Personalities in pigs

Individual characteristics and coping with environmental challenges

J.E. Bolhuis

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Jantina Elizabeth Bolhuis

Abstract

There are indications that pigs may have difficulty in adapting to the constraints of intensive housing conditions. Pigs show a wide variation in adaptive responses when exposed to the same stressful situation. Aim of this thesis was to investigate whether the behavioural coping responses of young piglets reflect and predict more general profiles of reactivity to challenges, often referred to as coping styles, under different rearing and housing conditions. For this purpose, pigs were characterized early in life as 'high-resisting' (HR) or 'low-resisting' (LR) on the basis of their resistance response in a so-called Backtest, in which they were manually restrained in supine position. The major part of the thesis focused on the interaction between these individual coping characteristics of pigs and their housing environment, which was either barren or enriched with straw bedding. The experiments described in this thesis show that HR pigs are more aggressive than LR pigs and less flexible in adapting their behaviour to environmental changes. As the two types of pig differed in response to the dopamine-agonist apomorphine, some initial evidence is provided for a neurochemical background of these behavioural differences. In addition, individual coping or personality characteristics of pigs were reflected in immune reactivity and in their home pen behaviour in barren and enriched environments. HR and LR pigs adapted differently to barren housing conditions. Moreover, individual characteristics modulated the effects of rearing and housing conditions on the behavioural response to novelty, immune reactivity, prevalence of gastric lesions and behavioural development. Remarkably, for almost all of the variables that were affected by housing environment, the impact was much larger for LR than for HR pigs. Thus, individual characteristics of pigs affect their performance in different environments and should be taken into account when studying the impact of housing on their behaviour and welfare. The knowledge of individual coping or personality characteristics could be extended and used for finding the optimal match between pigs and their social and physical environment in pig husbandry.

Keywords: pigs; behaviour; housing conditions; coping styles; personality; environmental enrichment; animal welfare; stress

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General introduction

The project described in this thesis was aimed at gaining insight in individual differences in adaptive responses to environmental challenges of growing-finishing pigs and the possible consequences for their health and welfare. Mismatches between the 'nature' of pigs and their environmental conditions in intensive pig husbandry appear to be the main source of adaptational problems. Therefore, this chapter starts with a brief biological characterization of pigs and a portrayal of some aspects of their species-specific behaviour that are relevant for (mal)adaptation to husbandry conditions, as well as a short description of recent developments in intensive pig production. It continues with a section on individual differences in stress responses. Finally, the aim and outline of the thesis are presented.

Pigs

Pigs were domesticated between 5000 and 10000 years ago and presumably are all descended from one species, the Eurasian wild boar, *Sus scrofa*, which is still common in Europe, Asia and North Africa (Clutton-Brock 1999; Hemmer 1983). Domestic pigs have been imported into other parts of the world and, as they easily feralise, large numbers of feral flocks are present in America and Australia (Graves 1984).

Domestication and selection have altered the anatomy and physiology of pigs. Modern breeds of domestic pigs differ largely from their wild ancestors in, for instance, skin hairiness, body form, fat disposal, rate of physical development, and litter size. Regarding behaviour, avoidance of humans, aggressiveness and motility are lower in domestic pigs than in wild boars (Hemmer 1983; Price 1999). Although wild and domestic pigs differ in many aspects, domestication has presumably not resulted in the loss of behaviours from the species-specific repertoire, i.e. behavioural differences between wild and domestic pigs appear to be of a quantitative rather than qualitative nature (Graves 1984; Price 1999). Studies on domestic pigs kept in semi-natural enclosures have indeed revealed that their behavioural repertoire still closely resembles that of their wild conspecifics (Jensen 1986; Newberry and Wood-Gush 1988; Petersen 1994; Stolba and Wood-Gush 1989).

Both wild and domestic pigs are gregarious animals. They make use of chemical marking, auditory and visual signals to communicate with conspecifics (Graves 1984; Kiley 1972; Von Klingholz 1979). The basic social unit of wild and feral pigs consists of one to several females and their juvenile offspring, near which 'peripheral individuals' (often (sub)adults associated with the females of the unit) are found. The animals within a basic unit have inter-individual bonds and usually synchronise their use of time and space. Female (wild and domestic) pigs preferably farrow in social isolation and construct a nest prior to parturition (Gundlach 1968; Jensen 1986).

Piglets enter the social group at about 10-14 days of age (Gundlach 1968; Jensen and Redbo 1987). The weaning process, although started early in life (Jensen et al. 1991), is

completed only when the pigs are about 13-17 weeks of age (Gundlach 1968; Jensen and Recén 1989). Adult males, which are commonly solitary but may also form all-male groups, approach the females when these exhibit sexual receptivity (Gundlach 1968; Mauget 1981; Meynhardt 1980).

Wild boars and feral pigs occupy a variety of habitats. Although pigs are not territorial, they do live in restricted areas, so-called home ranges (see Graves 1984; Mauget 1981). Activity periods of feral and wild pigs occur during both day and night, but it has been suggested that pigs shift to nocturnal activity only when predator (especially hunting) pressure is high (see Graves 1984; Mauget 1981). Wild and feral pigs can exploit an enormous variety of both vegetable and animal food sources (Gundlach 1968; Schley and Roper 2003). They spend the largest part of their active period, which ranges from 5-10 hours per 24h cycle, on feeding, foraging and exploratory behaviours (Gundlach 1968). Part of these exploratory behaviours, i.e. all activities concerned with gathering information about the environment (Birke and Archer 1983), are not specifically aimed at the immediate fulfilment of basic needs, but do play an important role in the animals' efforts to survive (Russell 1983; Wood-Gush and Vestergaard 1989). In spite of the fact that domestic pigs are generally supplied with basic necessities, such as food and shelter, they still appear to have an internal 'need' to perform explorative activities (Fraser and Broom 1997; Van Putten and Dammers 1976; Wood-Gush and Vestergaard 1991). Domestic pigs have been noted to spend 6-8 hours per day on exploration in a semi-natural enclosure, even when they were fed full rations of commercial feed (Wood-Gush et al. 1990a).

Pig husbandry

Since ancient times there have been two methods of keeping domestic pigs: firstly, the system of allowing groups of pigs to roam in forests under the eye of a swineherd, and secondly, the house or sty pigs in stables often with an outdoor area (Clutton-Brock 1999).

In the Netherlands, pig production changed rapidly from the sixties of the previous century onwards when intensive husbandry systems were developed for large-scale and efficient pork production. In order to increase efficiency, measures were taken that led to drastic alterations in both the social and physical environment of pigs. Over the past four decades, production costs have been reduced by increasing the number of animals per square meter, banning substrate and/or outdoor housing and introducing (partly) slatted floors. Productivity has been enhanced by provision of high quality feed, genetic selection for meat production traits and a decrease in the weaning age of piglets to 3-4 weeks in order to increase the number of litters per sow per year. Standard management procedures in pig husbandry comprise regrouping of pigs to obtain uniform cohorts for slaughter, ear tattooing, vaccination, teeth clipping, castration of the male piglets and tail docking.

Stress, coping and welfare

It has been suggested that housing conditions and management procedures in intensive pig husbandry cause stress in pigs. Although there are many definitions of 'stress', it is generally referred to as a state of threatened homeostasis. It can be viewed as a struggle between opposing forces, with on the one hand situations that are appraised by the individual as (potential) threats to its homeostasis (stressors), and on the other hand counteracting coping processes (after Lazarus and Folkman 1984). Stressors can be external stimuli that are perceived as threatening, but stress may also arise due to conflicts between internal (motivational) states of the individual and environmental conditions (Jensen and Toates 1997; Wiepkema 1987). Animals try to cope, i.e. to manage or alleviate stress (Lazarus and Folkman 1984), by alteration of behaviour and a variety of physiological responses, such as altered activity of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Acute stress responses basically concern the readjustment of behavioural and metabolic priorities and are normally adaptive, i.e. they involve learning processes that allow the individual to respond more adequately to (similar) future stressors (see Levine and Ursin 1991; McEwen 2001; Moberg 1985). If animals have difficulty in coping with persistent and severe stressors (i.e. chronic stress), sustained (over)activation of stress responsive systems may lead to a wide variety of behavioural and physiological disturbances and increased disease susceptibility (see Henry and Stephens-Larson 1985; McEwen and Stellar 1993; Moberg 1985; Wiepkema and Koolhaas 1993).

Stress and coping are crucial factors in many of the approaches used to describe and study welfare. This is most obvious for the 'biological functioning' approach, in which welfare of an individual is defined as 'its state as regards its attempts to cope with the environment' (see Broom 1986). According to this approach, the stronger the coping efforts of an individual, the poorer its welfare is assumed to be. In 'feelings-based' approaches welfare is seen as a balance between positive and negative subjective states or mental experiences (see Duncan and Fraser 1997; Simonsen 1996). Strong coping efforts and low success rates of these efforts are likely accompanied by negative emotions. Hence, sustained stress and coping difficulties can be considered a risk to welfare.

Pigs in pig husbandry

Many of the standard management procedures that growing-finishing pigs are subjected to induce behavioural and physiological changes that are indicative of short-term stress. The mixing of unfamiliar pigs, for instance, frequently results in temporary increases in cortisol levels, immune suppression and reduced growth (e.g. Ekkel et al. 1995; 1996; Hessing and Tielen 1994; Moore et al. 1994). It has been suggested that pigs may also suffer from sustained or chronic stress induced by the prolonged constraint on the possibility to perform

species-specific behaviour in intensive housing systems (e.g. De Jong 2000; Wiepkema and Koolhaas 1993). Most growing-finishing pigs in the Netherlands are housed in barren environments where bedding materials are rarely used and in which the performance of, for instance, explorative activities, such as chewing and rooting, is continuously thwarted. In view of the complexity of stress responses, it is not possible to determine whether animals experience sustained stress by measuring one single unambiguous variable (Moberg 1985). Several behavioural and physiological observations together indicate, however, that pigs may have difficulty in adapting to the limitations of modern pig housing conditions. For instance, many pigs cannot cope with these conditions without developing maladaptive behaviours, such as manipulative oral activities directed at pen mates, stereotypies or excessive aggression (Beattie et al. 1995; Hagelsø-Giersing and Studnitz 1996; Ödberg 1987; Schouten 1986). These behaviours may represent attempts to behave in an adaptive way in an environment to which complete adaptation is not possible (Mills 2003). In addition, changes in the (re)activity of the HPA-axis (Beattie et al 2000; De Jong et al. 2000) and the frequent occurrence of stress-related pathologies in intensively kept growing-finishing pigs (Hessing et al. 1992; 1994a) point to sustained stress.

It is often assumed that domestication and selection have resulted in pigs that are better able to cope with pig husbandry systems and procedures. Domesticated species may indeed adapt more readily to a relatively small, physically uniform environment than genetically wild stocks (Price 1999). However, the modern housing systems developed in the past few decades have a rather short history as compared with the history of the pig as a domestic animal, and it is likely that adaptation has not kept pace with the intensification of pig husbandry. Moreover, it has been stated that the strong genetic selection for high productivity has decreased the adaptive capacity of pigs. Thus, 'modern' pigs that allocate a high proportion of resources to production traits may have fewer resources left for responding adequately to environmental challenges (see Beilharz 1993; Rauw et al. 1998). In addition, limitations in the rearing conditions of pigs in early life may have long-lasting negative consequences for adaptive capacity at a later age (Schouten 1986; Wiepkema 1987). For instance, the restricted space in farrowing pens in current pig husbandry systems appears to hamper the development of sophisticated social skills that are necessary for coping with social challenges in later life (Lammers and Schouten 1985a; 1985b; Schouten 1986).

Individual differences in responding to stress
Individual pigs may adopt different reaction patterns when exposed to the same stressful situation (e.g. Lawrence et al. 1991; Von Borell and Ladewig 1992). Moreover, pigs show a large variation in the vulnerability to develop stress-related behavioural and physiological

disturbances, indicating differences in adaptive capacity (Schouten and Wiepkema 1991; Von Borell and Ladewig 1989). The identification of basic characteristics that are predictive of (mal)adaptation to various husbandry conditions could be relevant both for pig welfare and pig industry. Individual differences in coping reactions of pigs have therefore received growing attention (e.g. Janczak et al. 2003; Spoolder et al. 1996).

Individual response patterns have been most extensively studied in humans and laboratory animals. A general assumption is that every individual has more or less stable or enduring psychophysiological characteristics or traits that, besides the actual situation, influence how the individual reacts (see Eysenck 1981; Zuckerman 1984). The moderating influences of such characteristics are often most pronounced under conditions of stress (Budaev 1997; Stowell et al. 2001; Suomi 1987). In attempting to structure and study (sets of) these individual characteristics, a wide range of concepts and models have been used, with accompanying terms like 'temperament', 'personality', 'emotionality' or 'coping style' (see Erhard and Schouten 2001; Gosling and John 1999; Ramos and Mormede 1998; Schwarz et al. 1999; Zuckerman et al. 1984).

The search for stable individual characteristics in pigs has largely been influenced by the concept of diverging 'coping styles' or 'coping strategies', which is based on two basic modes of responding to challenges that have been described in the classical stress literature (see Benus et al. 1991b; Engel and Schmale 1972; Henry and Stephens 1977; Koolhaas et al. 2001). These are on the one hand the fight-flight response, characterized by active behaviour and enhanced sympathetic adrenal-medullary reactivity that prepares the individual to action (Cannon 1914), and on the other hand the conservation-withdrawal response, marked by behavioural immobility (Engel and Schmale 1972) and physiologically coupled to activation of the HPA-axis (Henry and Stephens 1977).

Both situational and individual factors appear to determine which of these patterns is most predominantly activated in response to stress (e.g. Cools and Ellenbroek 2002; Henry and Stephens 1977; Sluyter et al. 1996). The degree of (perceived) control over the situation appears to be an important factor in selecting a coping pattern (see Henry and Stephens 1977; Thoits 1995). Extensive research in male mice selected for the readiness to attack an intruder in their territory demonstrated that individuals might be biased towards one of the two modes of responding in threatening situations (Benus et al. 1991b; Koolhaas et al. 2001). Fast-attackers were more likely to adopt an active behavioural (fight/flight) response to several social and non-social challenges, whereas slow attackers generally 'preferred' a conservation-withdrawal or passive response (Benus et al. 1990a; 1992; Sluyter et al. 1996). Moreover, the two types of mice differed in the flexibility of responding to environmental changes (Benus et al. 1987; 1988; 1990b), indicating fundamental differences in the organization of behaviour with internal and external information (see Koolhaas et al. 2001).

Differences in the state and reactivity of central nervous and neuroendocrine mechanisms appear to underlie these individual differences (e.g. Bohus et al. 1987; Koolhaas et al. 1999; 2001). Divergent selection for attack latency in mice thus led to co-variation in associated features that determine the 'preferred' response pattern to several challenging situations, i.e. their 'coping style'. Similarly, in rats, selection for behavioural or physiological components of individual coping styles resulted in correlated selection for other traits. Examples are the Roman High and Low Avoidance rats, which diverge in active avoidance behaviour (see Driscoll and Bättig 1982), and the APO-SUS and APO-UNSUS rats, divergently selected for their stereotyped gnawing response to the dopamine-receptor agonist apomorphine (see Cools et al. 1990; 1993; 1994). The differences in apomorphine-susceptibility of rats with diverging coping styles reflect differences in functional activity of dopaminergic pathways, which are involved in the central response to stress (see LeMoal and Simon 1991; Puglisi-Allegra and Cabib 1990) and the organisation of behaviour (see Cools et al. 1980; 1990; 1994).

Besides rodents, in a growing number of other species differences between individuals in the 'preference' for one of the described coping responses have been identified (see Koolhaas et al. 1999 for review; Verbeek et al. 1994; 1996).

Backtest

It has been suggested that the predisposed coping style of an individual pig can be predicted early in life from its reaction pattern in a so-called Backtest (Hessing et al. 1993; Ruis et al. 2000). In the Backtest a piglet is manually restrained in supine position for 60 s on a table (or other surface) outside its home pen. The test is mostly carried out during the suckling period, at about 1-2 weeks of age (Geverink et al. 2002a; Hessing et al. 1994a; Van Erp-Van der Kooij et al. 2000). The behavioural response of piglets in the Backtest can range from uninterrupted immobility to excessive struggling. It has been hypothesised that a longer time spent struggling in this inescapable situation reflects an 'active' coping strategy whereas immobility corresponds with 'passive' coping (Hessing et al. 1993).

Classification of pigs is based on the number of escape attempts (i.e. bouts of struggles with mainly the hind legs) they display during the test. The number of escape attempts can be scored easily and without additional equipment, which allows classification of large numbers of piglets, even in commercial pig husbandry conditions. More importantly, the number of escape attempts is indicative of the overall reaction pattern of a pig in the Backtest, as it relates to the duration of escape behaviour, the number of vocalisations uttered, the total duration of escape behaviour and the cardiac response in this test (Hessing et al. 1994a; Ruis et al. 2000). The number of escape attempts ranges from 0 to about 10 and shows a unimodal distribution (Ruis et al. 2000; Van Erp-Van der Kooij et al. 2000; Bolhuis,

unpublished results). Usually, two 'types' of pig are identified at either end of this distribution: the ones that do not or hardly show resistance, from here on labelled 'low-resisters' (LR), versus the animals that show many escape attempts, referred to as 'high-resisters' (HR). To what extent these types actually are the extremes of a population of pigs depends on the exact characterization criteria, which have varied among different investigators since the original description of the Backtest (Hessing et al. 1993). For instance, in one study the batch of experimental pigs was simply divided into two groups (Schrama et al. 1997), whereas in another study sharper criteria were used that resulted in identification of roughly 50-60% of the tested population as either HR or LR, omitting a large intermediate group of pigs (40-50%) from analysis (Ruis et al. 2000; 2001). Others included all tested pigs in their studies and used a correlational approach (Van Erp - Van der Kooij et al. 2002).

The Backtest appears to be a promising test for grasping at least part of the individual variation in adaptive responses in pigs and possibly their vulnerability for developing stress-related behavioural and physiological disturbances, because reaction patterns in this test were predictive of some other responses in later life (Hessing et al. 1994a; 1994c; 1995).

From its first description as a challenge for assessing the coping style of individual pigs (Hessing et al. 1993), however, the Backtest has been criticised on methodological grounds. This criticism particularly concerns the 'classification approach', because selection of extremes is based on more or less arbitrary cut-off points (Jensen 1994; Jensen et al. 1995). Indeed, it should be kept in mind that HR and LR pigs are constructed classes of extremes that nevertheless coexist within a population. As it is unknown whether the selection variable, the number of escape attempts, is linearly related with the underlying characteristic(s) we are testing for (i.e. coping style or personality), we preferred to use a classification instead of a correlational approach. In the experiments described in this thesis, the classification criterion described by Hessing and co-workers, who reported to characterize about 80-90% of the tested population as either HR or LR (Hessing et al. 1994a), was used (see e.g. chapter 5 for a detailed description).

Aim and outline of the thesis

It is largely unknown whether and to what extent the Backtest response of piglets is predictive of their manner and capacity of adapting to intensive, potentially stressful, housing conditions. Moreover, both rearing conditions in early life and actual housing environment may affect adaptive responses of pigs. The impact of environmental conditions could be different for individuals with diverging coping characteristics.

The experiments described in this thesis investigated whether the characterization of piglets early in life reflects and predicts a more general profile of reactivity to challenges, often referred to as coping style, under different rearing and housing conditions. For this purpose

the concept of diverging coping styles was used as a framework, and the Backtest as a classification tool. The major part of this thesis focused on the interaction between housing environment and individual characteristics.

The experiment described in chapter 2 aimed at exploring the neurochemical background of the differences between LR and HR pigs. The possible involvement of dopaminergic pathways was investigated by measuring the behavioural response of pigs to the dopamine agonist apomorphine at 18 weeks of age.

In pig husbandry practice, the regrouping of unfamiliar pigs after weaning is a common procedure. Chapter 3 describes the behaviour of HR and LR pigs in response to this challenging social situation. We investigated whether Backtest classification of piglets is predictive of their fighting behaviour immediately after regrouping and the resulting social status.

Chapters 4-7 specifically studied the influence of housing environment on pigs with diverging coping characteristics. The physical environment in which pigs live is critical to their welfare. As argued above, the barren environments in which growing-finishing pigs are frequently housed may in particular constrain their internal 'need' to express explorative behaviours like rooting and chewing. In the experiments described in chapters 4-7, the environment of pigs was varied in one aspect: the availability of straw bedding. Straw bedding is thought to serve as a partial outlet for the explorative activities that are natural to pigs. Although we are aware that sole provision of straw is a relative enrichment, the contrasting housing conditions in this thesis are referred to as 'barren' and 'enriched'. In chapter 4, the flexibility of HR and LR pigs from different rearing conditions in responding to environmental changes (outside their home pen) was investigated. For this purpose, pigs were trained to find a food reward in one of the arms of a T-shaped maze, after which a change in the maze environment was introduced or the side of the food reward was reversed.

Chapter 5 was aimed at investigating the modulating effects of both housing environment (barren or enriched) and individual coping characteristics (HR or LR) on immune reactivity of pigs. Pigs were immunized with KLH-DNP and in vitro lymphocyte proliferation and antibody responses were determined.

The impact of housing environment on the behavioural development of pigs with different Backtest classifications was studied in chapter 6. Special attention was given to exploratory behaviours, injurious behaviours (i.e. oral manipulation of pen mates), aggression and play.

Because not only the actual housing environment, but also housing history (i.e. rearing environment) may influence the behaviour and physiology of pigs, in chapter 7 the effects of these two factors on the behaviour, growth and some pathological measures of LR and HR individuals were tested.

Finally, in chapter 8, the major findings of the studies are summarized and discussed.

Responses to apomorphine of pigs with different coping characteristics

JE Bolhuis, WGP Schouten, IC de Jong, JW Schrama, AR Cools, VM Wiegant

ABSTRACT

Classification of pigs based on the degree of resistance they display in a so-called Backtest seems, to a certain extent, predictive of their coping strategy. The present study examined whether, as found in rodents, the behavioural response to apomorphine of pigs relates to individual coping characteristics. During the suckling period pigs were subjected to the Backtest. In this test, each pig is restrained on its back for 1 min and the resistance (i.e. number of escape attempts) is scored. Pigs classified as low-resisting (LR, n=10) or high-resisting (HR, n=10) were selected. At 17-18 weeks of age they received a saline and an apomorphine injection (0.2 mg/kg s.c.) on two consecutive days in a balanced design. Behaviour was recorded until 120 min after injection. Apomorphine increased locomotion in all pigs and reduced standing, standing alert and defecating. In addition, apomorphine induced the occurrence of some peculiar activities, rarely seen in saline treated pigs, which seemed to represent either a transition between different postures or a conflict between hind and fore limb activities. Apomorphine-treated LR pigs performed significantly more of these activities than HR pigs. However, snout contact with the floor, an oral stereotypy, was significantly increased in apomorphine-treated HR pigs, but not in apomorphine treated LR pigs. In conclusion, the response to apomorphine of pigs relates to their behavioural response, HR vs. LR, in the Backtest. The contrasts in behavioural response to apomorphine suggest a difference in the dopaminergic system between HR and LR pigs.

INTRODUCTION

In rodent populations, two extreme types of individual have been described that adopt different behavioural and physiological response patterns, or coping strategies, when stressed (Benus et al. 1991b; Bohus et al. 1987; Cools and Gingras 1998; Cools et al. 1990). Diverging behavioural responses to challenges are reported for domesticated pigs as well (Erhard and Mendl 1999; Lawrence et al. 1991; Mendl et al. 1992; Schouten and Wiepkema 1991). An indication for the behavioural strategy of pigs can be obtained early in life (at 1-2 weeks of age) by measuring the degree of resistance displayed in a so-called Backtest (Hessing et al. 1993). In this test each pig is restrained on its back for 1 min and its reaction is scored by counting the number of escape attempts. The classification of pigs in this test as 'high-resisting' (HR) or 'low-resisting' (LR) is, to a certain extent, predictive of their behavioural responses to stressful events in later life. For instance, HR and LR pigs differ in level of aggression and reaction to a novel object (Hessing et al. 1993; 1994a). Since physiological and immune responses to challenges are different for HR and LR pigs as well (Hessing et al. 1995; Schrama et al. 1997), it has been suggested that the Backtest classification (LR versus HR) is an indicator of the coping style of individual pigs (Hessing et al. 1994a).

In rodents, a link has been found between individual coping characteristics and the

behavioural response to the dopamine-agonist apomorphine (Benus et al. 1991a; Cools et al. 1990; 1993; 1994; Durcan et al. 1984). Apomorphine induces stereotyped behaviour in several species (e.g. dogs: Nymark 1972, rats: McKenzie 1972, mice: Benus et al. 1991a, cats: Motles et al. 1989, cattle and sheep: Sharman and Stephens 1974) by stimulating cerebral dopamine receptors (Cooper and Dourish 1990; Kelly et al. 1975). The stereotyped syndrome is characterized by the performance of an invariant order of movements, initially accompanied by high levels of motor activity (Antoniou and Kafetzopoulos 1980; Costall and Naylor 1979; Kelly et al. 1975; Nymark 1972). As a function of time since injection and dose level, stereotypic behaviour becomes more and more inflexible and generally switches to the performance of compulsive oral activities such as sniffing, licking and gnawing in rodents (Lyon and Robbins 1975; McKenzie 1972) and cattle (Sharman and Stephens 1974), chewing and licking in sheep (Sharman and Stephens 1974) and snout rubbing, licking and apparent drinking in pigs (Fry et al. 1981; Terlouw et al. 1992). Rats and mice that adopt distinct strategies when challenged, differ in sensitivity to the stereotypic effects of apomorphine, which probably reflects a difference in functional activity of their dopaminergic system (Benus et al. 1991a; Cools et al. 1990; 1993; 1994; Durcan et al. 1984). Indeed, there is ample evidence implicating a connection between brain dopaminergic activity and responses to stress (see Cools et al. 1990; Piazza et al. 1991).

The behavioural effects of different doses of apomorphine have been studied systematically in pigs (Terlouw et al. 1992). Pigs also vary in their response to the drug (Fry et al. 1981; Terlouw et al. 1992). Whether the response to apomorphine in pigs is linked to individual coping characteristics, as has been found in rodents, is unknown. Therefore, the objective of the present study was to examine if variation in pigs' behavioural response to apomorphine relates to the degree of resistance (high or low) they display in the Backtest.

METHODS

Animals and housing

Pigs were subjected to the Backtest (Hessing et al. 1993) at 10 and 17 days of age. In this test, each pig is restrained on its back by placing one hand over the throat and the other loosely on the hind legs. The number of escape attempts (i.e. series of struggles of the hind limbs) is scored during 60 seconds. The number of escape attempts is, as it relates to the duration of resistance, the latency to the first escape attempt, the number of vocalisations and the cardiac response in the Backtest (Hessing et al. 1993; 1994a; Bolhuis, Schouten, Wiegant, unpublished results), indicative for the overall reaction pattern of a pig in this test.

A pig is classified as high-resisting (HR) if it performs more than four escape attempts in two Backtests, with a minimum of two attempts in one test. If a pig struggles less than four times in the two Backtests, with a maximum of two attempts in one test, it is labelled low-resisting (LR). From a pool of tested pigs 10 HR and 10 LR pigs were randomly selected; for both classifications the female to castrated male ratio was 2:3. Pigs were of the Great Yorkshire (GY, n=2), GY*Dutch Landrace (DL, n=3) or GY*(GY*DL) breed (n=15).

Pigs were weaned at 5 weeks of age and housed in groups of five; LR and HR mixed (2:3 or 3:2) within a pen. The floor of the pens, measuring 5 m^2, consisted of a straw-covered area and metal slats. Room temperature was 20°C. Lights were on between 7.00 and 19.00 h. Pigs were fed a commercial feed (crude protein content 13.2%) twice a day. Water was continuously available. The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments which complies with the ETS123 (Council of Europe, 1985) and the 86/609/EEC directive.

Procedure

At an age of 17-18 weeks (body weight 62.3 ± 1.8 kg), the behavioural response to apomorphine was observed. The dose of apomorphine hydrochloride (OPG, Utrecht, The Netherlands) used was 0.2 mg/kg BW. This dose was chosen based on the following considerations: (1) Starting with doses of 0.1 mg/kg s.c. hyperactivating effects of apomorphine (APO) are observed in pigs (Schouten, Schothorst and Wiegant, unpublished dose-response data; Terlouw et al. 1992, dose-response study). (2) With increasing doses of apomorphine administered the response repertoire narrows, i.e. the response becomes restricted to less and less behavioural elements (Robbins et al. 1990). In a recent study on pigs a dose of 1.0 mg apomorphine/kg s.c. induced large qualitative, rather than quantitative, differences in response. Although several pigs were engaged in oral stereotypies following APO injection, the type of oral activity displayed varied considerably among individuals (Terlouw et al. 1992). Such a heterogeneity in responses complicates an interpretation of the results in terms of sensitivity. The dose of 0.2 mg/kg used in the present study was considered to elicit (some of the) hyperactivating effects of the drug in pigs, without animals being 'trapped' in idiosyncratic response patterns.

APO was dissolved in 5 ml saline (0.9% NaCl) just before administration. The same volume of solvent was injected as a control. Individuals were their own controls, i.e. each pig received a saline and an apomorphine (APO) injection on two consecutive test days. The sequence of APO and saline (control) injection (APO followed by saline versus saline followed by APO) was balanced.

Post injection behaviour was recorded in a test room (floor 3.2 x 3.8 m, height circa 1.8 m) containing two food troughs. The floor of the room consisted of metal slats (2.0 x 3.8 m) and

a concrete area (1.2 x 3.8 m). Water was available from a drinking nipple and nipples above the food troughs; no food was available during the test. In order to habituate to the new environment, pigs were placed in the test room for 30 min, in pairs of pen mates, on the morning of their first test day. In the afternoon, an individual pig was removed from its home pen, injected subcutaneously (behind the ear) and immediately moved to the test room. Behaviour was videotaped until 120 min post injection.

Behavioural analysis

Behaviour was scored continuously until 120 min post injection using the Video Tape Analysis System Version 3.1 (Noldus, Wageningen, The Netherlands). The posture of the animal and the activity it performed were recorded, using the following ethogram:

Postures and locomotion: standing, locomotion (forward walking), lying or sitting on hind limbs. Other behavioural activities: standing alert (standing immobile with open eyes, raised head and pricked ears), defecating, snout contact with the floor (keeping rooting disk at or close to the floor), snout contact with objects (keeping rooting disk in contact with or close to walls, troughs, nipple or door), other (all other activities). Preliminary behavioural analysis indicated that APO strongly increased the occurrence of some peculiar activities, rarely seen in saline treated pigs, such as standing or walking with bent ankles or moving with the hind limbs while the fore foot was resting on the floor. Since each of these activities appeared to represent either a transition between different postures or a conflict between hind and fore limb activities, they were united into a single score, labelled 'discrepancy between fore and hind limb movement or posture' (DFH). This behavioural element was added to the ethogram.

All activities were recorded as proportions of time, with the exception of defecating, of which the frequency was scored. In order to facilitate analysis of time effects, the 120-min observation time was divided into 10 time periods of 12 min each.

Data analysis

Analysis was performed on square root transformations of frequencies and angular transformations of proportions of time spent on the various behaviours in 12-min time intervals, using the General Linear Methods procedure (Statistical Analysis System Institute Inc. 1989). The data of one pig were excluded from analysis because the animal escaped from the test room. Preliminary analyses showed no main or interaction effects of sex, breed, or sequence of treatments (saline followed by apomorphine or vice versa) on any behavioural element, so these factors were omitted from the final statistical model.

For each behaviour, the effects of and interactions between the factors treatment (saline or APO), Backtest classification (LR or HR) and time (ten 12-min periods) were assessed by

analysis of variance (ANOVA) using a split-split-plot model with values in time and treatment of individual animals taken as repeated measurements. When significant effects were found, least-square means were used to detect differences and p-values were adjusted using the Bonferroni correction. Data in the text are presented as means (over the whole 120-min observation period, unless mentioned otherwise) ± SEM.

RESULTS

The dose of APO used (0.2 mg/kg s.c.) induced hyperactivity: within 10 min post injection, most pigs started walking or even running around. We observed that during the first 30 min many pigs followed a fixed path and persistently performed the same behaviours in different parts of the room.

Often pigs kept snout contact with the floor while walking. In the period of 13-24 min post injection, some animals spent over 90% of their time on this behaviour. When snout contact with the floor became more sustained, it was often limited to the area where a metal strip covered the floor to separate the metal slats from the concrete area.

For all behavioural elements scored, significant time effects were found. Most of the described APO-induced behavioural changes appeared within a few minutes after drug administration and reached a peak between 13-24 min post injection.

Postures and locomotion

Percentages of time spent on different postures and locomotion after APO or saline injection are shown in Fig. 2.1. Treatment affected locomotion ($F_{(1,17)} = 34.0$, $p < 0.001$; 20.9 ± 1.6% for APO and 10.4 ± 0.8% for saline treatment). A treatment x time period interaction was found ($F_{(9,306)} = 13.7$, $p < 0.001$), with higher percentages of locomotion after APO than saline injection until 72 min post injection (Fig. 2.1). Standing was reduced after APO injection (58.3 ± 3.2%), as compared to saline injection (77.8 ± 3.4%), $F_{(1,17)} = 57.3$, $p < 0.001$ (Fig. 2.1). Following APO-injection animals spent more time lying or sitting (18.1 ± 4.2%) than following saline injection (10.6 ± 3.7%), $F_{(1,17)} = 7.0$, $p < 0.05$ (Fig. 2.1). Standing and lying/sitting were unaffected by treatment x time interactions.

DFH was rarely observed after saline treatment, and only while pigs explored the floor under the food troughs or while they made transitions from standing or walking to lying, or vice versa. Analysis of variance revealed an effect of treatment ($F_{(1,17)} = 46.4$, $p < 0.001$) and a treatment x time interaction ($F_{(9,306)} = 2.2$, $p < 0.05$) on DFH, with a higher percentage of DFH following APO injection (2.9 ± 0.6%) than following saline injection (0.6 ± 0.2%) during the largest part of the observation period (Fig. 2.1).
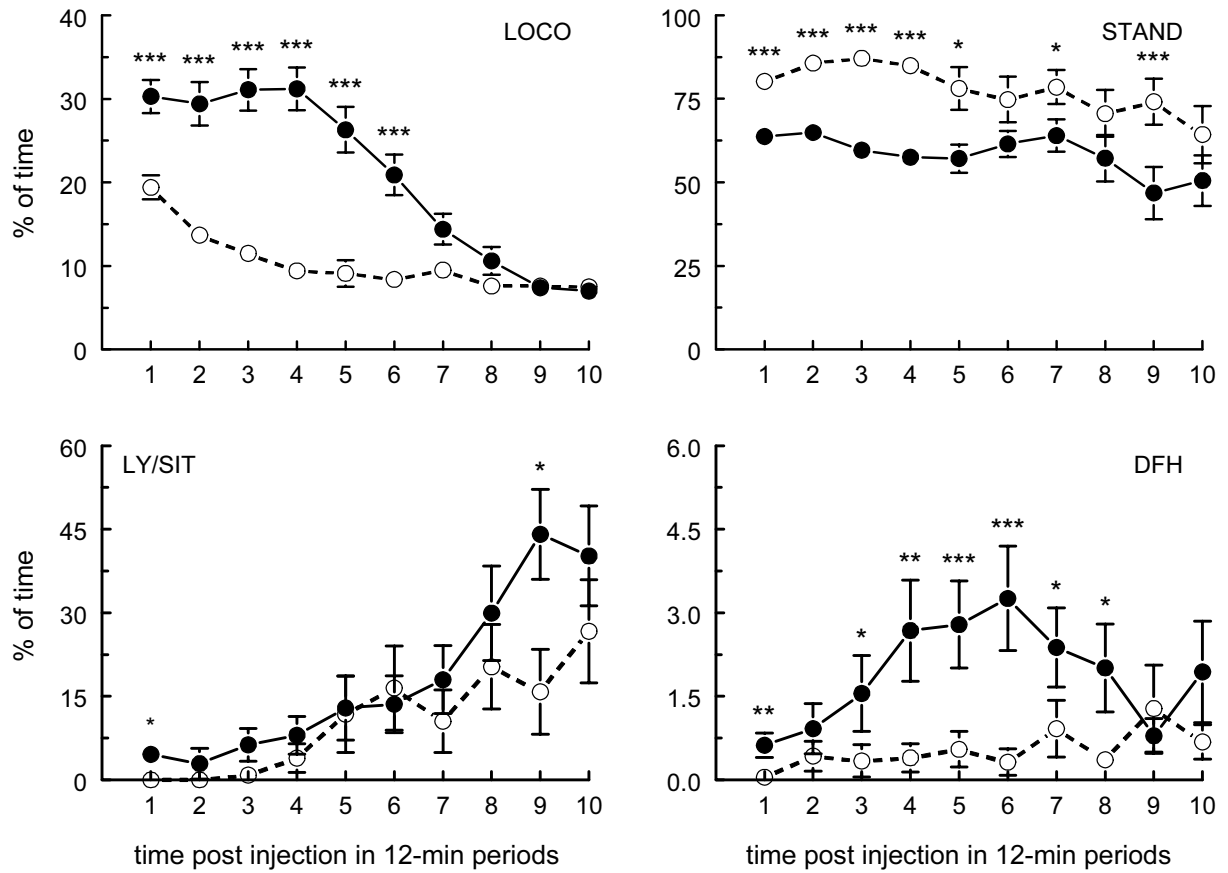
Fig. 2.1. Locomotion (LOCO), standing (STAND), lying/sitting (LY/SIT), and discrepancy between fore and hind limb movement or posture (DFH) after s.c. injection of saline (dotted lines, open symbols) or 0.2 mg/kg apomorphine (solid lines, closed symbols). Effects of treatment within a time period are indicated: *p < 0.05; **p < 0.01; ***p < 0.001. Note: different Y-axis scaling for LOCO, STAND, LY/SIT and DFH.

