The effect of a switch in housing systems on the welfare of gilts: does experience matter?

Effekten av en ändring i inhysningssystemet, på gyltors välfärd: spelar erfarenheter någon roll?

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I hope you will enjoy reading this thesis!

Anne-Marieke C. Smid

Abstract

In general, three approaches towards animal welfare exist: animal bodies, natures and minds. These concepts respectively emphasise physical health, natural behaviour and mental health. Each approach has its own corresponding welfare indicators. Knowledge in the area of especially the more persistent emotional states (mental health) of animals is lagging behind. This has to be improved, since the assessment of the emotional states of animals is a crucial step in improving their welfare.

This study investigated the welfare of gilts before and after a switch in housing conditions (pens with concrete floor and pens with a wood shavings bedding) was made with a specific focus on their mental welfare. The first aim of this thesis was to investigate if the welfare of pigs housed under particular housing conditions (barren or on wood shavings) is influenced by their previous experience of housing conditions. The short and longer term effects of the switch in housing conditions on the pigs' welfare were investigated. This was done by physical, behavioural *and* mental welfare indicators. The second aim of this thesis was to investigate whether the results of all three welfare indicators (animal body, nature and mind) lead to the same conclusions about animal welfare.

For this purpose, 212 purchased gilts were used. The animals were kept from an age of four weeks (weaning age) until slaughter at ±24-25 weeks at the experimental farm Carus of Wageningen University. The experiment was set up using a 2 x 2 factorial arrangement, with housing conditions from weaning until 10 weeks of age (barren vs. enriched with wood shavings) and housing conditions after 10 weeks of age (barren vs. enriched with wood shavings) as factors. This resulted in the following groups: pigs housed for the complete experimental period on wood shavings (EE) or in barren pens (BB), pigs switched from barren pens to pens with wood shavings (BE) and pigs which were switched the other way around (EB). The switch in housing conditions was set at 10 weeks of age since the pigs were also used for a study that investigated the prevalence of Osteochondrosis which required this specific timing of the switch. However, for the present study, this timing was also very interesting since for fattening pigs, the switch from nursery to the growing-finishing phase includes a change in environment and normally also takes place at an age of approximately 10 weeks.

With regard to the physical welfare (body approach), ulceration of the pars oesophageal region of the stomach was investigated post mortem, since stress is one of the causes of gastric ulceration. The behaviour of the pigs (nature approach) was investigated by continuous live observations for 4*10 min per pen per week when the animals were eight weeks old (before the switch), 10 weeks old (2-3 days after the switch) and 17 weeks old. The frequency of play (indicative of a more positive emotional state), manipulative and aggressive behaviours (indicative of a more negative emotional state) were scored. A startle response test, a gain of reward and a relative contrast test were used to investigate the emotional states of the animals in a more direct way (mind approach) compared to the nature approach. In the relative contrast test, for four consecutive days, the animals were given twice daily a reward with high incentive value (mix of yoghurt and chocolate raisins) followed by four consecutive days in which a reward with lower incentive value (their normal feed) was provided. Thereafter, for four consecutive days, the reward with high incentive value was again provided. This resulted in a total of 24 trials. A cue indicated that the treats were about to be provided to the pig in each trial. After a 15-sec time-interval in which the anticipatory response was investigated, the food reward was thrown into the pen. This was the start of the test phase, which lasted for 45 seconds, during which the response of the pigs to the reward was investigated. This test was conducted once, after the switch in housing conditions.

With regard to the stomach scores, it was shown that post switch B (i.e. EB and BB) pigs had higher stomach scores, i.e. more stomach wall damage than post switch E (i.e. BE and EE) pigs $F(1,28)=5.28$, $p=0.0292$. Also the interaction of pre*post switch housing conditions tended to affect stomach scores $F(1,28)=3.25$, $p=0.0823$. Post hoc analysis revealed that EB pigs had a higher score than EE pigs with levels of BB and BE in between. Therefore, from the body approach, actual housing conditions seemed to be most important with enriched housed pigs experiencing a higher physical welfare compared to barren housed pigs. Experience of a different housing condition tended to be important, but only for pigs that were switched from an enriched to a barren environment (negative switch).

The behavioural observations revealed that pigs housed in barren pens showed more negative (manipulative) and less positive (play) behaviour compared to pigs housed on wood shavings (all $P<0.05$). Also a slight effect of experience was present in some behaviours (pre*post or pre*post*week effects). However, no straightforward results were obtained in this regard, only minor indications of the importance of experience existed with regard to both a positive (steep decline in manipulative behaviour and delay of decrease of play behaviour) and negative (steep decline in play behaviour) switch. By means of behavioural observations, it was shown that from the nature point of view, indications existed that experience of either a barren or enriched housings system is important, but only for the short term. Actual housing seemed to be the most important determinant of the welfare of pigs with pigs housed on wood shavings experiencing a higher welfare compared to pigs housed in barren pens.

With regard to the startle, gain and relative contrast test, it was shown that the relative contrast test gave the best indication of the mental welfare of the pigs. Enriched housed pigs showed more play behaviour in the test compared to barren housed pigs. They also seemed to be better in accepting the loss of reward and seemed to be less reactive and therefore less bored. Barren housed pigs seemed to be more bored, indicated by their high reactivity towards the presence of the observers, indicative of a less positive emotional state. They also seemed less capable of accepting the situation since they kept searching for the reward when their normal pellet feed was provided (all $P<0.10$). It was also shown that pigs that were switched from enriched to barren housing (i.e. EB pigs) were in a more apathetic state compared to pigs that were always housed barren (i.e. BB pigs). Based on the animal mind approach it could be concluded that actual housing seemed most important with pigs housed on wood shavings experiencing a better mental welfare compared to pigs housed under barren conditions. Also the importance of experience of housing conditions was shown, but only for a negative switch in housing system.

Based on the results, it can be concluded that experience of housing systems does matter for the welfare of pigs. This was indicated by all three welfare indicators. It was shown that the results of the three different welfare indicators lead to the same conclusions. However, with regard to the body (stomachs) and mind (relative contrast test) approach: experience seemed to be mainly important when a negative switch was made, while the nature (behaviour) approach indicated that experience seemed to be important in both a positive and negative switch. Also, the time effect of the experience differed between the welfare indicators. The nature approach indicated that mainly a short term effect was present, while the body and mind approach indicated that also a longer term effect of experience was present. Therefore, it is recommended that all <u>three</u> welfare indicators are used to obtain a complete view of the welfare of an animal.

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Review: animal welfare

1. Introduction

There is an emerging view that animal welfare should be more than just the absence of suffering (Duncan, 2005), i.e. that it should also include pleasure (Boissy et al., 2007). A lot of valuable data with regard to pleasure has already been generated, however, there is still not much known about more persistent affective (emotional) states in animals (Boissy et al., 2007). This has to be improved, since the assessment of the emotional states of animals is a crucial step in improving their welfare (Dawkins, 2008).
Therefore, this thesis will investigate the welfare of gilts before and after a switch in housing conditions (pens with concrete floor and pens with wood shavings bedding) is made, with a specific focus on their mental welfare. For this purpose, first, different approaches regarding the concept of animal welfare are given (section 3). In general, three approaches of animal welfare exist, emphasising animal bodies, natures and minds (Duncan and Fraser, 1997). These will be described in sections 4, 5 and 6 respectively. Each approach has its own corresponding welfare indicators which will also be described in the same sections. It should be mentioned that the three welfare approaches do overlap each other, which is the reason why it is very difficult to describe them completely separate from each other.
Based on the investigation of the three approaches as provided below, it appears that the approaches are interconnected as will also be described below. For good animal welfare and animal welfare assessment, elements of all three approaches with their corresponding indicators are important. However, research in the area of animal minds (i.e. mental states, including the before mentioned emotional states) is not yet as developed as the research in the area of animal bodies or natures.
The first aim of this thesis is to investigate if the welfare of pigs housed under particular housing conditions (barren or on wood shavings) is influenced by their previous experience of housing conditions. Since experience can be regarded as purely a mental matter, by investigating the importance of *experience*, the focus of this thesis will be on the mental welfare of the pigs and will thereby contribute to research in the area of animal minds, especially to research in the area of emotional states of animals. The short and longer term effects of the switch in housing conditions on the pigs' welfare will be investigated. This will be done by physical, behavioural and mental welfare indicators. The second aim of this thesis is to investigate whether the results of all three welfare indicators (animal body, nature and mind) lead to the same conclusions about animal welfare. This will give an indication of the reliability of the three indicators and it will also show if all three welfare indicators have to be used or if reliable animal welfare assessment can also do with only one or two indicators.

1.1 The concept of animal welfare

Over the past decades, there has been a major change in the way production animals are kept in the Western world. Roughly, before the twentieth century, animals were kept in husbandry systems. These systems were characterised by the fact that small numbers of animals were kept, in an environment for which they had evolved in and later been selected for. If the animals' needs determined by their biological and psychological natures were not respected this would lead to decreased performance of the animals and possibly failure to survive. Therefore, in husbandry systems the essence was care of the animal by respecting its biological and psychological needs: the productivity of the animal was directly linked to the animals' welfare (Pond, 2012).
Nowadays, production animals are mainly kept in industrialized systems (Pond, 2012). The main characteristic of industrialized animal production is intensification (Fraser, 2008 in:

Harfeld, 2010). Intensification can be found in a number of different areas, including: housing (i.e. more animals on fewer square meters, increased indoor housing systems) and in the amount of desired output (e.g. more muscle mass and faster growth in pigs and broilers, more milk from cows) (Harfeld, 2010).

