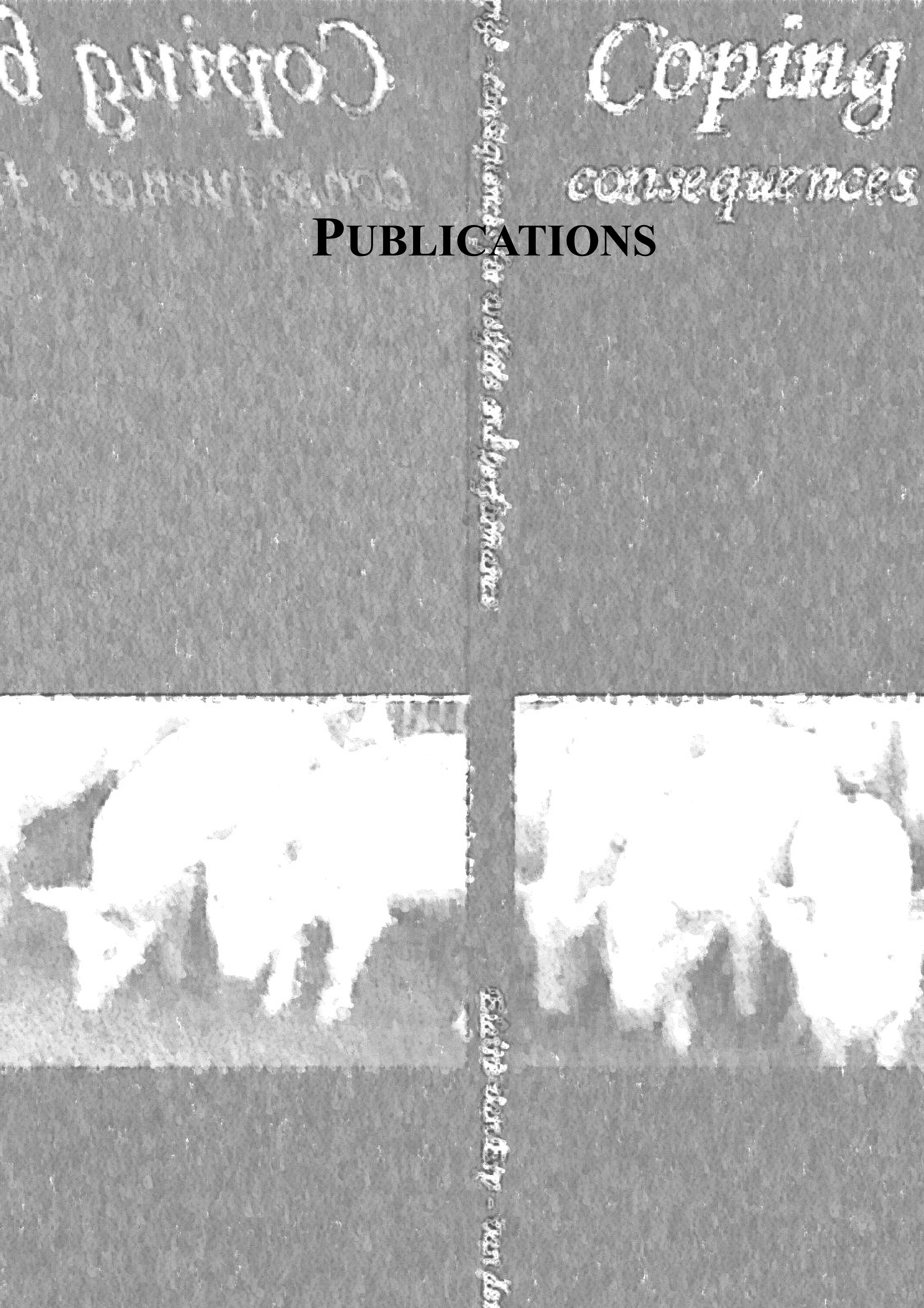
Voor varkens kan de rugtest gebruikt worden als maat voor (een dimensie van) de coping stijl. De rugtest wordt uitgevoerd bij jonge biggen (<4 weken oud). Een big wordt hierbij op de rug gelegd, en het aantal verzetspogingen gedurende 1 minuut wordt genoteerd. Het gemiddelde aantal verzetspogingen is 2 tot 3, variërend van 0 tot ongeveer 10. Een actief dier, met veel verzetspogingen, noemen we HR (‘high resisting’), een reactief dier, met weinig verzetspogingen, noemen we LR (‘low resisting’).