Other behavioural activities

Percentages of time spent on various behavioural activities and frequency of defecating after APO or saline injection are shown in Fig. 2.2. Treatment ($F_{(1,17)}$ = 32.2, $p < 0.001$) and the treatment x time period interaction ($F_{(9,306)}$ = 5.2, $p < 0.001$) affected standing alert, with lower levels of this behaviour for APO than for saline treatment until 84 min post-injection (120-min average 10.6 ± 1.6% for APO and 23.2 ± 4.1% for saline treatment; Fig. 2.2). Expressing the time spent on standing alert as a percentage of standing showed that the drug also reduced this behaviour relative to standing (data not shown).
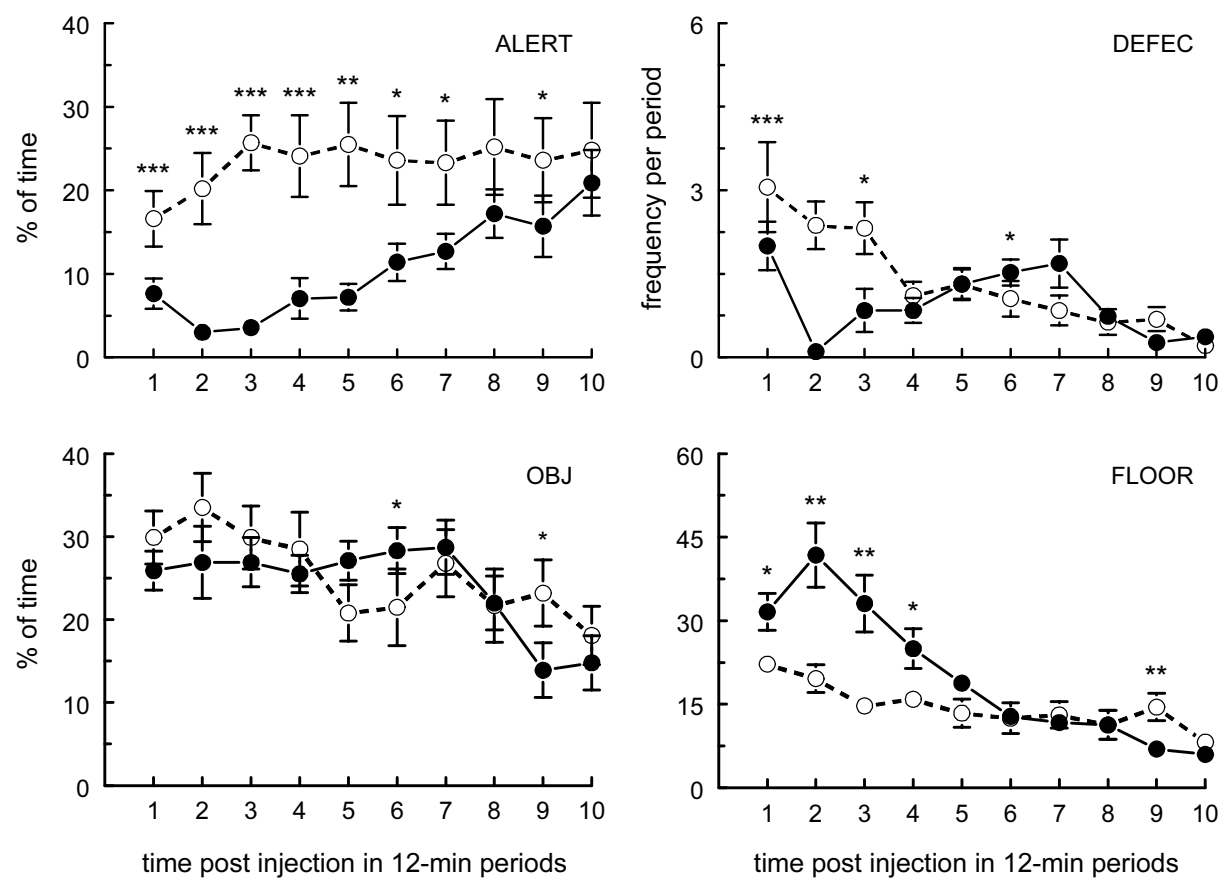
Fig. 2.2. Standing alert (ALERT), defecating (DEFEC), snout contact with objects (OBJ), and snout contact with the floor (FLOOR) after s.c. injection of saline (dotted lines, open symbols) or 0.2 mg/kg apomorphine (solid lines, closed symbols). Effects of treatment within a time period are indicated: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Note: different Y-axis scaling for ALERT, DEFEC, OBJ and FLOOR.

APO initially reduced frequency of defecating, with levels of almost zero in the period of 13-24 min post injection (Fig. 2.2). However, in the 61-72 min period the frequency of defecating was higher after APO than after saline injection. Analysis of variance revealed an effect of treatment ($F_{(1,17)} = 4.5$, $p < 0.05$) and a treatment x time interaction ($F_{(9,306)} = 6.5$, $p < 0.001$). Total frequencies of defecating were 13.6 ± 2.2 for saline and 9.7 ± 1.2 for APO treatment.

Time spent on snout contact with objects, such as the food troughs, door, nipple or walls, was not influenced by treatment (saline: 25.4 ± 3.1%, APO: 24.0 ± 1.8%; $p > 0.10$). However, the time course of this behavioural element differed between APO and saline treated pigs, which was indicated by a treatment x time interaction ($F_{(9,306)} = 2.3$, $p < 0.05$; Fig. 2.2).

Time spent on snout contact with the floor was affected by treatment ($F_{(1,17)} = 5.3$, $p < 0.05$) and the treatment x time interaction ($F_{(9,306)} = 6.9$, $p < 0.001$) with a higher percentage of snout contact with the floor until 48 min after APO injection, followed by a decrease relative to saline injection in the period of 97-108 min (Fig. 2.2). Percentages of snout contact with the floor were 14.5 ± 1.1% for saline and 19.6 ± 2.1% for APO treatment.
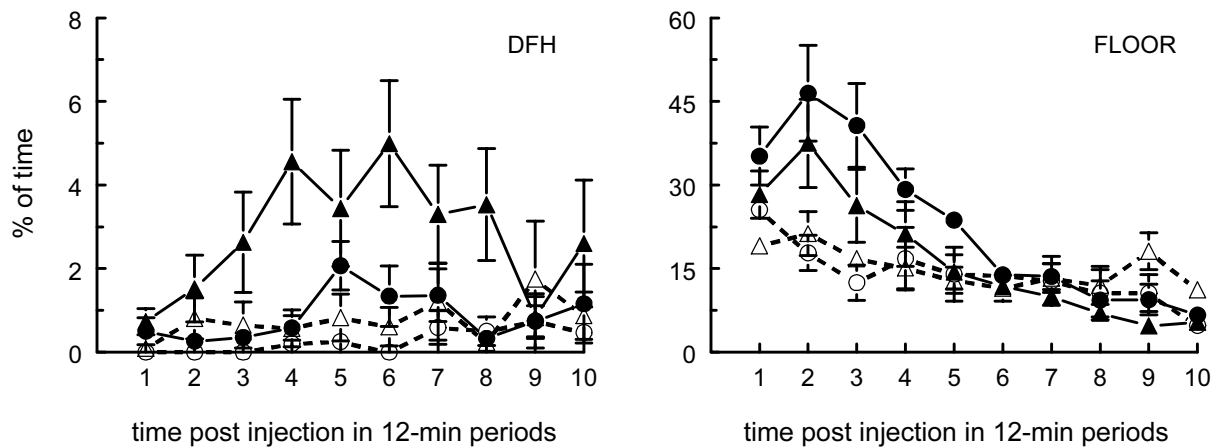
Fig. 2.3. Discrepancy between fore and hind limb movement or posture (DFH) and snout contact with the floor (FLOOR) of LR (triangles; n = 10) and HR pigs (circles; n = 9) after s.c. injection of saline (dotted lines, open symbols) or 0.2 mg/kg apomorphine (solid lines, closed symbols). Note: different Y-axis scaling for DFH and FLOOR.

Effects of Backtest classification

Behaviour of LR and HR pigs after saline or APO injection is summarized in Table 2.1 and Fig. 2.3. Locomotion and lying/sitting were unaffected by Backtest classification or its interaction with treatment and/or time. Standing, however, tended to be affected by an interaction between treatment and Backtest classification ($F_{(1,17)} = 3.7$, $p < 0.10$). Time spent on standing was more reduced after APO treatment, as compared to saline treatment, in LR pigs than in HR pigs (difference APO-saline treatment 24.4 ± 2.1% and 14.2 ± 4.4%, respectively).

Backtest classification ($F_{(1,17)} = 6.4$, $p < 0.05$) and treatment x Backtest classification interaction ($F_{(1,17)} = 6.0$, $P < 0.05$) influenced time spent on DFH. LR pigs showed more DFH under APO conditions than HR animals ($p < 0.05$), whereas the time spent on this behaviour during saline treatment was similar for HR and LR pigs (Table 2.1, Fig. 2.3).

Backtest classification or its interaction with treatment and/or time did not affect standing alert, defecating and snout contact with objects. A treatment x Backtest classification interaction was revealed for snout contact with the floor ($F_{(1,17)} = 5.9$, $p < 0.05$). When injected with saline, HR and LR pigs did not differ in time spent on snout contact with the floor. After APO injection this behaviour was, as compared to saline conditions, significantly increased in HR pigs ($p < 0.01$), but not in LR animals (Table 2.1). However, the time course of the action of APO on snout contact was similar for HR and LR pigs (Fig. 2.3); for instance, HR as well as LR pigs showed a peak of snout contact with the floor between 13-24 min post injection (46.5 ± 8.6% and 37.5 ± 7.9%, respectively).

Table 2.1. Behaviour of LR (n=10) and HR pigs (n=9) after s.c. injection of saline or 0.2 mg/kg apomorphine (APO). DFH = discrepancy between fore and hind limb movement or posture.

Behaviour	Saline		APO	
	LR	HR	LR	HR
Standing (%)	79.2 ± 4.2[a]	76.3 ± 5.5[a]	54.9 ± 4.6[b]	62.1 ± 4.6[b]
Locomotion (%)	10.8 ± 1.1[a]	9.9 ± 1.1[a]	20.3 ± 2.3[b]	21.5 ± 2.2[b]
Lying/sitting (%)	8.9 ± 4.4[a]	12.6 ± 6.4[a]	20.9 ± 5.9[b]	14.9 ± 5.9[b]
DFH (%)	0.8 ± 0.2[ab]	0.3 ± 0.2[a]	2.8 ± 0.5[c]	0.9 ± 0.2[b]
Snout contact floor (%)	15.1 ± 1.3[ab]	13.9 ± 1.9[a]	16.7 ± 2.7[ab]	22.8 ± 2.9[c]
Defecating (freq. per 12 min)	1.5 ± 0.4[a]	1.2 ± 0.2[a]	1.0 ± 0.2[b]	0.9 ± 0.2[b]
Snout contact objects (%)	26.5 ± 4.6	24.1 ± 4.3	22.7 ± 1.3	25.4 ± 3.0
Standing alert (%)	24.8 ± 6.2[a]	21.6 ± 5.5[a]	11.1 ± 2.8[b]	10.2 ± 1.7[b]

Within a row, means lacking a common superscript letter differ ($p < 0.05$).

DISCUSSION

The present study shows that pigs selected for divergent response in the Backtest at an early age, i.e. high-resisting (HR) versus low-resisting (LR), vary in the behavioural response to the dopamine agonist apomorphine (APO) later in life. The dose of 0.2 mg/kg s.c. used elicited hyperactivity, whereas standing alert and defecating were reduced. HR pigs, contrary to LR pigs, showed an increase in the time spent on snout contact with the floor when injected with APO. APO-treated LR pigs, however, performed more of the peculiar activities labelled DFH than HR pigs.

APO induced hyperactivity as described for other species (Costall and Naylor 1979; Fray et al. 1980; Ljungberg and Ungerstedt 1977; Motles et al. 1989; Nymark 1972; Sharman and Stephens 1974) and at similar doses in pigs (Terlouw et al. 1992), without confining pigs to highly invariant, individual-specific, responses. Although in the present study several APO-treated pigs performed more or less perseverant behaviour patterns, these patterns were observed for a shorter period and appeared less persistent than those described in an experiment on pigs where a higher dose of APO (1 mg/kg) was administered (Terlouw et al. 1992). The observation of such patterns agrees with the suggestion that APO has reinforcing effects and strengthens the behaviour that is performed at the onset of drug action (Broekkamp and Van Rossum 1974; Cools et al. 1977; Terlouw et al. 1992).

As the action of APO progresses, behaviour becomes less responsive to environmental change (Cooper and Dourish 1990), which may explain the substantial decrease in standing alert that was observed following APO injection. An influence of APO on the animal's attention towards the environment was suggested in other studies as well (Cheal 1980; Hård et al. 1985; Motles et al. 1989; Teitelbaum et al. 1990).

Contrary to other studies on APO-induced behaviour in pigs, in the present study hardly any licking, apparent drinking (Terlouw et al. 1992) or snout rubbing (Fry et al. 1981) was observed. This may, in the first place, be due to the relatively low dose used, as compulsive oral activities such as for instance licking appear to have higher threshold doses than increased locomotion or snout contact with the floor (Antoniou and Kafetzopoulos 1991; Fray et al. 1980; Terlouw et al. 1992). Second, as APO strengthens the behavioural activity performed at the onset of drug action (Broekkamp and Van Rossum 1974; Cools et al. 1977), features of the test environment may influence the type of stereotypy triggered by the drug. In the present study the metal strip covering the floor may have facilitated sustained snout contact with the floor at the expense of other (oral) activities.

In the present study APO strongly increased the occurrence of DFH, a group of activities representing an apparent disturbance in the coordination between hind and fore limb posture or movement. Although several peculiar movements have been described following APO

administration, such as limb flicking in cats (Motles et al. 1989), 'kicking out with hind legs' in cattle (Sharman and Stephens 1974), repetitive fore limb movements in rats (Ljungberg and Ungerstedt 1977), and disturbed hind limb stepping in rats (Cools and Jongen-Relo 1991; Cools et al. 1989), to our knowledge the behaviours here labelled DFH were not described previously. Other studies on apomorphine-induced behaviour of pigs reported none of these activities (Fry et al. 1981; Terlouw et al. 1992), although in one study pigs were described to behave 'uncoordinated' (Terlouw et al. 1992). We can only speculate about the significance of these movements. Dopamine is involved in the integration of various subcomponents of locomotion (Teitelbaum et al. 1990). It has been stated that (high) doses of APO, after initially inducing hyperactivity, gradually disintegrate motor behaviour in rats (Szechtman et al. 1980; 1985; Teitelbaum et al. 1990) by stimulating postsynaptic dopamine receptors of nigrostriatal neurons (Jaspers and Cools 1988). The DFH might be a reflection of (the recovery from) a similar process in pigs.

The finding that APO induces sustained snout contact with the floor in pigs agrees with other experiments (Terlouw et al. 1992). This activity likely parallels the behaviour described in rats as '(repetitive or stereotyped) sniffing' (Fray et al. 1980; McKenzie 1972), 'LS type of behaviour' (Ljungberg and Ungerstedt 1977), 'repeated sniffing on the floor' (Broekkamp and Van Rossum 1974), 'head down' (Fray et al. 1980), 'sliding snout contact with the ground' (Robbins et al. 1990) or 'snout contact fixation' (Szechtman et al. 1982).

Individual differences in the response to APO have mostly been interpreted as quantitative differences in susceptibility (Benus et al. 1991a; Cools et al. 1990; 1993; 1994; Durcan et al. 1984), although some investigators claimed to have found APO-induced response patterns in rats that differed in a qualitative manner, i.e. increasing or decreasing the dose level failed to change one type of response pattern into another (Ljungberg and Ungerstedt 1977). Variation in the response to APO may result from pharmacokinetic differences as well. In the present study, the time spent on snout contact with the floor, although not significantly increased in APO-treated LR pigs, showed a parallel time course and peaked in the same time period for both types of pig. The time patterns of the DFH response were also comparable for LR and HR pigs. Considering these kinetics, we tentatively suggest that (1) it is unlikely that the differences in response found between HR and LR pigs are due to pharmacokinetic variation between the two types of pig and (2) LR and HR differ in susceptibility to APO rather than in the type of response, but different dose levels should be tested to confirm the latter suggestion.

Sustained snout contact appears a sensitive indicator of the effect of APO as it is increased in a dose-dependent manner (Fray et al. 1980; Terlouw et al. 1992) and, at higher doses, is maintained as long as the drug acts (Szechtman et al. 1980; 1982). Therefore, it is tempting to suggest that the higher levels of this behaviour observed in APO-treated HR pigs, as

compared to LR pigs, reflect a higher susceptibility in this type of animal. This would fully agree with the finding in rodents that aggressive animals are more sensitive to apomorphine than non-aggressive ones (Benus et al. 1991a).

However, LR pigs showed more DFH behaviour when treated with APO. This seems paradoxical, but the possibility that different dopaminergic pathways mediate these two types of behaviour (snout contact with the floor and DFH) should be considered.

HR and LR pigs differ in response to apomorphine and thus probably vary in dopamine signalling in the brain, which may be reflected in different stress responses (Cools et al. 1990; 1993; Piazza et al. 1991). It is unknown to what extent these differences are fixed in the LR or HR classified animal or arise during their development, because both genetic and environmental factors (e.g. stress) influence dopaminergic functioning (Cabib et al. 1984; Cools et al. 1990; Piazza et al. 1991).

In conclusion, the response to apomorphine of pigs relates to their coping style, as assessed by the behavioural response in a so-called Backtest early in life. The underlying causes of these differences merit further research.

ACKNOWLEDGEMENTS

3

Individual coping characteristics, aggressiveness and fighting strategies in pigs

JE Bolhuis, WGP Schouten, JW Schrama, VM Wiegant

ABSTRACT

Individual pigs, *Sus scrofa*, considerably differ in the amount of aggressive behaviour they show during encounters with unfamiliar conspecifics. The present study examined whether individual coping characteristics of pigs were predictive of aggression during social encounters and the resulting social status. Piglets were subjected to the Backtest during the suckling period, as their behavioural response in this test seems to be predictive of their coping style. Each piglet was restrained in supine position for 1 min and the resistance (i.e. number of escape attempts) was scored. After weaning, 30 pigs classified as 'high-resisting' (HR) and 30 'low-resisting' (LR) pigs were regrouped with unfamiliar pigs of similar weight and their aggressive behaviour was recorded for 180 min. In addition, the social rank of each pig was assessed. HR pigs displayed more aggressive behaviour than LR pigs, i.e. they initiated more fights, started fighting earlier and spent more time on fighting during the observation period. HR and LR pigs did, however, not differ in achieved social rank. High levels of self-initiated fighting behaviour were correlated with a high social rank in LR pigs, but this relationship was not found in HR pigs. In conclusion, the Backtest response of pigs is related to their aggressive behaviour and the establishment of dominance relationships after mixing. Our results indicate that LR pigs are flexible in employing aggression, whereas the high level of aggression displayed by HR pigs irrespective of their success in encounters suggests that these animals are more rigid in their aggressive behaviour.

INTRODUCTION

The group structure of domesticated pigs living under farm conditions is, like in wild pigs, based upon a dominance hierarchy (McBride and James 1964; Meese and Ewbank 1973). Regrouping is a common procedure in pig production systems that causes the need for reestablishment of the social structure. When unacquainted pigs are brought together, generally a short period of vigorous fighting occurs that ends once dominance relationships have been settled (Meese and Ewbank 1973; Puppe and Tuchscherer 1994).

The assessment of relative fighting ability (resource holding potential) is an important factor driving fights between unacquainted pigs (Jensen and Yngvesson 1998; Rushen 1988a; 1988b). Body weight and size are known to influence a pig's fighting ability and social status: several studies have found that larger or heavier pigs are more likely to initiate (Arey 1999; McBride et al. 1965; Meese and Ewbank 1973) and win fights (Arey 1999; Scheel et al. 1977) than smaller ones. However, as pig farmers mostly prefer formation of uniform groups (Gonyou 2001), variation in size or weight of regrouped pigs is often small, which complicates the establishment of dominance relationships.

Several studies have documented, however, that individual pigs, irrespective of their weight

or size, differ considerably in the amount of aggressive behaviour they show after mixing (Hessing et al. 1993; Mount and Seabrook 1993). It has been suggested that aggressiveness, i.e. the propensity to display aggressive behaviour, is a rather stable trait of individual pigs (D'Eath and Pickup 2002; Erhard and Mendl 1997; Mount and Seabrook 1993).

Studies in male mice genetically selected for the readiness to attack an intruder in their territory have shown that aggressiveness is related to a set of behavioural and physiological responses to challenges, often referred to as 'coping style' or 'coping strategy' (e.g. Benus et al. 1991b). Aggressive mice are more likely to adopt a so-called (pro)active coping style, characterized by an active behavioural (fight/flight) strategy when challenged and a high propensity to develop behavioural routines, whereas non-aggressive individuals appear to prefer a so-called passive or reactive coping style, marked by a passive behavioural response and a high flexibility (see Koolhaas et al. 1999; 2001 for review). A similar link between aggressiveness and coping strategy was observed in great tits (Verbeek et al. 1996).

In attempting to assess the coping style of individual pigs, a so-called Backtest, though criticized (Jensen et al. 1995), has been used frequently (e.g. Geverink et al. 2003; Hessing et al. 1994a; Ruis et al. 2000; Van Erp-Van der Kooij et al. 2000). In this test, piglets are manually restrained in supine position and classified on the basis of their behavioural response, which can range from immobility to vigorous struggling (Hessing et al. 1993). Because pigs adopting one of the extreme reaction patterns, i.e. 'high-resisting' (HR) and 'low-resisting' (LR) individuals, were described to differ also in behavioural and physiological responses to a number of other stressful events (e.g. Geverink et al. 2002b; Ruis et al. 2000; 2001), it has been suggested that the Backtest classification of pigs is indicative of their coping style (Hessing et al. 1994a) or personality (Ruis et al. 2000).

In some studies a link between Backtest response and aggressiveness has been shown (Hessing et al. 1993; 1994a; 1994c; Ruis et al. 2000; 2002). HR pigs behaved more aggressively than LR pigs in 30-min social confrontation tests at one and two weeks of age (Hessing et al. 1993) and in food competition tests at 10 and 24 weeks of age (Ruis et al. 2000). Furthermore, groups solely made up of HR pigs showed higher levels of aggressive behaviour during the first hour after mixing than LR groups or groups containing both 'types' of pig (Hessing et al. 1994c). In other studies, however, no relationship between Backtest response and resident-intruder aggression was found (D'Eath and Burn 2002; Forkman et al. 1995). Thus, data on the relationship between aggressiveness and coping style in pigs are up till now inconclusive. Moreover, it is unclear whether pigs with diverging Backtest responses differ in the degree of success in aggressive encounters, indicated by the resulting social status. In the present study we investigated whether individual coping

characteristics of pigs, HR vs. LR as assessed early in life in the Backtest, were predictive of fighting behaviour after mixing and the resulting social status.

METHODS

The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments, which complies with the ETS123 (council of Europe 1985) and the 86/609/EEC directive. The Institutional Animal Care and Use Committee of the Wageningen University approved the experiment.

Animals and housing

The subjects of this study were 60 crossbred piglets, (Dutch Landrace x Yorkshire) x Yorkshire. Piglets were selected on the basis of their Backtest response and weight (see below) from a pool of litters. Before the start of the experiment, from birth until weaning, piglets were housed with their dams and littermates in farrowing pens (2.2 x 3.8 m). One week prior to the expected day of farrowing, their dams had been randomly assigned to either substrate-impoverished farrowing pens with a 65% concrete solid and 35% slatted floor (barren farrowing pens) or to identical, adjacent pens in which the solid part of the floor was covered with unchopped straw, approximately 4 kg per pen (enriched farrowing pens). Piglets received an iron injection and an ear tattoo on the day of birth. At three days of age, tails of the piglets were docked and male piglets were castrated following standard pig husbandry procedures. Water was continuously available and from 10 days of age creep feed was provided for the piglets. Lights were on between 7.00 and 19.00 h. All pens were cleaned out in the morning, between 8.00 and 9.00 h. Soiled straw was removed from the enriched farrowing pens and replaced with fresh unchopped straw.

Piglets were subjected to the Backtest (adapted from Hessing et al. 1993) at 10 and 17 days of age. The test procedure is described in detail elsewhere (Bolhuis et al. 2003). Briefly, each piglet was restrained on its back by placing one hand over the throat and the other loosely on the hind legs. Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 seconds (see Hessing et al. 1994c). Thirty high-resisting (HR) and 30 low-resisting (LR) pigs with similar body weights (7.3 ± 0.1 kg on the day before weaning) were selected; for both classifications the female to castrated male ratio was 2:1. Equal numbers of piglets from barren and enriched farrowing pens were selected.

In pig production systems, regrouping of pigs immediately after weaning is a common procedure. In our study pigs were weaned, relocated and mixed at 30.0 ± 0.2 days of age.

The 30 pigs that had been reared in barren farrowing pens were placed in five substrate-impoverished pens (7 m^2) with a 65% solid and 35% slatted floor (barren housing). The pigs from enriched rearing conditions were moved to five identical, adjacent pens enriched with deep straw bedding (enriched housing) where the slatted part of the floor was covered with a rubber mat. Per pen, three HR and three LR pigs (two gilts and one barrow of each Backtest classification) were grouped; these animals were not familiar with each other. Pigs of each group were placed into their new pen within two minutes.

In the new pens food (a standard commercial pelleted pig diet) and water were continuously available. Lights were on between 7.00 and 19.00 h. All pens were cleaned out in the morning, between 8.00 and 9.00 h. Soiled straw was removed from the enriched pens and replaced with fresh unchopped straw (replacement approximately 3.5 kg per pen per day).

Behavioural observations

Just before mixing pigs were sprayed on their backs with standard colour stock marker to allow identification of individuals during observations. Behaviour was recorded on videotape and scored afterwards using The Observer® and VideoTape Analysis® software packages (Noldus Information Technology b.v., Wageningen, The Netherlands). Fighting behaviour of each individual was scored for 180 min, as most fights between unfamiliar pigs occur during the first hours after mixing (Arey and Franklin 1995; McGlone 1986; Meese and Ewbank 1973), using focal sampling and continuous recording. In order to quantify the time spent on aggressive behaviour, two mutually exclusive states of the focal animal were scored: (1) fighting and (2) other activities. The focal animal was defined to be fighting if it was involved in a fight (see below), except when it did not retaliate at all during a fight or, approaching the end of a fight, it already had stopped fighting, retreated, turned away from its opponent or fled.

A fight, i.e., a 'bout' of fighting, was defined as a period of time lasting at least 10 seconds during which (1) two pigs showed close physical contact and (2) five or more head knocks or bites were displayed by either one of the pigs, or both. A fight was deemed to have ended when aggressive acts ceased after retreat of one or both pigs and pigs were separated for 60 s or more. For every fight the initiator, i.e. the pig that first bit or head knocked, if apparent, and its opponent were scored. In addition, the winner and loser of every 'decisive' fight were recorded. The pig that first stopped fighting, retreated, turned away from its opponent or tried to flee was considered to be the loser of the fight. Sometimes fights ended undecidedly, for instance when a third pig interrupted a fighting dyad, and were scored as such.

From these observations, the latency to fight, latency to initiate a fight, frequency of fighting and initiating fights, and duration of fighting and self-initiated fighting were calculated for each pig. A latency to initiate a fight of 180 min was assigned to the pigs that refrained from

initiating fights during the observation period. In addition, for each pig the average duration of fighting per encounter was calculated as (duration of fighting / frequency of fighting).

Social rank

Pigs appear to establish dominance relationships quickly; for instance, groups of four pigs were described to rank within 24 hours after mixing (McGlone 1986) and groups of eight within 48 hours (Meese and Ewbank 1973). Dominance relationships were based on outcomes of fights and, because several pairs of pig did not fight, shorter agonistic interactions during the first 48h after weaning.

Outcomes of observed encounters between all possible dyads were summed. The dyad member that won 67% (2:1) or more of the encounters was considered to dominate its opponent. Usually asymmetry in the outcome of dyadic agonistic interactions was much higher than 67%.

The position occupied by each pig in the dominance hierarchy was expressed by means of a social rank index as described previously (Lee et al. 1982). The index was calculated as ½(D-S+N+1), where D = the number of pigs dominated by the individual in question, S = the number of pigs dominating this individual and N = group size (6).

Data analysis

SAS version 6.12 was used for statistical calculations. Logarithmic, arcsine square root and square root transformations were applied for skewed distributions of duration, proportion and frequency, respectively. The effects of housing, Backtest classification, sex and their interactions on behavioural variables were studied with a mixed linear model allowing for random effects of pen within housing. Weight at weaning was introduced as a covariate in order to verify whether we had truly shut out possible effects of body weight on aggressive behaviour and social rank by selecting pigs of uniform weights. Effects of Backtest classification, sex and their interactions on social rank index values were studied using a mixed linear model with weight at weaning as a covariate. Data are presented as means ± SEM.

The effect of Backtest classification and sex on the proportion of pigs initiating fights was analysed with a G test (as described in Sachs 1984). For analysing relationships between behavioural variables and social rank index values Spearman correlation coefficients were calculated per Backtest classification.

RESULTS

Behavioural observations

Fighting behaviour during the observation period was unaffected by housing, sex or weight at weaning (data not shown). Aggressive behaviour of HR and LR pigs is summarized in Table 3.1. HR pigs showed a shorter latency to fight ($F_{(1,43)} = 5.8$, $p < 0.05$) and to initiate a fight ($F_{(1,43)} = 10.5$, $p < 0.01$) than LR pigs. Fifteen pigs refrained from initiating fights during the observation period. Also after omission of these animals (censored data) from analysis HR pigs showed a shorter latency to initiate a fight ($F_{(1,28)} = 6.1$, $p < 0.05$). Most HR pigs started fighting within 30 min, whereas for LR pigs the latency to initiate fights was more scattered over the observation period (see Fig. 3.1).

HR pigs also initiated more fights ($F_{(1,43)} = 5.8$, $p < 0.05$), tended to fight more often ($F_{(1,43)} = 3.3$, $p < 0.08$) and spent more time on fighting ($F_{(1,43)} = 5.8$, $p < 0.05$) and self-initiated fighting ($F_{(1,43)} = 7.0$, $p < 0.05$) than LR pigs. The average duration of fighting per bout was longer for HR pigs ($F_{(1,43)} = 6.6$, $p < 0.05$) than for LR pigs (Table 3.1). More LR (n=11; 36.7%) than HR pigs (n=4; 13.3%) did not initiate any fights during the observation period ($\hat{G} = 3.9$, $p < 0.05$).
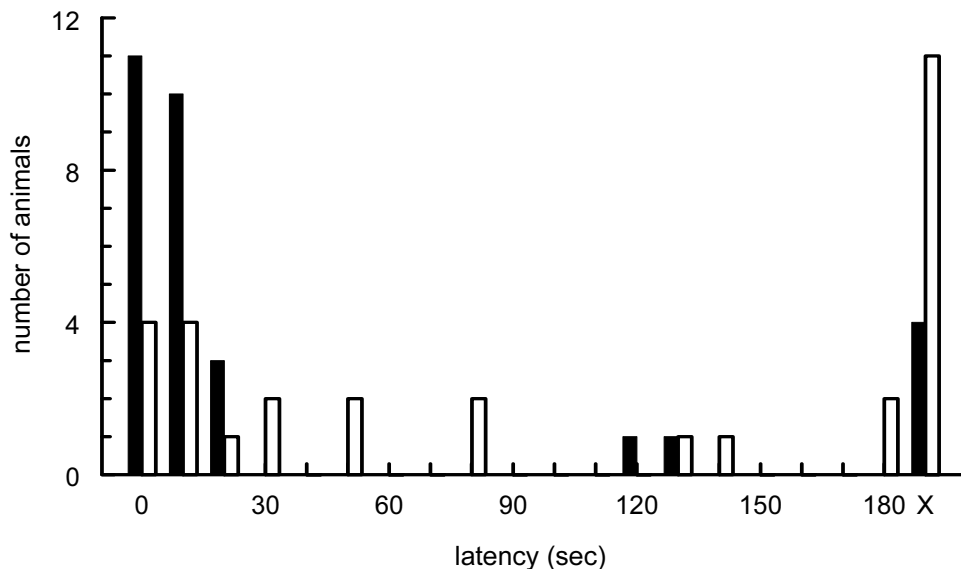
Fig. 3.1. Distribution of latency to initiate a fight of HR (solid bars) and LR (open bars) pigs. Data are grouped in classes per 10 min; X indicates pigs failing to initiate a fight during the 180 min observation period.

Table 3.1. Aggressive behaviour of HR and LR pigs during 180 min after regrouping.

Variable	HR	LR
Latency to initiate a fight (min)	41.4 ± 11.6^a	101.8 ± 14.0^b
Fights (freq)	7.5 ± 0.9^y	5.8 ± 1.1^z
Fights initiated (freq)	4.8 ± 0.7^a	2.9 ± 0.8^b
Fighting (% of time)	3.1 ± 0.6^a	1.9 ± 0.4^b
Fighting, self initiated (% of time)	2.2 ± 0.6^a	1.0 ± 0.3^b
Average duration (sec)	23.2 ± 4.6^a	15.2 ± 2.6^b

Within a row, means lacking a common superscript differ (a,b: $p < 0.05$; y,z: $p < 0.10$).

Social rank

The social rank index of HR pigs (3.7 ± 0.3) did not significantly differ from the index of LR pigs (3.3 ± 0.3). Spearman correlations between behavioural variables and the social rank of LR and HR pigs are shown in Table 3.2. In Fig. 3.2 one of these correlations is illustrated. For both types of pig a positive correlation between social rank and fighting frequency was found. The social rank of LR pigs was positively correlated with the number of fights they initiated ($p < 0.001$, Fig. 3.2), the time they spent on fighting and self-initiated fighting ($p < 0.001$), and the average duration of fighting per encounter ($p < 0.01$). The latency to initiate a fight was negatively correlated with social rank in LR pigs ($p < 0.001$, Table 3.2). After omission of the pigs that did not initiate fights during the observation period (ties), however, this correlation was not significant ($r_s = -0.39$, $p < 0.10$). In HR pigs no significant correlations between social rank and self-initiated aggressive behaviour were found (Table 3.2; Fig. 3.2).

Table 3.2. Spearman rank correlation coefficients between social rank and aggressive behaviour of HR and LR pigs.