Even though technological improvements brought advances in health care (e.g. better medical treatment of sick animals), there is no doubt about the current presence of all sorts of grave welfare problems (tail biting of fattening pigs, feather pecking of lying hens, lameness of cows and broilers etc.) in intensive animal husbandry systems (Harfeld, 2010). These problems originate from the fact that the change from husbandry based to industrial based animal production with their complete different characteristics as described before implied a major change in the core values of the traditional way of animal keeping: taking care of the animals' needs by respecting their natures. By the use of technological innovations like antibiotics, vaccines and the strong selection of animals to increase their genetic potential, animals could suddenly be kept under conditions that violated their natures, without a decrease in their productivity. By using technology, productivity became severed from animal welfare (Pond, 2012). Therefore, animal welfare became a concept of its own.

1.2 Approaches to animal welfare

Animal welfare is a very broad concept. It describes the state of an animal that can vary on a scale from very good to very bad and contains both physical and mental elements (Marchant-Forde, 2009). It is a characteristic of an animal, not something given to it (Appleby, 1999). The term animal welfare cannot be completely defined as a scientific concept because besides on information, it is based on values as well (Duncan and Dawkins, 1983). Therefore, to indicate the meaning of animal welfare, the underlying values should be set out instead of only defining a scientific term (Mason and Mendl, 1993).

Three main approaches exist in the description of animal welfare: animal bodies, animal natures and animal minds (Appleby, 1999). Each approach is based on different values. The different concepts will be explained below as well as their corresponding welfare indicators.

With regard to welfare indicators, it should be mentioned that animal welfare cannot be measured in the sense that it can be quantified like litres of milk, but it can be *assessed* by obtaining information to get insight into its various aspects and problems relating to them (Appleby and Hughes, 1997).

For both physical (body approach) and mental (nature and mind approach) welfare, a subdivision can be made between poor and good welfare. However, this only implies a rough distinction between good and poor welfare, since animal welfare varies on a continuous scale and the difference between poor and good welfare is sometimes difficult to draw. Poor welfare is associated with more obvious behavioural, physiological and pathological signs which is a reason why the assessment of poor welfare is more common than the assessment of good welfare (Broom and Johnson, 1993). Good welfare on the other hand is not defined by the absence of negative experiences but is rather the presence of positive experiences such as pleasure (Boissy et al., 2007). Since good welfare can only be obtained when poor welfare is absent, first poor welfare should be investigated to be able to prevent it. This is probably also an important reason why the assessment of poor welfare is more common than the assessment of good welfare.

With regard to welfare problems, Broom and Johnson (1993) made a subdivision of short and long term problems. Short term problems last a few hours, long term problems last a day or more. Some methods of trying to cope with problems are used for both short and long-lasting problems, but most methods are concerned principally with only the short or long term problems.

Sejian et al. (2011) propose the following four categories of welfare indicators: physical, and physiological indicators, production oriented indicators and behavioural welfare indicators, regarded by Marchant-Forde (2009) as all physical elements. However, this division leaves out the mental elements, including the emotional state of animals, which, considered by some, appears to be essential for good animal welfare assessment (Dawkins, 2008). Therefore, in this review, also indicators of mental welfare will be discussed.

2. Animal bodies

With regard to the concept of animal bodies, welfare is defined in terms of good physical health and functioning (growth, sustainable state of fitness) (Webster, 2005). It is based on the definition of Broom (1986): 'The welfare of an individual is its state as regards its attempts to cope with its environment.' It relies on the theory that welfare is concerned with the adaptive response of the animal to stress and the impact this has on its biological functioning (Marchant-Forde, 2009). In this approach, taking care of an animals' physical health will imply that its mental health is also taken care of (Appleby and Hughes, 1997).

A variety of definitions of the concept of animal health exists. Nordenfelt (2006) investigated these different definitions and concludes that complete health is the absence of disease, injury and defect. Disease is a state of the body and leads to a reduced function in relation to the biological goals of survival and reproduction. With regard to injuries and defects, the same is true.

The concept of animal bodies requires only physical welfare indicators to assess the welfare of animals, since according to this approach, good physical health equals good welfare. The physical welfare indicators could be direct and indirect (i.e. physiological) physical welfare as well as production related welfare indicators.

2.1 Physical welfare indicators

Impaired physical welfare arises when animals have diseases which are not (properly) cured and/or result in pain. It can also arise in the case when animals are selected for high production (e.g. fast growth, high milk yield, etc.) which can result in negative side effects. Since this thesis mainly focuses on the mental welfare of pigs, the physical welfare indicators will not be described as extended as the behavioural or mental welfare indicators.

2.1.1 Physical welfare indicators

Injuries cause pain and therefore lower the welfare of an animal. An inappropriate environment can cause injuries but also rough handling by animal handlers, or aggression between animals can lead to (severe) injuries. Injuries are in general easy to observe and can be used as indicator of impaired welfare.

(Chronic) stress is one of the risk factors of lesions of the pars oesophageal region of the stomach (Robertson, 2002). These lesions are also referred to as gastric ulcers and can be observed post mortem. The incidence of gastric ulcers can be used to indicate impaired welfare. However, it should be mentioned that other factors, such as feeding practices (ad libitum or not), fineness of feed particles (pelleted ration or not), infections with certain bacteria such as *H. heilmannii* type 1 (Queiroz et al., 1996) can affect the development of gastric ulcers too.

Also coping styles can influence gastric ulceration: pigs with a proactive coping style seem to be more sensitive to develop gastric ulcers compared to pigs with a reactive coping style (Hessing et al., 1994; Bolhuis et al., 2006).

Mortality rate can also be used as an indicator of poor welfare. Even though death itself is not a welfare problem for the animal that is dead (Webster, 1995), it is likely that its death has

been preceded by a period of poor welfare. Therefore, mortality rate can also be used as welfare indicator for a group of animals.
If an animal appears fit and healthy, is free from injuries and shows normal growth and function, this would, from the animal bodies' point of view, indicate good welfare. However, as mentioned above, the presence of e.g. a subclinical disease cannot be excluded based on observations alone. Subclinical diseases might not impair the welfare of an animal, however, they can develop into a clinical disease which negatively impacts the welfare of an animal. It is therefore important to also investigate possible subclinical diseases to prevent them from turning into clinical diseases. In this regard, physiological welfare indicators can provide additional information.

2.1.2 Physiological welfare indicators

With regard to physiological welfare indicators, a clear distinction can be made with regard to short and long term welfare problems. A distinction with regard to good and impaired welfare is less useful, since the physiological parameters themselves show if the welfare status of the animal is good (i.e. high heart rate variability) or bad (i.e. low heart rate variability) (McCraty et al., 1995). Examples of important physiological welfare indicators that can indicate short term welfare impairment have been used frequently in animal welfare studies are heart rate and some hormone levels. Heart rate increases when the level of physical activity of the animal, and therefore its metabolic rate, increases. Increases in heart rate can also occur before an action occurs (Broom and Johnson, 1993). Measurement of heart rate can be a useful measure of the emotional response of an animal to short-term problems, provided that the distinction is made between metabolic and emotional effects (Broom and Johnson, 1993). For example, heart rate increases when an animal runs from a predator, but also when it is engaging in a rewarding activity like mating (Appleby, 1999). Therefore, the context in which the heart rate is measured has to be taken into account. The measurement itself should also not have an effect on the animal (Broom and Johnson, 1993). It should also be mentioned that heart rate has a maximal level, caused by homeostasis (Beatty and Behnke, 1991). Therefore, a ceiling effect can therefore cause a problem. If e.g. two stressful events are compared and the animals show a maximal hearth rate in both situations, no conclusion can be made as to which event was perceived as more stressful.
Another characteristic of the heart rate which is also used as a welfare indicator, especially in more recent years is hearth rate variability (HRV). Healthy cardiac function has irregular time intervals between consecutive heart beats (Moss, 1995 in: von Borell et al., 2007). This is because heart rate is the net effect of the vagus nervus which decreases the heart rate and thereby increases the variability between consecutive heart beats and the sympathetic nerves which increases the heart rate. This variability between consecutive heart beats can be measured and thereby the vagal as well as the sympathetic activity can be measured (Rietmann et al., 2004). Cardiac vagal tone is influenced by the emotional state of an animal. It can be assessed by HRV analysis and it may be a more reliable indicator of negative and positive emotional states (Boissy et al., 2007) compared to heart rate measurements. However, in order to accurately analyse the complex characteristics of HRV, longer measurement periods are needed than for simpler heart rate measurements (Von Borell et al., 2007).

2.1.3 Production-related welfare indicators

As has already been mentioned, with the present technological innovations, the productivity of an animal is not a reliable indicator of good welfare anymore. On the other hand: it may be useful as *negative* evidence, to indicate poor welfare. If an animal shows lower production or stops to produce (e.g. in the case of pigs: if they show a lower growth rate or stop growing), something is definitely wrong (Appleby, 1999).

2.2 Animal bodies approach: an important basis

To summarize: physical health is the basis of good welfare. However, physical health does not equal good welfare. For example, an animal can be in a perfect physical condition, however, at the same time it can experience poor welfare because it is unable to carry out certain behaviours which will lead to frustration and therefore to impaired welfare. Therefore, in contrary to what this approach states, taking care of an animals' physical health not necessarily means that its mental health is also taken care of.

Duncan and Petherick (1991) go even further. They state that the presence of a disease does not necessarily imply poor welfare. This holds true, for example in the case of a subclinical disease of which the animal is not aware: it is pathologically sick but it can feel completely normal. Welfare is therefore more than solely physical health.