In ons onderzoek zijn rugtesten uitgevoerd op 3, 10 en 17 dagen leeftijd. In een validatie-experiment (hoofdstuk 2) hebben we uitgezocht dat het tijdstip waarop de test werd uitgevoerd en de volgorde waarin de dieren werden getest, geen invloed had op de testuitslagen. Opeenvolgende testuitslagen waren gecorreleerd (r=0,4) en standaardisatie van de uitgangspositie van waaruit biggen werden getest (wakker en lopend/staand) gaf geen verhoging van deze correlaties. De rugtest is relatief constant onder stabiele omstandigheden, maar uit onze studie waarin biggen werden overgelegd (hoofdstuk 4) is gebleken dat de dieren hun coping gedrag in de rugtest aanpassen wanneer uniforme hokken werden geformeerd op dag 3, op basis van rugtestuitslagen (alleen HR of alleen LR dieren in een hok).
We hebben geen verbanden gevonden tussen rugtestuitslagen en verhoging in speekselcortisol na spenen of mengen (hoofdstuk 4), maar dit kan te maken hebben met onze meetmethoden. We hebben 2 uur na de stressor speekselmonsters genomen, en voorbijgaande, kortdurende verschillen in cortisolresponsen tussen HR en LR dieren zouden we op deze manier gemist kunnen hebben.
Rugtestuitslagen waren voorspellend voor het gedrag in de ‘human approach test’ op 5-7 weken leeftijd (hoofdstuk 3), waarbij gemeten werd hoe snel elk dier een mens benaderde die in het hok was gestapt. HR dieren naderden de mens sneller dan LR dieren. Er werden geen verbanden gevonden tussen de rugtest en de andere gedragstesten op 5-7 weken, of met gedragstesten op 10-12 weken leeftijd. Mogelijk wordt dit verklaard doordat in de verschillende testen, verschillende aspecten van coping stijl of van de persoonlijkheid worden gemeten.
Een aantal relaties werden gevonden tussen rugtestuitslagen en immuunresponsen (hoofdstuk 7). HR dieren vertoonden een hogere cellulaire immuunrespons na het spenen en een lagere gevoeligheid voor een Salmonella-infectie. HR dieren lieten betere productieresultaten zien (hoofdstukken 5 en 6): ze groeiden harder in de vleesvarkenperiode (25-110 kg) en hadden een hoger vleespercentage en een betere karkassamenstelling bij slacht.

Uit dit onderzoek kan niet worden geconcludeerd dat een bepaalde coping stijl, hetzij actief, hetzij reactief, ‘beter’ is dan de andere. Dit zou ook vreemd zijn, aangezien ‘slechte’ coping strategieën door evolutie of selectie al verdwenen zouden moeten zijn. HR dieren hadden betere productieresultaten, maar uitsluitend in gemengde (HR/LR) groepen, en ze vertonen, zo blijkt uit andere studies, meer agressief gedrag. HR dieren hadden een hogere cellulaire afweerreactie en waren minder gevoelig voor een Salmonella-infectie, maar eenmaal geïnfecteerd zouden ze wel eens een minder goede humorale afweerreactie kunnen vertonen. In een groep is het waarschijnlijk voordelig om zowel actieve als reactieve dieren te hebben. Het op jonge leeftijd selecteren van HR dieren en deze in uniforme hokken bij elkaar zetten heeft weinig zin, omdat de coping stijl van de dieren dan kan veranderen, en in deze hokken wordt uiteindelijk niet beter gepresteerd. Wanneer op latere leeftijd (9 weken) wordt gemengd, wordt in gemengde hokken (HR/LR) beter geproduceerd dan uniforme (HR of LR) hokken, zo is uit ander onderzoek gebleken.

Omdat actief coping gedrag (HR) geassocieerd is met een hoog vleespercentage, waarop in de huidige varkensfokkerij geselecteerd wordt, verwachten we een stijging van het aantal HR dieren in de conventionele varkenspopulatie. In de biologische varkenshouderij, waarin meer stimuli geboden worden in de vorm van groepsgenoten, stro en buitenuitlopen, zullen waarschijnlijk de LR dieren bevoordeeld worden, omdat uit andere studies blijkt dat deze reactieve dieren beter om kunnen gaan met externe prikkels dan HR dieren. Bovendien zullen in deze en andere groepshuisvestingssystemen de meer actieve en agressieve HR dieren eerder worden uitgeselecteerd.