Variable	HR	LR
Latency to initiate a fight (min)	-0.15	-0.70***
Fights (freq)	0.40*	0.42*
Fights initiated (freq)	0.35+	0.80***
Fighting (% of time)	0.29	0.62***
Fighting, self initiated (% of time)	0.24	0.82***
Average duration (sec)	0.16	0.51**

+$p < 0.10$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
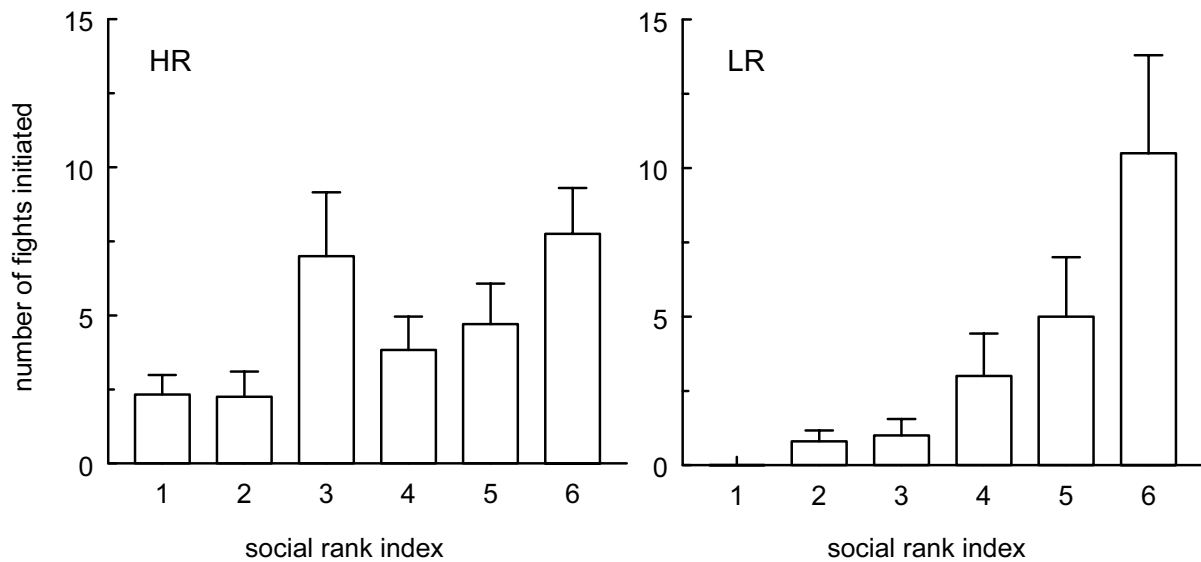
Fig. 3.2. Relationship between the number of fights initiated and social rank of HR (left panel) and LR pigs (right panel).

DISCUSSION

The aim of this study was to investigate whether individual characteristics of pigs, high-resisting (HR) vs. low-resisting (LR) as assessed early in life in the Backtest, were predictive of fighting behaviour after mixing and the resulting social status. For this purpose, 60 pigs of known Backtest classification were regrouped after weaning with unfamiliar conspecifics of similar weight and their fighting behaviour was recorded, as well as the social rank they achieved.

As expected, because relatively uniform pigs were selected, weight at weaning did not affect fighting initiative or social rank. Fighting behaviour was related to Backtest classification. HR pigs displayed more fighting behaviour than LR pigs, i.e. they initiated more fights, started fighting earlier and spent more time on fighting during the observation period. These results are in line with other studies showing a link between Backtest classification and aggressiveness in provocation experiments, such as social confrontation and food competition tests (Hessing et al. 1993; 1994a; Ruis et al. 2000). Others (D'Eath and Burn 2002), however, found no relationship between Backtest response and latency to attack an intruder (an unfamiliar pig) in the home pen. The discrepancy between the latter study (D'Eath and Burn 2002) and the present and other results (Hessing et al. 1993; 1994a; Ruis et al. 2000) could be due to differences in experimental set-up, i.e. dyadic resident-intruder tests vs. group-wise provocation experiments.

It has been suggested that HR pigs are most likely to adopt a (pro)active coping strategy,

whereas a LR-Backtest classification is indicative of a passive or reactive coping style (Hessing et al. 1994a; Ruis et al. 2000). If so, the present results agree with studies in other species demonstrating that individuals adopting an active behavioural strategy in stressful conditions, are more aggressive than animals with a passive/reactive coping strategy (rodents: Benus et al. 1991; Koolhaas et al. 1999; 2001; great tits: Verbeek et al. 1996; 1999). In these species aggressiveness has been mentioned as one of the key characteristics of the coping style of an individual (e.g. Koolhaas et al. 1999; 2001). In our study, however, Backtest classification was not unequivocally coupled with the tendency to display aggressive behaviour. Although LR pigs were on average less aggressive than HR pigs, some LR pigs showed high levels of aggressive behaviour.

The assessment of relative fighting ability is an important factor driving fights between unacquainted pigs (Jensen and Yngvesson 1998; Rushen 1988a; 1988b). If an individual is going to lose a fight it is better off to avoid it, and thus also avoid the potentially high cost associated with fighting (Fraser et al. 1995). Hence, as a result of previous defeats, pigs will lower the assessment of their own fighting ability and will be less likely to fight or to initiate fights on subsequent encounters (Rushen 1988b). Therefore, a relationship between fighting tendency and the degree of success, i.e. the resulting social rank, is to be expected. Some studies report indeed that most fighting is initiated by the individuals that go on to be high ranking (Arey 1999; Meese and Ewbank 1973). Others found, however, that aggressive pigs are not necessarily the most successful or dominant animals (D'Eath 2002; Scheel et al. 1977). In our study, the relationship between fighting behaviour and social rank differed markedly between HR and LR pigs. LR pigs managed to achieve - on average - the same social rank as HR pigs, even though a large proportion of LR pigs refrained from initiating fights and obtained a low social status. This indicates that the LR pigs that did fight were relatively successful. As the strength of the relationship between fighting tendency and success varied, depending on the 'type' of pig, LR and HR pigs appeared to differ in social strategy rather than in aggressiveness per se. In LR pigs, fighting effort (e.g. number of fights initiated, time spent on fighting and duration of fighting per encounter) and the obtained social rank were highly correlated. This suggests that LR pigs, as they appear to readily adjust their aggressive behaviour on the basis of their own success, are more flexible in social situations than HR pigs, which seemed to fight irrespective of the outcome of encounters. In line with this, in a study on pair-wise confrontations in pigs (Ruis et al. 2002) HR individuals were reported to persist in fighting, even beyond settlements of dominance relationships. In concordance with the current results, we recently found a relationship between Backtest response and behavioural flexibility in another (non-social) situation (Bolhuis et al. 2004). HR pigs were less successful in reversal learning than LR pigs, suggesting that they have a higher propensity to develop inflexible behavioural routines. It

thus appears that HR and LR pigs generally differ in the ability to adjust their behaviour to a changing situation.

Recently it has been suggested that the extent to which behaviour is controlled by actual environmental stimuli (feedback control) is fundamentally different between rodents with a (pro)active and reactive/passive coping style (Koolhaas et al. 1999; 2001). The characteristics of proactive and reactive coping styles with respect to behavioural flexibility may also be reflected in the aggressive behaviour of pigs after mixing. It is therefore tempting to suggest that the present differences in fighting behaviour of LR and HR pigs were a reflection of the proactive and reactive coping styles of the animals, rather than an expression of aggressiveness per se.

In conclusion, the Backtest response of pigs is related to their aggressive behaviour and the establishment of dominance relationships after mixing. Our results indicate that LR pigs adopt a flexible strategy in social confrontations, whereas the high level of aggression displayed by HR pigs irrespective of their success in aggressive encounters suggests that these animals rely on rather rigid behavioural routines.

ACKNOWLEDGEMENTS

Individual coping characteristics, rearing conditions and behavioural flexibility in pigs

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ABSTRACT

Several studies suggest that classification of piglets early in life based on the degree of resistance they display in a so-called Backtest may be indicative of their coping style at a later age. In the present study behavioural flexibility was investigated in pigs diverging for Backtest response and housing environment during rearing. Pigs were housed either without a rooting substrate (barren housing, B) or in identical pens enriched with deep straw bedding (enriched housing, E) from birth. During the suckling period piglets were subjected to the Backtest. Each piglet was restrained on its back for 1 min and the resistance (i.e. number of escape attempts) was scored. Pigs classified as 'high-resisting' (HR) or as 'low-resisting' (LR) were subjected to a simple (left/right) spatial discrimination (T-maze) task at 8 weeks of age. The effect of a single, subtle intramaze change was determined after acquisition of the task. In addition, pigs were subjected to reversal learning to assess their ability to modulate established behaviour patterns. Housing and its interaction with Backtest classification influenced the behavioural response to the intramaze change: E pigs were considerably more distracted than B pigs. Housing condition affected LR pigs more than HR pigs, as indicated by the interaction effects on various recorded behaviours. These interactions indicate that behavioural responding of pigs with diverging coping characteristics cannot simply be generalised across rearing conditions. Furthermore, HR pigs were less successful in reversal learning than LR pigs, suggesting that they have a higher propensity to develop inflexible behavioural routines.

INTRODUCTION

Pigs show a wide variation in adaptive responses when exposed to the same stressful situation (Lawrence et al. 1991; Schouten and Wiepkema 1991). Stable individual characteristics that may have a predictive value for a pig's adaptive capacity and its sensitivity to develop stress-related pathologies or abnormal behaviour have received increasing attention (e.g. Geverink et al. 2003; Thodberg et al. 1993). In attempting to identify individual reaction patterns in pigs, a so-called Backtest has been used frequently (e.g. Geverink et al. 2002a; Ruis et al. 2000; Van Erp-Van der Kooij et al. 2000). In this test, piglets are manually restrained in supine position for 1 min and their behavioural reaction, which can range from immobility to excessive struggling (Hessing et al. 1993), is assessed. Generally, two 'types' of pig are identified at either end of the distribution: the ones that struggle a lot, referred to as 'high-resisters' (HR), versus the animals that do not or hardly show resistance, the 'low-resisters' (LR). The 'classification approach' of the Backtest has been criticised because selection of extremes is based on more or less arbitrary cut-off points (Jensen et al. 1995). In spite of this methodological criticism, several research groups have used the Backtest and demonstrated that classification of pigs as HR or LR is predictive

of a range of responses measured at a later age, such as aggressiveness (Hessing et al. 1993; 1994a; Ruis et al. 2000; 2002, but see D'Eath and Burn 2002, who found no relationship between Backtest response and resident-intruder aggression), immune responsiveness (Bolhuis et al. 2003; Hessing et al. 1995; Schrama et al. 1997) and HPA-axis (re)activity (Geverink et al. 2002a; 2003; Ruis et al. 2001; 2002). Therefore, it has been suggested that the response of piglets in the Backtest may reflect a more general profile of behavioural and neuroendocrine reactivity (Hessing et al 1994a; Ruis et al. 2000).

Several studies (e.g. Ellenbroek and Cools 2002; Hessing et al. 1994a; Ruis et al. 2000) have pointed to a resemblance between response profiles of HR and LR pigs and the diverging reaction patterns, often referred to as 'coping styles' or 'coping strategies', that have been identified in rodents and a number of other species (see Benus et al. 1991b; Cools et al. 1990; Koolhaas et al. 1999; 2001 for review). The extreme phenotypes within a population have been labelled with different terms, such as manipulators and adjusters (Benus et al. 1991b; Sluyter and Van Oortmerssen 2000); high and low responders to novelty (Cools and Gingras 1998), proactive and reactive copers (Koolhaas et al. 1999; 2001), fast and slow attackers, active and passive copers (Benus et al. 1991b), and fast and slow explorers (Verbeek et al. 1994; 1996). Despite the use of different tests and terms for selection or characterization of extremes, the above-mentioned diverging reaction patterns essentially seem to reflect fundamental differences in the organization of behaviour with the help of internal and external information, resulting from variation in neurochemical make-up and reactivity of the brain (Benus et al. 1991a; Cools et al. 1990; 1994; Koolhaas et al. 2001).

Mice and rats selected for the readiness to attack an intruder in their home pen were reported to differ in the ability to adjust their behaviour to an unexpected change in an otherwise familiar situation (Benus et al. 1987; 1990b). One of these 'types' of rodent thus appears to have a relatively high propensity to develop rather unvarying, persistent patterns of behaviour that can be described as 'routine-like', 'habit-like' or 'rigid', whereas the other type remains more flexible and is able to readily adjust its behaviour to environmental changes (Benus et al. 1987; 1990; Koolhaas et al. 2001). Also in other species, alternative response patterns have been reported that reflect differences in the propensity to develop inflexible behavioural routines (e.g. meadow voles: Teskey et al. 1998; great tits: Marchetti and Drent 2000; Verbeek et al. 1994; 1996; geese: Pfeffer et al. 2002; humans: Patterson and Newman 1993).

It is unknown whether the Backtest classification of piglets early in life, as HR or LR, coincides with differences in behavioural flexibility. Based on the similarities between the reaction patterns of HR and LR pigs and the types of rodent classified as 'pro(active) and reactive copers' (Koolhaas et al. 1999) or 'high and low responders to novelty' (Cools et al.

1990), we hypothesized HR pigs to have a higher propensity to develop inflexible behavioural routines and consequently to be less reactive to environmental changes than LR pigs.

Behaviour patterns are also influenced by environmental factors, including the housing environment of animals. Domestic pigs are mostly reared and housed in stimulus-poor and restrictive environments that impose constraints on the development and expression of behaviour (Haskell et al. 1996; Schouten 1986; Wemelsfelder et al. 2000) and cognitive abilities (Sneddon et al. 2000). In rodents it has been shown that rearing environments lacking sufficient stimulation may in particular reduce behavioural flexibility (e.g. Morgan 1973; Renner and Rozenzweig 1987). Housing environment may have differential effects on animals depending on their reaction pattern, i.e., the effects of housing and 'type of animal' are not necessarily additive.

In the present study we investigated the effects of individual characteristics of growing pigs as assessed in the Backtest (HR or LR), housing condition (barren or substrate-enriched) and the interaction of these two factors on behavioural flexibility using a T-maze task. After acquisition of a simple (left/right) spatial discrimination, the tendency to alter an established behaviour pattern in response to a single change in the maze environment was determined. Subsequently, pigs were subjected to reversal learning to assess their ability to inhibit and modulate previously learned behaviour patterns.

METHODS

Subjects and housing

The experiment was carried out in four successive batches, using eight litters of crossbred pigs in total. Pigs were housed from birth in either standard (substrate-impoverished) farrow-to-finish pens (7 m^2) with a 65% solid and 35% slatted floor (barren environment, 'B') or in identical, adjacent pens enriched with deep straw bedding (enriched environment, 'E') of which the slatted part of the floor was covered with a rubber mat.

Per batch, two sows with approximately the same insemination date bred the experimental pigs. One week before the expected day of farrowing, the sows were randomly assigned to a barren or an enriched pen. Six litters (three B and three E) were Pietrain x (Large White x (Duroc x British Landrace)) and two litters (one B and one E) were Pietrain x (Great York x Dutch Landrace) crossbred.

At three days of age, tails were docked and male piglets were castrated following standard pig husbandry procedures, and litter sizes were set to a maximum of ten piglets. Pigs were weaned at four weeks of age by removing the sow from the pen. Pens contained two drinking

nipples and a food trough. Food (a standard commercial pelleted pig diet) and water were available ad libitum. Lights were on between 7.00 and 19.00 h. In the morning, between 8.00 and 9.00 h, all pens were cleaned out. Soiled straw was removed from the enriched pens and replaced with fresh unchopped straw (replacement approximately 3.5 kg per pen per day). The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments, which complies with the ETS123 (council of Europe 1985) and the 86/609/EEC directive. The Wageningen University Animal Care and Use Committee approved the experiment.

Backtest

Pigs were subjected to the Backtest (adapted from Hessing et al. 1993) at 10 and 17 days of age. The test was carried out on a table placed in the corridor near the home pen. The experimenter lifted a piglet from its pen and used the left hand to place the piglet on its back on a rubber mat. The right hand of the experimenter was then placed firmly on the thorax of the piglet, with the left foreleg of the piglet between the experimenter's thumb and index finger, and the right foreleg between index and middle finger. The experimenter's left hand was used to stretch and move the hind legs of the piglet downward at the start of the test, and subsequently remained loosely on the hind legs. Pigs were restrained in this supine position for 60 s. Each bout of struggling with at least the hind legs was counted as an escape attempt. A bout was regarded terminated when the piglet stopped struggling or paused by stretching or relaxing its hind legs.

Classification of pigs was based on the number of escape attempts they displayed during the test as described previously (Hessing et al. 1994c). Briefly, a pig was classified as high-resisting (HR) if it performed more than four escape attempts in the two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low-resisting (LR). The remaining pigs were characterized as 'intermediate' and not used for T-maze testing. Of the 76 pigs that were subjected to the Backtest, 41 were classified as HR (20 B and 21 E), and 23 as LR (10 B and 13 E). Each litter contained at least one HR and one LR pig. Averaged over the two Backtests, HR pigs showed 3.6 ± 0.2 escape attempts and LR pigs 1.1 ± 0.1.

T-maze

The T-maze is shown in Fig. 4.1. The maze, located in a separate room, was constructed of dark brown wooden walls, and measured approximately 6.5 x 6.2 x 1.0 m (width of corridors: 1.0 m). The floor of the maze was covered with black rubber mats. The maze was divided into ten imaginary sections and comprised a start box, an exit and a left and right arm. At the end of both arms a food trough was placed.
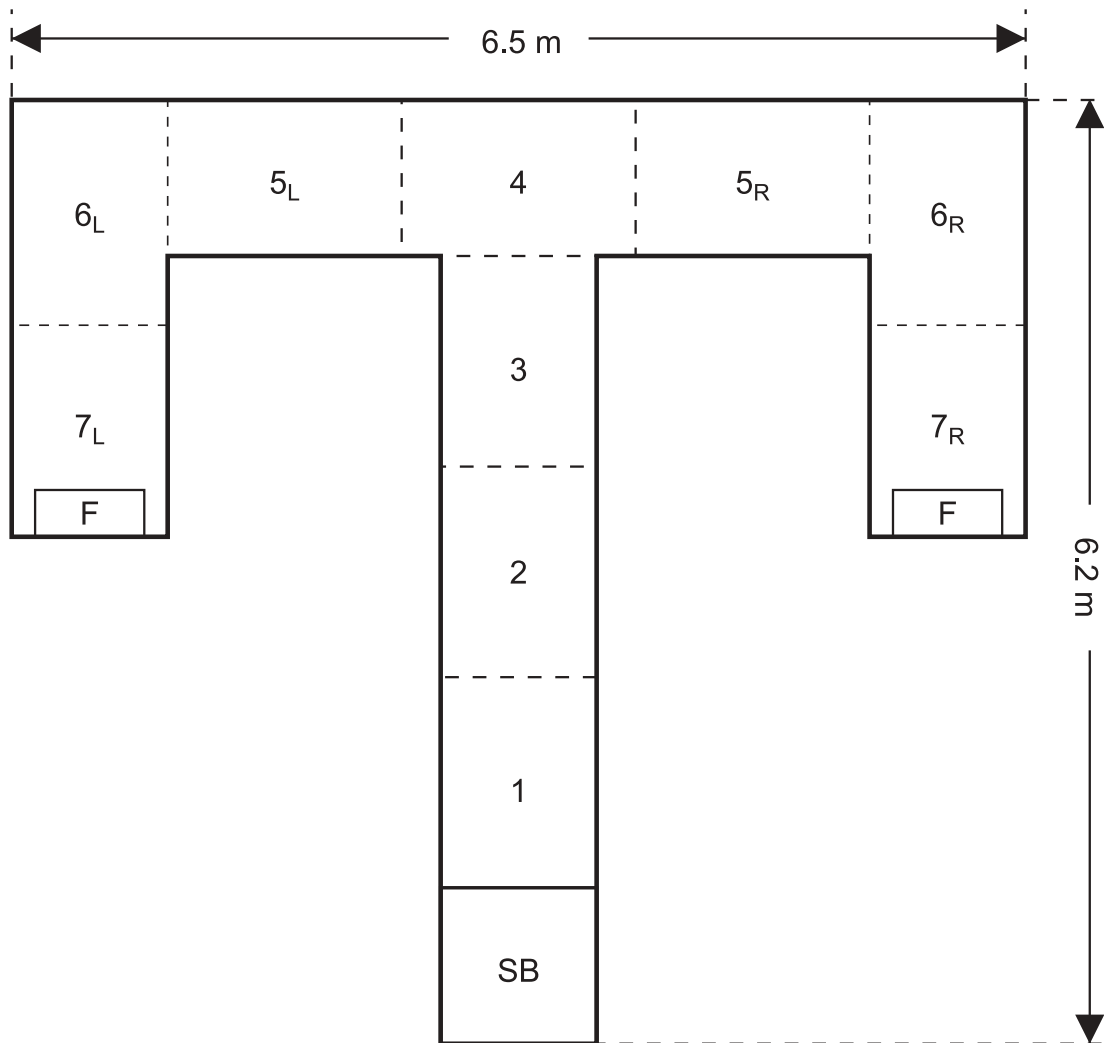
Fig. 4.1. Plan of the T-maze. Width of corridors was 1 m. F = food trough; SB = start box.

T-maze training procedure

Pigs were familiarized with the experimenters and the experimental room prior to the T-maze training, which started at 8 weeks of age. After food deprivation for approximately 12 h, all HR and LR pigs (n=64) were trained to find a food reward (40 g of their commercial pelleted pig feed) in one of the arms of the maze. For half of the pigs the food reward was placed in the food trough in the left arm of the maze. For the other half of the pigs, the right arm contained the food reward. The location of the food (left/right) was balanced for housing and Backtest classification. Buckets with fresh food out of reach of the pigs were present behind sections 7L and 7R (Fig. 4.1) to prevent the use of olfactory cues to locate the food reward. Prior to the first trial each pig was guided through the maze once; first to the arm with the empty food trough and then to the other arm where it was allowed to eat for 1 min.

B and E pigs were trained in an alternating schedule. When pigs of both litters had run the

maze once, the next training session started. Consequently, for individual pigs time between two subsequent trials on the same day was at least 1 h. Pigs were subjected to maze running for 2 - 5 trials per day, depending on the number of trials they needed to attain criterion for stable task performance (see below). For each trial, a pig was taken out of its home pen and led to the experimental room, where it was gently guided into the start box of the maze. After 5 sec the guillotine door of the start box was lifted manually and the pig was allowed 180 sec to find the food and then 1 min to eat. If a pig had not succeeded in finding the food reward within 180 sec, it was directed to the food, allowed to eat and given the maximum time score. After eating the food reward, the pig was returned to its home pen and another pig was brought to the experimental room.

Pigs could be observed from a monitor in the experimental room that was connected to a video camera placed above the maze. All trials were recorded on videotape. An error was scored if a pig crossed one of the imaginary lines with at least two legs in the wrong direction, i.e., away from the food reward. A trial was considered correct if the pigs ran the maze within 15 seconds without making any error and ate the food. Criterion for stable task performance was a series of nine consecutive correct trials (acquisition). Pigs that did not fulfil this criterion within 20 training trials were excluded from further testing.

Intramaze change

Following the trial in which criterion for stable task performance was attained a novel object was introduced in section 5 of the 'rewarding' arm, at the boundary with section 6, on the first trial of the next day. The novel object was an aluminium pan with a diameter of 20 cm, placed upside down. Pigs could easily pass the object on both sides. Pigs that did not reach the food reward within 300 sec during this intramaze change-trial were gently directed to the food, allowed to eat and given the maximum time score.

Reversal

After the intramaze change-trial five normal training trials followed. Subsequently, during six consecutive reversal trials, the food reward was placed in the opposite arm of the maze. Pigs that did not reach the food reward within 300 sec were gently directed to the food, allowed to eat and given the maximum time score. Criterion for correct reversal performance was a faultless maze run.

Measurements

During all trials, the latency of reaching the food reward, the number of vocalisations and the number of errors were scored, as well as the frequency of defecating. During the intramaze change, the latency to enter section 5 (where the novel object was placed), the time spent in

section 5, and the latency and duration of touching the object were recorded as well. The frequency and duration of snout contact with the food through that originally contained the food reward were recorded during the first reversal trial.

Data analysis

SAS versions 6.12 and 8.0 were used for statistical calculations. Preliminary analyses showed no effects of batch or breed, so these factors were not included in the final statistical model. Skewed distributions of frequency and duration (including latency measures) were square root and logarithmically transformed, respectively, to obtain homogeneity of variances. Effects of and interactions between the factors Backtest classification (LR or HR), housing (barren or enriched) and sex on performance during the T-maze test were assessed by analysis of variance. Effects of housing were tested against the random effect of litter within housing. Significant interaction effects were further analysed using post hoc tests with Bonferroni adjustments for multiple comparisons.

Performance of pigs during reversal trials was transformed into a single discrete (binary) score, with values 0 (incorrect performance) or 1 (correct performance). A generalised linear mixed model with a logit link and binomial distribution was used to analyse the effects of the factors housing, Backtest classification and sex on reversal performance; the random effect of litter within housing was included in this model. Data are presented as means ± SEM.

RESULTS

Training trials

Four pigs, one HR from the barren (B) environment and two HR and one LR from the enriched (E) environment did not fulfil criterion for stable task performance (nine consecutive correct trials) within 20 training trials and were excluded from further testing.

The 60 pigs that were successfully trained needed on average 12.1 ± 0.3 trials (range: 9 to 18) before they attained criterion for stable task performance. During correct trials, pigs reached the food in 8.1 ± 0.3 sec. Pigs never defecated during training trials, although 18 animals defecated during the pre-training run, in which they were guided through the maze. Pigs hardly vocalised during training trials (on average 0.8 ± 0.1 times per trial), and vocalisations uttered were usually barks or short grunts.

Performance during training trials was unaffected by housing, Backtest classification (see Table 4.1) or sex (data not shown).

Table 4.1. Performance of LR and HR pigs from barren (B) or enriched (E) housing during training trials.

Variable	HR		LR	
	B (n=19)	E (n=19)	B (n=10)	E (n=12)
Trials (number)[1]	12.3 ± 0.5	12.2 ± 0.5	11.8 ± 0.6	12.1 ± 1.0
Latency to reach food (s)[2]	8.1 ± 0.8	8.2 ± 0.5	7.7 ± 0.6	8.1 ± 0.6
Vocalisations (frequency)[2]	1.0 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	0.7 ± 0.2

[1]Before attaining criterion of 9 consecutive correct trials; [2]Average of the 9 correct trials.

Intramaze change

Pigs differed widely in their responses to the intramaze change, i.e. the unfamiliar object placed in section 5 of the maze. Some pigs did not alter their running speed at all, whereas others were held up by the object for several minutes. Seven pigs, one HR from barren housing (B) and three HR and three LR pigs from enriched housing (E) had to be guided to the food reward after 300 sec. Defecating was recorded for four animals (one B-HR, two E-HR and one E-LR) and 11 pigs (six B-HR, one B-LR, one E-HR and three E-LR) did not eat for the maximum time allowed (1 min).

The performance of differently housed LR and HR pigs during the intramaze change-trial is summarized in Table 4.2. E pigs vocalised more often (10.1 ± 2.5) than B pigs (2.9 ± 1.2), $F_{(1,6)} = 6.3$, $p < 0.05$. E pigs also tended to show a higher latency to reach the food (132.7 ± 23.1 vs. 40.7 ± 11.7 sec), $F_{(1,6)} = 4.7$, $p = 0.08$, and tended to make more errors than B pigs (14.1 ± 2.9 vs. 3.3 ± 1.5), $F_{(1,6)} = 5.9$, $p = 0.06$. In addition, the housing effect was dependent on Backtest classification, as indicated by effects of the interaction between Backtest classification and housing. The Backtest classification x housing-interaction affected the latencies to reach the food reward ($F_{(1,46)} = 4.6$, $p < 0.05$) and to enter section 5, where the object was placed ($F_{(1,46)} = 5.3$, $p < 0.05$), the number of errors ($F_{(1,46)} = 5.2$, $p < 0.05$), and tended to affect the number of vocalisations ($F_{(1,46)} = 3.2$, $p = 0.09$). HR pigs from barren housing were held up by the novel object for a longer time than LR pigs from barren housing, whereas in enriched housing LR pigs appeared more distracted than HR pigs. The contrast in behaviour of pigs from different housing environments was largest for LR pigs: pair-wise comparisons revealed that LR pigs from enriched housing showed longer latencies to reach the food and enter section 5, and a higher number of vocalisations and errors than LR pigs from barren housing, whereas differently housed HR pigs did not significantly vary in any of these measures (Table 4.2). Housing ($p = 0.93$), Backtest classification ($p = 0.72$), and their interaction ($p = 0.73$) did not affect the time spent in

section 5. The latency to touch the object and time spent in contact with the object were also unaffected by housing (p = 0.12 and 0.32, respectively), Backtest classification (p = 0.32 and 0.88) or the Backtest classification x housing-interaction (p = 0.25 and 0.39). Latency to reach the food was shorter for barrows (82.0 ± 18.6 sec) than for gilts (104.6 ± 24.5 sec), $F_{(1,46)} = 4.3$, $p < 0.05$. No other effects of sex were found (data not shown).

Four pigs (one from each housing environment and Backtest classification) made errors during the first normal trial following the change. In the four training trials that succeeded, all pigs ran the maze faultlessly and within 15 sec again (data not shown).

Table 4.2. Performance of LR and HR pigs from barren (B) or enriched (E) housing during the intramaze change-trial.

Variable	HR		LR	
	B (n=19)	E (n=19)	B (n=10)	E (n=12)
Latency to reach food (s)	48.9 ± 17.1[a]	125.5 ± 28.8[ab]	24.9 ± 9.3[a]	144.1 ± 40.0[b]
Vocalisations (frequency)	3.4 ± 1.7[ab]	9.1 ± 2.8[ab]	2.1 ± 1.3[a]	11.7 ± 4.9[b]
Errors (frequency)	3.6 ± 1.9[a]	11.6 ± 3.1[ab]	2.7 ± 2.2[a]	18.0 ± 5.6[b]
Latency to enter section 5 (s)	7.5 ± 2.6[ab]	8.5 ± 4.6[ab]	3.6 ± 0.3[a]	37.8 ± 24.8[b]
Presence in section 5 (s)	12.5 ± 3.2	18.1 ± 3.2	15.8 ± 7.1	13.8 ± 3.6
Latency to touch object (s)	85.2 ± 29.3	108.6 ± 30.0[a]	34.5 ± 29.5[a]	114.2 ± 40.2
Contact with object (s)	7.8 ± 2.2	4.3 ± 1.3	7.8 ± 2.7	3.1 ± 1.1

Within a row, means lacking a common superscript letter differ (p < 0.05).

Reversal

Pigs were trained for a total of six reversal trials. Performance during the first reversal trial was not affected by housing, Backtest classification (Table 4.3) or sex. One pig did not eat for the maximum time allowed and another pig defecated. Five pigs had to be guided to the food after 300 sec; one HR from barren housing, and one HR and three LR from enriched housing.

Table 4.3. Performance of LR and HR pigs from barren (B) or enriched (E) housing during the first reversal trial.

Variable	HR		LR	
	B (n=19)	E (n=19)	B (n=10)	E (n=12)
Latency to reach food (s)	105.2 ± 18.8	119.5 ± 18.3	115.8 ± 29.6	152.0 ± 31.9
Vocalisations (frequency)	18.4 ± 9.0	6.3 ± 2.3	14.1 ± 6.1	13.3 ± 6.0
Errors (frequency)	10.7 ± 2.3	10.7 ± 2.0	12.0 ± 3.9	14.6 ± 3.6
Visits 'old' trough (frequency)	2.3 ± 0.4	2.5 ± 0.3	2.3 ± 0.7	2.8 ± 0.4
Contact with 'old' trough (s)	28.0 ± 7.6	31.9 ± 5.9	30.8 ± 8.3	32.9 ± 10.6

During the subsequent five reversal trials, pigs found the food reward amply within the maximum time but not always faultlessly. In the second reversal trial, all pigs rapidly entered the previously rewarded, incorrect arm (see Fig. 4.2a and 4.2b). Starting from the third reversal trial, most pigs either faultlessly entered the 'new' arm, or first turned into to the previously rewarded ('old') arm, resulting in three errors. Because the number of errors showed a binomial distribution (most pigs scored 0 or 3), performance of pigs during reversal trials was transformed into a discrete (binary) score: correct (i.e., reaching the food faultlessly) or incorrect.
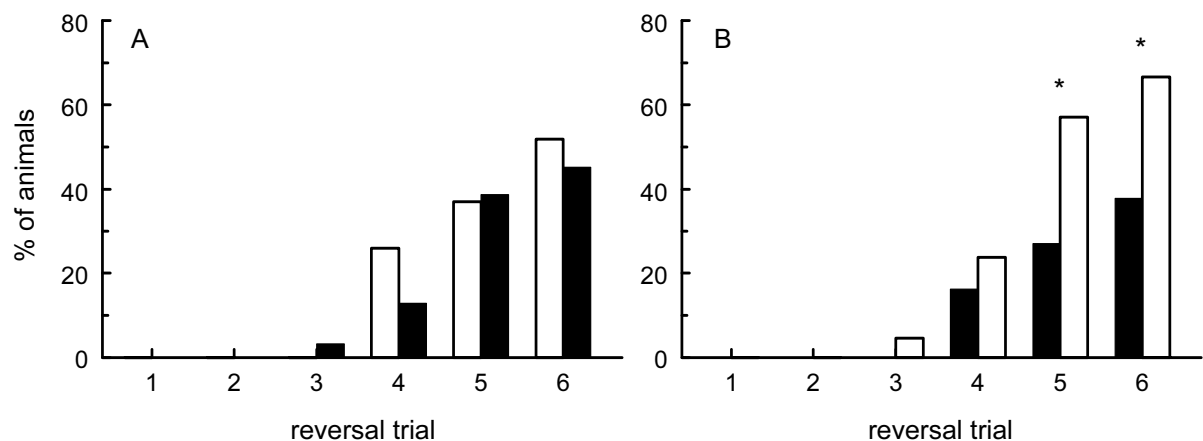
Fig. 4.2. Proportion of pigs with correct reversal performance. Left panel (A): barren (open bars) versus enriched housing environment (solid bars). Right panel (B): HR (solid bars) versus LR (open bars) pigs. Differences within a trial are indicated: *p < 0.05.

The proportion of pigs with correct reversal performance is shown in Fig. 4.2a and 4.2b. Housing (Fig. 4.2a) and the interaction between housing and Backtest classification did not affect performance during reversal trials (e.g., $p = 0.78$ and 0.37, respectively, for the sixth reversal trial), and no effects of sex were found. Reversal performance was related to Backtest classification. Starting from the fifth reversal trial the proportion of LR pigs with correct performance was significantly higher than the proportion of successful HR pigs, $p < 0.05$ (Fig. 4.2b). Latencies to reach the food were, however, unaffected by housing, Backtest classification or sex, except for the third trial in which HR pigs were faster than LR pigs, $p < 0.05$ (Fig. 4.3).
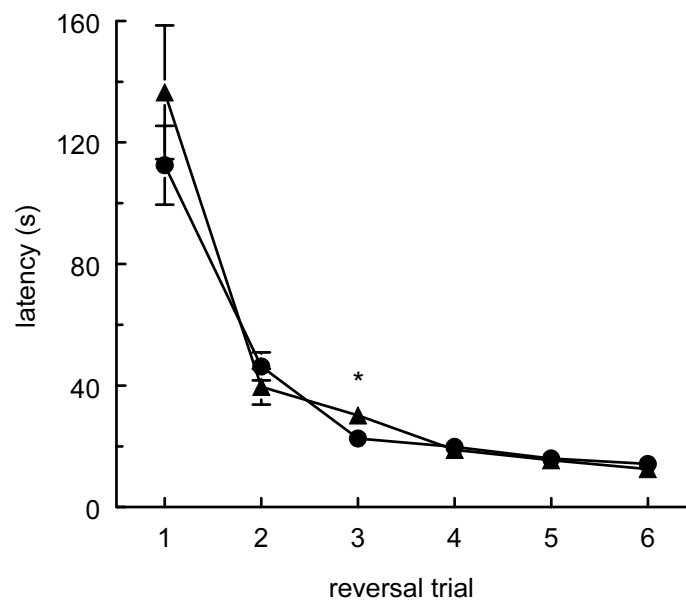
Fig. 4.3. Latency to reach food reward in reversal trials of LR (triangles) and HR pigs (circles). Differences within a trial are indicated: *$p < 0.05$.

DISCUSSION

Behavioural flexibility was studied in pigs diverging for Backtest response, high-resisting (HR) or low-resisting (LR), and housing environment during rearing, barren (B) or substrate-enriched (E), using a spatial discrimination (T-maze) task. The effect of a single, subtle environmental change in the maze environment was determined after acquisition of the task. In addition, pigs were subjected to reversal learning to assess their ability to inhibit and modulate previously learned behaviour patterns.

We found no effects of Backtest classification or housing condition on acquisition of the maze

response. Housing and its interaction with Backtest classification influenced the behavioural response to the intramaze change. The novel object distracted E pigs considerably more than it did pigs from the barren environment. Furthermore, housing condition affected LR pigs more than HR pigs, as indicated by the interaction effects on various recorded behaviours. Finally, reversal learning was related to Backtest classification. LR pigs were more successful than HR pigs in acquiring the reversal task, irrespective of their housing environment.