The concept of coping, which forms the basis of this approach, is also not as straightforward as it may seem to be. According to Duncan (2005) the term 'coping' suggests that it is explaining something, however, this is not true: a failure of an animal to cope with its environment will result in impaired welfare and if nothing is done, the animal will die. However, the converse is not true. An animal that is coping cannot be said to experience good welfare. An example is a lame pig which lies down much longer than non-lame pigs to avoid the use of its lame leg, which causes pain. The animal is coping by adjusting its behaviour, it is coping, however, its welfare is definitely impaired.

With regard to the physical welfare indicators, especially the physiological indicators, most of them are only useful when the context they are taken in is also taken into account. Direct physical welfare indicators can provide the wrong information, in the case of e.g. a subclinical disease. The behaviour of an animal can provide valuable additional information about its welfare status. The fact that behaviour depends on the context does not pose a problem here, since the behaviour itself will reflect the welfare experienced by an animal in a certain situation, thereby showing how aversive or appropriate a certain context is. Behaviour and its relation to welfare forms the basis of the second approach to animal welfare: animal natures.

3. Animal natures

From the point of view of the animal natures approach, the welfare of an animal depends on the possibilities to perform natural behaviour and to live under natural conditions (Appleby and Hughes, 1999). According to Rollin (in: Appleby and Hughes, 1997) each animal species has an inherent, genetically encoded nature, its: 'telos', which should be respected in order to obtain good welfare. It is an Aristotelian concept which indicates the nature of animals: 'the pigness of the pig, the dogness of the dog – 'fish gotta swim, birds gotta fly' (Appleby, 1999). Kiley-Worthington (1989) supports this view. She states that: 'If we believe in evolution, then in order to avoid suffering, it is necessary over a period of time for the animal to perform all the behaviours in its repertoire.' According to Fraser (2008), natural behaviour falls, roughly, into three types: behaviours that animals want to do (e.g. eating, playing), some things they want to do only if the conditions require it (in the case of pigs: e.g. wallowing) and behaviour that animals generally not want to do (e.g. shivering in the cold, fleeing from predators). In this regard, the concept of ethological needs is important. This term indicates normal patterns of behaviour, which, if animals are unable to carry them out, will result in frustration and therefore in impaired welfare (Hughes and Duncan, 1988).

3.1 Ethological needs

Two different types of needs exist: ultimate and proximate (Dawkins, 1983). Ultimate needs are needs which, if they are not met, result in reproductive failure and in the extreme: death of the animal. Examples of ultimate needs are food and water. Failure to meet proximate needs do not result in death or loss of reproductive success, but it may cause the animal to suffer nevertheless. Behavioural needs are proximate needs (Dawkins, 1983). The term behavioural need is used to imply that frustration will result if a behaviour is prevented that an animal is motivated to perform (Appleby and Hughes, 1997).

In order to determine how behavioural deprivation affects animal welfare it is important to know if the factors which govern the behaviour are internal or external. It is also important to determine if the factors that reduce the motivation to perform the behaviour require the performance of the behaviour itself or only the functional consequences of the behaviour (Appleby and Hughes, 1997).

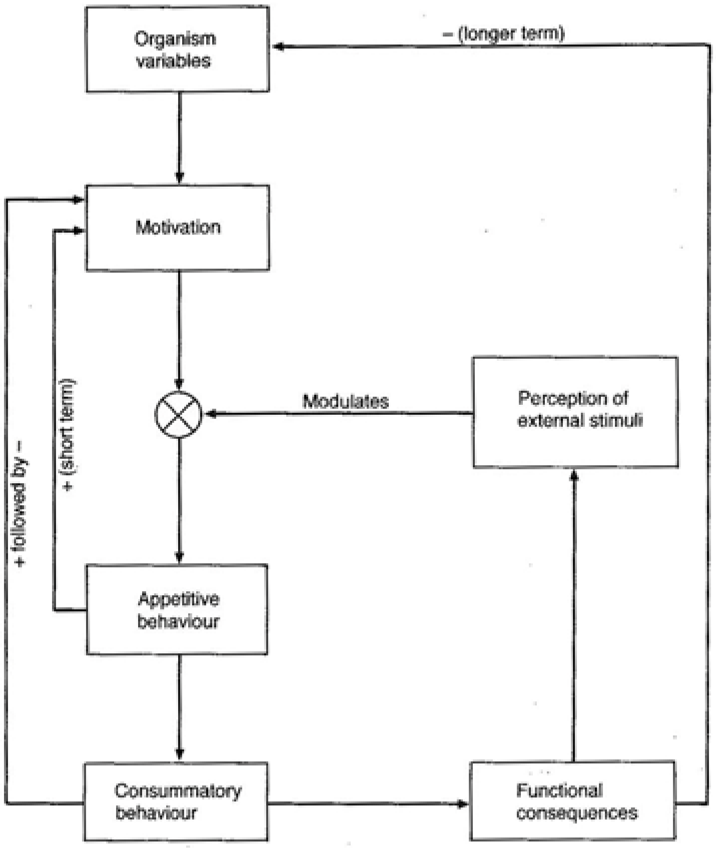

Figure 1. Model of motivation (Hughes and Duncan, 1988)

This can both be explained with help of the ‘model of motivation’ constructed by Hughes and Duncan (1988) (Figure 1). In this model, the functional consequences of the behaviour act as a negative feedback, switching off the motivation of the animal to perform a particular behaviour. This negative feedback acts through organism variables such as physiological changes (Appleby and Hughes, 1997).

With regard to behaviours that are triggered by external factors, the animal may get into a closed loop from which it cannot escape in the case when it cannot obtain the functional consequences of its behaviour. This is the cause of inappropriate behaviour, as for example bar biting in pigs. Inappropriate behaviour has become divorced from its functional consequences and can be characterized as being repetitive, stereotyped and/or persistent (Appleby and Hughes, 1997).

For any behaviour which is largely governed by internal factors, motivation levels will at some point increase above threshold. This will trigger appetitive behaviour, which is the exploratory, goal seeking phase of behaviour that normally precedes the consummatory behaviour when the goal of the behaviour is reached (McFarland, 1981 in: Hughes and Duncan, 1988). However, in intensive environments it is impossible to proceed to the consummatory sequence. Therefore, the appetitive behaviour will continue, sometimes in an abbreviated or incomplete form, which will increase the motivation further and the animal is again in a loop from which it cannot escape (Appleby and Hughes, 1997).

Therefore, the welfare of animals is impaired in situations where motivation reaches high levels but the consummatory behaviour cannot be performed and/or when the interaction of the appetitive behaviour with the environment does not provide the appropriate functional consequences to the animal (Appleby and Hughes, 1997). This will lead to abnormal or damaging behaviours, which are indicative of impaired welfare.

With regard to food directed behaviours, pigs in barren pens cannot perform the appetitive behaviour (e.g. rooting), however, they can nevertheless move on to the consummatory stage (e.g. ingesting of the food). Therefore, in this case, the interaction of the appetitive behaviour with the environment does not give them the appropriate functional consequences, which is frustrating for pigs since they show a high motivation to root (Studnitz et al., 2007).

The motivation to perform rooting behaviour is therefore redirected towards pen mates or occurs as vacuum activity (Hughes and Duncan, 1988). If straw or other rooting materials are present, the rooting behaviour is directed towards these materials, even though the animal already obtained the functional consequences (i.e. a full stomach).

It has been shown by Ladewig and Matthews (1996) that pigs are willing to work quite hard to obtain access to wood shavings, sawdust, straw bedding etc., which are all materials by means of which they can perform rooting behaviour in a reinforcing way. It also appears that the slope of the demand functions of these materials is very low, indicating that the importance of these materials is very high for pigs.

Among others based on these observations, it can be concluded that rooting is a behavioural need for pigs.

3.2 Behavioural welfare indicators

Behaviour is what animals do to change and control their environment. It therefore provides information about their needs, preferences and internal states (Appleby and Hughes, 1997).

3.2.1 Behaviours indicative of impaired welfare

The changes in the husbandry and housing environments of pigs have occurred relatively rapidly, both with regard to evolutionary time and with regard to the domestication history of the pig. This has led to welfare problems because the pigs' behavioural needs cannot be fulfilled by the environment they are, in general, kept in (Marchant-Forde, 2009). An important finding is that even modern bred pigs show a complete behavioural repertoire when released into extensive environments (Marchant-Forde, 2009). Stolba and Wood-Gush (1989) showed that intensively farmed pigs show different patterns of behaviour from those of wild or free-ranging pigs, generally expressing a smaller behavioural repertoire, which includes apparently functionless activities such as stereotypic behaviours as bar biting and excessive drinking, which are probably the inappropriate expression of frustrated patterns of natural behaviour. A satisfactory environment for an animal is an environment in which it can perform constructive acts to control its environment. In general, the source of suffering arises not so much from the primary stress but from the fact that the animal is denied the opportunity to control it. This results in frustration and stress. Behavioural patterns associated with frustration, and thus indicative of impaired welfare, include: displacement behaviour, rebound behaviour, stereotypic behaviour, learned helplessness and redirected behaviour (Webster, 1995).

3.2.1.1 Displacement behaviour

This type of behaviour is indicative of short term welfare problems (Webster, 1995). Displacement behaviours are activities that are displayed in a conflict or in a frustrating situation (i.e. when animals are unable to perform certain behaviours). The behaviours are apparently irrelevant and out of context with the activity immediately preceding or following them (Wood-Gush et al., 1975 in: Appleby and Hughes, 1997).

An example of this with regard to pigs is bar biting. Pigs are highly motivated to perform foraging behaviours, especially rooting. When they are not able to perform this behaviour they become frustrated and may develop displacement behaviours such as bar biting. Displacement behaviours might develop into stereotypic behaviours. They are used in welfare assessment as evidence that an animal is motivated to perform a type of behaviour that the environment does not permit, which will lead to impaired welfare (Fraser, 2008).