PUBLICATIONS



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DANKWOORD

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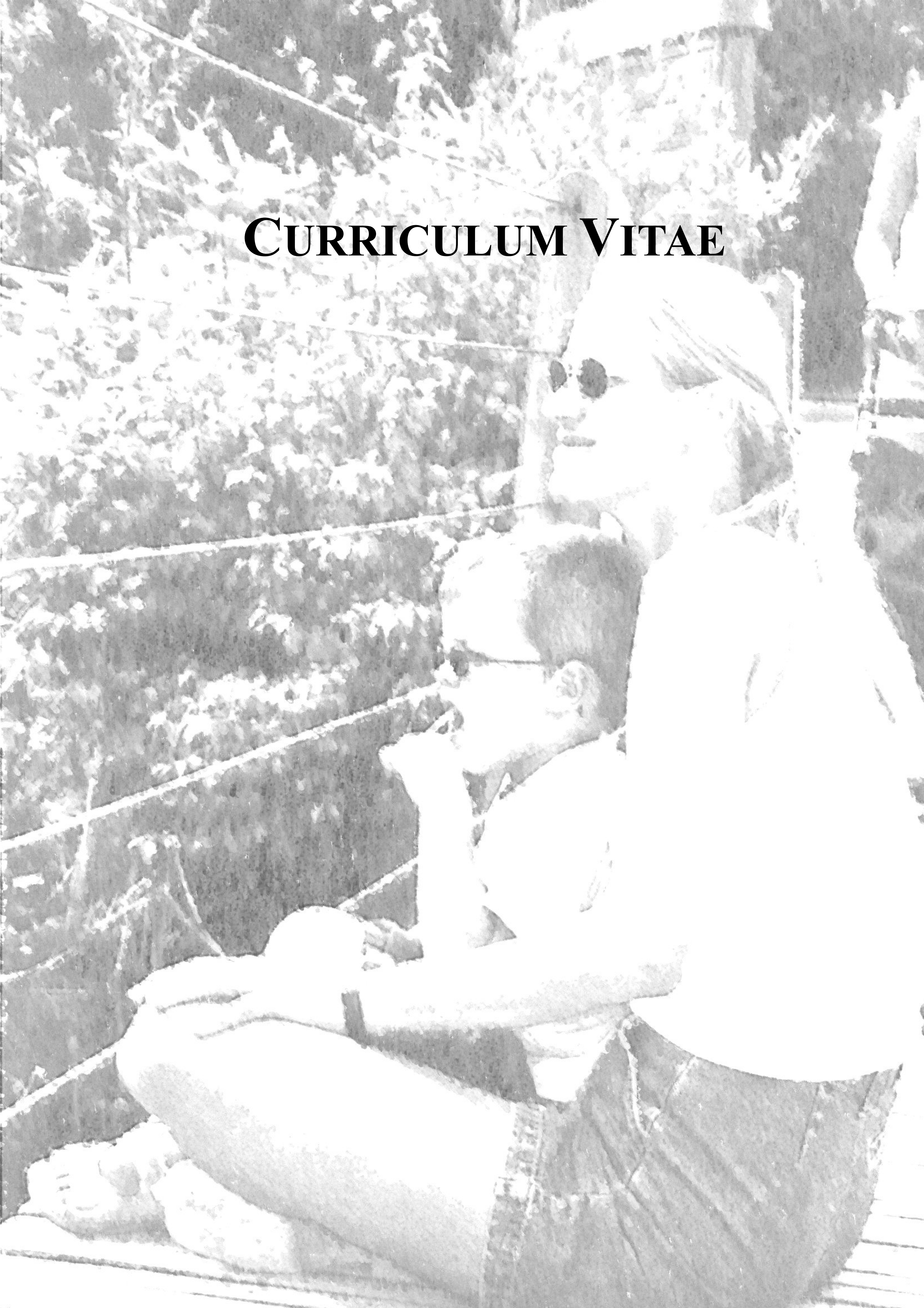
CURRICULUM VITAE



CURRICULUM VITAE

Lenny van Erp-van der Kooij werd op 5 april 1971 geboren in Den Haag als Elaine van der Kooij, dochter van Bram en Jannie van der Kooij. In 1989 behaalde zij het gymnasium-β diploma aan het Eerste Christelijk Lyceum te Haarlem. In datzelfde jaar begon zij in Wageningen aan de studie zoötechniek. Tijdens haar studie heeft Lenny een afstudeeronderzoek uitgevoerd in Ouwehand's Dierenpark, naar oestrusdetectie bij de Aziatische olifant, en een stage aan de Veterinaire Faculteit in Uppsala, Zweden, waar zij een onderzoek uitvoerde naar endotoxine verontreiniging van FSH preparaten voor pinken. Haar interesse voor varkens werd gewekt tijdens een afstudeervak over eenzijdige longontsteking bij vleesvarkens. Al voor haar afstuderen kwam Lenny in 1994 in dienst bij de toenmalige Vakgroep Veehouderij van de Universiteit Wageningen onder Jos Noordhuizen, waar ze eerst Lisette Graat en later Klaas Frankena ondersteunde met allerhande werkzaamheden, variërend van praktisch stalwerk tot het uitvoeren van statistische analyses. In januari 1995 studeerde ze af en na nog een aantal maanden bij Veehouderij werd Lenny aangenomen bij het Productschap voor Vee, Vlees en Eieren. Onder leiding van Jan Odink werkte ze daar als beleidsmedewerker aan de PVE Regeling Scharrelvarkens. In 1996 kwam Lenny in dienst bij de Faculteit Diergeneeskunde, eerst bij de Vakgroep Bedrijfsdiergeneeskunde en Voortplanting op de afdeling Huisvesting en Verzorging onder leiding van Martin Tielen, later bij de afdeling Varken van de Hoofdafdeling Gezondheidszorg Landbouwhuisdieren onder leiding van Jos Verheijden. Na twee jaar met vooral onderwijstaken werd in 1998, naast het geven van onderwijs, gestart met promotieonderzoek, onder begeleiding van Martin Tielen, Frank van Eerdenburg, Willem Schouten en Victor Wiegant, met dit proefschrift als resultaat.
Lenny is getrouwd met Ton van Erp, en samen hebben zij twee zoons, Martijn (16-4-1999) en Daan (30-5-2002).