We assumed all pigs to be well-habituated to the maze environment prior to the intramaze change and reversal, as they had run the maze for at least ten times (pre-training run plus acquisition criterion of nine correct trials). Accordingly, high-pitched vocalisations and defecating, which may indicate fear or stress (Fraser 1974; Kiley 1972), never occurred during training trials. Acquisition of the initial spatial discrimination task and performance during training trials were not affected by housing environment or Backtest classification, indicating that all groups of pigs had the same starting point.

Housing affected behaviour in the intramaze change-trial; its impact, however, depended on individual coping characteristics and was greater in LR than in HR pigs. LR pigs from enriched housing (E-LR pigs) hesitated longer before entering the section where the novel object was placed, vocalised more often, and showed a higher latency to reach the food reward than LR pigs from barren housing (B-LR pigs). Moreover, E-LR pigs also made more errors than B-LR pigs, by visiting the other sections of the maze before passing the object. Several studies in rodents (e.g. Morgan 1973; Renner and Rozenzweig 1987; Van Woerden 1986) have shown that rearing or housing environments lacking sufficient stimulation may reduce behavioural flexibility, i.e., the capacity of an animal to alter its behaviour when confronted with novel challenges (Fagen 1982). Correspondingly, it has been proposed that the barren, substrate-impoverished conditions in which pigs are frequently housed may cause a similar shift towards a more restricted, inflexible organization of behaviour in this species (Haskell et al. 1996; Wemelsfelder et al. 2000). The finding that LR pigs from enriched housing were more distracted by the change than LR pigs from barren housing, which was also found for the HR pigs, albeit less evidently and not significantly (Table 4.2), might be interpreted as a sign of reduced behavioural flexibility in barren housed pigs.

Housing did, however, not affect reversal learning, which argues against a general reduction in behavioural flexibility in pigs from the barren environment. The absence of a housing effect on reversal learning appears to oppose results of rodent studies showing a profound positive influence of environmental enrichment on the ability to reverse previously learned response patterns (e.g. Morgan 1973; Renner and Rozenzweig 1987; Van Woerden 1986). In these studies, however, social (group vs. isolated housing) and physical enrichment (inanimate enrichment) were often confounded. In fact, Morgan (1973) showed that enrichment of the

physical environment alone did not affect reversal learning in rats. Similarly, others (Mendl et al. 1997) found no effect of enrichment of the (physical) housing environment on the ability of individually housed pigs to reverse a maze task. Hence, the presence or absence of substrate in the home environment does not seem to affect spatial reversal learning in pigs, even though it markedly influences the expression and diversity of behaviour (Beattie et al. 2000; Haskell et al. 1996; Schouten 1986; Wemelsfelder et al. 2000).

The intramaze change and the reversal tests were both carried out to assess the propensity of pigs to develop inflexible response patterns or routines. Unlike the reversal trials, however, in the intramaze change trial pigs were confronted with a novel object that may have elicited a variety of emotional responses. Several studies have shown indeed that pigs from different housing conditions vary in their reaction to novelty, although the results are inconsistent. Pigs from barren housing environments have both been reported to be less (De Jong et al. 1998; Olsson et al. 1999; Stolba and Wood-Gush 1980) and more (Beattie et al. 2000; Wemelsfelder et al. 2000) reluctant to approach and explore novel objects than pigs from more complex environments. Housing-related differences in responses to novelty have been attributed to variation in a range of supposed underlying factors, such as, for instance, motivation to explore (Pearce and Paterson 1993; Stolba and Wood-Gush 1980), fearfulness (Beattie et al. 2000), 'cautiousness', i.e. 'the ability to recognise and evaluate potentially risky situations' (Marchant-Forde et al. 2003), habituation (Zimmermann et al. 2001), alertness and anxiety (Van de Weerd et al. 1994). Some of these factors may have influenced the reaction to the intramaze change in the present study. For instance, E-LR pigs appeared more fearful than B-LR pigs by showing a longer latency to enter the section where the novel object was placed. In addition, the high number of errors displayed by pigs from enriched housing (and in particular by the LR individuals), indicating renewed exploration of the maze environment, could be interpreted as enhanced alertness, 'cautiousness' or 'motivation to explore'. The differential reactions to the intramaze change of pigs with different environmental background may therefore, besides behavioural (in)flexibility, reflect variation in a range of underlying emotions and motivations.

It has been suggested (Hessing et al. 1994a; Ruis et al. 2000) that LR pigs preferably adopt a so-called 'passive' or 'reactive' coping style that is characterized by a high attention for subtle changes in a familiar situation and behavioural flexibility (for review, see Koolhaas et al. 1999; 2001). Behaviour patterns of HR pigs, on the other hand, would resemble those of '(pro)active copers' which become, with repeated experience, relatively routine-like and resistant to distracting stimuli (Benus et al. 1987; 1990b). E-LR pigs were indeed more distracted by the intramaze change than HR pigs from enriched housing. For pigs from the barren environment, however, an opposite effect of Backtest classification on the behavioural response to the intramaze change was found. If this response was a reflection of behavioural

flexibility, its supposed reduction by barren housing was most evident in LR pigs.

As argued above, the response to the intramaze change may also have reflected underlying differences in the appraisal of and responses to novel stimuli. It has been suggested that LR pigs are more reluctant to approach novel stimuli (Hessing et al. 1994a) and unfamiliar persons (Ruis et al. 2000) than HR pigs. If that is indeed the case, the present results indicate that the relationship between Backtest classification and behavioural responses to novelty does not generalise across different tasks and environmental backgrounds. Interactions between the effects of environmental factors and 'individual response profiles' have also been reported in rodents (e.g. Cools and Ellenbroek 2002; Ellenbroek and Cools 2002; Fernandez-Tereul et al. 1997; Van de Weerd et al. 1994).

Acquisition of the reversal task was related to Backtest classification, irrespective of housing. HR pigs were less successful in reversal learning than LR pigs, as the proportion of LR pigs with correct reversal performance was higher than the proportion of faultlessly performing HR pigs. Because Backtest classification did not affect acquisition or performance of the original maze task, this effect is unlikely related to general differences in learning ability or motivational factors. Operationally, reversal learning only differed from the original maze task in the additional requirement to inhibit an established response pattern. Indeed, the great majority of pigs with incorrect reversal performance did not randomly walk around in the maze, but repeatedly made the same perseverative set of errors: they spurted into the previously rewarded arm and then turned quickly to the other, correct, arm. In fact, HR pigs reached the food just as fast as – and in the third reversal trial even faster than – LR pigs, but they had more difficulty in inhibiting their previously reinforced response, which suggests that the types of pig may have used different mechanisms to solve the learning task. Thus, during training trials HR pigs may have developed more routine-like or habitual action patterns that are relatively resistant to change (cf. Benus et al. 1990b). Several studies make mention of distinct neural mechanisms for processing and storing information that operate simultaneously to influence behavioural output. Amongst these, 'less cognitive, more rigid' memory processes, that are referred to as '(stimulus)-response', 'non-declarative', 'implicit', 'procedural' or 'habit' learning have been distinguished, as opposed to more 'cognitive', 'explicit', 'reflective' or 'declarative' processes (see Packard and Knowlton 2002, Poldrack and Packard 2003; Toates 1998; White 1997; White and McDonald 2002 for review). In a maze task in which food is always found in a fixed direction from the animal's starting position, as in our experiment, both habitual (response learning) and cognitive processes (place learning) can be used to reach the food. Cross-maze studies in rats have revealed that with extended training, the weight attributed to the habitual, stimulus-response processes for controlling behaviour increases relatively to cognitive processes (Packard and McGaugh 1996). So, the results of the present study might suggest that the differences in

reversal learning between HR and LR pigs reflect differences in the relative contribution of these behavioural control mechanisms.

Clinical and behavioural studies suggest that the striatum is the brain centre for habit formation and performance of routinised behaviour (Graybiel 1995, Jog et al. 1999; Packard 1999; Packard and Knowlton 2002; Packard and McGaugh 1996; White 1997). HR pigs have recently been found to show a stronger stereotypic response to the dopamine-agonist apomorphine than LR pigs (Bolhuis et al. 2000a). Furthermore, they also tended to display higher levels of stereotypic chain biting after transfer to individual stalls than LR pigs (Geverink et al. 2003). This suggests differences between the two types of pig in functional activity of the striatum, as this brain structure is involved in the display of both pharmacologically (Cabib 1993; Kelly et al. 1975) and environmentally induced stereotypies (Garner and Mason 2002).

The results agree with studies in rodents and great tits demonstrating that individuals that preferably adopt an active behavioural strategy when stressed, develop, with repeated experience, most easily inflexible behavioural routines (Benus et al. 1991a; Koolhaas et al. 1999; 2001; Verbeek et al. 1994; 1996). Also in humans, individual differences in perseveration of previously rewarded response patterns have been described to co-vary with a broader set of characteristics, including an active behavioural style (see Patterson and Newman 1993).

In conclusion, our data indicate that individual coping characteristics of pigs, as assessed by the behavioural response in a so-called Backtest (HR versus LR) early in life, are related to behavioural flexibility in later life. HR pigs were less successful in reversal learning than LR pigs, suggesting that they have a higher propensity to develop inflexible behavioural routines. The disrupting effect of a single, subtle environmental change on task performance depended, however, on the interaction between individual coping characteristics and rearing conditions. This indicates that behavioural responding of pigs with diverging coping styles cannot simply be generalised across rearing conditions.

ACKNOWLEDGEMENTS

Effects of housing and individual coping characteristics on immune responses of pigs

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ABSTRACT

The impact of environmental factors on immune responses may be influenced by coping characteristics of the individuals under study. The behavioural response of pigs in a so-called Backtest early in life seems indicative of their coping style at a later age. The present study investigated the effects of housing, barren versus enriched, and coping style, as assessed by Backtest classification, on immune responses of pigs. Pigs were housed either without a rooting substrate (barren housing) or in identical pens enriched with deep straw bedding (enriched housing) from birth. During the suckling period pigs were subjected to the Backtest. Each pig was restrained on its back for 1 min and the resistance (i.e., number of escape attempts) was scored. Pigs classified as 'high-resisting' (HR) or as 'low-resisting' (LR) were immunized with KLH-DNP at 9 weeks of age. Blood samples were drawn prior to immunization (day 0) and weekly thereafter, until day 35. KLH-specific lymphocyte proliferation following immunization was higher for HR pigs than for LR pigs. Housing did not affect proliferative responses. Housing and coping style interacted in their effect on KLH-specific humoral immune responses. LR pigs from barren housing showed higher KLH-specific antibody titres than LR pigs from enriched housing. Differently housed HR pigs, however, showed similar antibody titres. These findings support other research indicating that individual coping styles of pigs are reflected in their immune responses. More important, the present study demonstrates that effects of housing on humoral immune responses of pigs may differ for pigs with divergent coping styles.

INTRODUCTION

In intensive farming conditions, growing pigs are usually housed in barren environments with restricted possibilities for the performance of important behaviours. Behavioural and physiological features of barren housed pigs, as compared to those of pigs in an enriched environment, indicate that pigs may have difficulties to adapt to the limitations of intensive housing conditions (Beattie et al. 1995; De Jong et al. 1998; 2000; Schouten 1986).
Besides affecting behavioural and physiological characteristics, housing conditions can influence the immune reactivity of animals (Barnett et al. 1987; Griffin 1989; Kelley 1980). The described effects of housing on immune function, however, show a large variation both within and between studies (see Barnard et al 1996; Griffin 1989; Karp et al. 1993; Kelley 1980). The impact of environmental factors on immune responses can be influenced by characteristics of the individuals under study, which may explain part of this variation (Cohen 1999; Kemeny and Laudenslager 1999). Studies in humans (Kropiunigg 1993; McEwen and Stellar 1993; Miller et al. 1999), non-human primates (Capitanio and Mendoza 1999; Line et al. 1996) and rodents (Bohus et al. 1991; Kavelaars et al. 1997; 1999) have clearly demonstrated the significance of stable individual characteristics, such as personality traits or

coping style, for immune processes.

Individual pigs show different adaptive behavioural and physiological responses when exposed to challenging circumstances (Erhard and Schouten 2001; Mendl et al. 1992; Schouten and Wiepkema 1991). The behaviour piglets display early in life in a so-called Backtest appears to be related to coping responses at a later age. In the Backtest, a piglet is manually restrained on its back for 1 min and its escape behaviour is recorded. Pigs adopting one of the extreme reaction patterns in this test, i.e. high-resisting (HR) and low-resisting (LR) animals, were described to vary in a set of behavioural, physiological and neurochemical characteristics, such as aggressiveness (Hessing et al. 1993; 1994c; Ruis et al. 2000; 2002), responses to novel environments (Hessing et al. 1994a; Ruis et al. 2001), susceptibility to the dopamine agonist apomorphine (Bolhuis et al. 2000a) and (re)activity of the sympathetic nervous system (Geverink et al. 2002b; Hessing et al. 1994a; Ruis et al. 2001) and hypothalamic-pituitary-adrenal axis (Geverink et al. 2002a; Ruis et al. 2000; 2001). Therefore, it has been suggested that the Backtest classification of individual pigs is indicative of their coping style (Hessing et al. 1994a; Ruis et al. 2000).

Individual coping styles of pigs appear to be reflected in some immune parameters as well (Hessing et al. 1995; Schrama et al. 1997). It is unknown, however, whether and how individual coping characteristics of pigs interact with the effects of housing on immune reactivity. Therefore, we examined the effects of housing conditions, barren versus enriched with straw, on parameters of humoral and cellular immune reactivity of pigs with divergent Backtest responses, high-resisting (HR) and low-resisting (LR) pigs.

METHODS

Animals and housing

Four litters of commercial crossbred pigs (Dutch Landrace x Great Yorkshire) were used (n=38). Two litters (n=18) were housed from birth in substrate-impoverished, farrow-to-finish pens (7 m^2) with a 65% solid, concrete and 35% slatted floor (barren housing, 'B'). The other two litters (n=20) were housed in identical, adjacent pens enriched with deep straw bedding (enriched housing, 'E').

In the morning, pens were cleaned and fresh straw was supplied to the enriched pens. Water and food were available ad libitum. Lights were on between 7.00 and 19.00 h. At three days of age, tails were docked and male piglets were castrated following standard procedures at the experimental farm. Animals were weaned at four weeks of age; the sow was removed from the pen. The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments, which complies with the ETS123 (council of

Europe 1985) and the 86/609/EEC directive. The Wageningen University Animal Care and Use Committee (Wageningen, The Netherlands) approved the experiment.

Backtest

Pigs were subjected to the Backtest (adapted from Hessing et al. 1993) at 10 and 17 days of age and classified as described before (Bolhuis et al. 2000a). The test was carried out on a table placed in the corridor near the home pen. The experimenter lifted a piglet from its home pen and used the left hand to place the piglet on its back on a mat. The right hand of the experimenter was then placed on the thorax of the piglet, with the left foreleg of the piglet between thumb and index, and the right foreleg between index and middle finger. The experimenter's left hand was used to stretch and moved the hind legs of the piglet downward at the start of the test and then the hand remained loosely on the hind legs. Pigs were restrained in this supine position for 60 s. Each bout of struggling with at least the hind legs was counted as an escape attempt. A bout was terminated when the piglet stopped struggling or paused by stretching or relaxing its legs. In addition, duration of escape behaviour, latency to the first escape attempt and number of vocalisations were recorded.

Classification of pigs was based on the number of escape attempts they displayed during the test. The number of escape attempts is indicative of the overall reaction pattern of pigs in the Backtest, as it is generally related to the total duration of escape behaviour (Ruis et al. 2000), the latency to the first escape attempt (Hessing et al. 1993; 1994a), the number of vocalisations (Hessing et al. 1993; 1994a; Ruis et al. 2000) and the cardiac response (Hessing et al. 1994a) in this test. A pig was classified as high-resisting (HR) if it performed more than four escape attempts in two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low-resisting (LR). Thirty-eight pigs were tested, yielding 19 HR (10 B and 9 E) and 10 LR (5 B and 5 E) pigs.

Immunization and blood sampling procedure

At 9 weeks of age (body weight 28.7 ± 0.9 kg) HR and LR pigs (n = 29) were immunized intramuscularly in the neck with 1.0 mg of di-nitrophenyl-conjugated keyhole limpet haemocyanin (DNP-KLH, Calbiochem, San Diego, USA) in a volume of 1 ml phosphate buffered saline (PBS) mixed with 1 ml incomplete Freund adjuvant (Difco, Detroit, USA). Pigs were restrained by a nose sling to collect blood samples by venipuncture before immunization (day 0), and on day 7, 14, 21, 28 and 35 afterwards. Blood samples (2 x 5 ml; one heparinized) were collected within 2 min after restraining the pigs using two vacutainer tubes (Venoject, Omnilabo, Breda, The Netherlands). Heparinized blood samples were analysed on the day of collection. Serum samples were stored at -20°C until analysis.

In vitro lymphocyte proliferation

Con A. Mitogen-induced lymphocyte proliferation was evaluated with a lymphocyte stimulation test (LST) following a previously described method (Joling et al. 1993) optimised for whole blood. Heparinized blood samples, diluted 1:10 in culture medium (RPMI-1640 with L-glutamine (ICN, Aurora, USA) supplemented with 2 g/l $NaHCO_3$, 100 IU penicillin and 100 µg /ml streptomycin) were cultured in sterile round-bottom microtiter plates (Greiner, Nürtingen, Germany) in a volume of 200 µl containing Concanavalin A (Con A, Sigma, St. Louis, USA), 5 µg/ml or medium only (control cultures). The cultures, all set up in triplicate, were incubated for 96 h at 37°C, 5% CO_2 in humidified air. Eighteen hours before harvest, 0.4 µCi of methyl-[^{3}H] thymidine (Amersham, Bucks, UK) was added to each well. Blood cells were harvested onto filter paper using a semiautomatic cell harvester. Incorporation of [^{3}H] thymidine was determined by liquid scintillation counting. Results were expressed as stimulation index (SI) scores per sampling day per animal, with SI = mean counts per minute in stimulated cultures / mean counts per minute of control cultures.

KLH. KLH-specific lymphocyte proliferation was evaluated with a similar LST, using a concentration of 10 µg/ml of KLH and applying an incubation period of 120 h.

Humoral immune response

KLH-specific antibody titres were routinely determined by a two-step enzyme-linked immunosorbent assay (ELISA) as described before (Schrama et al. 1997). Briefly, flat-bottom microtiter plates (Greiner) were coated overnight at room temperature (RT) with KLH dissolved in $NaHCO_3$ buffer (pH 9.6) to 1 µg/ml. Plates were washed twice with tap water containing 0.05% Tween 20. Subsequently, serial (^{2}log) dilutions of serum were added and incubated for 1 h at RT. After washing as before, plates were incubated for 1 h at RT with a 1:8000 diluted peroxidase (PO) conjugated rabbit antibody directed to swine IgG$_{FC}$ (RαSw-IgG$_{FC}$/PO, Nordic, Tilburg, The Netherlands) or to swine IgG$_{H+L}$ (RαSw-IgG$_{H+L}$/PO Cappel, Gaithersburg, MD, USA) to detect IgG antibody and total antibody (total Ig), respectively. For detection of IgM antibody, plates were incubated with 1:5000 diluted goat anti-swine IgM$_{FC}$ (GαSw-IgM$_{FC}$/PO, Nordic, Tilburg, The Netherlands). After washing, tetramethylbenzidine (Sigma) was added as a substrate. Colour formation was stopped after 10 min with 2.5 N H_2SO_4 and the absorbance was measured at 450 nm in a Titertek Multiskan (Labsystems, Helsinki, Finland). All absorbances were expressed relatively to the absorbance of a standard positive control serum and antibody titres were assessed as previously described (Schrama et al. 1997).

Data analysis

SAS version 6.12 was used for all statistical calculations. Effects of and interactions between the factors Backtest classification (LR or HR), housing (barren or enriched) and sex on performance during the Backtest were analysed with a mixed linear model (Statistical Analysis System Institute Inc. 1996). Effects of housing were tested against the random effect of litter within housing. Logarithmic, angular and square root transformations were applied for skewed distributions of duration (latency to the first escape attempt), proportion (time spent on escape behaviour) and frequency (number of escape attempts and vocalisations), respectively.

The effects of housing, Backtest classification, sex and sampling day and their interactions on stimulation indices and antibody titres were studied with a mixed linear model allowing for random effects of litter and animal. Preliminary analyses showed sex differences in the proliferative response to Con A (data not shown) and therefore this factor was included in the model. Sex effects did not interfere with effects of housing or Backtest classification; non-significant interactions with sex were removed from the final model. Stimulation indices showed increasing variance with increasing mean and were logarithmically transformed prior to analysis. In addition, mixed linear models with housing, Backtest classification, sex and interactions as fixed effects and litter as random effect were used to assess effects on stimulation indices and antibody titres at separate sampling days. Data are presented as means ± SEM.

RESULTS

Backtest classification

HR pigs performed 4.5 ± 0.3 escape attempts during the Backtest and LR pigs 0.7 ± 0.2 ($p < 0.001$), average of two tests. Latency to the first escape attempt was 12.7 ± 1.9 sec for HR pigs and 47.8 ± 4.1 sec for LR pigs ($p < 0.001$). HR pigs spent more time (20.9 ± 1.6%) on escape behaviour than LR pigs (3.6 ± 0.7%, $p < 0.001$), and vocalised more often during testing (48.4 ± 2.9 vs. 19.6 ± 5.9, $p < 0.001$), average of two tests. Behaviour during the Backtest was unaffected by housing (Table 1).

In vitro lymphocyte proliferation

Con A. Con A-stimulated lymphocyte proliferation was affected by time (sampling day, $p < 0.001$), and by the interaction between Backtest classification and time ($p < 0.05$). HR pigs showed higher stimulation index (SI) scores than LR pigs on the last two sampling days (Fig. 5.1). Housing and its interaction with Backtest classification (Table 5.1) or time did not affect

SI scores for Con A.

KLH. KLH-induced lymphocyte proliferation was affected by time ($p < 0.001$). Backtest classification tended to affect SI scores for KLH ($p < 0.07$). Omission of day 0 from the model showed that the effect of Backtest classification was significant over the post immunization period ($p < 0.05$, Fig. 5.1). No (main or interaction) effects of Housing were found (Table 5.1).

Table 5.1. Behaviour in the Backtest and lymphocyte proliferation (Stimulation Index; SI) in response to Con A and KLH after immunization with DNP-KLH of HR and LR pigs in barren and enriched housing.

Variable	HR		LR	
	Barren	Enriched	Barren	Enriched
Backtest[1]				
Number of escape attempts	4.7 ± 0.4^a	4.3 ± 0.3^a	0.7 ± 0.12^b	0.6 ± 0.3^b
Escape behaviour (% of time)	23.5 ± 2.7^a	17.9 ± 0.8^a	4.0 ± 0.3^b	3.2 ± 1.4^b
Latency to 1st escape attempt	10.1 ± 1.8^a	15.7 ± 3.4^a	49.8 ± 4.8^b	45.7 ± 7.2^b
Number of vocalisations	49.8 ± 5.0^a	46.9 ± 2.7^a	16.2 ± 9.7^b	22.9 ± 7.6^b
Lymphocyte proliferation[2]				
SI Con A[3]	253.5 ± 15.3	224.8 ± 42.7	182.8 ± 27.6	265.9 ± 26.2
SI KLH before immunization	1.3 ± 0.3	1.7 ± 0.5	2.5 ± 1.6	1.0 ± 0.1
SI KLH after immunization[4]	11.8 ± 3.6^a	13.2 ± 4.4^a	7.66 ± 3.1^b	6.1 ± 3.3^b

Within a row, means lacking a common superscript letter differ (a, b: $p < 0.05$). [1]Average of two tests; [2]Background cpm (non-stimulated control cultures) were 142.0 ± 17.3; [3]Average of all sampling days; [4]Average day 7 - 35.

Antibody response

Humoral immune responses to KLH (total antibody (Ig) titres, and titres of IgM and IgG isotypes) of differently housed LR and HR pigs are shown in Fig. 5.2. Total Ig, IgM and IgG responses to KLH were affected by time ($p < 0.001$) with increasing titres from day 7 on.

Barren and enriched-housed pigs differed in the time course of total Ig titres directed to KLH (housing x time interaction, $p < 0.01$). The effect of Housing on the kinetics of the total Ig response to KLH depended, however, on the type of pig (HR vs. LR), which was demonstrated by a Backtest classification x housing x time interaction ($p < 0.01$). LR pigs in barren housing showed higher total Ig titres than LR pigs in enriched housing ($p < 0.05$), whereas antibody titres of barren and enriched housed HR pigs did not differ.
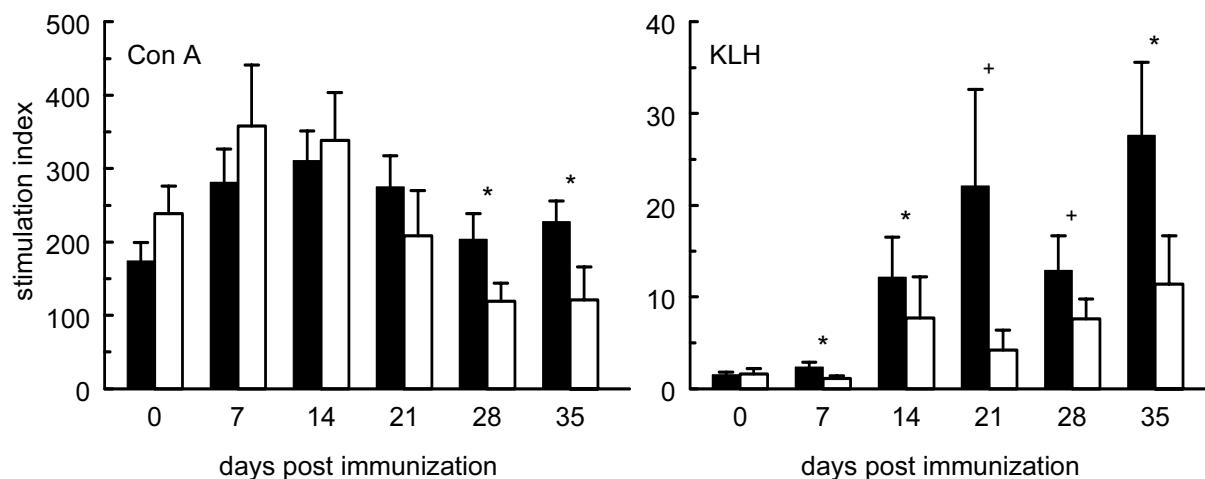
Fig. 5.1. Whole blood lymphocyte proliferation (Stimulation Index) in response to Con A and KLH after immunization with DNP-KLH of HR pigs (solid bars) and LR pigs (open bars). Differences between LR and HR pigs within a time period are indicated: $*p < 0.05$; $+p < 0.10$. Background cpm (non-stimulated control cultures) were 142.0 ± 17.3. Note: different Y-axis scaling for Con A and KLH.

The time course of the IgM response to KLH differed for barren and enriched housed pigs (housing x time interaction, $p < 0.05$). In addition, a tendency for a Backtest classification x housing x time interaction was found ($p < 0.09$). At the peak of the IgM response (day 7) titres were higher for barren LR pigs than for enriched housed LR pigs, whereas differently housed HR pigs showed similar IgM titres.

Housing affected the kinetics of KLH-specific IgG response (housing x time interaction, $p < 0.01$). The interaction between Backtest classification and housing tended to affect IgG titres ($p < 0.08$). Again, titres of differently housed LR pigs differed (barren housed LR pigs > enriched housed LR pigs, $p < 0.05$) whereas barren housed HR pigs and enriched housed HR pigs showed similar IgG titres in between.
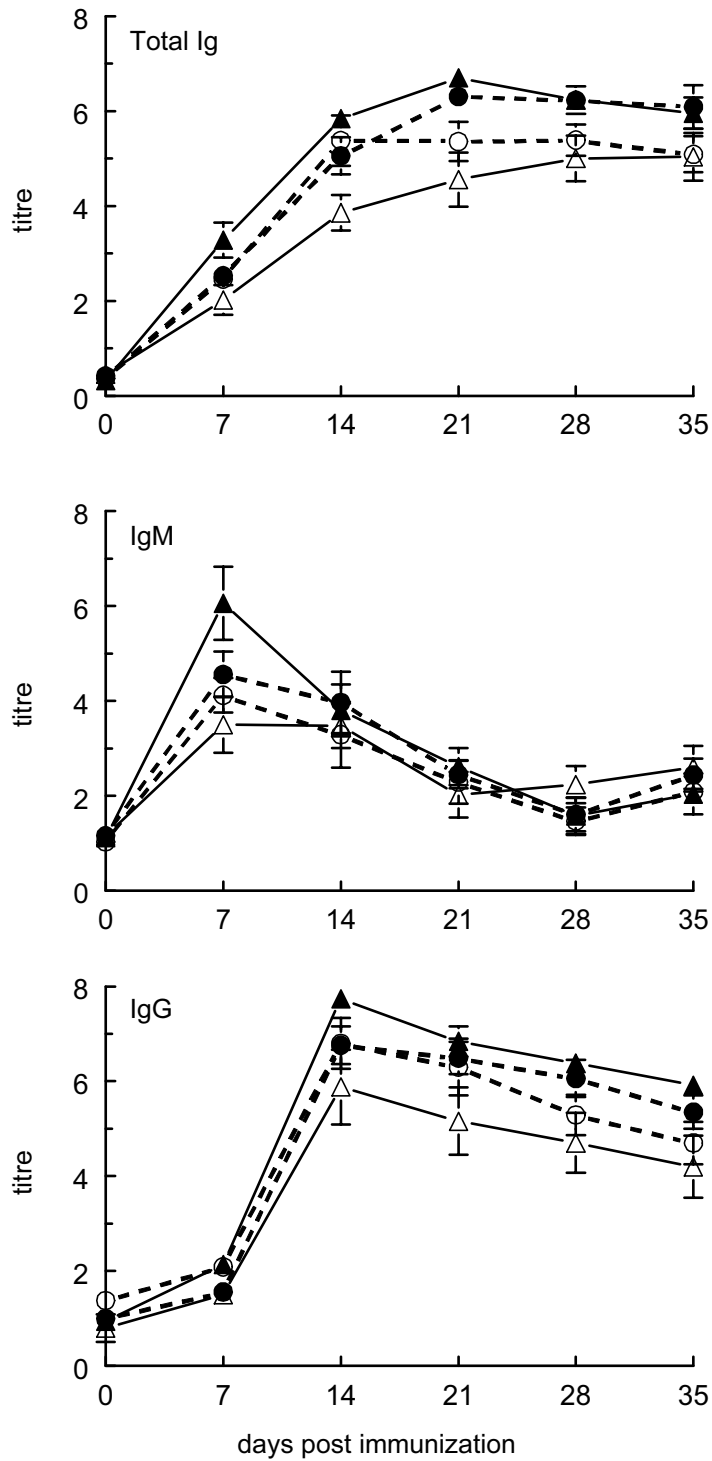
Fig. 5.2. KLH-specific antibody titres (Total Ig, IgG and IgM) of HR (circles, dotted lines) and LR pigs (triangles, solid lines) in barren (closed symbols) or enriched housing (open symbols).

DISCUSSION

The present study demonstrates for the first time that the effects of housing, barren versus enriched, on immune responses of pigs depend on individual coping characteristics. Pigs selected for divergent responses in the Backtest at an early age, i.e. high-resisting (HR) versus low-resisting (LR), varied in KLH-induced lymphocyte proliferation and in the impact of housing on antibody responses to KLH.

Housing did not affect Con A- or KLH-induced lymphocyte proliferation. Others (De Groot et al. 2000) reported, to the contrary, a higher Con A-induced proliferation of lymphocytes from enriched housed pigs than from barren housed pigs after a similar culture period. In their study, however, the contrast in housing conditions was larger, as the barren and enriched environments not only differed in the availability of straw, but also in space allowance. It should also be noted that the sample size in the present study was relatively small.

Lymphocyte proliferation was influenced by coping characteristics. HR pigs showed a higher Con A-induced lymphocyte proliferation than LR pigs on two sampling days and displayed higher proliferative responses to KLH following immunization. The latter is consistent with a previous study on Backtest-typified pigs reporting a higher KLH-induced lymphocyte proliferation for HR pigs than for LR pigs on day 21 post immunization (Hessing et al. 1995). It is not clear how Backtest responses and lymphocyte proliferation are related. As lymphocyte proliferation was evaluated using whole blood assays and T-cell numbers in the blood were not determined, we cannot conclude whether the differences in proliferation are due to variation in numbers of circulating T-cells or the functional capacity per cell. Whole blood assays may, however, reflect the in vivo situation of immune responsiveness more closely than assays on isolated cells. Several studies state that cortisol reduces cell-mediated immune responses, including in vitro lymphocyte proliferation, in pigs (Klemcke et al. 1990; Westly and Kelley 1984). Pigs with a high adrenocortical response when stressed display a low mitogen-induced lymphocyte proliferation (Brown-Borg and Klemcke 1993). LR pigs have been reported to show a higher adrenocortical reactivity (Ruis et al. 2000; 2001) than HR pigs, which is in accordance with the lower proliferative responses observed in this type of pig. As HR pigs are more aggressive than LR pigs, which has been demonstrated in food competition tests (Ruis et al. 2000) and social confrontation tests (Hessing et al. 1993; 1994a; Ruis et al. 2002), the results also correspond with studies reporting a higher mitogen- or antigen-induced lymphocyte proliferation for more aggressive individuals (rodents: Bohus et al. 1991; Petitto et al. 1999; pigs: Hessing et al. 1995).

The present experiment shows that barren vs. enriched housing can affect humoral immune responses of pigs, contrary to another study (De Groot et al. 2000), in which no effect of a similar contrast in housing conditions on antibody responses was found. Our results

demonstrate, however, that the effects of housing on humoral immunity may depend on individual coping styles. The results indicate that barren housed pigs showed overall higher or earlier antibody responses to KLH than enriched housed pigs, but this effect was merely found for the LR animals: antibody titres of differently housed LR pigs varied, whereas humoral responses of barren and enriched housed HR pigs were similar. Previous studies (Hessing et al. 1994a; Ruis et al. 2000) have suggested that LR pigs are predisposed to adopt a so-called 'passive' or 'reactive' coping style, that is characterized by a high dependency on environmental cues, whereas HR pigs operate relatively independent of external factors; a feature of a '(pro)active' coping style (see Koolhaas et al. 1999). The present results indicate that LR pigs may also be more reactive to a long-term environmental factor, i.e. housing.

Behavioural and physiological studies show that the restrictions of a barren housing environment constitute a long-term challenge for growing pigs (Beattie et al. 1995; 2000; De Jong et al. 2000). It is often stated that long-term stress has a suppressive effect on the immune system (Glaser et al. 1987; Herbert and Cohen, 1993; Kiecolt-Glaser et al. 1994). The higher humoral immune responses in barren housed LR pigs, as compared to responses of enriched-housed LR pigs, seem to contradict this supposed immunosuppressive effect of long-term stress (Glaser et al. 1987; Kiecolt-Glaser and Glaser 1994; Vedhara et al. 1999). Recent studies suggest, however, that sustained stress, rather than suppressing the immune system as a whole, may induce a shift in the balance between the cellular (T-helper-1 (Th1) mediated) versus humoral (T-helper-2 (Th2) mediated) immune responses to an antigenic challenge by favouring cytokine patterns that enhance antibody production (Agarwal and Marshall 1998; Chrousos 2000; Marshall and Agarwal 2000; Marshall et al. 1998). One of the neuroendocrine systems involved in mediating this shift appears to be the hypothalamic-pituitary-adrenal (HPA) axis, as glucocorticoids are important determinants of the ultimate balance between Th1 and Th2 cytokines (Chrousos 2000; Mason 1991; Wilckens and De Rijk 1997). LR pigs have been reported to display a higher adrenocortical reactivity when challenged (Ruis et al. 2000; 2001), and show higher basal levels of cortisol in barren housing than HR pigs (Hessing et al. 1994a). As glucocorticoids enhance Th2-mediated responses (Chrousos 2000; Mason 1991; Wilckens and De Rijk 1997), the high HPA-axis reactivity of LR pigs in barren housing could have contributed to a cytokine balance that leads to high antibody titres. As yet, effects of housing on cytokine balances in pigs with different coping styles have to be determined.

Other neuroendocrine factors (Ader et al. 1995; Jamner and Leigh 1999; Jamner et al. 1998; Moynihan et al. 1994; Savino and Dardenne 1995) might also have been involved in mediating the effects of housing or coping characteristics of pigs on their immune reactivity. For instance, LR and HR pigs have been suggested to vary in functioning of the

dopaminergic system (Bolhuis et al. 2000a) and the autonomic nervous system (Geverink et al. 2002b; Hessing et al. 1994a; Ruis et al. 2001), which have immunomodulatory properties (Basu and Dasgupta 2000; Madden and Livnat 1991).