3.2.1.2 Rebound behaviour

Rebound behaviour is indicative of a long term welfare problem (Webster, 1995). When an animal is prevented from performing a particular behaviour to satisfy a behavioural need, this will cause it to perform the behaviour with increased intensity and for an unusually long time once it has the opportunity to perform it again: the rebound effect (Appleby and Hughes, 1997; Webster, 1995). This rebound is often taken as the evidence of an internal motivation that continues to increase while the animal is unable to perform the necessary behaviour (Appleby and Hughes, 1997). For pigs, rooting behaviour is one of the behaviours that can rebound after it has been prevented for a while.

However, McFarland (1989 in: Appleby and Hughes, 1997) suggested that animals may show an exaggerated behavioural response just because the stimulus is re-presented after a period of time. If this holds true, there would have been no increase in motivation during the period of deprivation and welfare would therefore not have been impaired, which would imply that this is not a good welfare indicator.

3.2.1.3 Stereotypic behaviour

Stereotypies are generally defined as unvarying, repetitive behaviour patterns that have no obvious goal or function (Fox, 1965). They are not part of the normal functional systems of the animal and they might be a pathological sign of a method of coping with difficult conditions (Broom, 1983), which is supported by the fact that stereotypies have been associated with barren and restrictive environments (Levy, 1944; Hediger, 1955; Morris, 1964; Meyer-Holzapfel 1968; Hinde, 1970 in: Mason, 1991) in which not all necessary behaviours can be performed.

With regard to pigs, stereotypies especially occur in sows which are kept in individual stalls (normal practice in e.g. the USA) or farrowing pens (normal practice in intensive systems all around the world) and/or are restricted-fed. They develop oral stereotypies such as chewing and biting their chain and bars of the stall, sham chewing (chewing without anything in their mouths) and manipulations of their drinker (Lawrence and Terlouw, 1993; Marchant-Forde, 2009).

If stereotypies indeed result from the frustration of specific motivational systems (Rushen et al., 1993), then their display indicates that an animal is still motivated to perform the behaviour. The results of a study by Keiper (1969) with caged birds support this. In this study with caged birds it was found that stereotypies such as route-tracing were reduced when the birds were kept in a very large aviary cage and spot-picking, another stereotypy, was reduced when canaries were required to work for their food. Therefore, stereotypies can be taken as evidence for behavioural deprivation. They develop over time, which is why they are indicative of longer term welfare problems.

3.2.1.4 Learned helplessness

Learned helplessness defines the state of mind of an animal that ‘has given up’ (Webster, 1995). It is a response to chronic stress (Friend, 1991). The key feature of this state it is that the animal will no longer even attempt to control its environment, even though the means of such control might be available to it (Curtis and Stricklin, 1991).

Farm animals may have all the necessities to survive, in contrary to wild animals, but are very likely to experience real suffering as a consequence of the inability to make any constructive contribution to the quality of their own existence. This is because they cannot generate a meaningful response towards the perceived information of their surroundings (Appleby and Hughes, 1997; Webster, 1995). This has been described as adaptation to barren environments and referred to as: learned apathy or learned helplessness (Webster, 1995). Such a situation deprives animals of the very core on which their existence is based, namely: the ability to act. This may lead, simultaneously or alternately, to apathy and high levels of anxiety (Rowan, 1988; Wood-Gush and Vestergaard, 1989). Environmental challenge thus appears important for welfare, its absence may render animals unable to cope (Appleby and Hughes, 1997). According to Webster (1995), the lack of opportunity to control challenges may be one of the greatest insults to the welfare of animals.

Intensive pig production systems are very monotonous and may give rise to chronic apathy and boredom (Newberry et al., 1988; Wood-Gush and Vestergaard, 1989). Inactivity might be an example of learned helplessness in pigs (Marchant-Forde, 2009). Therefore, the level of activity might be an indicator to assess the welfare of pigs. However, according to Schouten (1985) a higher level of restlessness is found in piglets living in an impoverished environment, among others due to a higher incidence of manipulative behaviour. Therefore, when using this parameter, the causes of restlessness should be taken into account and should be eliminated when analysing activity levels.

3.2.1.5 Redirected behaviour

Redirected behaviours are normal activities that are redirected towards an unusual or inappropriate substrate. It arises in captive environments that do not provide a full range of foraging materials. If animals redirect their normal foraging behaviours towards their conspecifics, severe welfare problems can result for the recipients (Webster, 2011).

Manipulative behaviour such as tail biting and belly nosing are redirected behaviours. According to Schouten (1985), manipulative behaviour depends mainly on the *actual* situation of the animal. Bolhuis et al. (2006) found that pigs that switched from enriched to barren housing conditions showed an increase in the time they spent on manipulative behaviour directed at their pen mates as compared to pigs that were always housed barren. In the case of pigs that, particularly in barren environments, tend to redirect their exploratory behaviour towards pen mates (Petersen et al., 1995), this behaviour can result in severe welfare problems including infections, abscesses and even death of the recipients. Therefore, (the amount of) non-nutritive oral manipulative behaviour of pigs can both be a symptom and cause of reduced welfare in pigs and can thus be used as a welfare indicator (Olsen et al., 2002). This oral manipulative behaviour can result in skin, tail and ear lesions. The lesion score (amount and severity of the lesions) can therefore be used as behavioural and physical welfare indicator (since the lesions cause pain).

3.2.2 Behaviours indicative of good welfare

From the point of view of the animal natures approach, good animal welfare includes the complete absence of behavioural patterns indicative of impaired welfare (as described above) and the presence of behaviours indicative of good welfare (i.e. play, which will be described below). This means that pigs are given the opportunity to carry out behaviours which they are highly motivated to perform.

The main behaviour that is indicative of good welfare is play behaviour. The level of synchrony of activities between pen mates (Schouten, 1985) might also be indicative of good welfare.

3.2.2.1 Play

Several hypotheses with regard to the biological functions of play have been made. A common aspect of these hypotheses is that they all state that playing as young animal results in better performance of some serious, more adult form of behaviour later in ontogeny and a better capacity to cope with sudden losses of control (Martin and Caro, 1985; Spinka et al., 2001).

Play behaviour might only be performed when all primary needs (such as food, rest, safety, etc.) are satisfied (Newberry et al., 1988). This is supported by the results of several studies. The study of Barnes et al. (1976) induced a malnourished state in piglets. These piglets spent less time playing with objects than their well-nourished conspecifics. Muller-Schwarze et al. (1982) showed that White-tailed deer reduced play by 35 percent when experimentally 33 percent milk shortage was induced. However, even though there was a reduction in play behaviour, the fact that play still persisted under conditions of suboptimal nutrition shows the importance of play. This finding therefore cautions the use of solely this behaviour as indicator of good welfare. Panksepp et al. (1985) deprived rat pups of food and showed that food deprivation of 24 hours or more reduced the levels of play. However, only a single meal appeared to be sufficient to return the play behaviour to baseline levels. This also shows the importance of play behaviour. An observational study by Barrett et al. (1992) showed that gelada baboons decreased both the quantity and quality of play behaviour during the dry season, due to lower dietary quality and more widely dispersed food resources.

It follows from the previous that a reduction of play behaviour might be indicative of some form of stress (Dybkjaer, 1992) since it is sensitive to adverse physical and environmental conditions (Donaldson et al., 2002).

Play behaviour is more observed in enriched environments and in environments which provide more space and freedom of movement (Johnson et al., 2001; Chaloupková et al., 2007), which result in less stress experienced by the animal and therefore a higher welfare status of animals kept under these conditions. It is therefore that play behaviour seems to be an explicit indicator of enjoyment (Newberry et al., 1988) and indicative of a positive affective state (Spinka et al., 2001). This is why the frequency of play behaviour might be indicative of good welfare (Buchenauer, 1981 in: Newberry et al., 1988).

3.3 Animal natures approach: a valuable additional approach

Summarizing, the animal natures approach states that the welfare of an animal is good if it can perform natural behaviour and lives under natural conditions.

However, good welfare does not necessarily require that animals can display their *full* behavioural repertoire. Running away from a predator is part of a prey animals' behavioural repertoire, however, it is very likely that the display of this behaviour does not lead to improved welfare, it is likely to even lead to (short term) impaired welfare.

Also: an animal that lives under natural conditions and that can display its natural behaviour but is severely underweight is not likely to experience a good welfare either. The mental and physical welfare of an animal are in general related: a disease has an effect on an animals' body but also on its mind (Appleby and Hughes, 1997). It also works the other way around: a stressed animal is more susceptible to disease (Biondi and Zannino, 1997).

It follows from the previous that the animal bodies and animal nature approach have added value towards each other and their corresponding indicators can be used together to obtain a more complete indication of the welfare status of an animal.

In this regard, it can also be stated that the physical welfare of an animal *and* the possibilities to perform certain behaviours and live under natural conditions determines how it *feels*. It might therefore be that solely the mental welfare of animals is important for their overall welfare and therefore only has to be investigated in order to be able to give a complete and valid conclusion about an animals' welfare. This is exactly what the animal minds approach proposes.