KLH-DNP is a model, but complex, antigen. An advantage of using KLH-DNP is that it 'eliminates' genetic (MHC-related) differences in immune responses. It is known, however, to induce Th2-type responses mainly (Bliss et al. 1996; Bradley et al. 1995). Therefore, until further extended to other antigens, the effects found in the present study are only accurate for this type of response. Whether and how housing conditions and individual characteristics of pigs are related to immune reactions to other types of antigen, remains to be shown.

In humans, individual differences in stress-reactions and immune function have been reported to be of clinical relevance (Marsland et al. 2002). Social rank-related variation in Aujeszky Disease Virus (ADV)-induced lymphocyte proliferation in pigs was reported to be linked to differences in morbidity after ADV-challenge (Hessing et al. 1994b). In addition, selection of pigs for immune responsiveness was suggested to result in improved health and growth (see Wilkie and Mallard 1999). The implications of the presently reported immune differences for disease resistance of pigs merit further research. This is true both for effects of coping style and its interaction with housing on the magnitude as well as on the kinetics of the immune response.

In conclusion, individual coping characteristics of pigs, as assessed by the behavioural response in the Backtest, relate to their immune reactivity. Moreover, the present study shows for the first time that the effects of housing, barren versus enriched, on humoral immune responses can differ for pigs with divergent coping styles. More studies are needed to further investigate these interactions and their effects on different types of immune response. Although the present study was based on a limited number of pigs and only two types of housing were compared, our results imply that the effects of individual coping characteristics on immune responses of pigs found in a given environment might not necessarily apply to other housing conditions. Conversely, when studying the effects of housing factors on immune reactivity and health of pigs, the importance of individual characteristics of the animals under study should be considered.

ACKNOWLEDGEMENTS

Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions

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ABSTRACT

Characterization of piglets based on the degree of resistance they display in a so-called Backtest is, to a certain extent, predictive of their coping responses to several short-term challenges in later life. It is unknown, however, whether these individual coping characteristics of pigs are also reflected in their behavioural development in different environments. The present study investigated the behavioural development of pigs with diverging Backtest responses, high-resisting (HR) and low-resisting (LR) pigs, in both barren housing and housing enriched with straw-bedding. During the suckling period pigs were subjected to the Backtest. Pigs classified as HR (n = 30) or LR (n = 30), were selected and after weaning housed in groups of 6 (3 LR and 3 HR) in either barren or enriched pens. Home pen behaviour was recorded at 5, 9, 15 and 19 weeks of age for 8 hours per week using 2-min instantaneous scan sampling. Housing environment largely influenced behaviour of pigs. Barren-housed pigs were less active, showed less explorative and play behaviour, and spent more time on oral activities directed at pen mates than pigs from enriched housing. HR pigs showed more aggressive behaviour than LR pigs and they tended to be less active in both environments. LR pigs spent more time on manipulating pen mates, in particular in barren housing. In addition, they played more than HR pigs in enriched pens. In conclusion, an enriched housing environment, i.e. availability of straw bedding, appeared to improve welfare as play behaviour was increased and manipulative oral behaviour directed at pen mates decreased in comparison with barren housing conditions. Individual coping characteristics of pigs, as assessed in the Backtest, were also reflected in their home pen behaviour. Notably, the effects of housing on both manipulative and play behaviour were most obvious in LR pigs. Further research will have to elucidate whether LR pigs are more responsive than HR pigs to housing conditions in general, or whether they are more susceptible to the specific effects of an exploratory substrate in their home environment.

INTRODUCTION

In intensive farming conditions growing pigs are generally housed in barren and stimulus-poor environments that impose constraints on the development and expression of normal species-specific behaviour (Schouten 1986; Wemelsfelder et al. 2000). In such environments injurious behavioural activities may appear, such as, for instance, manipulative oral behaviours directed at conspecifics (Beattie et al. 1995; Dybkjaer 1992; Schouten 1986; Wiepkema and Koolhaas 1993). Besides aberrant behaviours, pigs in intensive husbandry systems have been reported to show physiological and pathological signs of stress and poor welfare (Beattie et al. 2000; De Jong et al. 2000; Hessing et al. 1992; 1994a).

Pigs vary largely in behavioural and physiological reactions when exposed to the same stressful situation (Lawrence et al. 1991; Schouten and Wiepkema 1991). Individual differences in adaptive or coping reactions of pigs have received growing attention, as the

identification of basic characteristics that predict (mal)adaptation to husbandry conditions could be relevant both for pig husbandry and pig welfare (Spoolder et al. 1996). In the search for such characteristics, a so-called Backtest, in which young piglets are restrained in supine position for 1 min, has been used frequently (Bolhuis 2003; Hessing et al. 1993; Ruis et al. 2000). The behavioural reaction of piglets in this test is thought to reveal (part of) their 'coping style' or 'personality' (Hessing et al. 1994a; Ruis et al. 2000).

Some piglets, usually referred to as 'high-resisters' (HR), struggle a lot during the Backtest, whereas piglets at the other end of the distribution tend to respond with immobility, the so-called 'low-resisters' (LR). At a later age, HR and LR pigs have been shown to differ in their behavioural and neuroendocrine reactions to novel environments and objects (Hessing et al. 1994a; Ruis et al. 2000) and social isolation (Geverink et al. 2003; Karman 2003; Ruis et al. 2001) as well as in aggressiveness in social provocation tests (chapter 3; Hessing et al. 1993; 1994a; Ruis et al. 2000; 2002, but see D'Eath and Burn 2002). Moreover, the Backtest response of piglets was found to relate to lean meat percentage post mortem (Van Erp-Van der Kooij et al. 2000; 2003), immune reactivity (Bolhuis et al. 2003; Schrama et al. 1997), and the behavioural response to the dopamine-agonist apomorphine (Bolhuis et al. 2000a).

Reaction patterns of HR and LR pigs have thus been shown to diverge in a series of, rather short-term, challenging test situations. In addition, HR and LR pigs differ in behavioural and physiological responses during long-term social isolation (Geverink et al. 2003; Karman 2003).

It is unknown, however, whether individual coping or personality characteristics of growing pigs are also reflected in their behaviour patterns in everyday life in common (group) housing systems. Do HR and LR pigs differ in the extent or way of adapting to intensive housing conditions? From several studies it is known that enrichment materials, such as straw, may strongly influence the behavioural activities of pigs and alleviate behavioural problems found in barren housing, such as the oral manipulation of pen mates (Beattie et al. 1995; Fraser et al. 1991; Schouten 1986). The effects of environmental complexity on behavioural development of pigs might, however, be different for individuals with diverging coping or personality characteristics. Aim of the present study was to investigate differences in the development of behaviour of HR and LR growing pigs in two different housing environments, barren and enriched with straw bedding.

METHODS

The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments, which complies with the ETS123 (council of Europe 1985)

and the 86/609/EEC directive. The Institutional Animal Care and Use Committee of Wageningen University approved the experiment.

Subjects

The experiment was carried out in two successive batches, using 60 crossbred pigs in total, selected on the basis of their Backtest response and weight (see below) from a pool of pigs. Per batch, subjects were bred from 21 Dutch Landrace x Yorkshire sows, inseminated at the same date with commercially supplied sperm of Yorkshire boars. During gestation, sows were housed in groups of three or four individuals.

Pre-weaning housing environment

One week prior to the expected day of farrowing, sows were randomly assigned to either substrate-impoverished Danish farrowing pens (2.2 x 3.8 m) with a 65% solid and 35% slatted floor (barren environment) or to identical, adjacent pens in which the solid part of the floor was covered with unchopped straw, approximately 4 kg per pen (enriched environment).

On the day of birth, piglets received an iron injection and an ear tattoo. Tails of the piglets were docked and male piglets were castrated at three days of age following standard pig husbandry procedures. Water was continuously available and from 10 days of age piglets were provided with creep feed. Lights were on between 7.00 and 19.00 h. Pens were cleaned out in the morning, between 8.00 and 9.00 h. Soiled straw was removed from the enriched farrowing pens and replaced with fresh unchopped straw.

Backtest and selection of subjects

Piglets were subjected to the Backtest (adapted from Hessing et al. 1993) at 10 and 17 days of age. The test procedure is described in detail elsewhere (Bolhuis et al. 2003). Briefly, each piglet was restrained on its back by placing one hand over the throat and the other loosely on the hind legs. Classification of pigs was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 seconds (see Hessing et al. 1994c) A pig was classified as high-resisting (HR) if it performed more than four escape attempts in the two tests, with a minimum of two attempts in one test. If a pig struggled less than four times in two tests, with a maximum of two attempts in one test, it was labelled low-resisting (LR). Thirty HR and 30 LR pigs with similar body weights (7.3 ± 0.1 kg on the day before weaning) were selected for the present experiment. For both classifications the female to castrated male ratio was 2:1. Half of the selected pigs were from barren and the other half from enriched farrowing pens.

Post-weaning housing environment

Pigs were weaned, mixed and relocated at 4 weeks of age. The 30 pigs from barren farrowing pens were placed in five substrate-impoverished pens (7 m^2) with a 65% solid and 35% slatted floor (barren environment). The pigs from enriched farrowing pens were placed in five similar, adjacent pens enriched with deep straw bedding (unchopped straw, approximately 4 kg per pen) where the slatted part of the floor was covered with a rubber mat (enriched environment). Per pen, six pigs, three HR and three LR pigs (two gilts and one barrow of each type) were grouped; these pigs were not familiar with each other.

During the whole experiment, food (commercial pelleted pig feeds) and water were continuously available. Lights were on between 7.00 and 19.00 h. In the morning, between 8.00 and 9.00 h, all pens were cleaned out. Soiled straw was removed from the enriched pens and replaced with fresh unchopped straw (replacement approximately 3.5 kg per pen per day).

Observations

At 5, 9, 11, 15 and 19 weeks of age behaviour of individual pigs was recorded for 8 h per week using 2-min instantaneous scan sampling. Observations were carried out on two days per week (4 h a day) and started at approximately 9.00, 11.00, 13.30 and 15.30 h. The Observer® software package (Noldus Information Technology b.v., Wageningen, The Netherlands) installed on a Psion Organiser II LZ64 was used for behavioural recordings. Individual pigs could be identified by an ear tag and a number sprayed on their backs with colour stock marker.

The ethogram, partly based on descriptions of behavioural elements from other studies on domestic pigs (Beattie et al. 1995; Newberry et al. 1988; Schouten 1986) and wild boars (Gundlach 1968), is given in Table 6.1. All oral activities directed to pen mates that could potentially harm the receiver (except the aggressive behaviours) were included in the category 'manipulative behaviour'. The category 'play behaviour' comprised behavioural elements that comply with the description of play by Martin and Caro (1985), i.e. 'locomotor activity performed postnatally that appears to an observer to have no obvious immediate benefits for the player, in which motor patterns resembling those used in serious functional contexts may be used in modified forms', and have been identified as 'play markers' in wild and domestic pigs (Beattie et al. 1995; Gundlach 1968; Newberry et al. 1988).

Table 6.1. Ethogram.

Behaviour	Description
Inactive	
Lying with closed eyes	Lying on side or belly with eyes closed
Lying with open eyes	Lying with eyes open, without performing any other described behaviour
Sitting	Sitting or kneeling without performing any other described behaviour
Standing	Standing without performing any other described behaviour
Locomotion	Walking without performing any other described behaviour
Ingestive behaviour	
Eating	Eating from food trough or chewing feed
Drinking	Drinking from water nipple
Eliminating	Defecating or urinating
Comfort behaviour	Rubbing body against objects or pen mates, scratching body with hind leg or stretching (part of) body
Social behaviour	
Nosing head	Touching or sniffing any part of the head of a pen mate
Nosing body	Touching or sniffing any part of the body of a pen mate except the head
Mounting	Placing both front hoofs on the back of a pen mate
Aggressive behaviour	
Head knocking	Ramming or pushing pen mate with the head, without biting
Biting	Ramming or pushing pen mate with head, with biting
Fighting	Mutual pushing or ramming, or lifting pen mate
Explorative behaviour	
Nosing floor	Sniffing, touching or rooting (substrate on) floor
Scraping floor	Scraping the floor with one of the fore legs
Nosing fixtures	Sniffing, touching or rooting part of the pen above floor level
Manipulating fixtures	Nibbling, chewing or biting part of the pen above floor level
Chewing	Chewing particles other than feed (e.g. straw, faeces) or vacuum chewing
Manipulative behaviour	
Belly nosing	Rubbing belly of a pen mate with up and down movements of the snout
Manipulating ears	Nibbling, sucking or chewing an ear of a pen mate
Manipulating tails	Nibbling, sucking or chewing the tail of a pen mate
Manipulating other	Nibbling, sucking or chewing any part of a pen mate, except tail or ears
Play behaviour	
Gambolling	Running across the pen, occasionally accompanied by nudging pen mate gently
Pivoting	Jumping and turning around the body axis
Rolling	Lying on back and moving from side to side on the floor
Shaking object	Shaking of head while holding material (e.g. straw) that protrudes from mouth

Data analysis

SAS (Statistical Analysis System Institute Inc. 1989) version 6.12 was used for statistical calculations. The behavioural element 'mounting' was excluded from analysis because it was seen very rarely (0.1% of observation time in week 5; 0% in weeks 9 - 19). Eating, drinking and eliminating were summed and presented as a single behavioural element: 'ingestive behaviour and elimination'. Prior to analysis, proportions of (observation) time spent on various behaviours were averaged per animal per week. Skewed distributions of proportion were arcsine square root transformed to obtain homogeneity of variances.

Effects of and interactions between the factors Backtest classification (LR or HR), housing (barren or enriched) and week on behaviour were analysed with a mixed linear model; batch and sex were included in the model. Effects of housing environment and batch were tested against the random effect of pen. Effects of Backtest classification and sex were tested against the random effect of animal. Because we could not be sure whether all pigs were observed to the same extent in their active periods, we also analysed the above-mentioned effects on behaviours expressed as a proportion of 'active time' (i.e. proportion of observations spent on any behavioural element except lying).

Significant interaction effects were further analysed using post hoc tests with Bonferroni adjustments for multiple comparisons. For brevity, only main effects and significant interaction effects are reported in the text. Data are presented as means ± SEM. Pigs from barren and enriched housing are referred to as B and E pigs, respectively.

RESULTS

Home pen behaviour was unaffected by sex, with the exception that gilts showed more (21.3 ± 1.2%) exploratory behaviour ($F_{(1,47)} = 6.1$, $p < 0.05$) than barrows (18.7 ± 1.6%). This was mainly due to a difference in nosing the floor, 12.2 ± 0.8% vs. 10.1 ± 0.9% of observations ($F_{(1,47)} = 8.1$ $p < 0.01$).

Inactivity

Inactivity was affected by housing environment ($F_{(1,7)} = 50.1$, $p < 0.01$; Fig 6.1) and did not change with age. B pigs displayed both more lying with open ($F_{(1,7)} = 34.8$, $p < 0.001$) and closed eyes ($F_{(1,7)} = 5.7$, $p < 0.05$) than E pigs (Table 6.2). HR pigs tended to be less active than LR pigs ($F_{(1,47)} = 2.8$, $p = 0.10$; Fig 6.1). Inactivity was not affected by the interaction between housing and Backtest classification ($p = 0.94$).
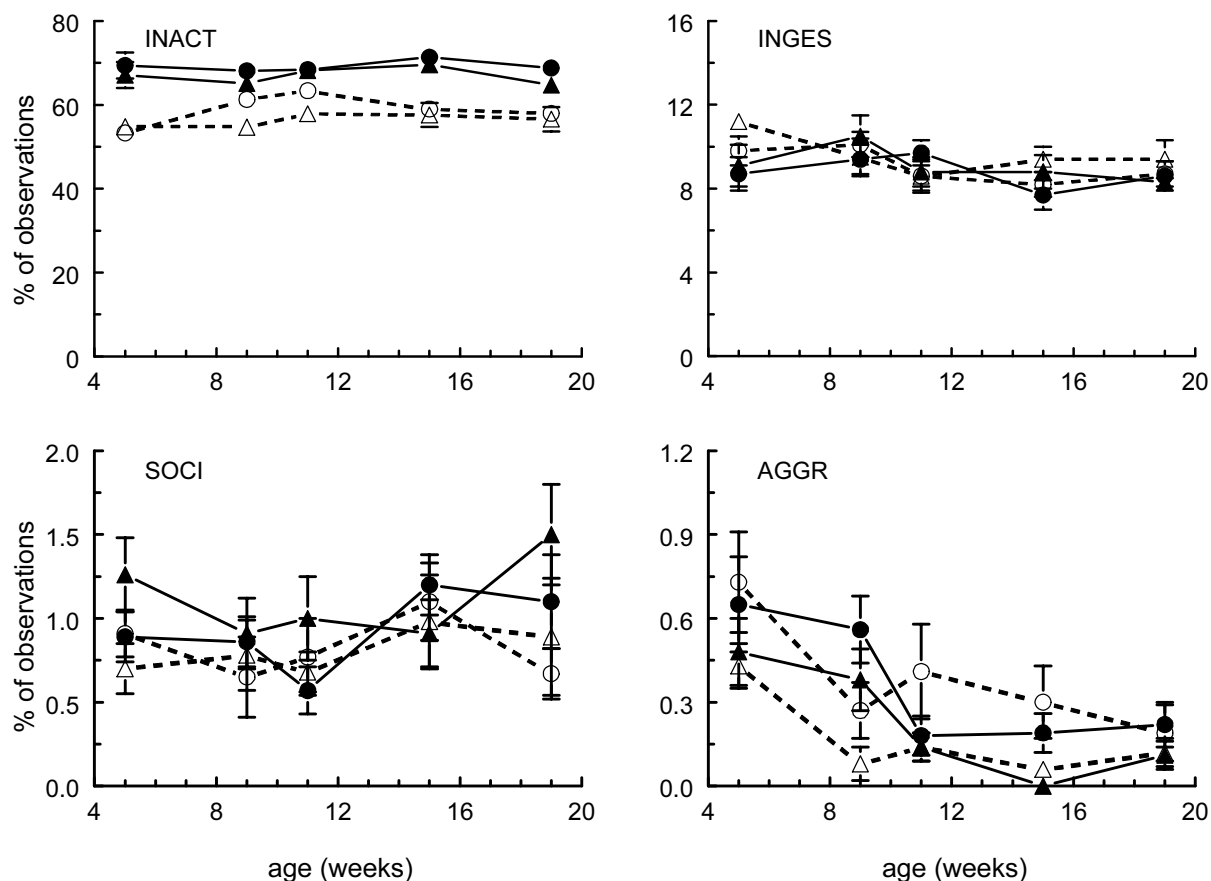
Fig. 6.1. Percentages of time spent on inactive behaviour (INACT), ingestive behaviour and elimination (INGES), social (SOCI) and aggressive behaviour (AGGR) of HR (circles) and LR (triangles) pigs in barren (solid line, closed symbols) and enriched housing (dotted line, open symbols). Note: different y-axis scaling for INACT, INGES, SOCI and AGGR.

Sitting, standing and locomotion

Sitting gradually increased with age, ($F(4,222) = 34.7$ p < 0.001), from 0.4 ± 0.1% of observations in week 5 to 2.0 ± 0.2% in week 19. Standing decreased ($F(4,222 = 28.1$, p < 0.001) from week 5 (2.9 ± 0.2%) to week 19 (1.3 ± 0.2%). Locomotion also decreased ($F(4,222) = 24.2$, p < 0.001), from week 5 (2.1 ± 0.2%) to week 19 (0.9 ± 0.1%). Proportions of time spent on sitting, standing or locomotion were not affected by Backtest classification or housing (Table 6.2).

Table 6.2. Behaviour (average of all observations) of HR and LR pigs in barren or enriched housing.

Behaviour (% of observed time)	Barren housing		Enriched housing		Effects[1]		
	HR	LR	HR	LR	H	B	HB
Inactive	69.2 ± 1.7^a	66.9 ± 1.6^a	58.8 ± 1.4^b	56.5 ± 1.5^b	**	+	ns
Lying closed eyes	47.7 ± 1.8^a	46.3 ± 1.4^{ab}	43.8 ± 1.3^{ab}	42.2 ± 1.7^b	*	ns	ns
Lying open eyes	21.5 ± 0.5^a	20.5 ± 0.5^a	15.0 ± 1.0^b	14.3 ± 0.8^b	***	ns	ns
Sitting	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.1	ns	ns	ns
Standing	2.4 ± 0.3	2.4 ± 0.2	1.5 ± 0.2	2.7 ± 0.7	ns	ns	ns
Locomotion	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	ns	ns	ns
Ingestive behaviour	8.8 ± 0.4	9.1 ± 0.4	9.1 ± 0.3	9.6 ± 0.4	ns	ns	ns
Comfort behaviour	0.12 ± 0.02^a	0.17 ± 0.07^a	0.23 ± 0.04^b	0.29 ± 0.04^b	***	ns	ns
Social behaviour	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	+	ns	ns
Aggressive behaviour	0.36 ± 0.05^a	0.22 ± 0.04^b	0.38 ± 0.07^a	0.17 ± 0.03^b	ns	**	ns
Explorative behaviour	14.2 ± 1.0^a	15.4 ± 1.3^a	26.0 ± 1.3^b	26.3 ± 1.2^b	***	ns	ns
Nosing floor	7.8 ± 0.7^a	8.3 ± 0.9^a	15.1 ± 1.0^b	14.9 ± 0.9^b	***	ns	ns
Scraping floor	0.01 ± 0.01^a	0.06 ± 0.06^a	0.17 ± 0.03^b	0.26 ± 0.05^b	***	ns	ns
Nosing fixtures	3.3 ± 0.4	3.7 ± 0.4	2.6 ± 0.2	2.9 ± 0.3	ns	ns	ns
Manipulating fixtures	0.28 ± 0.04^a	0.27 ± 0.05^a	0.14 ± 0.03^b	0.09 ± 0.03^b	**	ns	ns
Chewing	2.8 ± 0.3^a	3.0 ± 0.3^a	7.9 ± 0.4^b	8.1 ± 0.4^b	***	ns	ns
Manipulative behaviour	1.19 ± 0.20^a	2.14 ± 0.47^b	0.31 ± 0.06^c	0.45 ± 0.10^c	***	*	+
Belly nosing	0.10 ± 0.05^a	0.56 ± 0.38^b	0.07 ± 0.03^a	0.06 ± 0.02^a	ns	ns	ns
Manipulating ears	0.38 ± 0.05^a	0.38 ± 0.07^a	0.09 ± 0.02^b	0.17 ± 0.08^b	**	ns	ns
Manipulating tails[2]	0.08 ± 0.04	0.06 ± 0.02	0.00	0.00	-	-	-
Manipulating other	0.63 ± 0.10^a	1.13 ± 0.13^b	0.15 ± 0.05^c	0.21 ± 0.04^c	***	**	*
Play behaviour	0.10 ± 0.02^a	0.11 ± 0.02^a	0.37 ± 0.06^b	0.52 ± 0.07^c	**	*	+
Gambolling	0.08 ± 0.02^a	0.08 ± 0.02^a	0.17 ± 0.04^{ab}	0.23 ± 0.04^b	*	ns	ns
Pivoting	0.02 ± 0.01^a	0.01 ± 0.01^a	0.05 ± 0.02^{ab}	0.09 ± 0.02^b	**	ns	+
Rolling	0.01 ± 0.01^a	0.03 ± 0.01^a	0.13 ± 0.03^b	0.13 ± 0.02^b	***	ns	ns
Shaking object[2]	0.01 ± 0.01	0.00	0.02 ± 0.1	0.06 ± 0.02	-	-	-

Means with different superscript letters differ significantly ($p < 0.05$). Effects of and interactions with observation week are described in the text. [1]Significance of effects of housing (H), Backtest classification (B) and their interaction (HB) is indicated: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; +$p < 0.10$; ns = non-significant. [2]No statistical analysis.

Ingestive behaviour and comfort behaviour

The proportion of time spent on ingestive behaviour and elimination (sum of eating, drinking and eliminating) gradually decreased with age (week-effect: $F(4,222) = 2.6$, $p < 0.05$; Fig. 6.1) and was unaffected by housing or Backtest classification (Table 6.2). E pigs showed more comfort behaviour than B pigs ($F(1,7) = 45.1$, $p < 0.001$).

Social behaviour

Proportions of time spent on social behaviour (sum of nosing head and nosing body) were unaffected by housing, Backtest classification or week (Fig. 6.1; Table 6.2).

Aggressive behaviour

Aggressive behaviour consisted of head knocking (56% of total observations on aggressive behaviour), biting (13%) and fighting (30%). Time spent on aggressive behaviour was affected by week ($F(4,222) = 15.1$, $p < 0.001$), with highest levels in week 5, and by the housing x week interaction ($F(4,222) = 3.2$, $p < 0.05$): in week 9, B pigs displayed more aggression than E pigs (Fig. 6.1). HR pigs showed, irrespective of housing environment, more aggressive behaviour than LR pigs ($F(1,47) = 10.4$, $p < 0.01$; Table 6.2). Analysis per behavioural element showed that HR pigs displayed more head knocking ($F(1,47) = 17.1$, $p < 0.001$) and biting ($F(1,47) = 5.7$, $p < 0.05$) than LR pigs, whereas the two types of pig did not differ in time spent fighting ($p = 0.84$. Data not shown).

Exploratory behaviour

Pigs spent a substantial proportion of their time on exploratory behaviour (see Table 6.2). Total time spent on exploration (sum of nosing floor, scraping floor, nosing fixtures, manipulating fixtures and chewing) was affected by housing ($F(1,7) = 83.0$, $p < 0.001$) and increased with age in B pigs mainly (week effect: $F(4,222) = 3.8$, $p < 0.01$; housing x week effect: $F(4,222) = 2.5$; $p < 0.05$; Fig. 6.2). Especially chewing increased with age ($F(4,222) = 49.3$, $p < 0.001$) in a housing-dependent manner (housing x week interaction, $F(4,222) = 6.3$, $p < 0.001$; Fig. 6.2). Analysis per behavioural element revealed that E pigs displayed more nosing floor ($F(1,7) = 73.8$, $p < 0.001$), scraping floor ($F(1,7) = 22.4$, $p < 0.01$) and chewing than B pigs ($F(1,7) = 95.3$, $p < 0.001$), whereas B pigs showed more manipulating fixtures ($F(1,7) = 14.1$, $p < 0.01$). Housing did not affect the time spent on nosing fixtures ($p = 0.18$; Table 6.2). Total time spent on exploratory behaviour was not affected by Backtest classification (Table 6.2). Nosing floor was affected by the interaction between Backtest classification and week ($F(4,222) = 2.5$, $p < 0.05$). HR pigs showed more nosing floor in week 5 than in the subsequent weeks of age, whereas in LR pigs time spent on this behaviour was not affected by week (data not shown).
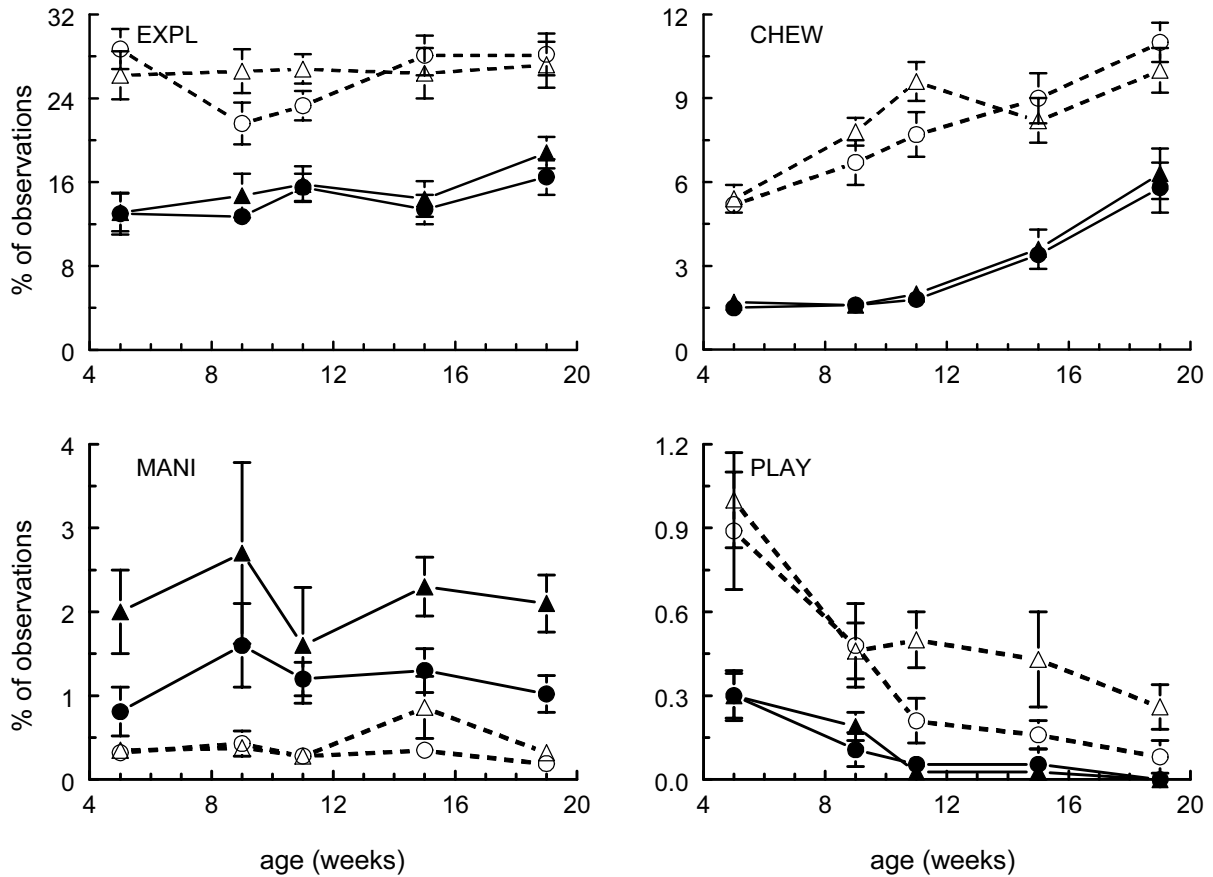
Fig. 6.2. Percentages of time spent on total exploratory behaviour (EXPL), chewing (CHEW), manipulative (MANI) and play behaviour (PLAY) of HR (circles) and LR (triangles) pigs in barren (solid line, closed symbols) and enriched housing (dotted line, open symbols). Note: different y-axis scaling for EXPL, CHEW, MANI and PLAY.

Manipulative behaviour

Manipulative oral behaviour directed at pen mates consisted of belly nosing (19% of total observations on manipulative behaviour), ear biting (24%), tail biting (4%) and manipulating other parts (52%). Behaviours scored as 'manipulating other parts' were mainly nibbling or chewing a pen mate's belly and paw chewing. Time spent on manipulative behaviour was unaffected by age. However, the different elements in this behavioural category showed different courses in time. Belly nosing peaked at 5 and 9 weeks of age and thereafter sharply decreased (week-effect: $F(4,222) = 2.3$, $p < 0.10$), whereas ear biting and manipulating other parts increased with age (week effect: $F(4,222) = 4.8$ and 4.3, respectively, $p < 0.01$); data not shown. Tail biting was scored only rarely and never in the enriched pens. Proportion of time spent on manipulative behaviour was affected by housing ($F(1,7) = 67.2$, $p < 0.001$) and Backtest classification ($F(1,47) = 5.8$, $p < 0.05$), and tended to be affected by the housing x

Backtest classification interaction (F(4,222) = 3.0, p < 0.10). LR pigs, and mainly those in barren housing, showed more manipulative behaviour than HR pigs (Fig. 6.2 and Table 6.2).

Play behaviour

Play behaviour consisted mainly of gambolling (50% of total observations on play), which was usually displayed by more than one pig at the same time. Pivoting (15%), rolling (27%) and shaking object (8%) were scored less frequently. The different elements of play behaviour often occurred in sequence. Time spent on play behaviour decreased with week (F(4,222) = 19.4, p < 0.001, Fig. 6.2). Play behaviour was affected by Backtest classification (F1,47) = 6.0, p < 0.05) and housing (F(1,7) = 29.4, p < 0.001), and tended to be affected by the interaction between these two factors (F(1,47) = 3.9, p < 0.10). LR pigs displayed more play behaviour than HR pigs, and this was most obvious in enriched housing conditions from week 11 on (Fig. 6.2).

Time spent on various behaviours as a proportion of 'active time'

B and E pigs differed in activity. Because we could not be sure whether all pigs were observed to the same extent in their active periods, we re-analysed the data with all behaviours expressed as a proportion of 'active time' (instead of observation time). Effects of housing, Backtest classification and week were similar for all behavioural elements described (means and significance given in Table 6.3), with the exception that B pigs spent more of their active time on nosing fixtures (F(1,7) = 14.0, p < 0.01), ingestive behaviour (F(1,7) = 25.3, p < 0.01) and social behaviour (F(1,7) = 17.3, p < 0.001) than E pigs (Table 6.3).

Table 6.3. Behaviour as a percentage of active time of HR and LR pigs in barren or enriched housing.

Behaviour (% of active time)	Barren housing		Enriched housing		Effects[1]		
	HR	LR	HR	LR	H	B	HB
Sitting	4.1 ± 0.6	3.6 ± 0.5	3.0 ± 0.5	2.8 ± 0.3	ns	ns	ns
Standing	7.6 ± 0.6[y]	7.2 ± 0.6	3.7 ± 0.4[z]	6.2 ± 1.4	+	ns	ns
Locomotion	4.1 ± 0.4	3.6 ± 0.3	2.9 ± 0.3	3.1 ± 0.3	ns	ns	ns
Ingestive behaviour	29.5 ± 1.7[a]	28.1 ± 1.7[a]	22.3 ± 0.8[b]	22.1 ± 1.0[b]	**	ns	ns
Comfort behaviour	0.4 ± 0.1[a]	0.5 ± 0.2[a]	0.5 ± 0.1[ab]	0.7 ± 0.1[b]	*	ns	ns
Social behaviour	2.9 ± 0.3[ab]	3.4 ± 0.3[a]	2.1 ± 0.2[b]	1.8 ± 0.3[b]	**	ns	ns
Aggressive behaviour	1.2 ± 0.1[a]	0.7 ± 0.1[bc]	0.9 ± 0.2[ac]	0.4 ± 0.1[b]	ns	***	ns
Explorative behaviour	45.8 ± 1.5[a]	45.7 ± 2.3[a]	62.9 ± 1.3[b]	60.6 ± 1.8[b]	***	ns	ns
Nosing floor	24.9 ± 1.3[a]	24.3 ± 1.6[a]	36.4 ± 1.3[b]	34.3 ± 1.6[b]	***	ns	ns
Scraping floor	0.02 ± 0.02[a]	0.24 ± 0.24[a]	0.40 ± 0.07[b]	0.58 ± 0.10[b]	***	ns	ns
Nosing fixtures	10.5 ± 1.0[a]	11.0 ± 0.9[a]	6.4 ± 0.4[b]	6.6 ± 0.7[b]	**	ns	ns
Manipulating fixtures	0.94 ± 0.15[a]	0.79 ± 0.13[a]	0.33 ± 0.07[b]	0.20 ± 0.06[b]	***	ns	ns
Chewing	9.4 ± 1.0[a]	9.4 ± 1.1[a]	19.3 ± 0.8[b]	18.9 ± 0.9[b]	***	ns	ns
Manipulative behaviour	3.8 ± 0.6[a]	6.6 ± 1.4[b]	0.8 ± 0.2[c]	1.0 ± 0.2[c]	***	*	+
Belly nosing	0.33 ± 0.15[a]	1.77 ± 1.16[b]	0.17 ± 0.07[a]	0.12 ± 0.05[a]	ns	ns	ns
Manipulating ears	1.20 ± 0.15[a]	1.13 ± 0.20[a]	0.22 ± 0.06[b]	0.38 ± 0.17[b]	**	ns	ns
Manipulating tails[2]	0.26 ± 0.12	0.21 ± 0.07	0.00	0.00	-	-	-
Manipulating other	1.99 ± 0.28[a]	3.47 ± 0.40[b]	0.40 ± 0.12[c]	0.48 ± 0.08[c]	***	**	*
Play behaviour	0.36 ± 0.08[a]	0.34 ± 0.06[a]	0.87 ± 0.12[b]	1.18 ± 0.14[c]	**	*	+
Gambolling	0.27 ± 0.07[a]	0.24 ± 0.06[a]	0.40 ± 0.09[ab]	0.52 ± 0.09[b]	+	ns	ns
Pivoting	0.06 ± 0.03[a]	0.01 ± 0.01[a]	0.11 ± 0.04[ab]	0.21 ± 0.05[b]	*	ns	+
Rolling	0.02 ± 0.02[a]	0.09 ± 0.03[a]	0.30 ± 0.06[b]	0.30 ± 0.05[b]	***	ns	ns
Shaking object[2]	0.02 ± 0.02	0.00	0.05 ± 0.02	0.15 ± 0.05	-	-	-

Means with different superscript letters differ significantly (a,b,c: $p < 0.05$; y,z: $p < 0.10$). Effects of and interactions with observation week are described in the text. [1]Significance of effects of housing (H), Backtest classification (B) and their interaction (HB) is indicated: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; +$p < 0.10$; ns = non-significant. [2]No statistical analysis.