4. Animal minds

The concept of animal minds states that welfare is solely dependent on what animals feel (Duncan and Petherick, 1991). Therefore, welfare is a term which can only be applied to sentient animals, i.e. animals capable of feeling. Good animal welfare in this view implies mental satisfaction or, at least, freedom from mental distress (Webster, 2005). It implies the absence of strong, negative, subjective emotional states, together referred to as: suffering. It includes the absence of states as pain, fear, frustration, deprivation and in some species: boredom. Positive emotional states, referred to as 'pleasure', also exist (Duncan, 2005) and are important for good welfare.
Dawkins (1988) states a similar view: 'to be concerned about animal welfare is to be concerned with the subjective feelings of animals, particularly the unpleasant subjective feelings of suffering and pain.' Duncan and Petherick (1991) state that when an animal is ill, it will also feel ill. Therefore, taking care of an animals' mental health (i.e. how it feels) will automatically imply that its physical health is also taken care of.
However, knowledge in the area of especially the more persistent emotional states (mental health) of animals is lagging behind. Some promising approaches/techniques exist, some of which are adapted from human psychology.

4.1 Indicators of mental states

Behavioural and physiological indicators form the basis for most current indicators of the emotional states of animals. However, there are some shortcomings of these indicators. They often are a good measure of arousal, which is the intensity of emotions (how activated an animal is), but are less useful to indicate emotional valence (i.e. whether the emotional state is positive or negative (Watson et al., 1988; Russell, 2003). If the indicators provide a good indication of emotional valence, most of them are only useful to indicate negative valence. Even though of increasing interest, assessment of positive emotional states, by means of indicators which can investigate positive valence, is lagging behind. Therefore, also other, additional approaches to assess the emotional state of animals should be investigated. Examples of such approaches are the relative contrast test and startle test. These will both be described below.

4.1.1 Relative contrast tests (gain and loss of reward)

A relative contrast test makes use of the fact that a certain reward is valued according to its relative value in comparison with other rewards (Freet, 2010). Therefore, the effect of a given reward on the behaviour of an animal is, in part, relative to the animals' experience with rewards of different amounts or qualities. Relativity effects occur when changing abruptly from an expected reward to a differently valued one (successive contrast).
Two different contrast effects can be made: either a successive negative or positive contrast effect (SNC and SPC respectively). A SNC and SPC effect describes the change in the behaviour of an animal following respectively a decrease and increase in the qualitative or quantitative value of an expected reward. The response changes must fall below that of control animals which have only ever experienced the lower or higher reward value in order for the effect to be considered an SNC or SPC (Flathery, 1996). The induction of a positive contrast can be seen as a gain of reward and the induction of a negative contrast as a loss of reward.

Burman et al. (2008) state that sensitivity to reward loss and gain may reflect emotional valence in animals. Human studies have indicated that people in negative affective states show stronger behavioural responses to loss (Hajcak et al., 2004; Chiu and Deldin, 2007; Tucker and Luu, 2007 in: Burman, 2008). A similar relationship in animals would result in a stronger aversive response to loss if animals are in a negative affective state, which was observed in the study of Burman et al. (2008). They showed that rats which were housed in barren cages, likely to experience a lower welfare and therefore likely to be in a more negative emotional state, displayed a prolonged response to a decrease in anticipated food reward, indicative of an increased sensitivity to reward loss.
The study of Rosas et al. (2007) showed that more fearful rats showed a greater SNC than less fearful rats. Barr and Philips (2002) found that rats which experienced the negative effects of drug withdrawal showed an increased and prolonged SNC response. Animals in a negative affective state may have a lower expectation that the reward will return to its original size (Paul et al., 2005) which lengths the response to reward loss (Burman, 2008).
Therefore, sensitivity to reward reduction (and possibly also gain) may be indicative of animal emotion and welfare. A relative contrast test might therefore provide a reliable indication of the mental states of animals. In general, more studies show a negative rather than a positive contrast effect. Negative contrast effects seem to be a more robust phenomenon that is easier to detect. One of the reasons of the better detection of negative contrast effects is the fact that ceiling effects might confound the positive contrast effects. In a typical contrast experiment an animal is trained to perform a certain task by using reinforcers. When the reward is changed, the animal changes its behaviour, the response is slower/faster or more/less vigorous than that of the control group. If e.g. the latency to reach a certain reward is used to measure the effect of an increase of reward, an animal that is running at top speed to a certain reward is unable to increase its speed if the reinforcement is increased (Boissy et al., 2007). This poses a problem which can be circumvented with the quantitative use of behaviours that are displayed in response of the loss or gain of reward which themselves are indicative of positive emotional states (e.g. play) and negative emotional states (e.g. animals that kept lying down in the test). Therefore, in this thesis the response of the gilts to a certain cue and subsequent a certain food reward will be investigated by means of the scoring of these behaviours both in anticipation time and in response to the rewards (i.e. test time). This way, the animals do not have to run and no ceiling effect exists which can influence the results.

4.1.2 Startle response test

The startle reflex is part of a complex reaction of an animal in response to changes in its environment. The exposure to a new, sudden stimulus halts the animals' current activity and initiates the display of an orienting response. The most pronounced symptoms of this response are: increased blood pressure, muscle contraction and looking in the direction of the stimulus (Pavlov 1927, Sokolov, 1963 in: Blaszczyk and Tajchert, 1996). These symptoms seem to prepare the animal for a certain response which is essential for the animals' survival. Depending on the behavioural context of the stimulus this response will either be a freezing or escape response (Blaszczyk and Tajchert, 1996).
This startle reflex appears to be influenced by the emotional state of the organism. Emotions can be defined as action dispositions and are founded on brain states that organize behaviour along a basic appetitive-aversive dimension. All affects are presumed to be associated with either a behavioural set that favours the more positive behaviours: approach, attachment and consummatory behaviour, or a set disposing the organism to display more negative behaviours such as: avoidance, escape and defence behaviour. It is presumed that the efferent system of the body is tuned according to the current status of this central affect-motivational

organization, also referred to as emotional state. Reflexes that have the same valence as the current emotional state are increased whereas reflexes with another valence are inhibited. It follows from this that the startle reflex, which is in itself an aversive reflex, is enhanced during negative emotional states and diminished during positive emotional states (Lang et al., 1990). Enhanced in this regard means that the startle response occurs faster and with a greater amplitude (Lang et al., 1998).

Not very much experimental data exists so far with regard to the effect of the emotional state of an animal on its startle reflex. A study conducted on humans by Vrana and Lang (1990) however clearly shows that the amplitude of the startle response was significantly higher in students during the processing of sentences that evoked fear responses than during the processing of neutral sentences. A similar result was found in the study of Lang et al (1998) showing that rodents reacted with a startle response under negative emotional states faster and with a greater amplitude.

4.2 Animal mind approach: completion of the concept of animal welfare

According to the animal mind approach, animal welfare depends only on the feelings of animals. Good animal welfare consists of the absence of suffering and the presence of pleasure. When using this approach, the welfare of an animal only has to be assessed with mental welfare indicators, since, according to Duncan and Petherick (1991) good mental health will automatically imply good physical health.

However, an animal that is suffering from a subclinical disease for example, of which it is not (yet) aware, feels perfectly fine but pathologically seen it is sick. Therefore, the use of solely mental welfare indicators to assess the welfare of animals, will not lead to valid conclusions about animal welfare, at least not for the longer term.

The assessment of an animal’s mental welfare does, however, provide valuable additional information to the information obtained from physical and behavioural welfare assessment. Duncan (2005) states that even though variables to determine good biological functioning are quite easy to measure in an objective way, they are not sufficient in welfare assessment. The objective determination of feelings, which are poorly defined and can only be communicated indirectly, is much more difficult but essential to give a valid conclusion about an animals’ welfare status (Duncan 2005; Webster, 1995).

Therefore, it can be concluded that all three approaches provide important added value to each other. Animal welfare assessment should thus be based on a combination of physical, behavioural and mental welfare indicators to obtain a complete view of the welfare status of an animal. It is therefore that, especially in the field of mental welfare assessment of animals (especially the assessment of their emotional state) still a lot of progress has to be made. This, as already mentioned, forms the basis of this thesis. In the next section research in the area of mental welfare will be described, with a specific focus on the influence of the experience of certain housing conditions on emotional states of animals.

5. Experience and animal welfare

Pigs housed in enriched environments, especially if the enrichment concerns suitable rooting materials, such as straw or peat, have a better overall welfare than pigs housed in barren pens (Lyons et al., 1995; van de Weerd and Day, 2009). This is because rooting materials provide a stimulus and outlet for exploratory and manipulative behaviour which involves the snout and mouth. An example is rooting, a behaviour that pigs are highly motivated to perform. Kelly et al. (2000) showed that pigs housed in pens with straw showed less pig-directed and pen-directed behaviour, associated with increased welfare, relative to pigs housed in barren pens. In the absence of rooting materials, exploratory and manipulative behaviour is often redirected to pen-mates (Fraser et al., 1991). Therefore, pigs housed in a barren environment will likely be in a more negative affective state whereas pigs housed in pens that contain rooting materials will be in a more positive affective state.

In the study of Douglas et al. (2012), the affective states of pigs were assessed by means of a cognitive bias test. The affective state of an organism has an effect on its cognitive processes, generally referred to as 'cognitive bias' (Mendl et al., 2009). In a cognitive bias test, positive, negative and ambiguous cues are presented and the subjects' response are recorded. From human psychology, it is known that positive states are associated with an increased likelihood of judging ambiguous information positively (optimistic cognitive bias). Douglas et al. (2012) showed that also pigs have indeed more optimistic judgement biases when housed in enriched environments compared to pigs housed in barren environments, indicative of a more positive affective state and a better welfare. It appears that experience also plays an important role in this view: pigs that have spent time in an enriched environment react more negatively to the absence of enriching material when they are subsequently housed in a barren environment then pigs that have always been housed in a barren environment. Day et al. (2002a) found that pigs that were housed in a strawed pen and were moved to an unstrawed accommodation, showed a higher occurrence of pen-mate-directed behaviour compared to pigs housed in unstrawed pens with no prior experience with straw. Also other studies indicate that the rearing history of animals influences their welfare. In the study of Bolhuis et al. (2006) the environmental conditions of pigs were changed at 10 weeks of age (barren pens and pens with straw bedding). It was observed that the actual absence or presence of straw largely determined the behaviour of the pigs. However, pigs that were switched from enriched to barren pens showed a higher inactivity level compared to the inactivity level of pigs which were raised and kept in barren pens. Since inactivity might be a reflection of learned helplessness in pigs (Marchant-Forde, 2009), higher levels of inactivity might be indicative of impaired welfare. Since the pigs housed on straw beds have generally a higher overall welfare compared to pigs housed in barren environments, this higher level of inactivity supports the view that higher levels of inactivity are indicative of impaired welfare. Another observation made by Bolhuis et al. (2006) was that the impact of the rearing history appeared to differ between coping styles. The impact of the rearing history on chewing, manipulative behaviour and play behaviour later in life appeared to be larger for low resisting pigs compared to high resisting pigs. A study conducted by Latham and Mason (2010) found that mice in a barren environment that were previously housed in an enriched environment developed more severe stereotypic behaviour and showed a higher motivation to access enrichments than their conspecifics that were raised from birth in barren environments. This indicates that mice that have experienced a good quality environment might have lasting motivational differences from mice raised under barren conditions. This suggests sustained behavioural effects which are related to the frustration of the loss of enrichment. If this is indeed true and if this effect can be parallelized to pigs, this can have significant implications for the welfare of pigs in the pig industry, since in the progressive stages of the life of pigs there is sometimes a reduction in environmental complexity (Douglas et al., 2012).

Therefore, based on the observation that research in the area of emotional states of animals is still lagging behind and the possible consequences of a loss of enrichment for the emotional state of pigs, this thesis will investigate the effect of a switch in housing systems (barren or on wood shavings) on the welfare of gilts, with a specific focus on their emotional state. Differences in the welfare of the pigs in the post switch environment compared to pigs that do not switch housing conditions will be caused purely by their experiences. This will probably result in different emotional states of these pigs compared to pigs that do not switch conditions.

6. Aim and research questions

Based on the previous, this thesis has two aims. First of all, to investigate whether the welfare of pigs housed under certain housing conditions (barren or on wood shavings) is influenced by previous experience of housing conditions. The short and longer term effects of the switch in housing conditions on their welfare will be investigated. This will be done by physical, behavioural *and* mental welfare indicators. The second aim of this thesis is to investigate if the mental welfare indicators give consistent results with regard to the physical and behavioural indicators.
With regard to the first aim: the physical health of the pigs will be assessed by measurements of skin lesions, damage to ears and tail, and, post mortem, gastric ulceration will be scored. Also records of diseases will be made. The behavioural welfare indicators will be obtained by means of live behavioural observations which will investigate the manipulative, aggressive and play behaviour of the pigs. The startle, relative contrast and gain of reward test will investigate the mental welfare of the pigs, i.e. they will investigate their emotional state. The second aim is to investigate if the results of all three welfare indicators (animal body, nature and mind) point in the same direction with regard to animal welfare. For this purpose, the conclusions about animal welfare derived from the three welfare indicators will be compared to see if they give consistent results.

The following research questions are stated to investigate these aims:

- Do pigs housed on wood shavings display more play behaviour and less manipulative and aggressive behaviours compared to pigs housed under barren conditions? Is there a change in the frequency of these behaviours when pigs are switched from barren to wood shavings housing or vice versa?
- Do pigs react more positively to a gain in reward (a jute sack is provided as a replacement of a less preferred toy), indicated by a higher frequency of interaction with the jute sack and more play behaviour, when housed on wood shavings compared to pigs housed under barren conditions? Is this reaction changed when a switch in treatment is made?
- Do pigs react more negatively to a loss of reward (chocolate raisins are provided several times after which they are replaced by a less preferred feed) and a subsequent gain, indicated by their behaviours during anticipation and provision of the reward, when housed under barren conditions compared to pigs housed on wood shavings? Is this reaction changed when a switch in treatment is made?
- Do pigs housed on wood shavings show a less intense startle reaction (longer latency to react and shorter freeze/escape response) when a loud acoustic stimulus is given compared to pigs housed under barren conditions? Is this reaction different for the groups that switched housing conditions?
- Do pigs housed on wood shavings have a better health score indicated by a lower incidence of gastric lesions and ulcers compared to pigs housed under barren conditions? Is there a change in these scores when pigs are switched from barren to wood shavings housing or vice versa?
- Do the results of the physical, behavioural and mental welfare indicators lead to the same conclusions with regard to the welfare of pigs?

It is hypothesised that the welfare of pigs kept on wood shavings for the complete experimental period is better than the welfare of pigs housed under barren conditions for the same period. It is therefore hypothesised that pigs housed on wood shavings for the whole experimental period score better for all of the parameters compared to pigs housed under barren conditions for the same period. This means that they are expected to display more play behaviour and less manipulative and aggressive behaviours compared to pigs housed under barren conditions. The pigs housed on wood shavings are expected to react more positive to a gain of reward and less negative to a loss of reward and are expected to show a less intense startle reaction and also to have a better physical health score. The pigs housed on wood shavings are expected to be more active and more synchronised in their behaviour. The pigs kept on wood shavings are therefore expected to be in a more positive emotional state in contrast to pigs kept in barren pens. They are expected to be in a more negative emotional state.

With regard to the pigs that will change housing conditions it is hypothesised that a switch from wood shavings to barren conditions is the worst for their welfare compared to the other groups. Therefore, this group is expected to score the worst for all parameters after the switch. This means that they are expected to display the least play behaviour and the most manipulative and aggressive behaviour of all groups. They are also expected to react the least positive to a gain of reward, the most negative to a loss of reward and they are expected to show the most intense startle reaction and to have the worst health score. It is expected that they are the least active of all groups and also the least synchronised in their behaviour. They are expected to be in the most negative emotional state of all the treatment groups. The group that switched from barren to wood shavings housing is expected to score the best for all parameters after the switch in housing conditions, indicating that these pigs have the highest level of welfare of all groups after the switch and this group is therefore hypothesised to be in the most positive emotional state.

With regard to the different welfare indicators, it is hypothesised that all three welfare indicators lead to the same conclusions about animal welfare. It is also hypothesised that all three welfare indicators are needed for complete animal welfare assessment.

7. Materials and Methods

The Animal Care and Use Committee of Wageningen University approved the experiment. The experiment was set up as a 2 x 2 factorial arrangement, with housing conditions from weaning until 10 weeks of age (barren vs. enriched with wood shavings) and housing conditions after 10 weeks of age (barren vs. enriched with wood shavings) as factors (see Figure 2). A total of 32 pens, each containing six (12 pens) or seven (20 pens) pigs were formed. The pens were distributed over four different stables (balanced for treatment).
The gilts were also used for another experiment, which investigated the effects of different flooring systems on the prevalence and severity of osteochondrosis (OC) during the rearing period of gilts. The switch in housing conditions was set at 10 weeks of age. This way, pigs were subjected to both housing systems during the vulnerable period of OC development which is between seven and thirteen weeks of age (Ytrehus et al., 2004; van Grevenhof et al., 2012). However, for this study, this timing was also very interesting since for fattening pigs, the switch from nursery to the growing-finishing phase includes a change in environment and normally takes place at an age of approximately 10 weeks. The groups which did not switch housing conditions were also relocated to another pen with the same housing conditions to control for a possible effect of a switch in home pen.

Group	**Pre**	**Switch**	**Post**
1.	Wood shavings	→	Wood shavings (EE)
2.	Wood shavings		Wood shavings (BE)
3.	Barren		Barren (EB)
4.	Barren	→	Barren (BB)

Figure 2. Overview of treatment groups

7.1 Animals

For this study 212 gilts were used. The animals were purchased from Topigs breeding company Veldhuizen, located in Wehl. The animals were offspring of 32 Great Yorkshire/Large White type sows of different parities which were crossed with Dutch landrace boars. The resulting crossbred is a typical crossbred for commercially kept sows in The Netherlands that give birth to fattening pigs. Pigs could be identified by an ear tag and number sprayed on their backs. The pigs were kept from an age of four weeks (weaning age) until slaughter at ± 24-25 weeks of age at the experimental farm Carus of Wageningen University, located in Wageningen.

7.2 Housing and management

At arrival at Carus the gilts were distributed over the 32 pens. No littermates were assigned to the same pen and littermates were equally divided over treatments. Body weight of the gilts was balanced for treatments. Half of the gilts were housed in barren pens of which 60% of the floor was slatted (synthetic coated expanded metal slats) and 40% was solid (epoxy-coated solid concrete). This flooring is also used in conventional commercial pig housing. The other half of the gilts were housed in pens of which wood shavings covered the complete surface. The height of the wood shaving bedding was slightly less than 25 cm at the start of the experimental period and was increased till approximately 50 cm when the pigs grew older. All pens had the same dimensions being 465 cm x 180 cm, resulting in a surface of 8.4 m^2 per pen. To prevent the wood shavings from leaking into the adjacent barren pens, boards were

placed between the pens. Therefore, pigs of adjacent pens could not see each other. The pens were cleaned three times a week.
Lights were automatically switched on at 07.00 h and switched off at 19.00 h. At the same time the lights were on also the radio was on. At the beginning of the experimental period, environmental temperature in the stables was kept at ±26°C and was gradually lowered to 22°C. All animals received the same commercial pelleted feed which was provided ad libitum in one feeder per pen. They received four different feeds (Agruniek Rijnvallei, Wageningen), adjusted for their stage of life. Water was also provided ad libitum via one drinking nipple per pen inside a drinking bowl. Both feed and water were checked each day by the animal caretakers of Carus.
Each pen contained two toys, such as chains, solid plastic balls, rubber mats or hessian bags, which were changed every three days.
The health of the animals was checked every day by the animal caretakers of the Carus facility. Animals with health problems were treated according to their illness. When animals exhibited severe health problems they were removed from the experiment and euthanized (when they were ≤10 weeks of age) or emergency slaughtered (when >10 weeks of age) at Carus. All remarks with regard to the animals were written down in a log book.
At the end of the experiment the gilts were slaughtered at a commercial but small (emergency) slaughterhouse (Diepeveen B.V., Herveld). They were slaughtered via the conventional way (electrical stunning followed by exsanguination by incision of the carotid artery).