DISCUSSION

In the present study behaviour patterns of pigs with diverging Backtest responses early in life, high-resisting (HR) and low-resisting (LR), were studied from 5 to 19 weeks of age in two housing environments: barren and enriched with straw bedding. Our results demonstrate that individual coping or personality characteristics of pigs, as assessed in the Backtest, are also reflected in their home pen behaviour in different environments. HR pigs displayed more aggressive behaviour than LR pigs and tended to spend more time inactive, irrespective of housing environment. LR pigs showed more manipulative behaviours directed at pen mates than HR pigs, especially in barren housing conditions. LR pigs in enriched housing spent more time on play behaviour than HR pigs. The effects of housing on both manipulative and play behaviour were most obvious in LR pigs.

Effects of housing environment

The availability of straw strongly influenced behavioural activities of the experimental pigs. During observations, B pigs were less active than E pigs, in line with other studies describing a lower activity of pigs in barren housing conditions (Beattie et al. 1995; 2000; Fraser et al. 1991). Pigs in semi-natural conditions dedicate the greatest part of their active time to foraging and exploratory behaviours (Newberry and Wood-Gush 1988; Petersen 1994; Stolba and Wood-Gush 1989), such as nosing, grazing, rooting and chewing. Also in intensive husbandry pigs appear to be highly motivated to exhibit explorative activities, even when food is abundantly available (Fraser and Broom 1997; Van Putten and Dammers 1976; Wood-Gush and Vestergaard 1991). Indeed, in the present study, both B and E pigs spent most of their active time on nosing, rooting and chewing. Time spent chewing increased with age, even in the B pigs that were not provided with a suitable substrate and in which chewing probably reflected vacuum or sham chewing. B pigs showed more manipulation of pen fixtures than E pigs, whereas E pigs displayed more nosing floor and chewing. Total time spent on explorative activities was higher for E pigs than for B pigs (cf. Beattie et al. 2000; Kelly et al. 2000, Schouten 1986), presumably because the exploration of pen fixtures and bare floor was less satisfying than the manipulation of straw, as pigs appear to prefer 'chewable', 'destructible', 'rootable' and 'deformable' materials for their exploratory behaviour (Van de Weerd et al. 2003). B pigs did not restrict their explorative activities to inanimate materials, but, in comparison with E pigs, spent more time on manipulative oral behaviours directed at pen mates, such as the chewing, nibbling and sucking of ears, paws and tails (cf. Beattie et al. 1996; Fraser et al. 1991; Kelly et al. 2000; Schouten 1986). Although several factors may underlie the development and display of these manipulative behaviours, also classified as 'harmful social' or 'disturbed' behaviours, the restricted possibility to practise

normal exploratory behaviour is generally considered the most crucial one (Van Putten 1979; Van Putten and Dammers 1976; Wiepkema and Koolhaas 1987). Furthermore, the observation that pigs housed in barren environments explore novel environments and objects longer and more intensively than pigs from enriched housing is another indication that barren housed pigs possess an unsatisfied motivation for exploration, which may affect their welfare negatively (De Jong et al. 1998; Stolba and Wood-Gush 1980; Wood-Gush et al. 1990b).

Play behaviour sharply decreased from 5 to 9 weeks of age and thereafter gradually declined, consistent with studies on pigs in semi-natural environments noting a peak in play between 2 and 6 weeks of age (Newberry et al. 1988; Stolba and Wood-Gush 1989). Although pigs spent only a small proportion of time on playful activities, the effect of housing environment was evident: E pigs showed more than three times as much play behaviour as B pigs. The housing effect on play behaviour is similar to that of another study reporting a three-fold increase in playful activities in deep straw housing as compared with barren flatdeck pens of identical floor surface (Kelly et al. 2000). Most playful activities scored, such as gambolling and pivoting, were so-called individual and parallel locomotor play behaviours that appear not to be directly related to the presence of straw. The difference between B and E pigs in this type of play is remarkable in view of the fact that sizes of barren and enriched pens were identical. Play is known, however, to be reduced in adverse physical and environmental conditions and following severe or prolonged negative emotions (Donaldson et al. 2002; Martin and Caro 1985; Spinka et al. 2001). For this reason, a reduction in play behaviour as found in B pigs in the present study may indicate endangered health or welfare (cf. Buchenauer 1982; Fraser and Broom 1997; Lawrence 1987; Newberry et al. 1988). Reduced or altered play behaviour may also negatively affect the development of behaviour (e.g. decrease social skills and behavioural flexibility) and thus have consequences for adaptive capacity and welfare in the longer term (Donaldson et al. 2000; Fagen 1982; Spinka et al. 2001; Van den Berg et al. 1999).

Time spent on social behaviour and ingestive behaviour were not affected by housing environment, consistent with other studies (Beattie et al. 1996; Schouten 1986). Housing had only a minor effect on aggressive behaviour. Although aggression is often reduced in environments that provide both rooting material and extra space (Beattie et al. 2000; De Jonge et al. 1996) as compared with barren environments, the availability of straw per se does not seem to affect the occurrence of aggression much (this study; Fraser et al. 1991, Schouten 1986).

Effects of Backtest classification

Based on the positive correlation between resistance in the Backtest and lean meat percentage at slaughter in ad libitum fed pigs, it has been suggested that HR pigs have a

higher basic activity level than LR pigs (Van Erp-Van der Kooij et al. 2000; 2003). We found, however, no evidence for a higher basal activity level of HR pigs. Quite the contrary, HR pigs tended to be less active than LR pigs in both housing environments. The difference between HR and LR pigs in activity induced by acute stress, i.e. an active vs. passive behavioural response in the Backtest and restraint test (Bolhuis and Schouten 2002), is thus not accompanied by or attributable to differences in basic activity.

HR pigs displayed more aggressive behaviour than LR pigs in all observation weeks. In other studies, HR pigs have been described to behave more aggressively in confrontations with unfamiliar pigs (chapter 3; Hessing et al. 1994c; Ruis et al. 2002). A novel finding from the present study is that HR pigs also have a higher propensity to display aggressive behaviour than LR pigs in stable social conditions, as in the present study groups were formed at 4 weeks of age and thereafter remained unchanged. Aggressive behaviour in stable social groups may occur for a variety of reasons, such as, for instance, attempts to change the rank order. The observed aggressive activities in the present study did, however, not appear to be associated with dominance fights, as most of the aggressive acts scored were unilateral (series of) head knocks and bites rather than mutual fighting. Furthermore, HR and LR did not differ in time spent on fighting, but only in the display of head knocking and biting. Aggression can, however, also be elicited by discomfort, irritability, annoyance or frustration (Arnone and Dantzer 1980; Hagelsø Giersing and Studnitz 1996; Ruis et al. 2002). Further research is necessary to elucidate whether HR pigs are more likely to take negative emotions out on pen mates.

LR pigs showed more manipulative oral behaviours directed at pen mates than HR pigs, especially in barren housing. As argued above, the frustration of normal exploratory behaviour appears to be a crucial factor in the development of these injurious behaviours, which are therefore generally considered to be redirected exploratory behaviours. The difference in the amount of manipulative behaviour between the two types of pig might indicate that LR pigs either react differently, or more intensively to the lack of exploratory stimuli in their housing environment than HR pigs. LR and HR pigs did not differ in time spent on exploratory behaviour in the present study, though. Results from several other studies suggest, however, that the two types of pig might differ in the thoroughness of their explorative behaviour. For instance, LR pigs spent more time on explorative behaviour than HR pigs when transferred to a novel environment individually (Ruis et al. 2001) or in pairs (Bolhuis, unpublished results), and they explored a novel object more slowly and thoroughly than HR pigs (Hessing et al. 1994a). The possibility that LR pigs might have a higher motivation to display explorative behaviour than HR pigs, and therefore show more manipulative behaviour directed at pen mates when no adequate substrate is available, merits further research.

LR pigs spent more time on playful activities than HR pigs, particularly in enriched housing. This difference is remarkable as three HR and three LR pigs were housed together in a pen and the performance of play behaviour appeared to be socially facilitated. Individual differences in the amount of play behaviour have, to our knowledge, not been reported before in pigs. We can therefore only speculate about the significance of these individual differences. Although there is no general agreement concerning the exact function(s) and effects of play behaviour (Bekoff and Byers 1981; Martin and Caro 1985; Smith 1982), most studies suggest that play contributes to the development of adaptive emotional, social and motor skills used in interacting with the physical and social environment (Fagen 1982; Spinka et al. 2001). Play experience may thus lead to enhanced coping ability and behavioural flexibility (Donaldson et al. 2000; Fagen 1982). This is an interesting point since LR pigs were reported to react more flexibly in changing situations than HR pigs, both at a young age in social (chapter 3) and non-social challenges (Bolhuis et al. 2004) and as adults (Geverink et al. 2004a). The observed differences in play behaviour could also reflect variation in behavioural development.

It has been suggested (Hessing et al. 1994a; Ruis et al. 2000) that LR pigs are predisposed to prefer a so-called passive or reactive coping style, whereas HR pigs are most likely to adopt a (pro)active coping style. The differences found between HR and LR pigs in home pen behaviour appear to reflect different coping styles, as level of aggressiveness, explorative strategy and behavioural flexibility are important distinguishing characteristics of individuals with diverging coping styles in other species (Koolhaas et al. 2001; Verbeek et al. 1994; 1996).

An enriched housing environment, i.e. availability of straw bedding, appeared to improve welfare as play behaviour increased and manipulative oral behaviour directed at pen mates decreased as compared with barren housing conditions. Notably, the effects of housing on both manipulative behaviour and play behaviour were most obvious in LR pigs. Recently we found that the effects of barren vs. straw-enriched housing on humoral immune reactivity (Bolhuis et al. 2003) and the behavioural reaction to a novel object in a familiar maze task (chapter 3) were also much larger for LR pigs than for HR pigs. Further research will have to elucidate whether LR pigs are more responsive than HR pigs to housing conditions in general, or whether they are more susceptible to the specific effects of an exploratory substrate in their home environment.

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Effects of rearing and housing environment on pigs with different coping characteristics

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ABSTRACT

The availability of straw bedding may profoundly affect behaviour and welfare of pigs. Apart from their actual housing environment, also the conditions present in early life and individual characteristics may influence behaviour and welfare of pigs. The present study investigated the relative importance of the housing environment during rearing and the actual housing environment on behaviour during the finishing phase, pathological lesions of the heart and stomach wall and weight gain in pigs with diverging coping characteristics. Pigs were reared either without a rooting substrate (barren, B) or in identical pens enriched with straw bedding (enriched, E). During the suckling period piglets were subjected to the Backtest. The Backtest classification of pigs is, to a certain extent, predictive of their coping style. Each piglet was restrained in supine position for 1 min and its resistance (i.e. the number of escape attempts) was scored. Sixty high-resisting (HR) and 60 low-resisting (LR) pigs were selected. Hhalf of these pigs were from barren and the other half from rearing environments. They were housed in groups of 6 (3 HR and 3 LR) after weaning. At 10 weeks of age, environmental conditions (B or E) were switched for half of the pens. Behaviour patterns of pigs during the finishing phase were largely determined by the actual presence or absence of straw bedding. Pigs that changed from enriched to barren pens (EB pigs), however, showed an increased inactivity beyond the inactivity levels of pigs with a barren rearing history (BB pigs). The impact of rearing history on later behaviour was larger for LR than for HR pigs. The availability of straw reduced the occurrence of gastric lesions in LR, but not in HR pigs. Feed intake tended to be lower in EB pens than in EE pens, but this was reflected in the growth rate of HR pigs only: HR-EE pigs showed a higher weight gain than HR-EB pigs. In conclusion, influence of rearing history and actual housing on home pen behaviour, occurrence of gastric lesions and weight gain depended on coping characteristics (LR or HR) of the pigs under study. When investigating the effects of environmental factors on pigs, the modulating influence of individual characteristics should be considered.

INTRODUCTION

In intensive pig husbandry, pigs are predominantly housed in stimulus-poor environments in which the possibilities for expression of species-specific behaviours are limited (De Jonge et al. 1996; Schouten 1986). Because of a lack of suitable substrates, barren housing environments may in particular frustrate the performance of explorative activities like rooting and chewing, which are considered important elements in the behavioural repertoire of pigs (Fraser 1988; Fraser and Broom 1997; Van Putten and Dammers 1976; Wood-Gush and Vestergaard 1991). This frustration of normal exploratory behaviour has been suggested to lead to the development of several maladaptive oral behaviours, such as nibbling, biting and rooting the tails, ears and other parts of pen mates, and vacuum chewing (Schmidt 1982; Schouten 1986; Van Putten and Dammers 1976). Indeed, there is ample evidence that the

availability of a rooting substrate reduces abnormal oral behaviours in growing-finishing pigs, and, as indicated by both behavioural and physiological data, improves their welfare substantially (Beattie et al. 1996; 2000; De Jong et al. 2000; Fraser et al. 1991; Kelly et al. 2000; Schouten 1986).

However, apart from the actual housing environments in which pigs are kept, also the conditions present in early life may have profound effects on later behaviour and welfare. For instance, space allowance during the socialisation period may crucially affect the development of social skills needed in later life (Lammers and Schouten 1985a; 1985b; Schouten 1986). Studies investigating specifically the immediate and long-lasting effects of exposure to a rooting substrate on behaviour and welfare of pigs are rather scarce and have yielded different results (e.g. Day et al. 2002a; Ruiterkamp 1985; Schouten 1986). Thus, it was reported that it is mainly the actual presence or absence of a rooting substrate that determines the behavioural activities of pigs (Day et al. 2002a; Schouten 1986), whereas others concluded that the provision of a rooting substrate (straw) in early life affected the behaviour and adaptive capacity of pigs at a later age (Beattie et al. 1995; Ruiterkamp 1985). Recently we have shown that the impact of environmental conditions on behaviour differs for pigs with diverging responses in a so-called Backtest (chapter 6). In the Backtest, piglets are manually restrained in supine position during the suckling period and characterized on the basis of their behavioural response (Hessing et al 1993). Pigs adopting one of the extreme reaction patterns in the Backtest, i.e. high resisting (HR) and low-resisting (LR) pigs, have been reported to differ in their behavioural and neuroendocrine responses to a variety of challenging situations (e.g. Bolhuis 2003; Bolhuis et al. 2004; Hessing et al. 1994a; Geverink et al. 2003; 2004a; Karman 2003; Ruis et al. 2000). The Backtest response of piglets is therefore thought to reveal part of a more general profile of responding, i.e. 'coping style' or 'personality' (Hessing et al. 1994a; Ruis et al. 2000).

Backtest characteristics of pigs were also reflected in the effect of housing conditions on the development of their behaviour (chapter 6; Geverink et al. 2003). In pens enriched with straw bedding, LR pigs spent more time on play behaviour than HR individuals. When housed in pens without a suitable substrate for explorative activities, LR pigs showed more oral manipulation of pen mates than HR pigs, suggesting that the two types of pig differ in the ability or manner of adapting to barren housing conditions (chapter 6). Whether these effects are a result of the actual availability of straw bedding or arise early in life is unknown. The present study investigated the relative importance of rearing history and actual housing in influencing behaviour and welfare during the finishing phase of pigs with diverging coping characteristics. For that purpose, pigs with diverging Backtest responses (LR and HR pigs) experienced either a straw-enriched or a barren environment during their first 10 weeks of life, after which environmental conditions were switched for half of the experimental animals,

and behaviour during the finishing phase, pathological lesions of the stomach and heart, and weight gain were assessed.

METHODS

The established principles of laboratory animal use and care were followed as well as the Dutch law on animal experiments, which complies with the ETS123 (council of Europe 1985) and the 86/609/EEC directive. The Institutional Animal Care and Use Committee of the Wageningen University approved the experiment.

Subjects

The experiment was carried out in two successive batches, using 120 Great Yorkshire x (Great Yorkshire x Dutch Landrace) pigs in total that were selected on the basis of their Backtest response (see below) and body weight before weaning. Half of these pigs were also part of another study investigating the effects of rearing environment on the development of behaviour of growing-finishing pigs from 5 to 19 weeks of age (chapter 6).

Per batch, subjects were bred by 21 sows that were inseminated at approximately the same date. During gestation, sows were housed in groups of three or four individuals at the experimental farm "De Haar" of the Wageningen University. One week prior to the expected day of farrowing, sows were randomly assigned to either substrate-impoverished Danish farrowing pens (2.2 x 3.8 m) with a 65% solid and 35% slatted floor (barren environment) or to identical, adjacent pens in which the solid part of the floor was covered with unchopped straw, approximately 4 kg per pen (enriched environment). Sows farrowed within five days per batch. On the day of birth, piglets received an iron injection and an ear tattoo. Tails of the piglets were docked and male piglets were castrated following standard pig husbandry procedures at 3 days of age.

Pigs were subjected to the Backtest (adapted from Hessing et al. 1993) at 10 and 17 days of age. The test procedure is described in detail elsewhere (Bolhuis et al. 2003). Briefly, each pig was restrained on its back by placing the experimenter's right hand over the throat and the left hand loosely on the hind legs. Classification of pigs as 'high resisting' (HR) or 'low resisting' (LR) was based on the number of escape attempts (i.e. bouts of struggling with at least the hind legs) they displayed during 60 seconds (see Hessing et al. 1994c). Sixty HR and 60 LR pigs with similar body weights (7.3 ± 0.07 kg on the day before weaning) were selected for the present experiment. For both classifications the female to castrated male ratio was 2:1. Half of the selected pigs were from barren and the other half from enriched farrowing pens.

Post-weaning housing environment

Pigs were weaned, mixed and relocated at 29.0 ± 0.14 days of age. The 60 pigs from barren farrowing pens were placed in barren pens (7 m^2) with a 65% solid and 35% slatted floor (B pens). The pigs from enriched farrowing pens were placed in similar, adjacent pens enriched with deep straw bedding (unchopped straw, approximately 4 kg per pen) where the slatted part of the floor was covered with a rubber mat (E pens). Per pen, six pigs, three HR and three LR pigs (two gilts and one barrow of each type) were grouped; these pigs were not familiar with each other.

During the whole experiment, water was continuously available from two drinking nipples and pigs had ad libitum access to normal commercial feeds (9.7 MJ NEv/kg and 16.6% CP from weaning until 11 weeks of age; 9.2 MJ NEv and 16.8% CP from 11 to 16 weeks of age; 9.2 MJ NEv and 14.7%nCP from 16 weeks until slaughter). Lights were on between 7.00 and 19.00 h. Temperature and ventilation within the building were thermostatically controlled. Temperature was set at 25°C when the weaned pigs entered the building, and thereafter gradually lowered (approximately 1°C per week) until a set temperature of at least 18°C during the finishing period was reached. In the morning, between 8.00 and 9.00 h, all pens were cleaned out. Soiled straw was removed daily from the E pens and replaced with fresh unchopped straw (replacement approximately 3.5 kg per pen per day).

Environmental change

At 10 weeks of age, housing conditions were changed for half of the pens during daily cleaning. In five B pens, the slatted part of the pen was covered with a rubber mat and straw bedding was provided (BE treatment) and in five E pens the straw bedding and rubber mat were removed (EB treatment). In the other five B and five E pens, housing conditions remained unchanged (BB and EE treatment, respectively).

Observations

At 15 and 19 weeks of age behaviour of individual pigs was recorded for eight hours per week using 2-min instantaneous scan sampling. Observations were carried out on two days per week and started at 9.00, 11.00, 13.30 and 15.30 h. Individual pigs could be identified by an ear tag and a number sprayed on their backs with standard colour stock marker. The Observer® software package (Noldus Information Technology b.v., Wageningen, The Netherlands) installed on a Psion Organiser II LZ64 was used for behavioural recordings. The ethogram is given in Table 7.1.

Table 7.1. Ethogram.

Behaviour	Description
Inactive	
Lying with closed eyes	Lying on side or belly with eyes closed
Lying with open eyes	Lying with eyes open, without performing any other described behaviour
Sitting	Sitting or kneeling without performing any other described behaviour
Standing	Standing without performing any other described behaviour
Locomotion	Walking without performing any other described behaviour
Ingestive behaviour	
Eating	Eating from food trough or chewing feed
Drinking	Drinking from water nipple
Eliminating	Defecating or urinating
Comfort behaviour	Rubbing body against objects or pen mates, scratching body with hind leg or stretching (part of) body
Social behaviour	
Nosing head	Touching or sniffing any part of the head of a pen mate
Nosing body	Touching or sniffing any part of the body of a pen mate except the head
Aggressive behaviour	
Head knocking	Ramming or pushing pen mate with the head, without biting
Biting	Ramming or pushing pen mate with head, with biting
Fighting	Mutual pushing or ramming, or lifting pen mate
Explorative behaviour	
Nosing floor	Sniffing, touching or rooting (substrate on) floor without exerting much force
Rooting floor	Moving the snout over the floor while rooting disc exerts some force
Exploring fixtures	Sniffing, touching, chewing or rooting part of the pen above floor level
Chewing	Chewing particles other than feed (e.g. straw, faeces) or vacuum chewing
Manipulative behaviour	
Belly nosing	Rubbing belly of a pen mate with up and down movements of the snout
Manipulating ears	Nibbling, sucking or chewing an ear of a pen mate
Manipulating tails	Nibbling, sucking or chewing the tail of a pen mate
Manipulating other	Nibbling, sucking or chewing any part of a pen mate, except tail or ears
Play behaviour	
Gambolling	Running across the pen, occasionally accompanied by nudging pen mate gently
Pivoting	Jumping and turning around the body axis
Rolling	Lying on back and moving from side to side on the floor
Shaking object	Shaking of head while holding material (e.g. straw) that protrudes from mouth

Weight gain

Pigs were weighed several times during the experiment. Body weights on the day before weaning, the day before the change of environment and the day before slaughter were used for calculating average daily (live) weight gains (ADWG). Feed intake was calculated per pen by registering the amount of feed given and weighing residual feed at several time points. Because two pigs died during the experiment, feed intake was expressed per pig per day.

Pathological examination

Pigs were slaughtered on two successive days per batch, 126 or 127 days after weaning (155.4 ± 0.16 days of age). Groups of pen mates were transported in separate compartments of a lorry to the abattoir where they were placed in separate pens. Heart, liver and stomach were macroscopically examined after slaughter. The judging of pathological changes was done without knowledge of the previous treatment of the pigs. The heart was checked for pericarditis and the occurrence of subepicardial and subendocardial lesions, inflammations and scar tissue. In pigs, gastric lesions occur most commonly in the non-glandular pars oesophagea, a small area of the stomach near the oesophagus lined by squamous epithelium (Pfeiffer 1992). Stomach wall damage at this area was scored using a 6-point scale as described in detail elsewhere (Hessing et al. 1992). Briefly, 0 = normal pars oesophagea, 1 = minor hyperkeratosis, i.e. less than 50% of the surface; 2 = severe hyperkeratosis, i.e. more than 50% of the surface; 3 = hyperkeratosis and small lesions, less than 5 and shorter than 2.5 cm; 4 = hyperkeratosis and more than 4 lesions or lesion(s) longer than 2.5 cm; 5 = hyperkeratosis and more than 10 lesions, lesion(s) longer than 5 cm or ulcer with or without bleeding or occlusion (stenose) of the oesophagus into the stomach.

Data analysis

SAS (Statistical Analysis System Institute Inc. 1989) versions 6.12 and 8.0 were used for statistical calculations. Prior to analysis, proportions of time spent on various behaviours were averaged per animal per week. Skewed distributions of proportion were angularly transformed to obtain homogeneity of variances (Sachs 1984). Effects of and interactions between the factors Backtest classification (LR or HR), pre-change rearing environment (barren or enriched), post-change housing environment (barren or enriched) and week (15 or 19) on behaviour were analysed with a mixed linear model which included batch, sex and the random effects of pen and animal. Only few interactions between week and the other factors were found (these are reported in the results section); therefore, behavioural data are presented as means over the two observation weeks.

Effects of Backtest classification (HR and LR), rearing environment and post-change housing on weight gain and stomach score were analysed with a mixed linear model in which batch,

sex and random effects of pen were included. Effects of rearing environment and post-change housing on feed intake and feed-to-gain ratio were analysed at pen level with a mixed linear model. A generalised linear mixed model with a logit link and binomial distribution was used to analyse the effects of Backtest classification, rearing environment, housing and sex on the occurrence of gastric lesions (stomach wall damage score > 2).

Significant interaction effects were further analysed using post-hoc tests with Bonferroni adjustments for multiple comparisons. Data are presented as means ± SEM. In the results section the pre-change and post-change housing environment will be referred to as 'rearing environment' and '(actual) housing', respectively.

RESULTS

Effects of sex on behaviour

Inactivity was higher ($F_{(1,94)}$ = 7.8, $p < 0.01$) for barrows (67.2 ± 1.4% of observations) than for gilts (63.2 ± 1.3%). Gilts showed more exploratory behaviour ($F_{(1,94)}$ = 18.0, $p < 0.001$) than barrows (22.2 ± 1.1% vs. 18.1 ± 1.3%, respectively). This was mainly due to differences in nosing the floor (11.0 ± 0.6% for gilts vs. 7.9 ± 0.7% for barrows; $F_{(1,94)}$ = 30.4, $p < 0.001$) and exploring fixtures (3.4 ± 0.2 vs. 2.9 ± 0.3%; $F_{(1,94)}$ = 4.6, $p < 0.05$). Barrows spent more time (9.2 ± 0.4%) on ingestive behaviour and elimination ($F_{(1,94)}$ = 5.2, $p < 0.05$) than gilts (8.1 ± 0.3%). No other effects of sex on behaviour were found.

Effects of rearing environment, housing and Backtest classification on behaviour

The main behavioural elements that were affected by rearing environment, actual housing or Backtest classification are shown in Table 7.2.

Inactivity. Inactivity was affected by actual housing ($F_{(1,15)}$ = 78.9, $p < 0.001$) and tended to be affected by the rearing environment x actual housing interaction ($F_{(1,15)}$ = 3.5, $p < 0.08$): EB pigs were less active than all other groups, including BB pigs. Lying with closed eyes was influenced by the rearing environment x actual housing interaction ($F_{(1,15)}$ = 5.9, $p < 0.05$). EB pigs spent more time lying with closed eyes (51.4 ± 1.6%) than all other groups (BB: 42.6 ± 1.4%; BE: 39.9 ± 1.6%; EE: 39.3 ± 1.7%). Lying with open eyes was affected by actual housing only ($F_{(1,15)}$ = 38.9, $p < 0.001$): barren housed pigs (BB and EB; 21.9 ± 0.8%) spent more time on this behaviour than pigs from enriched housing (BE and EE; 20.5 ± 7.5%).

HR pigs (21.9 ± 0.8%) showed more lying with open eyes than LR pigs (20.5 ± 7.5%), irrespective of their rearing or housing environment ($F_{(1,94)}$ = 4.4, $p < 0.05$). Backtest classification did not affect lying with closed eyes or total time spent inactive.

Table 7.2. Behaviour of HR and LR pigs from barren (B) and enriched (E) rearing and actual housing conditions.

| Behaviour | Barren (actual) housing | | | | Enriched (actual) housing | | | | Effects | | | | | | |
| | Barren rearing | | Enriched rearing | | Barren rearing | | Enriched rearing | | | | | | | | |
(% of obs)	HR BB	LR BB	HR EB	LR EB	HR BE	LR BE	HR EE	LR EE	R	H	RH	B	BR	BH	BRH
Inactivity	70.1 ± 2.1	67.1 ± 1.8	72.9 ± 1.5	75.0 ± 1.6	58.7 ± 1.8	57.1 ± 2.9	58.0 ± 2.1	57.1 ± 2.5	ns	***	+	ns	ns	ns	ns
Lying cl. eyes	43.3 ± 2.3	41.9 ± 1.8	50.0 ± 2.2	52.8 ± 2.2	39.9 ± 1.8	39.9 ± 2.8	38.9 ± 2.4	39.8 ± 2.6	*	**	*	ns	ns	ns	ns
Lying op. eyes	26.8 ± 0.9	25.2 ± 1.0	22.9 ± 1.7	22.2 ± 1.5	18.8 ± 1.1	17.3 ± 0.9	19.1 ± 1.7	17.3 ± 1.5	+	***	ns	*	ns	ns	ns
Aggression	0.20 ± 0.07	0.05 ± 0.02	0.19 ± 0.06	0.11 ± 0.06	0.38 ± 0.09	0.22 ± 0.05	0.24 ± 0.08	0.09 ± 0.04	ns	+	ns	**	ns	ns	ns
Exploration	15.0 ± 1.4	16.6 ± 1.4	13.3 ± 1.0	11.2 ± 1.0	27.3 ± 1.3	28.8 ± 2.2	28.2 ± 1.8	26.8 ± 1.9	ns	***	ns	ns	+	ns	ns
Nosing floor	6.8 ± 0.9	7.4 ± 0.8	5.2 ± 0.6	5.2 ± 0.7	13.2 ± 1.1	13.5 ± 1.1	14.7 ± 1.3	13.9 ± 1.2	ns	***	*	ns	ns	ns	ns
Rooting floor	0.11 ± 0.06	0.24 ± 0.14	0.08 ± 0.03	0.06 ± 0.02	0.35 ± 0.09	0.71 ± 0.15	0.44 ± 0.07	0.27 ± 0.08	+	***	ns	ns	**	ns	*
Expl. fixtures	3.5 ± 0.4	4.0 ± 0.5	3.3 ± 0.4	2.7 ± 0.3	3.1 ± 0.4	2.9 ± 0.3	3.0 ± 0.3	3.5 ± 0.6	ns	ns	ns	ns	ns	ns	ns
Chewing	4.6 ± 0.6	4.9 ± 0.7	4.7 ± 0.6	3.3 ± 0.5	10.6 ± 0.7	11.6 ± 1.4	10.0 ± 0.6	9.1 ± 0.7	ns	***	ns	ns	*	ns	ns
Manip. behaviour	1.2 ± 0.2	2.2 ± 0.2	1.0 ± 0.2	1.6 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	ns	***	ns	*	ns	+	*
Play behaviour	0.03 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.15 ± 0.05	0.12 ± 0.05	0.12 ± 0.03	0.35 ± 0.10	ns	**	ns	ns	*	ns	+

Significance of effects of rearing environment (R), actual housing (H), Backtest classification (B) and their interactions (RH, BR, BH and BRH) is indicated: ***p < 0.001; **p < 0.01; *p < 0.05; +p < 0.10; ns = non-significant. Effects of and interactions with week are described in the text.

Sitting, standing and locomotion. Sitting increased from 15 (1.5 ± 0.1% of observations) to 19 weeks of age (1.9 ± 0.2%; F(1,111) = 7.3, p < 0.01). Percentages of time spent sitting (1.5 ± 0.1%), standing (1.4 ± 0.1%) and locomotion (0.8 ± 0.05%) were unaffected by rearing environment, actual housing or Backtest classification.

Ingestive and comfort behaviour. Percentage of time spent on ingestive behaviour and elimination (8.5 ± 0.2%) was unaffected by rearing environment, actual housing or Backtest classification. Enriched housed pigs (EE and BE) showed more comfort behaviour (0.17 ± 0.03% vs. 0.07 ± 0.01% of observations) than pigs from barren actual housing (BB and EB; F(1,15) = 10.8, p < 0.01).

Social behaviour. Total time spent on social behaviour (0.91 ± 0.06%) was not affected by rearing environment, actual housing or Backtest classification.

Aggressive behaviour. HR pigs displayed more aggression than LR pigs (F(1,94) = 10.1, p < 0.01), irrespective of rearing or actual housing environment (Table 7.2). Pigs from enriched actual housing (0.23 ± 0.04%) tended (F(1,15) = 3.4, p < 0.10) to show more aggressive behaviour than pigs from barren actual housing (0.14 ± 0.03%).

Exploratory behaviour. Pigs spent the major part of their active time on exploration. Exploratory behaviour was affected by actual housing (E > B, F(1,15) = 116.6, p < 0.001) and tended to be affected by the rearing environment x Backtest classification interaction (F(1,94) = 3.3, p < 0.08). Post-hoc analysis revealed that LR pigs with a barren rearing history tended to spent more time on explorative behaviour than LR pigs from enriched rearing; this difference was most pronounced within barren actual housing (Table 7.2).

The rearing environment x actual housing interaction (F(1,15) = 6.0, p < 0.05) influenced nosing the floor. The time spent on this behaviour was similar for EE and BE pigs, whereas EB pigs showed less nosing the floor than BB pigs (p < 0.05).

Rooting the floor was affected by the rearing environment x actual housing x Backtest classification interaction (F(1,94) = 5.6, p < 0.05). LR pigs that had changed from barren to enriched housing (LR-BE) showed more rooting than the other pigs.

Chewing increased from 15 (6.2 ± 0.4%) to 19 weeks (8.5 ± 0.5%) of age (F(1,111) = 50.1, p < 0.001). Pigs from barren actual housing showed less chewing than enriched housed pigs (F(1,15) = 56.8, p < 0.001). Chewing was also affected by the rearing environment x Backtest classification interaction (F(1,94) = 4.1, p < 0.05): LR pigs from the barren rearing environment, and mainly the LR-BE pigs (Table 7.2), spent more time on chewing than LR pigs from enriched rearing conditions.

Time spent exploring fixtures (3.3 ± 0.1%) was not affected by rearing environment, actual housing or Backtest classification.

Manipulative behaviour. Manipulative oral behaviour directed at pen mates mainly consisted of ear biting (30% of total observations on manipulative behaviour) and

manipulating other parts, such as the paw or flank of a pen mate (58%), whereas belly nosing (8%) and tail biting (4%) were observed less frequently. Manipulative behaviour was affected by actual housing (B > E; $F_{(1,94)}$ = 71.1, $p < 0.001$), Backtest classification (LR > HR; $F_{(1,94)}$ = 6.4 $p < 0.05$), and the interaction between rearing environment, actual housing and Backtest classification ($F_{(1,94)}$ = 4.4 $p < 0.05$). HR-EB and HR-BB pigs did not differ in time spent on manipulative behaviour, whereas LR-BB pigs showed more manipulative behaviour than LR-EB pigs.

Play behaviour*. Play behaviour consisted of gambolling (28% of total observations on play), frisking (14%), rolling (39%) and shaking object (28%). Time spent on play was affected by actual housing ($F_{(1,15)}$ = 14.6, $p < 0.01$), the interaction between rearing environment and Backtest classification ($F_{(1,94)}$ = 5.2, $p < 0.05$) and tended to be influenced by the rearing environment x housing x Backtest classification interaction ($F_{(1,94)}$ = 3.4, $p < 0.07$). LR pigs from an enriched rearing environment, and in particular the LR-EE pigs, spent more time on play behaviour than the other groups.

Weight gain and feed intake

Rearing environment and Backtest classification did not affect average daily weight gain (ADWG) from weaning until the change of environment at 10 weeks of age (HR-B: 577.9 ± 16 g; HR-E: 590.2 ± 19 g; LR-B: 583.2 ± 18 g; LR-E: 561.2 ± 12 g). ADWG in this period was higher for barrows (600.2 ± 16) than for gilts (567.1 ± 10 g), $F_{(1,97)}$ = 4.1, $p < 0.05$. Feed intake until the change was not affected by rearing environment (B: 939.7 ± 58 g per pig daily; E: 900.9 ± 47 g).

ADWG from weaning to slaughter was affected by the interaction between rearing environment, actual housing and Backtest classification ($F_{(1,93)}$ = 4.5, $p < 0.05$) and ADWG from change to slaughter tended to be affected by this interaction ($F_{(1,93)}$ = 3.7, $p < 0.06$). Post-hoc analysis revealed that ADWG in both periods was higher for HR-EE pigs than for HR-EB pigs, whereas the other pigs showed intermediate weight gains (Table 7.3). Barrows showed a higher ADWG than gilts ($F_{(1,93)}$ = 20.4 and 21.2, $p < 0.001$ for the periods weaning–slaughter and change–slaughter, respectively; data not shown).

Feed intake from weaning until slaughter was affected by actual housing ($F_{(1,15)}$= 4.9, $p < 0.05$) and tended to be affected by the interaction between rearing environment and actual housing ($F_{(1,15)}$= 3.7, $p < 0.08$). Feed intake from the change of environment (10 weeks of age) until slaughter also tended to be affected by actual housing ($F_{(1,15)}$= 4.2, $p < 0.06$) and the interaction between rearing environment and actual housing ($F_{(1,15)}$= 3.3, $p < 0.10$). Feed intake in EE pens was higher than in EB pens, whereas feed intake in BB and BE pens did not differ from the other pens. Feed intake to weight gain ratios did not differ between treatments (Table 7.4).

Table 7.3. Weight gain and stomach wall damage of HR and LR pigs from barren (B) and enriched (E) rearing and actual housing conditions.

Variable	Barren (actual) housing				Enriched (actual) housing				Effects						
	Barren rearing		Enriched rearing		Barren rearing		Enriched rearing								
	HR BB	LR BB	HR EB	LR EB	HR BE	LR BE	HR EE	LR EE	R	H	RH	B	BR	BH	BRH
S weight (kg)	105.0 ± 2.3	103.1 ± 1.4	101.1 ± 2.3	104.4 ± 2.2	103.8 ± 2.7	104.9 ± 3.2	112.3 ± 2.3	105.7 ± 2.2	ns	ns	ns	ns	ns	ns	*
ADWG W-S (g)	772 ± 17	756 ± 12	744 ± 18	767 ± 18	764 ± 21	770 ± 25	8 31 ± 18	779 ± 17	ns	ns	ns	ns	+	ns	*
ADWG C-S (g)	887 ± 19	847 ± 13	844 ± 22	868 ± 25	855 ± 27	863 ± 27	933 ± 25	887 ± 21	ns	ns	ns	ns	ns	ns	+
Stomach score[1]	3.4 ± 0.4	3.4 ± 0.3	2.9 ± 0.4	3.1 ± 0.3	1.8 ± 0.4	0.8 ± 0.3	2.3 ± 0.4	1.7 ± 0.3	ns	***	*	ns	+	*	ns
Gastric lesions[2]	60.0	73.3	57.1	73.3	33.3	13.3	40.0	28.6	ns	**	ns	ns	+	*	ns

S = slaughter; W = weaning; C = change of environment at 10 weeks of age. [1]For description of stomach wall damage scores, see Method section; [2]Percentage of animals with stomach wall damage score > 2. Significance of effects of rearing environment (R), actual housing (H), Backtest classification (B) and their interactions is indicated: ***p < 0.001; **p < 0.01; *p < 0.05; +p < 0.10; ns = non-significant. Effects of and interactions with week are described in the text.

Table 7.4. Effects of rearing environment and housing conditions from 10 weeks of age on feed intake and feed-to-gain ratio.

Variable[1]	BB (n = 5)	EB (n = 5)	BE (n = 5)	EE (n = 5)
FI W-S per pig daily (kg)	1.78 ± 0.07^{ab}	1.72 ± 0.04^{a}	1.79 ± 0.07^{ab}	1.88 ± 0.05^{b}
FI C-S per pig daily (kg)	2.18 ± 0.07^{yz}	2.13 ± 0.04^{y}	2.19 ± 0.06^{yz}	2.33 ± 0.06^{z}
FI W-S / kg gain	2.34 ± 0.07	2.28 ± 0.06	2.34 ± 0.05	2.32 ± 0.06
FI C-S / kg gain	2.55 ± 0.09	2.51 ± 0.06	2.57 ± 0.05	2.54 ± 0.04

FI = feed intake; W = weaning; C = change of environment at 10 weeks of age; S = slaughter at 22 weeks of age; BB = barren rearing, barren actual housing; EB = enriched rearing, barren actual housing; BE = barren rearing, enriched actual housing; EE = enriched rearing, enriched actual housing. Within a row, means lacking a common superscript letter differ (a,b: $p < 0.05$; y,z: $p < 0.10$).
[1]Feed intake was measured and analysed at pen level.

Pathological examination

Pathological examination after slaughter revealed one pig (0.8%) with pericarditis. No liver alterations were found. Table 7.3 presents the average stomach wall damage scores for all treatment groups. About half of the pigs, 52.5%, had no (code 0, 14.4 %) or light damage (code 1 or 2; 38.1%) of the mucosal surface, whereas 47.5% of the pigs had severe damage including lesions (code 3, 4 or 5). The proportion of pigs with gastric lesions (code 3, 4 or 5) was affected by post-change housing ($p < 0.01$) and the interaction between housing and Backtest classification ($p < 0.05$). The proportion of LR pigs with severe lesions was significantly lower in enriched housing than in barren housing, whereas in HR pigs proportions were not significantly affected by housing environment (Fig. 7.1). Stomach wall damage scores were affected by actual housing ($F(1,15) = 29.2$, $p < 0.001$), the rearing environment x actual housing interaction ($F(1,15) = 5.0$, $p < 0.05$) and the Backtest classification x actual housing interaction ($F(1,93) = 6.3$, $p < 0.05$; see Table 7.3).
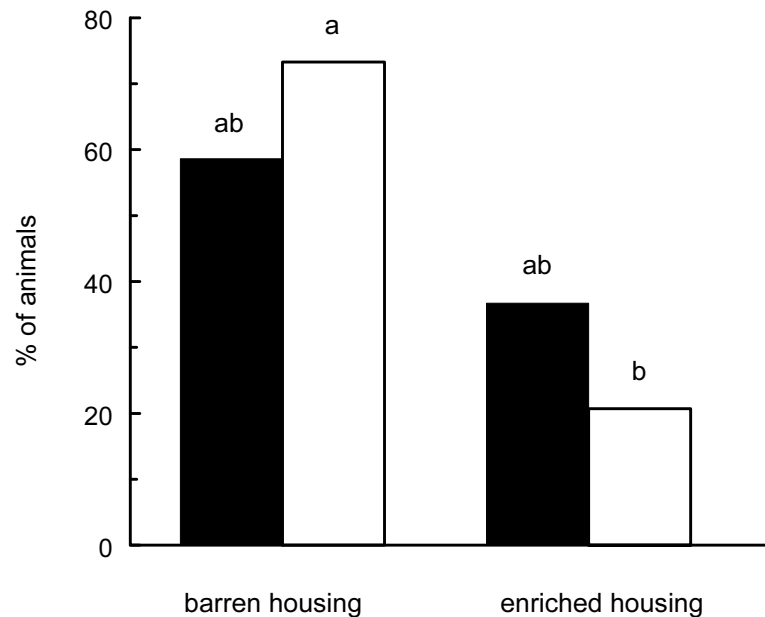
Fig. 7.1. Percentages of HR (solid bars) and LR (open bars) pigs in different housing conditions with gastric lesions (stomach wall damage score > 2). Means lacking a common superscript letter differ (p < 0.05).

DISCUSSION

In the present study pigs with diverging Backtest responses (LR and HR pigs) experienced either a straw-enriched or a barren environment during their first 10 weeks of life, after which environmental conditions were switched for half of the experimental animals. Our study shows that the relative importance of rearing history and actual housing on behaviour in the finishing phase, pathological lesions of the stomach wall and weight gain depended on the individual coping characteristics (LR or HR) of the pigs under study.

Behaviour

Housing environment during the finishing period significantly influenced the behaviour of pigs. Barren housed pigs were less active, showed less play behaviour and explorative activities like chewing, nosing and rooting, and they spent more time on the oral manipulation of pen mates than pigs in enriched housing, which is largely in line with other studies (chapter 6; Beattie et al. 1996; Fraser et al. 1991; Kelly et al. 2000; Lyons et al. 1995).

HR pigs tended to spent more time on lying with open eyes and displayed more aggression than LR pigs, irrespective of their rearing or housing environment. LR pigs showed more manipulation of pen mates than HR pigs, in particular in barren housing, and more play behaviour when kept in enriched pens. The effects of housing environment were most

obvious in LR pigs. These results agree with previous findings on differently housed HR and LR pigs (chapter 6).

Pigs with a barren rearing history changed their behaviour markedly when they were supplied with straw during the finishing phase (BE pigs). Activity, play and explorative behaviour directed at the environment were increased, whereas the oral manipulation of pen mates was reduced as compared with pigs that remained in barren housing conditions until slaughter (BB pigs). Hence, behaviour patterns of pigs with a barren (BE) and an enriched rearing history (EE pigs) housed in enriched pens during the finishing period were largely comparable, i.e. it was mainly the actual availability of straw that determined the activities of the pigs (cf. Day et al. 2002a; Schouten 1986). Others described, however, that pigs originating from barren pens when subjected to an enriched environment at a later age displayed more rooting (Kelly et al. 2000) or general straw-directed behaviour (Bøe 1993; Ruiterkamp 1985) throughout the finishing phase than pigs reared in straw-bedded pens. This was interpreted as a 'catching up' or 'rebound' effect indicating a heightened motivation for exploration in the pigs that had been thwarted in the expression of this behaviour (Ruiterkamp 1985). Although the BE treatment in the present study induced similar effects in the short term (Bolhuis et al., in preparation), no clear 'rebound' effect was found (any more) for the total time spent on exploration at 15 and 19 weeks of age. However, LR pigs that had switched from barren to enriched housing displayed more chewing and rooting than all other pigs during the finishing period. Time spent on play behaviour in the enriched environment was, on the other hand, higher in LR pigs from enriched rearing conditions than in barren reared LR pigs.

In barren housing conditions, the effect of rearing history on behaviour was more clear. Pigs that had switched from enriched to barren conditions (EB pigs) showed a decrease in exploration, play and general activity as compared with EE pigs, and an increase in the time spent on manipulative activities directed at pen mates. In comparison with BB pigs, however, EB pigs were less active (i.e. they spent more time lying with closed eyes during observations) and showed less nosing the floor. Thus, the inactivity induced by barren housing (cf. Beattie et al. 1995; Lyons et al. 1995; Wood-Gush and Beilharz 1983) was even higher in pigs with an enriched rearing history. Reduced activity and exploration have also been reported to occur in pigs that were regularly subjected to unpleasant handling during the growing phase (Pearce et al. 1989). Although difficult to interpret, increased inactivity in the home pen has been suggested to represent an apathetic 'cut-off' response by which pigs distance themselves from an aversive environment and thus may be indicative of reduced welfare (cf. Pearce et al. 1989; Ruiterkamp 1985; Wood-Gush and Beilharz 1983).

Studies investigating the relative importance of early exposure to a barren or enriched environment for the expression of injurious manipulation of pen mates have yielded different

results. Thus, manipulation of pen mates has been reported to be a consequence of the actual situation rather than of early experience (Schouten 1986), whereas others showed an influence of rearing environment on the occurrence of some manipulative behaviours (Day et al. 2002a; Ruiterkamp 1985). Concerning the effects of rearing, on the one hand, some pig-directed manipulative behavioural elements were shown more frequently or longer within barren finishing pens by pigs with a barren rearing background, indicating that part of these behaviours may be learned early in life (cf. Day et al. 2002a; Ruiterkamp 1985). The same studies reported, however, that some other manipulative behaviours occurred more frequently in pigs reared in enriched pens, and it was suggested that this could be due to altered needs caused by prior experience (Day et al. 2002a; Ruiterkamp 1985).

In the present study, the availability of straw during the first 10 weeks of life did not prevent pigs from redirecting their explorative behaviours to pen mates when exposed to a barren environment at a later age. The relative contribution of rearing history to the expression of these behaviours, however, depended on individual characteristics of the experimental animals. LR-EB pigs manipulated their pen mates less than LR-BB pigs, whereas rearing conditions did not affect the manipulative behaviour of HR pigs in barren housing. Notably, some other effects of rearing environment were most evident in LR pigs as well. LR pigs that changed from barren to enriched housing displayed more chewing and rooting than all other pigs during the finishing period, and, conversely, a change from enriched to barren housing decreased these explorative behaviours most markedly in LR pigs. This indicates that not only the effects of actual (in)availability of straw bedding on behaviour are more obvious in LR than in HR pigs (chapter 6), but also the effects of early experience. These different effects on individuals with diverging coping characteristics could partly explain why previous studies on the impact of early experience with bedding material on later behaviour have revealed varying results.

Weight gain and feed intake

EE groups tended to have a higher feed intake than EB groups, whereas groups subjected to the other treatments (BE or BB) had intermediate values. Feed-to-gain ratios did not significantly differ between treatments. Although all groups consisted of 3 HR and 3 LR pigs, the combined effect of rearing and housing environment on feed consumption was mainly reflected in the average daily weight gain (ADWG) of HR individuals. Thus, the ADWG of HR-EE pigs was substantially (around 10%) higher than that of HR-EB pigs, whereas in LR pigs ADWG was similar for all rearing and housing combinations. In another study, pigs in housing systems with straw ate 6% more food and grew 7% faster than pigs in conditions without straw when kept at temperatures within their thermoneutral range (Lyons et al. 1995). It was suggested that the sustained stress of barren housing contributed to a reduction in

food intake and live weight gain in pigs without straw (Lyons et al. 1995). This is supported by a study showing that also pigs provided with inedible enrichment (toys) tended to grow faster than pigs in barren housing (Schaefer et al. 1990), although others found no effects of toys on weight gain (Pearce et al. 1989; Pearce and Paterson 1993). Our study shows that the effect of environmental enrichment on weight gain may differ for pigs with diverging coping styles.

Studies investigating the growth rate of pigs that differ in Backtest response have not revealed consistent effects. In one study, pigs with higher Backtest scores (HR pigs) showed a higher weight gain from 9 weeks to slaughter in barren pens than LR pigs (Van Erp-Van der Kooij et al. 2003), whereas in another study no clear relationship between Backtest response and weight gain was found (Van Erp-Van der Kooij et al. 2000). The present results suggest that particularly HR pigs, although their behaviour was less influenced by rearing and housing than that of LR pigs (see also chapter 6), might respond to a 'negative' change in environmental conditions by a reduction in weight gain (and likely also feed intake).

Pathology

The prevalence of gastric lesions in pigs varies largely (see Nielsen and Ingvartsen 2000b; Pfeiffer 1992). Many factors have been linked to the occurrence of lesions in the porcine stomach wall, including infectious agents, dietary factors and stress (Geverink et al. 2003; Guy et al. 2002; Hessing et al. 1992; Lawrence et al. 1998; Nielsen and Ingvartsen 2000b; Pfeiffer 1992). In the present study, the availability of straw bedding during the finishing period reduced the occurrence of gastric lesions as compared with barren housing, but this effect was only significant for LR pigs.

Also in other studies straw has been reported to reduce gastric lesions in pigs (Guy et al. 2002; Nielsen and Ingvartsen 2000a), although Day et al. (2002b) found no effect of the daily supply of half a bucket of unchopped straw per pen on stomach wall damage. The availability of straw may influence erosion of the stomach wall in different ways. First, straw intake may increase the stomach content firmness, which is known to reduce the risk of lesions and ulceration (Nielsen and Ingvartsen 2000b). Second, as stress is clearly a risk factor in the development of gastric lesions (e.g. Geverink et al. 2003; Hessing et al. 1992; Lawrence et al. 1998), the stress induced by long-term frustration of the expression of several behavioural 'needs' may have increased the vulnerability for gastric erosion in barren housed pigs.

In any case, the stomach wall of LR pigs appeared to be more sensitive to the (in)availability of straw bedding than that of HR pigs. The proportion of LR pigs with severe lesions was significantly lower in enriched than in barren housing, whereas in HR pigs proportions were not significantly affected by environmental conditions. This indicates that LR pigs either react differently or more intensively to the absence or presence of straw bedding in their home

environment than HR pigs, or both. In support of the first possibility, it has been suggested that LR pigs, given their physiological mode of responding to stress (see Hessing et al. 1994a), are more likely to develop gastric lesions in aversive conditions than HR pigs (Hessing 1994), but the results of previous studies are so far inconclusive. Thus, the incidence of gastric lesions was higher indeed in groups composed of only LR pigs as compared to heterogeneous LR-HR groups and homogeneous HR groups (Hessing et al., 1994c). Others found, however, no effect of Backtest classification on stomach wall damage in growing-finishing pigs housed in barren pens (Hessing et al. 1994a) or in adult gilts subjected to long-term individual housing (Geverink et al. 2003).

Besides for home pen behaviour and stomach wall damage, also for other variables, such as reactivity to novel stimuli (Bolhuis et al. 2004) and antibody response to KLH (Bolhuis et al. 2003) the effects of environmental enrichment were much larger in LR than in HR pigs. This is in support of the above-mentioned second possibility, i.e. a stronger influence of the (in)availability of exploratory stimuli on LR pigs. However, although LR pigs unambiguously showed behavioural and pathological signs of reduced welfare in barren pens, particularly when they were switched from enriched to barren housing, this was not reflected in their growth rate. Conversely, rearing and housing influenced behaviour and gastric pathology of HR pigs less obviously, but their combined effects were clearly reflected in the growth rate of these animals. It is therefore difficult to interpret the diverging effects of rearing and housing environment on pigs with different coping characteristics in terms of welfare.

In conclusion, the relative importance of rearing history and actual housing on home pen behaviour, gastric lesions and weight gain depend on coping characteristics (LR or HR) of the pigs under study. When investigating the effects of environmental factors on pigs, the modulating influence of individual coping characteristics should be considered.

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8

Summarizing discussion

Aim of this thesis was to investigate whether the characterization of piglets early in life reflects and predicts a more general profile of reactivity to challenges, often referred to as coping style, under different rearing and housing conditions. For this purpose, the Backtest, a manual restraint in supine position, was used as a classification tool. As it is unknown whether the impact of environmental conditions differs for pigs with diverging characteristics, the major part of the thesis focused on the interaction between housing environment and Backtest classification of pigs.

The experiments described in this thesis show that pigs with diverging Backtest responses, high-resisting (HR) and low-resisting (LR) pigs, differ in aggressiveness (chapters 3, 6, and 7), social strategy (chapter 3) and behavioural flexibility (chapters 3 and 4). The results of chapter 2 provide some initial evidence that these differences in behaviour may be related to neurochemical properties of the brain. In addition, individual coping characteristics of pigs were also related to their immune reactivity (chapter 5) and reflected in their home pen behaviour in barren en enriched environments (chapters 6 and 7). Moreover, the effects of housing conditions on response to novelty (chapter 4), behavioural development (chapters 6 and 7), immune reactivity (chapter 5) and the prevalence of gastric lesions (chapter 7) depended strongly on individual coping characteristics. In this chapter, it is described whether and to what extent the response of pigs in the Backtest is related to reaction patterns in various other situations. Subsequently, the impact of individual coping characteristics of pigs on the performance in their social and physical environment will be discussed, as well as the possible implications for their health, welfare and productivity.

CHARACTERIZATION OF LR AND HR PIGS

Active and passive coping

The classification of young piglets as HR or LR expresses their tendency to show an active (i.e. escape) or passive (i.e. immobility) behavioural response in the Backtest. When restrained in a cage (Bolhuis and Schouten 2002; Table 8.1) or isolated in an unfamiliar environment (Hessing et al. 1994a; Ruis et al. 2001) at a later age, HR pigs also displayed more escape behaviour than LR pigs. Similarly, pigs showing no or only short immobility in a so-called Tonic Immobility (TI) test (see Erhard and Mendl 1999), which to some extent resembles the Backtest, displayed more struggling behaviour when being held for an injection than pigs with long immobility scores (Erhard et al. 1999). Pigs thus appear to be rather consistent in their 'preference' for either an active (fight-flight) or a passive (conservation-withdrawal) mode of responding in mild (restraint) stress situations, irrespective of their housing environment (Table 8.1).

Table 8.1. Behaviour of HR and LR pigs at 8 weeks of age during a 1-min restraint test.

Variable	HR	LR
Escape behaviour (latency in s)	18.3 ± 3.0[a]	29.6 ± 4.9[b]
Escape behaviour (% of time)	44.7 ± 4.6[a]	21.7 ± 5.1[b]
Immobility (% of time)	31.6 ± 4.5[a]	45.6 ± 6.4[b]

Within a row, means lacking a common superscript differ ($p < 0.05$). Behaviour in the test was unaffected by housing. For description of pigs, see chapter 4; for methods, see Bolhuis and Schouten 2002.

It should be noted, though, as the terms 'active coping' and 'passive coping' have led to confusion (e.g. Breuer et al. 2002; Van Erp-Van der Kooij 2003; Van Erp-Van der Kooij et al. 2003), that differences in stress-coping behaviour in HR and LR pigs appear not to be accompanied by (or even attributable to) differences in basic activity. Chapters 6 and 7 reveal that HR pigs do not show a higher activity than LR pigs in their home pen. Moreover, HR pigs even tended to be less active than LR pigs, both in barren and enriched environments.

A passive coping style has been related to a high (re)activity of the hypothalamic-pituitary-adrenal (HPA) axis (Henry and Stephens 1977; Koolhaas et al. 1997; 1999; Veenema et al. 2003; but see Bohus et al. 1987 who reported differently), whereas an active coping response is accompanied by a high sympathetic reactivity in several species (Fokkema et al. 1988; 1995; Korte et al. 1992; Sgoifo et al. 1996). Although data on pigs are not always consistent (e.g. see Geverink et al. 2002b; Van Erp-Van der Kooij et al. 2003), several studies point to a higher HPA-axis (re)activity for LR pigs under challenging conditions. For instance, LR pigs have been reported to display higher salivary cortisol responses than HR pigs to social isolation, routine weighing, ACTH administration and novel environment tests (Ruis et al. 2000; 2001) and a higher weight of the adrenals at slaughter (Hessing et al. 1994a). In addition, 6-month old LR gilts showed higher basal levels of salivary cortisol than HR pigs during the light period of the 24h-cycle in group housing (Geverink et al. 2002a). Five months later, however, no differences were found between the two types of pig in group housing. Yet, after four months of individual stall housing, LR pigs did show higher basal cortisol levels than HR pigs at the same age, i.e. 11 months (Geverink et al. 2003). Recently it has been shown that LR and HR pigs also show divergent adaptations to chronic stress, induced by long-term individual stall housing, at the hypothalamic level. HR, but not LR, pigs showed an increase in vasopressin in the paraventricular nucleus of the hypothalamus, whereas only LR pigs displayed an increase in CRH mRNA expression (Karman 2003). This

divergence in neuroadaptation to chronic stress indicates a fundamental difference in the brain circuitry that governs HPA-axis function (Karman 2003).

A suddenly introduced novel object within an unfamiliar environment induced a tachycardia in HR pigs, whereas LR pigs showed a lower increase in heart rate, or even a bradycardia in response to this event (Hessing et al. 1994a). It was therefore suggested, although other data in support of this suggestion are scarce (e.g. Geverink et al. 2003; Ruis et al. 2001), that LR and HR pigs differ in reactivity of the autonomic nervous system (Hessing et al. 1994a; Ruis et al. 2001). Thus, both behavioural and physiological data point to an active coping style in HR pigs, and a passive one in LR individuals.

Aggressiveness and social strategy

In most commercial pig husbandry systems, regrouping of pigs is a common procedure that causes the need for establishment of a new hierarchy. Pigs usually fight vigorously for several hours when introduced to unfamiliar conspecifics, although some individuals appear to refrain from showing aggression. In the experiment described in chapter 3, the tendency to display aggressive behaviour after regrouping was related to Backtest classification. HR pigs were more likely to initiate fights, spent more time on fighting and fought more often than LR pigs during the first hours after weaning and mixing. Moreover, the average duration of fighting per encounter was longer for HR pigs, indicating that these pigs have difficulty in inhibiting their aggressive behaviour. The results of chapter 3 are in line with other studies reporting HR pigs to behave more aggressively than LR pigs in provocation experiments, such as social confrontation tests and food competition tests, at several ages (Hessing et al. 1993; 1994a; Ruis et al. 2000; 2002).

A novel finding, described in chapters 6 and 7, is that also outside a clear provocative context, under relatively stable social conditions (i.e. up to three months after formation of groups), HR pigs displayed more aggressive behaviour in the home pen than LR pigs. Thus, aggressiveness and the tendency to display an active or passive response in threatening conditions appear to be related in pigs, like in several other species such as great tits, mice and rats (see Koolhaas et al. 1999 for a review). In these species aggressiveness has been mentioned as one of the key characteristics of the coping style of an individual (e.g. Koolhaas et al. 1999; 2001).

In chapter 3, however, Backtest classification and aggressiveness were not unequivocally coupled. Although LR pigs were on average less aggressive than HR pigs, some LR pigs showed high levels of fighting behaviour after being confronted with unfamiliar pigs. The LR pigs that did show a lot of fighting were, however, relatively successful, as illustrated by the positive correlation between self-initiated fighting behaviour and social rank, which was absent in HR individuals (chapter 3). Notably, in a recent study similar differences in social

strategy were described in sows: some individuals were more flexible in social situations than others. These individuals explored but also avoided others more and won a larger proportion of fights (Andersen et al. 2002). The results of chapter 3 suggest that HR and LR pigs do adopt different social strategies that can not just be explained as differences in aggressiveness per se, but rather in the flexibility of applying aggressive behaviour. HR pigs initiate aggressive responses quickly and may have difficulty in inhibiting fighting behaviour once started, whereas LR pigs, as they seem to 'tune' their fighting behaviour, appear to be more flexible in social encounters.

Response initiation

The differences between HR and LR pigs in response initiation and behavioural flexibility (chapter 3) do not seem to be restricted to agonistic encounters. Also in non-social challenges, particularly in response to novel stimuli, HR pigs have been reported to act more quickly than LR pigs. For instance, HR pigs showed shorter latencies to touch a novel object (Hessing et al. 1994a) or a human (Ruis et al. 2000), and entered an unfamiliar environment more readily (Ruis et al. 2000) than LR pigs. Similarly, pigs showing no immobility in the TI-test displayed a shorter latency to enter an unfamiliar arena than pigs that did show immobility (Erhard and Mendl 1997). These differences in the readiness to approach novel stimuli, also referred to as 'risk-taking behaviour', 'novelty seeking', 'initiative to explore', 'response initiative', 'uninhibited behaviour', 'proactivity' or 'boldness' have also in other species been described to be a distinguishing factor between individuals differing in coping patterns or personality traits (Benus 2001; Benus and Röndigs 1997; Campbell et al. 2003; Cools and Gingras 1998; De Boer et al. 2003; Gosling 2001; Kagan et al. 1987; Koolhaas et al. 1999; Mendl and Paul 1991; Verbeek et al. 1994; Wilson 1998).

It should be noted, though, that in several studies on pigs no clear relationship was found between responses in the Backtest or similar tests, like the TI-test, and reaction to novelty (Janczak et al. 2003; Ruis et al. 2001; 2002; Van Erp-Van der Kooij et al. 2002). Responses to novel stimuli in pigs are, however, strongly influenced by the complexity of their (physical) housing environment. Pigs from barren housing conditions have frequently been reported to be less inhibited to approach novel stimuli than pigs from enriched environments, probably due to an unsatisfied motivation for exploration (De Jong et al. 2000; Olsson et al. 1999; Pearce and Paterson 1989; Stolba and Wood-Gush 1980; Wood-Gush et al. 1990b). This effect of housing may be most pronounced in LR pigs. In chapter 4, the latency to approach and pass a novel object that was placed in a familiar maze was markedly reduced in barren housed LR pigs as compared with LR pigs from enriched housing, whereas in HR pigs no significant effect of housing environment was found. In line with this, 6-week old LR pigs were more reluctant than HR pigs to touch a novel object and an unfamiliar human in their

home pen when housed in an enriched environment (Table 8.2; unpublished results). In barren housing, however, contact latencies of LR pigs were considerably reduced and did not differ from those of HR individuals (Table 8.2). This indicates that, in pigs, measures of reaction to novelty may not be very distinctive, as their housing environment clearly seems to affect the balance between motivation to approach or avoid novel stimuli.

Table 8.2. Latencies to touch a human and a novel object in the home pen at 6 weeks of age of LR and HR pigs from barren (B) or enriched (E) housing.

Variable	HR		LR	
	B (n=30)	E (n=30)	B (n=30)	E (n=30)
Latency to touch novel object (s)	52.0 ± 8.6^a	55.1 ± 11.9^a	52.9 ± 10.8^a	74.0 ± 12.3^b
Latency to touch human (s)	24.3 ± 4.0^a	55.4 ± 21.5^a	55.1 ± 18.7^a	125.2 ± 28.2^b

Within a row, means lacking a common superscript letter differ ($p < 0.05$). For description of pigs and housing conditions, see chapter 7.

Routines and behavioural flexibility

HR and LR pigs differed in behavioural flexibility in aggressive encounters (chapter 3) as well as in a non-social context. In chapter 4 pigs were subjected to a simple (left/right) spatial discrimination task in a T-maze. HR and LR pigs did not differ in acquisition of this task, which involved choosing the side with a food reward at the end of the arm. However, when the rewarding side of the maze was reversed after a series of successful maze runs, HR pigs had more difficulty in changing their response pattern than LR pigs (chapter 4).

The results of chapter 3 and 4 suggest that during the repeated execution of a particular sequence of behaviours within a specific context, HR pigs more readily develop routine-like or habit-like action patterns. These patterns seem to be relatively independent of actual information and rather difficult to inhibit when circumstances have changed. The behaviour of LR pigs, on the other hand, appears to remain more flexible, as it is easily modified by changing conditions.

This difference between HR and LR pigs may be a result of differences in the balance of neural systems that control their behavioural output (cf. Toates 1998; see chapter 4). Several studies make mention of distinct mechanisms for processing and storing information that operate simultaneously to influence behaviour. In these studies procedural, stimulus-response memory processes have been distinguished that lead to routinisation or automatisation of behaviour (i.e. habits), as opposed to more declarative or cognitive

processes that allow flexible responding (see Poldrack and Packard 2003; Toates 1997; 1998; White and McDonald 2002 for review). A role for the basal ganglia, and in particular the dorsal striatum, in habit formation and the performance of routinised behaviour has been suggested, whereas the hippocampus appears to be the primary component of the cognitive form of learning (see Gerdeman et al. 2003; Poldrack and Packard 2003, Toates 1998).

Regarding behavioural control, it can be hypothesised that HR pigs, with experience in a certain context, attribute relatively more weight to stimulus-response mechanisms as opposed to cognitive processes, whereas LR pigs rely more on the latter type of information processing and base their behavioural output more on the actual situation. Indeed, results of other studies indicate that LR pigs have a higher tendency to monitor the environment and probably update information more frequently than HR pigs. For instance, LR pigs spent more time on explorative behaviour than HR pigs when relocated individually (Ruis et al. 2001) or in pairs (Bolhuis, unpublished results) and they explored a novel object longer and more thoroughly (Hessing et al. 1994a).

In a number of other species, similar differences between individuals with diverging coping responses in habit formation and behavioural flexibility have been described (e.g. Benus et al. 1987; 1992; Cools et al. 1994; Verbeek 1998). For instance, great tits characterized as 'slow explorers' (SE) with an accompanying preference for a passive coping style (see Verbeek 1998) took longer to develop a foraging habit when trained than 'fast explorers' (FE) with an active coping style. However, the SE birds responded more quickly to changes in food distribution than the FE individuals, because they continuously explored other feeding options (Marchetti and Drent 2000; Verbeek et al. 1994). Similarly, actively coping male mice (SAL), after being exposed several times to a male intruder, failed to change their behaviour appropriately when a familiar female was subsequently presented (i.e., they attacked the female by mistake), whereas passive copers (LAL) that consistently attacked male intruders shifted their behaviour readily when the familiar female was introduced (Benus et al. 1990b). It has recently been shown that SAL and LAL mice differ in the expression of several cytoskeleton genes in the hippocampus, possibly leading to differences in morphology and neuronal plasticity of this brain structure. This could partly explain the divergent behavioural flexibility of the two types of mice (cf. Feldker et al. 2003a; 2003b).

The use of external and internal information to organise behavioural responses thus appears to be an essential distinguishing feature of individuals with different coping styles in several species (cf. Cools et al. 1990; Koolhaas et al. 2001; Toates 1998), including pigs (chapters 3 and 4).

It has been suggested (Carere 2003) that the distinction between active and passive 'copers' may, to some extent, be the animal equivalent of variation in the introvert-extravert axis in humans (see Eysenck 1973). According to Brebner (2001) extraversion may reflect a general

tendency to process little stimulus information and organise responses rapidly. Although a comparison with human personality traits seems far-fetched, some of the characteristics attributed to extraverts would fit the HR-type of pig well: extraverts have also been reported to tend to unchecked action, i.e. they have shorter reaction times than introverts under challenging conditions and are less apt to pause and reflect on the situation. They also generalise from past experience whereas introverts update their information more frequently (see e.g. Brebner 2001; Patterson and Newman 1993).

Apomorphine susceptibility

The experiment described in chapter 2 provides evidence for a link between the behavioural response in the Backtest and the neurochemical state of the brain. In this experiment, pigs were injected at 18 weeks of age with a low dose of apomorphine, a dopamine agonist that induces stereotyped behaviour by stimulating dopamine receptors in the striatum (Cooper and Dourish 1990; Kelly 1975). In rodents, sensitivity for the stereotypic effects of apomorphine and coping style appear to be related. For instance, divergent genetic selection of Wistar rats for apomorphine-induced (stereotypic) gnawing resulted in coupled differences in behavioural and neuroendocrine reactions to challenging conditions (see Cools et al. 1990; 1993; 1994; Cools and Gingras 1998, for review). Conversely, mice lines selected for long (LAL) and short attack latency scores (SAL) accompanied by a predisposition for an active or passive coping style, respectively (see Benus 1988), differed in response to apomorphine (Benus et al. 1991a; Sluyter et al. 1994).

HR pigs showed a stronger stereotypic response to apomorphine than LR pigs, which may indicate a difference in striatal dopaminergic functioning between the two types of pig. As the striatum is also involved in the display of environmentally induced stereotypies (Garner and Mason 2002), another indication that the types of pig differ in functional activity of this brain structure is the recent finding that HR pigs tend to develop higher levels of stereotypic chain biting than LR pigs when housed in individual stalls (Geverink et al. 2003).

Studies in rodents have revealed differences in many brain areas and neurotransmitter systems between individuals diverging in coping style (see Cools et al. 1994; Koolhaas et al. 2001). The difference in apomorphine susceptibility of HR and LR individuals (chapter 2) is a first possible indication for a neurochemical background of coping styles in pigs. It is not sure whether the difference in apomorphine reactivity is causally linked with the behavioural characteristics of HR and LR pigs. It should be noted, though, that the striatum has a role in the initiation and patterning of a wide variety of behaviours, and also appears to be the brain centre for habit formation (see previous section). The nigrostriatal physiology underlying habit formation appears to involve dopamine, as dopamine receptors in the striatum are required for the consolidation of stimulus-response learning in rats (Packard and Knowlton

2002) and are implicated in the ability to arbitrarily switch behaviour (Cools et al. 1994).

Immune reactivity

In view of the proposed differential neuroendocrine background of diverging stress-coping responses (e.g. Bohus et al. 1987; Cools et al. 1990; Koolhaas et al. 1999) and the bi-directional communication between the immune system and the brain (see Ader et al. 1991; Savino and Dardenne 1995), different coping styles may also be reflected in immune parameters. In chapter 5 the immune response to KLH-DNP of HR and LR pigs from different housing environments was tested. HR pigs showed higher in vitro lymphocyte proliferation than LR pigs, in line with other studies (Bolhuis et al. 2000b; Hessing et al. 1995). It has been shown that HR pigs, as compared with LR pigs, also display a higher in vivo cell-mediated immunity (Hessing et al. 1995; Van Erp-Van der Kooij 2003). LR pigs, on the other hand, were reported to show faster or higher specific antibody responses to various antigens than HR pigs (Hessing et al. 1995; Schrama et al. 1997). A difference in the balance between Th1- and Th2-mediated immune responses (see Mosmann and Sad 1996) was hypothesized to underlie these differences in immune reactivity between the two types of pig (Hessing 1994; Hessing et al. 1995).

The results of chapter 5 indicate, however, that the effects of individual coping characteristics on immune responses of pigs found in a given environment may not necessarily apply to other housing and rearing conditions. Barren housed LR pigs showed higher KLH-specific antibody titres than LR pigs from enriched pens, whereas differently housed HR pigs showed similar antibody responses, with levels in between those of the barren and enriched housed LR pigs. Thus, although coping styles of pigs are reflected in their immune responses (chapter 5; Bolhuis et al. 2002b; Hessing et al. 1995; Schrama et al. 1997; Van Erp-Van der Kooij 2003, but see Geverink et al. 2004b who reported differently), these individual characteristics may show complex interactions with environmental variables, such as housing, in modulating immune reactivity.