7.3 Physical welfare

The physical welfare of the animals was investigated by a daily check of their general physical health. All remarks were written down. Post mortem, stomach lesions and ulceration were scored.

7.3.1 Stomach lesions and ulceration

The animals were slaughtered on four separate days, balanced for treatments. The incidence of lesions of the pars oesophageal region of the stomach was investigated post mortem using a six-point scoring system (Table 1), as described by Hessing et al. (1992). Occasionally, a score in between the stated categories was given. All stomachs were examined within 10 min of exsanguination in the slaughterhouse. Scoring of the stomach was done without knowledge of the previous treatment of the pigs. A score of three or higher indicated that lesions were present in the pars oesophagus.

Table 1. Score of the pars oesophagea of the porcine stomach wall

Category	Definition
0	Normal pars oesophagea
1	Minor hyperkeratosis (< 50% of the surface)
2	Severe hyperkeratosis (> 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 and shorter than 5 cm
5	Hyperkeratosis and more than 10 lesions or lesion(s) longer than 5 cm or ulcer with or without bleeding or occlusion (stenose) of the oesophagus into the stomach

7.4 Mental welfare

The mental welfare of the pigs was investigated by live behavioural observations, a startle response test, a gain in reward test and a relative contrast test. For all observations and tests, the animals were individually marked with a number from one to six or seven (depending on the amount of animals per pen) on their back using a blue colored stock marking spray. All observations and tests took place while the pigs were in their home pen. Observations of the pigs during the different tests was either done live (all behaviours recorded during the relative contrast test) or via video recordings (latency of relative contrast test, gain of reward and startle response test). When video recording were used, scoring of the behaviours was done with The Observer XT 10.0 (Noldus Information Technology B.V., Wageningen, The Netherlands).

7.4.1 Live behavioural observations

7.4.1.1 Pilot study

In advance of the 'real' observation sessions, pigs of two pens (one barren pen and one pen with wood shavings) were individually marked and observed. Based on these observations and existing literature the ethogram as shown in Table 2 was constructed.

7.4.1.2 Live observations

The frequencies of play and damaging behaviours (manipulative and aggressive behaviours) of each individual pig were recorded by continuous live observation for 4*10 min per pen per week when the animals were eight weeks old (before the switch), 10 weeks old (2-3 days after the switch) and 17 weeks old. For one observation day, the observation sessions were conducted in the morning from 08:00 until 11:40 h and in the afternoon from 14:00 until 17:40 h, as pigs appear to be relatively inactive during midday. The distribution of observation blocks over pens was balanced for treatment; i.e. n=2 pens per treatment per 80-min observation block in the morning and afternoon, and the distribution over days and sessions was balanced for treatment and pen. In the week after the switch, the observations were spread over 2 days and carried out by two observers of which one also conducted the other two observation sessions. Frequencies were averaged per pen per week before analysis. From the last observation session (at 17 weeks of age) only 3*10 min per pen per week were used for analysis since the pigs appeared to be distracted by the presence of the observer at the first day of the session. The relative contrast test (a test in which the pigs received a reward in the form of chocolate and yoghurt raisins – see later section) had been conducted six weeks earlier and the animals might have been triggered by the presence of the observer the first observation day since they might have been expected the treats again. The second day of observation the animals seemed more or less as undisturbed, as they had in the previous observation sessions.

Table 2. Ethogram behavioural observations

Behaviour	Description
Manipulative	
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 part of the body of a pen mate, except tail or ears
Manipulating environment	Nibbling, sucking or chewing the pen
Aggressive	
Biting	Biting any part of another pig
Chasing	Actively pursuing another pig
Fighting	Mutual pushing or ramming, or lifting pen mate
Head knocking	Ramming or pushing pen mate with head, without biting
Play	
Locomotor play (=individual play)	
Scamper*	A sequence of at least two forward hops in rapid succession
Turn	Rapid turning around the body axis on the spot
Toss head	Vigorous latero-rotationary movements of the head
Flop	A rapid drop from an upright position to sternal or lateral recumbence
Rolling	Lying on back and moving from side to side on the floor
Social play (= play of two or more pigs)	
Pushing	Pushing the opponent with the head or shoulder
Gambolling*	Running across the pen, occasionally accompanied by nudging pen mate gently
Explorative	
Towards object	
Interacting with a toy	Sniffing, nibbling, pushing forward, pulling, biting a toy with or without using a paw
Towards pen mate (social)	
Nudging	Gentle snout contact of one pig to another pigs body

Ethogram is partly based on Bolhuis et al. (2006) and Keeling (personal communication, 2013)

**When the pigs were clearly startled by a certain event and started to run, this was not scored as play behaviour (gambolling or scampering).*

7.4.2 Startle response test

The startle response test was conducted once in week 12, nine days after the switch in treatment. For this purpose, the air-conditioning of the stables was turned off two hours before the startle response test in order to allow the pigs to clearly hear the explosion of the balloon and to allow them to get used to the quietness. Four persons entered one of the four different units at the same time (10:00 h) with synchronized stopwatches. The persons walked to the end of each unit and back again, until halfway of the unit. There, he/she stood for ± 2.5 min to

habituate the pigs to their presence. When the stopwatch indicated: 10:03 h, the persons punctured a balloon in all four units simultaneously while the behaviours of the pigs were recorded by camera.
The posture of the individual pigs immediately before the balloon was punctured (standing, sitting or lying) was scored. This way it could be investigated if the position of a pig shown before the acoustic stimulus influenced the startle reaction of the pig. Also, the reaction of the pigs to this acoustic stimulus was observed from video recordings (for 60 sec) using the ethogram as shown in Table 3. If a pig stopped freezing and started freezing again within 5 seconds, the freezing response was scored again. When this was not the case (i.e. the pig did not freeze within 5 seconds again) the next freezing response (if it occurred) was not scored again. Latency to startle response was observed by video recordings and was scored as soon as an individual pig showed a reaction to the noise by a startle reflex.

Table 3. Ethogram startle response test

Body positions and behaviour	Description
Body positions	
Standing	Standing on all four legs
Sitting	Sitting on the tail with forelegs stretched under the body
Lying	Lying down either on the belly or on the side
Behaviour	
Running*	Running around in the pen
Startle	A quick alarm reaction, indicated by a shock of the body
Freeze*	Immobility of body and head, usually accompanied by erect ears

Ethogram is partly based on Blackshaw et al. (1998) and Krohn et al. (2000)
**Duration was scored instead of frequency*

7.4.3 Gain of reward test

The sensitivity to a so called ‘reward gain’ was tested by observing the behaviour of the pigs when a less preferred toy was replaced by a hessian bag. A hessian bag appears to be perceived by pigs as a more than average popular toy (personal communication, De Koning) and can therefore be seen as a gain of reward. This test was carried out when the pigs were nine weeks old (before the switch), and when the pigs were 11 weeks old (four days after the switch). The hessian bag was placed in the pen from Friday 12:00 until Monday 8:00. The behaviour of the individual pigs in the two min following the provision of the hessian bag was observed from video recordings using the ethogram shown in Table 2. In addition, also the total duration of each individual pig interacting with the hessian bag was scored for these two minutes. By scan sampling, the proportion of pigs interacting with the hessian bag was scored for eight different time points. These time points were: Friday at 13:30 and 18:00, Saturday and Sunday at 09:00, 13:30, 18:00 h.
The hessian bag had not been provided to the pigs for at least 12 days before these behavioural observations were made.

7.4.4 Relative contrast test

Since, to the best of our knowledge, no information about group relative contrast tests for pigs exists, a new paradigm was designed. This paradigm was first investigated in a pilot study (Appendix I). Exact measurements and timings were determined during this pilot study and led to the final relative contrast test. The test was conducted in the home pen of the pigs and started at 14 weeks of age.

In Figure 3 a schematic overview of the experimental set up is provided. Two cameras were used. One of the cameras was fastened to the ceiling at the back of each pen. The other camera was placed on a trolley and moved and placed in front of each pen every time it was observed. The test was conducted by two observers.

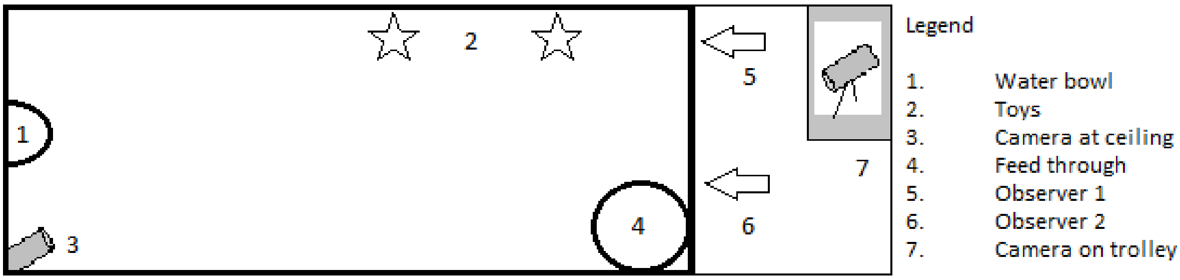

Figure 3. Schematic overview of the experimental setup of the relative contrast test. The pen containing the pigs is to the left, surrounded by the thick black line and the observation corridor is to the right.