It can be imagined that the differential immune profiles in animals with diverging coping styles affect disease susceptibility. In rodents, individual coping styles and the associated variation in immune reactivity have indeed been related to differences in vulnerability for inflammatory diseases, such as periodontitis (Breivik et al. 2000) and experimental autoimmune encephalomyelitis (Kavelaars et al. 1997; 1999) and infectious diseases, like parasitic helminth infections (Kavelaars et al. 1997). The implications of the differences between HR and LR pigs in immune reactivity for disease susceptibility and health are not clear. Results of a recent study indicate, though, that resistance to salmonella, which depends primarily on cell-mediated immunity, is higher in HR pigs than in LR pigs (Van Erp-Van der Kooij 2003).

SOCIAL ENVIRONMENT

Probably the most challenging and complex part of the environment of pigs is the social group in which they live. The group structure of pigs is based upon a dominance hierarchy (McBride and James 1964; Meese and Ewbank 1973). In pig husbandry the direct benefits or costs accompanying a particular social rank are often small as humans usually control the access to resources (e.g. food). Social relationships may, however, have a crucial effect on health and welfare (e.g. DeVries et al. 2003; Kiecolt-Glaser 1999; Sachser et al. 1998). The social environment can be a source of social support, i.e. stable, positive social bonds can supply animals with an improved capacity to cope with environmental demands (see Sachser et al. 1998; Seeman and McEwen 1996). On the other hand, long-term instability in social relationships and a low predictability and controllability of interactions with conspecifics may lead to an aversive situation, in particular for submissive individuals (e.g. Tuchscherer et al. 1998).

Formation of groups

Individual coping characteristics of pigs may influence the formation of a social hierarchy after regrouping. As HR pigs are more aggressive than LR pigs and appear to have difficulty in inhibiting aggression (chapter 3), mixing of HR pigs likely results in more intensive and prolonged fighting and hence a slower settling of dominance relationships. Indeed, formation of homogeneous HR pairs or groups has been reported to result in higher levels of aggression as compared to formation of homogeneous LR groups or heterogeneous LR/HR groups (Hessing et al. 1994b; Ruis et al. 2002). Fighting also persisted longer, and more skin lesions were found in confrontations of two HR individuals and in HR/LR pairs where the HR pig became dominant. In addition, the gain-to-feed ratio was significantly lower during the first week after mixing in pairs with a dominant HR gilt (Ruis et al. 2002). The mixing of HR pigs thus appears to be unfavourable for both welfare and productivity.

Social status

Social status is not a trait of an individual, but a relative position that results from many factors (Drews 1993). In their model, Henry and Stephens (1977) suggested stress response profiles to be related to social status, with the fight-flight pattern being typical for high-ranking individuals, and the conservation-withdrawal response for subordinate ones. Chapter 3 shows, however, that LR and HR pigs did not differ in the achieved social rank when mixed with unfamiliar pigs with comparable physical characteristics (i.e. body weight). Similarly, in pair-wise confrontations, LR and HR individuals had almost equal chances of becoming dominant or submissive (Ruis et al. 2002).

The strategy to cope with a low social status might, however, be more important than the actual position in the hierarchy (e.g. Mendl et al. 1992; Stefanski 1998). Submissive sows that displayed relatively high levels of aggressiveness in spite of their low social status, had a lower total weight of piglets born alive than dominant sows. The reproductive success of less aggressive submissive sows did, however, not significantly differ from that of dominant group members (Mendl et al. 1992). Similarly, in rats the consequences of having a low social status were reported to depend on individual characteristics. Only those low-ranking rats that adopted a proactive coping style accompanied with high levels of aggression were at risk for developing a high blood pressure and cardiovascular disease (Fokkema et al. 1995). A low level of aggressiveness may thus be beneficial for individuals with a low social rank (Mendl and Deag 1995).

Interestingly, it has been shown, in pairs of pigs, that the aggressive behaviour of submissive pigs may also be influenced by the coping characteristics of the individual that dominates them. Dominant LR pigs appeared to attenuate aggression in submissive HR pigs, whereas pairs of HR pigs provoked high levels of mutual aggression during the first three weeks after mixing (Ruis et al. 2002). These complex interactions between 'type of pig' and social status in affecting social behaviour merit further research.

Social stability

Excessive aggression can be a significant problem for welfare as well as production in confined systems for pigs (Hagelsø-Giersing and Studnitz 1996). It is clear from the preceding paragraphs that the high level of aggressiveness and low flexibility of HR pigs may complicate and slow down the establishment of dominance relationships, and hence induce social friction in the short term. However, avoiding social stress requires not only the settling of a clear hierarchy shortly after mixing, but also a subsequent state of social stability. Little is known about the effects of individual coping characteristics on social relationships in the long term. It has been found that the composition of groups of finishing pigs affected their average daily weight gain, lean meat percentage and carcass quality: homogeneous HR groups performed worse than heterogeneous (LR/HR) groups (Hessing et al. 1994c). Although pigs were only observed for one hour after group formation, it was assumed that the lower performance of HR groups could be attributed to long-term social stress (Hessing et al. 1994c). The results of chapters 6 and 7 provide some support for this assumption: HR pigs displayed more aggressive behaviour over the whole finishing period, hence groups solely made up of HR pigs probably experience social friction in the long term.

It has been proposed that forming groups of only low-aggressive animals would be beneficial for welfare (Erhard et al. 1997). However, Hessing et al. (1994c) stated that homogeneous groups of LR finishing pigs also experience social stress, because, in their study, these

groups showed a lower carcass quality and higher prevalence of gastric lesions at slaughter than heterogeneous LR/HR groups. It is questionable, however, whether this lower performance of LR groups can solely be attributed to social stress. Other studies have shown that also in heterogeneous (HR/LR) groups, LR pigs have a lower carcass quality (Van Erp-Van der Kooij et al. 2000; 2003) and appear to be more sensitive to develop gastric lesions in barren housing (Hessing et al. 1994a; chapter 7). It is possible that the relatively high levels of manipulative oral behaviour directed at pen mates displayed by LR pigs in barren environments (chapters 6 and 7), which may induce stress and restlessness in their victims, also negatively affect the performance of groups in such environments. Long-term analysis of how individuals within LR and LR/HR groups perform in different physical environments is needed to clarify how pigs with different coping characteristics contribute to social (in)stability.

Implications

Studies carried out so far thus strongly suggest that individual coping characteristics affect both the formation and stability of social relationships in pigs. Measures that could improve the quality of social relationships in pigs and thereby their welfare, health and productivity could involve the (genetic) selection of animals. However, apart from selecting the optimal (combination of) individuals, many other factors may improve the capacity of pigs to cope with their social environment. The conditions under which pigs are reared, and in particular space allowance during the socialisation period early in life, are crucial for developing social skills (De Jonge et al. 1996; Lammers and Schouten 1985a; 1985b; Schouten 1986). It is unknown whether and to what extent the social behaviour of different types of pig can be optimised by early life experiences. Furthermore, also in later life measures could be taken to enhance social stability and reduce social stress, such as, for instance, avoiding the mixing of unfamiliar pigs when possible, providing conditions that facilitate the use of assessment instead of fighting to resolve conflicts (Andersen et al. 2000; Jensen and Yngvesson 1998; Mendl 2001), and supplying escape or avoidance opportunities like 'hide areas' within the housing environment (McGlone and Curtis 1985).

PHYSICAL ENVIRONMENT

Not only the social environment, but also the physical environment in which pigs live is critical to their welfare. In many studies evaluating different housing conditions for pigs, space allowance (i.e. floor space per animal) and environmental complexity (e.g. bedding) were simultaneously varied. These two factors may have differential effects on the behaviour

and welfare of pigs (see e.g. Beattie et al. 1996; Schouten 1986). In the experiments described in this thesis (chapters 3-7), the physical environment of pigs was varied in one aspect only: the availability of straw bedding. Barren housing environments may in particular constrain the internal 'need' of growing-finishing pigs to express explorative behaviours like rooting and chewing (Fraser and Broom 1990; Van Putten and Dammers 1976; Wood-Gush and Vestergaard 1991). Straw is thought to serve, besides as a bedding material and nutritional stimulus, as a partial outlet for the explorative activities that are natural to pigs (Fraser et al. 1991). Provision of straw can thus be considered a *relative* enrichment, i.e. a modification in the environment that improves the biological functioning of animals (Newberry 1995).

Availability of straw

The availability of straw indeed strongly influenced behavioural activities of the experimental pigs (chapters 6 and 7), in concordance with other studies (Beattie et al. 1995; 2000; Fraser et al. 1991; Ruiterkamp 1985; Schouten 1986). Pigs spent the major part of their active time on exploratory behaviour, although levels of exploration were lower in barren pens. Generally, in the enriched pens, much exploration was directed to the straw bedding, whereas in barren pens, besides the bare floor and pen fixtures, pen mates became targets for manipulative activities like nibbling, rooting and chewing. In addition, barren housed pigs were frequently observed to be vacuum or sham chewing (chapter 6). The explorative motivation of pigs in a barren environment is, however, not fully satisfied by using these alternatives (cf. Ruiterkamp 1985; Schouten 1986; Wood-Gush et al. 1990b).

Chapter 7, in which housing conditions were changed for half of the experimental pigs at 10 weeks of age, reveals that it is mainly the actual availability of straw that determines the target of exploratory activities, rather than previous experience (cf. Day et al. 2002a; Schouten 1986). Thus, pigs with a barren housing history drastically reduced the oral manipulation of pen mates when subjected to an enriched environment. Conversely, the availability of straw during the first weeks of life did not prevent pigs from redirecting their explorative behaviours to pen mates when exposed to a barren environment at a later age. Within barren pens, however, LR pigs with a barren rearing history manipulated their pen mates more than LR pigs with previous experience in an enriched environment (chapter 7).

The high occurrence of oral manipulative activities directed at pen mates in barren conditions is clearly indicative of an inadequate housing environment. Moreover, these activities, also referred to as 'manipulative social', 'adverse social' or 'harmful social' behaviour, may lead to serious wounds (or even cannibalism), long-term restlessness and stress in the victims (Beattie et al. 1995; 2001; Schouten 1986). Pigs housed without a rooting substrate often have more skin lesions and injuries than pigs in enriched housing (Beattie et al. 2001; Lyons

et al. 1995). The reduction in play behaviour in barren housed pigs, as compared with the enriched animals (chapters 6 and 7), is another indication that barren environments may negatively affect the welfare of pigs (cf. Buchenauer 1982; Fraser and Broom 1997; Lawrence 1987; Newberry et al. 1988). This is also supported by physiological data. For instance, pigs in barren housing were reported to have a blunted circadian rhythm in salivary cortisol (De Jong et al. 2000; Bolhuis, unpublished results) and heavier adrenals at slaughter (Beattie et al. 2000). Although Karman (2003) revealed that barren housing, in contrast with long-term individual housing, does not seem to induce alterations in the HPA-axis at the hypothalamic level, changes in functional activity of the hippocampus of barren housed pigs as compared with pigs in enriched housing have been suggested (Kallivretaki et al. 2001; Van der Beek et al. 2000; in preparation). In addition, barren housed pigs have been reported to show impaired long-term spatial memory (De Jong et al. 2000) and cognitive performance (Sneddon et al. 2000) as compared with pigs in enriched housing. These observations together strongly suggest that barren housing environments induce sustained stress in pigs and have negative implications for their welfare (cf. De Jong 2000; Schouten 1986).

Coping characteristics and housing conditions

Individual coping characteristics of pigs were reflected in their home pen behaviour in different housing environments (chapters 6 and 7). HR pigs were more aggressive and tended to be less active than LR pigs both in barren and enriched pens, whereas LR pigs showed more play behaviour, in particular in enriched housing. In addition, the detrimental effects of barren housing conditions appeared to depend on individual coping characteristics. LR pigs spent more time on manipulating pen mates in barren pens than HR pigs (chapters 6 and 7). Moreover, the effect of housing on the prevalence of gastric lesions was most pronounced in LR individuals (chapter 7).

The effects of rearing environment were most evident in LR pigs as well. Within barren pens, LR pigs with a barren rearing history manipulated their pen mates more than LR pigs with previous experience in an enriched environment, whereas rearing conditions did not affect the manipulative behaviour of barren housed HR pigs. In addition, LR pigs that had changed from barren to enriched housing at 10 weeks of age displayed more rooting and chewing than all other pigs during the fattening period, and, conversely, a change from enriched to barren housing decreased explorative behaviour most markedly in this type of pig (see chapter 7).

The physical environment not only affected the home pen behaviour of HR and LR pigs differently, but also their reactivity to novel stimuli (chapter 4; Table 8.2), the humoral immune response to KLH (chapter 5) and the prevalence of gastric lesions (chapter 7). These

interactions between housing environment and type of pig imply that the relationships between individual coping characteristics and responses of pigs found in a given environment may not necessarily apply to other conditions, i.e. some 'features' are idiosyncratic to particular circumstances (chapters 4 and 5; Table 8.2). Conversely, when studying the effects of housing conditions, and possibly other environmental factors, the modulating influence of individual characteristics of the animals under study should be considered.

An important finding is that for almost all of the variables that were affected by housing environment, the impact was much larger for LR pigs than for HR pigs (chapters 4, 5, 6, and 7). This difference between the two types of pig indicates that LR pigs either react more intensively, or differently to the lack of exploratory stimuli in their housing environment than HR pigs. The behavioural effects appear to be in line with the suggestion that LR pigs have a higher tendency to monitor their environment than HR pigs (see previous section; chapters 6 and 7). It is, however, difficult to interpret these effects of coping characteristics in terms of welfare. Manipulation of pen mates is frequently used as an indicator of housing inadequacy and poor welfare, but the performance of this behaviour may, to some extent, fulfil the manipulators' need to perform explorative behaviour. Moreover, although the behavioural effects of housing were less pronounced in HR pigs, in these individuals the combined effects of housing and rearing environment were reflected in their growth rate (chapter 7). Hence, pigs with diverging coping characteristics differ in their behavioural and physiological reactions to intensive housing conditions, which may have implications for the assessment of pig welfare.

PERFORMANCE IN PIG HUSBANDRY

It has been shown in other species that the alternative coping styles may both be equally successful in counterbalancing stress. The relative 'success' of one or the other style depends, however, on a 'match' or 'mismatch' between the predisposed individual characteristics and the actual situation (Benus et al. 1991b; Cools et al. 1994; Van Oortmerssen et al. 1985). The higher tendency of active copers to develop routines and to generalise from past experience may be advantageous under stable conditions, whereas passive copers with a high behavioural flexibility may be better off in complex or changing environments (Benus et al. 1991b; Koolhaas et al. 1999; Van Oortmerssen et al. 1985). There are some indications that this may hold for pigs with diverging coping styles as well. Restrictively fed HR and LR gilts did not differ in average daily weight gain from 2 to 13 months of age, both in individual stalls and in group housing (Geverink et al. 2002a).

However, during the first week following relocation or relocation plus splitting up groups, LR pigs showed a higher weight gain and metabolisability than HR gilts, and a tendency for a higher protein retention (Geverink et al. 2004a). This difference in metabolic response to mild stress suggests that LR pigs may adapt faster or more readily to changes in their physical and social environment than HR pigs (cf. chapters 3 and 4; Heetkamp et al. 2003). This could also explain why the effects of Backtest classification on growth are so variable and inconsistent (e.g. Geverink et al. 2002a; 2004a; Ruis et al. 2000; Van Erp-Van der Kooij et al. 2002; 2003), as, in husbandry practice, on the one hand, pigs are subjected to rather stable conditions, but, at some points in their life, also to major changes in their physical and social environment.

HR pigs appear to be the best performers in pig husbandry, as they have been reported to have a higher lean meat percentage and a more favourable carcass classification than LR pigs when fed ad libitum, at least in barren housing conditions (Van Erp-Van der Kooij et al. 2000; 2003). However, these animals also display higher levels of aggressive behaviour, both in barren and enriched environments (chapters 6 and 7) and are not able to maintain a high performance when housed with other HR pigs only (Hessing et al. 1994c). LR pigs may, on the other hand, affect group performance in barren housing conditions negatively because of their higher tendency to show harmful manipulative behaviour directed to pen mates. In more complex and varying environments, however, such as, for instance, outdoor production systems, these flexible pigs might have a higher adaptive capacity, but more research is needed to test this.

Due to selection on meat percentage the number of HR fattening pigs in pig husbandry appears to increase steadily (Van Erp-Van der Kooij et al. 2000; 2003), which may in the long term have negative effects for social stability, and, thereby, welfare and productivity. The Backtest or other characterization tests could be used as tools to monitor the (side) effects of selection for meat percentage on individual coping or personality characteristics of pigs in breeding lines.

CONCLUDING REMARKS

There is no doubt that both situational and individual factors influence coping responses in pigs. We are fully aware that the classification of individual pigs as opposite extremes on the basis of their response in a single test is, like any other attempt to structure individual characteristics, a simplification of reality. We do not claim that these extremes represent distinct categories of pigs, or that only two 'types' of pig exist. However, by using the passive-active coping concept as a framework and the Backtest as a tool, we have shown that the

characterization of pigs early in life has a predictive value for their mode of responding at a later age under different housing conditions. Pigs with diverging responses in the Backtest, LR and HR pigs, differed in aggressiveness, social strategy and behavioural flexibility. The differential response to apomorphine in the two types of pig provides some initial evidence for a neurochemical background of these behavioural differences. In addition, the results of the present thesis clearly indicate that LR and HR pigs adapt differently to barren housing conditions and, moreover, individual coping characteristics of pigs modulate the effects of rearing and housing conditions on behavioural responses, immune reactivity and pathology. In view of the impact on responses of pigs in different social and physical environments, individual characteristics of pigs should be taken into account when studying their behaviour and welfare. The knowledge of individual coping or personality characteristics in pigs could be extended and used for finding the optimal match between pigs and their social and physical environment.

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Samenvatting

Persoonlijkheden van varkens

Individuele karaktereigenschappen en omgaan met stressvolle omstandigheden

Inleiding

De varkenshouderij in Nederland heeft zich vanaf de jaren zestig van de vorige eeuw ontwikkeld van een relatief extensief tot een intensief productiesysteem. De maatregelen die werden genomen om de efficiëntie van varkensvleesproductie te verhogen hebben geleid tot grote veranderingen in de sociale en fysieke omgeving van vleesvarkens. De meeste varkens worden tegenwoordig gehouden in vrij 'kale' hokken waarin de mogelijkheden om soortspecifiek gedrag uit te voeren beperkt zijn. In zulke hokken worden varkens geblokkeerd in de uitvoering van bijvoorbeeld exploratieve (onderzoekende) gedragingen, zoals wroeten en kauwen. De beperkingen in intensieve huisvestingsomstandigheden kunnen leiden tot lange-termijn stress in varkens, en zouden dus een negatief effect op hun welzijn kunnen hebben.

Varkens vertonen echter een grote individuele variatie in hun reactie op stressvolle omstandigheden, wat suggereert dat ze verschillen in de mate waarin of de manier waarop ze zich aanpassen aan stress (coping). In dit proefschrift werden individuele reactiepatronen, die ook wel worden aangeduid met termen als 'persoonlijkheid', 'coping style' of 'karakter', bestudeerd, waarbij een zogenaamde 'rugtest' als hulpmiddel werd gebruikt. In de rugtest werden biggen op hun rug gelegd, gedurende een minuut vastgehouden (Fig. 9.1) en getypeerd op grond van hun gedragsstrategie, actief of passief, in deze stressvolle situatie. De biggen met een actieve gedragsrespons (veel ontsnappingspogingen) werden aangeduid als 'high-resisters' (HR) en de biggen met een passieve respons (geen of weinig ontsnappingspogingen) als 'low-resisters' (LR).

Fig. 9.1. De rugtest

In dit proefschrift werd onderzocht of de typering van jonge biggen op grond van hun reactie in de rugtest een voorspellende waarde heeft voor hun reactiepatronen (hun 'coping style') op latere leeftijd onder verschillende huisvestingsomstandigheden. Varkens werden ofwel 'kaal' gehuisvest, in hokken met deels beton-, en deels roostervloer, of 'verrijkt', in soortgelijke hokken waarin de vloer bedekt was met stro. Stro fungeert als een 'uitlaatklep' voor de natuurlijke behoefte van varkens om exploratief gedrag te vertonen, en kan dus worden gezien als een (relatieve) verrijking.

Individuele verschillen

In de varkenshouderij worden biggen vaak na het spenen verplaatst en gehergroepeerd. Als varkens geconfronteerd worden met onbekende soortgenoten, volgt er vaak een korte periode met heftige gevechten, waarvan de uitkomst gebruikt wordt om een nieuwe rangorde vast te stellen. Individuele varkens verschillen echter in de mate waarin ze agressief gedrag vertonen na confrontatie met andere varkens. In hoofdstuk 3 werd onderzocht of de coping style van varkens, zoals bepaald in de rugtest, een voorspellende waarde heeft voor het agressieve gedrag dat ze vertonen na hergroepering en voor de resulterende sociale status. HR varkens begonnen meer gevechten dan LR varkens en vochten bovendien eerder en langer tijdens de eerste 3 uren na mengen. Uit het experiment in hoofdstuk 6 (zie hieronder) bleek bovendien dat HR varkens ook in stabiele groepen meer agressief gedrag vertoonden dan LR varkens, van spenen tot slacht. De reactie in de rugtest en agressiviteit lijken dus deels gekoppeld. Het gedrag van LR varkens na mengen was echter variabel: sommige LR varkens vochten veel, terwijl andere helemaal niet vochten. Bij LR, maar niet bij HR dieren, waren zelf-geïnduceerd vechtgedrag en rangorde sterk gerelateerd, dus de LR dieren die veel vochten, waren ook relatief succesvol. Uiteindelijk verschilden HR en LR dieren, ondanks de hogere mate van agressie in HR dieren, niet in de sociale status die ze verkregen. Deze studie geeft aan dat LR varkens flexibel zijn in het toepassen van agressie, terwijl de hoge mate van agressiviteit in HR varkens, ongeacht hun succes, erop wijst dat deze dieren meer rigide zijn in hun agressieve gedrag. Deze verschillen in sociale strategie zouden consequenties kunnen hebben voor het vaststellen van een sociale rangorde en voor de sociale stabiliteit op de langere termijn.

In hoofdstuk 4 werd de flexibiliteit in reactie op omgevingsveranderingen getest van HR en LR dieren met verschillende opgroeicondities (kaal of verrijkt). Hiertoe werden varkens getraind om een voerbeloning te vinden in een van de armen (links of rechts) van een T-vormige doolhof. Na een serie van negen foutloze 'runs' werd op het pad naar de voerbeloning een onbekend object geplaatst. Vervolgens werd de kant van de voerbeloning veranderd ('reversal'). Huisvesting en coping style hadden geen invloed op het aanleren van deze ruimtelijke leertaak. Verrijkt gehuisveste dieren veranderden hun gedragspatroon meer

in reactie op het onbekende object dan kaal gehuisveste dieren, maar dit huisvestingseffect was alleen significant voor de LR dieren. Dit geeft aan dat de gedragsreacties van varkens met verschillende karaktereigenschappen afhangen van hun huisvestingscondities. Verder hadden HR dieren meer moeite om hun gedragspatroon te veranderen in de reversal: ze bleven naar de kant rennen waar de voerbeloning eerst lag, terwijl LR dieren al vrij snel hun gedrag aanpasten.

De grondslag voor deze verschillen tussen LR en HR dieren moet waarschijnlijk gezocht worden in de organisatie van hun gedrag. De resultaten van hoofdstuk 3 en 4 samen suggereren dat HR dieren eerder geneigd zijn om routines te ontwikkelen, dat zijn gedragspatronen die relatief onafhankelijk zijn van actuele informatie en die dan ook moeilijk te onderdrukken zijn onder veranderende omstandigheden. Het gedrag van LR varkens daarentegen lijkt flexibeler te blijven, waardoor het gemakkelijk te wijzigen is als de situatie verandert.

In hoofdstuk 2 werd gezocht naar aanwijzingen dat de verschillen tussen HR en LR varkens ook een neurochemische achtergrond hebben. De mogelijke invloed van het dopaminerge systeem werd onderzocht door de varkens te injecteren met apomorfine, een stof met een soortgelijke werking als de neurotransmitter dopamine, en hun gedragsreactie op deze drug te bepalen. Apomorfine-injectie resulteerde onder andere in ongecoördineerde bewegingen bij voornamelijk de LR dieren. Verder induceerde apomorfine stereotiep gedrag, wat bij de behandelde varkens tot uiting kwam in 'snout contact fixation', het voortdurend op de grond houden van de snuit. Dit stereotiepe gedrag werd vooral vertoond door de HR dieren. Deze verschillen tussen HR en LR varkens in gedragsrespons op apomorfine wijzen op een verschil in dopaminerge activiteit in bepaalde hersengebieden. Omdat dopaminerge systemen betrokken zijn bij de reactie op stress en de organisatie van gedrag, lijken de verschillen in lijn met het idee dat de rugtest-typering van varkens gerelateerd is aan hun 'coping style'.

Het doel van hoofdstuk 5 was om de invloed van zowel huisvesting (kaal of verrijkt) als coping style (HR of LR) op immuunreactiviteit te testen. Varkens werden geïmmuniseerd met KLH-DNP, een antigen (lichaamsvreemde stof). Uit de wekelijks genomen bloedmonsters werden de in vitro lymfocytproliferatie en de antilichaamrespons op KLH bepaald. HR varkens vertoonden een hogere lymfocytproliferatie in reactie op KLH dan LR dieren. Huisvesting en coping style beïnvloedden de antilichaamrespons op een interactieve manier. LR varkens uit kale hokken vertoonden een hogere antilichaamrespons dan LR varkens uit verrijkte hokken, terwijl antilichaamtiters van HR dieren niet beïnvloed werden door hun huisvesting en tussen die van de verschillend gehuisveste LR dieren in lagen. Dit geeft aan dat ook immunologische reacties van varkens met verschillende coping styles niet voor alle huisvestingscondities gelden. En, andersom, wanneer men effecten van omgevingsfactoren

op immuunreactiviteit en ziektegevoeligheid van varkens bestudeert, zou rekening gehouden moeten worden met de invloed van individuele persoonlijkheidskenmerken of 'coping styles'.

Varkens en hun omgeving

In hoofdstuk 6 werd de ontwikkeling van gedrag van HR en LR varkens bestudeerd in kale en verrijkte huisvesting. De aanwezigheid van stro had een grote invloed op gedrag. Varkens die de beschikking hadden over stro vertoonden meer activiteit, spelgedrag en exploratief gedrag dan dieren uit kale huisvesting. In de kale hokken richtten de varkens een deel van hun exploratieve activiteiten op de vloer en de inrichting van het hok, maar ook hokgenoten werden het doelwit voor exploratie: er werd vaker beschadigend gedrag, zoals staart-, oor- en pootbijten en het beknabbelen van flanken gezien, en bovendien meer looskauwen dan in verrijkte hokken. De verschillen in met name beschadigend gedrag en spelgedrag tussen kaal en verrijkt gehuisveste dieren geven aan dat kale huisvesting een negatief effect heeft op het welzijn van varkens.

In hoofdstuk 7 werd onderzocht of gedrag vooral afhangt van de actuele aan- of afwezigheid van stro, of van de condities (kaal of verrijkt) waaraan de dieren op jongere leeftijd waren blootgesteld. Dit werd gedaan door op 10 weken leeftijd de helft van de kaal opgegroeide dieren stro te geven, en de helft van de verrijkt opgegroeide dieren in een kale omgeving te huisvesten. Uit de observaties bleek dat, hoewel de opgroeiconditie van dieren in sommige gedragingen tot uiting kwam, de actuele huisvesting het meest bepalend was voor het gedrag van varkens.

De invloed van actuele huisvesting en opgroeicondities op gedrag hing echter af van het type varken. LR dieren uit kale hokken vertoonden veel meer beschadigend gedrag dan HR dieren, terwijl in verrijkte hokken LR dieren meer tijd aan spelgedrag besteedden dan HR dieren (hoofdstuk 6 en 7). Bovendien hadden LR dieren uit kale huisvesting vaker ernstige maagwandbeschadigingen en maagzweren bij de slacht dan LR dieren uit verrijkte hokken, terwijl huisvesting minder invloed had op de maagwand van HR varkens (hoofdstuk 7).

Ook de invloed van opgroeicondities was groter in LR varkens. LR varkens die van kale naar verrijkte huisvesting waren gegaan, vertoonden meer wroeten en kauwen dan alle andere dieren, en, andersom, een verandering van verrijkte naar kale huisvesting gaf de sterkste afname in exploratief gedrag in de LR dieren.

Huisvesting beïnvloedde niet alleen het 'thuishok' gedrag van HR en LR dieren op een andere manier, maar ook de reactie op onbekende objecten (hoofdstuk 4), hun immuunreactiviteit (hoofdstuk 5) en het vóórkomen van maagwandbeschadigingen (hoofdstuk 7). Voor al deze kenmerken was het effect van huisvesting het meest opmerkelijk in de LR dieren. Toch waren de gecombineerde effecten van opgroei- en huisvestings-condities op groei juist het grootst in de HR dieren: HR dieren die op stro waren opgegroeid

en gehuisvest, groeiden harder dan de HR dieren die van verrijkte naar kale hokken waren gegaan (hoofdstuk 7). Varkens met uiteenlopende karaktereigenschappen verschillen dus in hun reactie op intensieve huisvestingscondities.

Tot slot

Hoewel de typering van varkens als HR of LR op grond van hun gedrag in één test, de rugtest, natuurlijk een versimpeling van de werkelijkheid is, kunnen uit de experimenten beschreven in dit proefschrift een aantal belangrijke conclusies getrokken worden. Om te beginnen heeft karakterisatie van varkens op een jonge leeftijd een voorspellende waarde voor de manier van omgaan met stressvolle omstandigheden in hun latere leven. Varkens met uiteenlopende reacties in de rugtest verschilden in agressiviteit en flexibiliteit in gedrag. Het verschil in respons op apomorfine tussen de twee typen varkens is een eerste aanwijzing dat deze gedragsverschillen een neurochemische achtergrond hebben. Verder is aangetoond dat LR en HR dieren verschillen in de manier waarop ze zich aanpassen aan intensieve huisvestingscondities. Bovendien moduleren individuele coping styles of persoonlijkheidskenmerken van varkens de effecten van omgevingsfactoren op hun gedragsontwikkeling, immuunreactiviteit en het vóórkomen van maagwandbeschadigingen. Opvallend genoeg was de impact van huisvesting (kaal of verrijkt) op de meeste bestudeerde variabelen het grootst voor LR dieren.

Individuele karaktereigenschappen van varkens beïnvloeden dus hun functioneren in verschillende (fysieke en sociale) omgevingen en zouden dus meegenomen moeten worden in studies naar de invloed van huisvesting op hun gedrag en welzijn. De kennis van individuele coping styles of persoonlijkheid van varkens zou kunnen worden uitgebreid en gebruikt om tot een optimale 'match' te komen tussen varkens en hun sociale en fysieke omgeving in de varkenshouderij.

Curriculum vitae

PERSONALIA

Jantina Elizabeth (Liesbeth) Bolhuis werd geboren op 14 maart 1974 te Groningen en groeide op in Stedum (tot 1977) en Uithuizermeeden. In juni 1991 behaalde zij haar VWO-diploma aan het Fivelcollege te Delfzijl. In september 1991 begon ze aan de studie Zoötechniek, met als oriëntatie Veehouderij, aan de (toenmalige) Landbouwuniversiteit te Wageningen. Deze studie werd in maart 1997 cum laude afgerond, met afstudeervakken Gezondheidsleer & Reproductie, Ethologie en Fysiologie van Mens & Dier en een stage bij het Schweizerischer Verband für künstliche Besamung in Zollikofen (Zwitserland). Van juli 1997 tot mei 2003 werkte ze bij de leerstoelgroepen Ethologie en Fysiologie van Mens & Dier van Wageningen Universiteit als onderzoeker in opleiding aan het in dit proefschift beschreven onderzoek, een onderdeel van het door NWO en LNV ondersteunde programma 'Grenzen aan Welzijn en Dierlijke Productie'. Vanaf mei 2003 was ze een aantal maanden aangesteld als (part time) toegevoegd docent bij de leerstoelgroep Ethologie van Wageningen Universiteit. Per 1 februari 2004 is ze werkzaam als post-doc bij de leerstoelgroep Adaptatiefysiologie van Wageningen Universiteit.

LIST OF PUBLICATIONS

Bolhuis JE, Schouten WGP, De Jong IC, Wiegant VM (1998). Individual differences in pigs: behavioural response to apomorphine. Proceedings of the 32nd International Congress of the International Society for Applied Ethology, Clermont-Ferrant, France, p 82 (abstract).

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Bolhuis JE (2003). Coping styles in pigs. In: Wiseman J, Varley M, Kemp B (eds). Perspectives in Pig Science. Nottingham University Press, Nottingham, pp 217-240.

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Bolhuis JE, Schouten WGP, Schrama JW, Wiegant VM. Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. Submitted.

Bolhuis JE, Schouten WGP, Schrama JW, Wiegant VM. Effects of rearing and housing environment on pigs with different coping characteristics. Submitted.

Gerrits WJJ, Rijnen MJA, Bolhuis JE, Schrama JW (2003). Influences of dietary fibre on physical activity and behaviour, and on its energy value for growing pigs. In: Wiseman J, Varley M, Kemp B (eds). Perspectives in Pig Science. Nottingham University Press, Nottingham, pp 475-490.

Koene P, Bokkers EAM, Bolhuis JE, Rodenburg TB, Urff EM, Zimmerman PH (2003). The open-field test: test of emotionality or an approach-avoidance conflict? Proceedings of the 37th International Congress of the International Society for Applied Ethology, Abano Terme, Italy, p 86 (abstract).

Van der Beek EM, Bolhuis JE, Karman AG, Van Eerdenburg FJCM, Wiegant VM (2000). Housing condition affects the correlation between volume of the dentate gyrus and the cortisol response to stress in pigs. Abstracts Society for Neuroscience, vol. 26, part 1, 30th Annual Meeting, New Orleans, LA (abstract).

EDUCATION AND TRAINING - Training and Supervision Plan, Graduate School WIAS

The Basic Package (3.0 cp)

WIAS Common Course (2001)

WIAS Course on Philosophy and Ethics (2001)

Scientific Exposure (12.0 cp)

International conferences, seminars, workshops and presentations

The 32nd International Congress of the International Society for Applied Ethology (ISAE), Clermont-Ferrant, France (1998) - oral presentation

WIAS Science Day (1998, 1999, 2002) - oral presentation

The 33rd International Congress of the ISAE, Lillehammer, Norway (1999) - poster presentation

Najaarsbijeenkomst van de Nederlandse Vereniging voor Gedragsbiologie, Dalfsen (1999, 2002)

The 34rd International Congress of the ISAE, Florianopolis, Brazilie (2000) - poster presentation

The 10th International Congress on Animal Hygiene, Maastricht (2000) - oral presentation

WIAS seminar 'Studies on Stress and Metabolic Adaptation', Wageningen (2001)

ISAE Benelux meeting 'Coping Styles in Pigs: Facts or Fiction?', Lelystad (2001) - oral presentation

Perspectives in Pig Science, Nottingham, United Kingdom (2002) - oral presentation

The 36th International Congress of the ISAE, Egmond aan Zee (2002) - poster presentation

Eindsymposium 'Grenzen aan Welzijn en Dierlijke Productie', Utrecht (2003) - oral presentation

The 37th International Congress of the ISAE, Venice, Italy (2003) - oral presentation

In-Depth Studies (3.1 cp)

Disciplinary and interdisciplinary courses, advanced statistics courses and discussion groups

TOPIG - Discussion group of WIAS PhDs working on pigs (1998-2000)

Projectgroep Stress en Welzijn Varkens - Researchers of UU, ASG/WUR working on pig welfare (1998-2003)

Course Behaviour and Genes, SLU, Skara, Sweden (2002)

WIAS Course Design of Animal Experiments (2002)

Professional Skills Support Courses (1.4 cp)

WIAS Course Techniques for Scientific Writing (1999)

Supervising MSc thesis work, OWI, Wageningen University (2000)

Guide to Digital Scientific Artwork, Wageningen University (2002)

Didactic Skills Training (10.6 cp)

Lecturing supervising practicals and excursions, supervising MSc theses

Discussiecolleges Integratievak Ethologie (1999, 2000, 2002)

Practicum Integratievak Ethologie (1999, 2000, 2002)

Capita selecta Gezondheidsleer en Reproductie (2000, 2001)

Supervision of 9 MSc students, 3 BSc students (1997-2002)

Management Skills training (1.0 cp)

Organization of seminars and courses

Member Organizing Committee of the 36th International Congress of the ISAE, Egmond aan Zee (2002)

Colophon