For 12 consecutive days, twice daily the animals were either given a reward with high incentive value (a mix of chocolate and yoghurt raisins) or a reward with lower value (their normal pellet feed). This resulted in a total of 24 trials. Table 4 shows the order in which the different feed rewards were provided. Each trial lasted for 1 min which was divided in two parts: an anticipatory part (first 15 sec) and a test part (subsequent 45 sec; after provision of the food reward).

In addition to the 24 trials, a 25th trial was conducted approximately 1 week before slaughter, when the pigs were 24-25 weeks of age. In this trial, only the behaviours of the pigs in the anticipation part of the test were scored. This last trial was conducted to investigate if there was any difference between the treatment groups with regard to their anticipation response 8 weeks after the last trial.

Table 4. Feed rewards provided in the relative contrast test per phase

Phase 1: Mix of chocolate and yoghurt raisins

Day	Mon		Tue		Wed		Thu	
Trial	1	2	3	4	5	6	7	8
Session	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon

Phase 2: Normal pellet feed

Day	Fri		Sat		Sun		Mon	
Trial	9	10	11	12	13	14	15	16
Session	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon

Phase 3: Mix of chocolate and yoghurt raisins

Day	Tue		Wed		Thu		Fri	
Trial	17	18	19	20	21	22	23	24
Session	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon

During each session, all of the 32 pens were tested. Order of testing was balanced for the four units. The order of testing the pens within a unit remained the same. Two units were always tested beginning at the first pen and two units were always tested beginning at the last pen. In each session, the pens were tested in the following manner: two observers stood in front of the pen of which observer 1 scored the behaviours (Table 5) and stood in front of the trolley, to have the best overview of the pen (Figure 3). The behaviours were scored per individual pig.

Observer 2 scored the vocalizations (Table 5) and threw the reward into the pen.
At the start of the observation minute observer 1 shouted 'ja' after which observer 2 put a white basket on top of the fence of the pen. This indicated the start of the anticipation time. After a 15-sec time-interval in which the anticipatory response was investigated, observer 1 again shouted: 'ja' and observer 2 threw the food reward into the pen, between 0.5-1.5 m from the fence, to allow all pigs to approach and obtain a reward. This was the start of the test phase, which lasted for 45 seconds. Per pig three chocolate/yoghurt raisins were provided, and an equal weight of normal pellet feed in phase two was provided. However, not all the pigs got (the same amount of) the reward because of competition of their pen mates.
The end of the minute was indicated by observer one shouting: 'stop'! The pilot study showed that the pigs needed approximately four trials to make a good association between the cue (white basket) and the reward. To counteract a possible olfactory effect, both food rewards were placed in the bucket.

Table 5. Ethogram relative contrast test

Behaviour	Description
Behaviour	
Standing ready*[1]	Standing with head in upright position in the direction of the observers at or close to the fence
Running up*[1]	Walking or running towards the fence behind which the observers are positioned
Keep lying down*[1]	Remain lying on the floor of the pen
Locomotor play	Individual play
Social play	Play of two or more pigs
Biting	Aggressively biting any part of another pig
Chasing	Actively pursuing another pig
Fighting	Mutual pushing or ramming, or lifting pen mate
Head knocking	Ramming or pushing pen mate with head, without biting
Exploration[1]	Sniffing, touching or routing the floor with the snout
Defecating[1]	Defecating
Urinating[1]	Urinating
Vocalizations	
Grunts	Short - or long grunts and grunt-squeals
Barks	A low tone that sounds like ´woof´

* Behaviours only recorded during anticipatory phase
** Behaviours were scored on video, the other behaviours were scored live
[1] Behaviours were scored as number of pigs per pen that showed the behaviour, otherwise behaviours were scored as frequency of display per individual pig

7.5 Other measurements

For the whole experimental period, every two weeks the weight of the animals was measured. The animals from one pen were taken out of their home pen to the corridor. Every gilt was locked individually in the weighing scale. They were not longer locked than 30 sec.
Five times, a health check was performed. In this check, the amount of lesions on the body of the pigs was scored. Two observers stood in the pen of the pigs and scored the lesions. These lesions were either the result of aggressive interactions between the pigs but could also be caused by manipulative behaviour.

7.6 Statistical analysis

SAS (Statistical Analysis System Institute and Inc.) versions 9.2 and 9.3 were used for the statistical analysis of the results. With regard to the analysis of the stomach scores, scores were averaged per pen prior to analysis. Stomach ulceration scores were analyzed using a mixed linear model with pre, post, unit and date as class variables included in the model.

Effects and interactions between housing (class variables: pre, post, unit and pen) and stomach lesions were investigated using a general linear mixed model. In this model, a random pen effect was included. Analysis was based on individual pigs.

With regard to the analysis of the behavioural observations, startle response test, gain and relative contrast test, prior to analysis, the frequency of behaviours displayed by individual pigs was averaged per pen. All observations and tests were analyzed using a mixed linear model with the same class variables as previously stated. If different sessions (behavioural observations and gain of reward test) and different phases (relative contrast test) were tested, this was included as ‘repeated’ in the model.

Significant effects were analyzed using post hoc tests. All data are presented as (untransformed) means ± SEM. F-statistics were used and results are presented with corresponding degrees of freedom and p-values.

8 Results

8.1 Stomach health

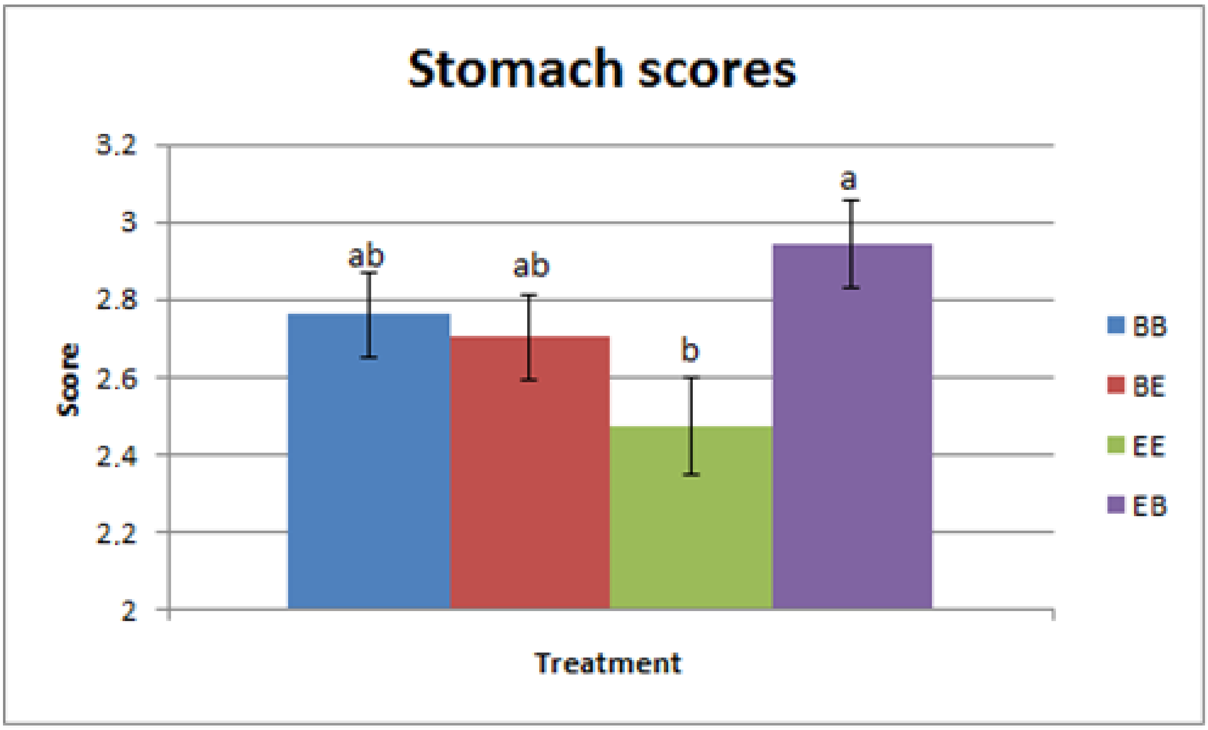

Figure 4. Mean stomach scores (0=completely healthy to 5=severely damaged) including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), pigs housed in wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). Means lacking a common superscript (a,b) differ ($P<0.10$).

8.1.1 Stomach scores

Stomach scores were affected by post switch housing conditions $F(1,28)=5.28$, $p=0.0292$ with higher scores for post switch B (i.e. EB and BB) pigs than for post switch E (i.e. BE and EE) pigs (see Fig. 4).

Also to the interaction between pre*post switch housing conditions tended to affect stomach scores $F(1,28)=3.25$, $p=0.0823$ with EB pigs tending to have a higher score than EE pigs with levels of BB and BE in between.

In Appendix III pictures of stomachs with accompanying scores are provided.

8.1.2 Gastric lesions

The proportion of pigs with gastric lesions (i.e. stomach score ≥ 3) tended to be affected by the interaction between pre*post switch housing conditions $F(1,25)=3.71$, $p=0.0656$. Analysis per pre switch housing condition revealed that a higher proportion of EB pigs (62%) suffered from lesions than EE pigs (35%), whereas proportions of BB (44%) and BE pigs (52%), with levels in between.

8.2 Behavioural observations

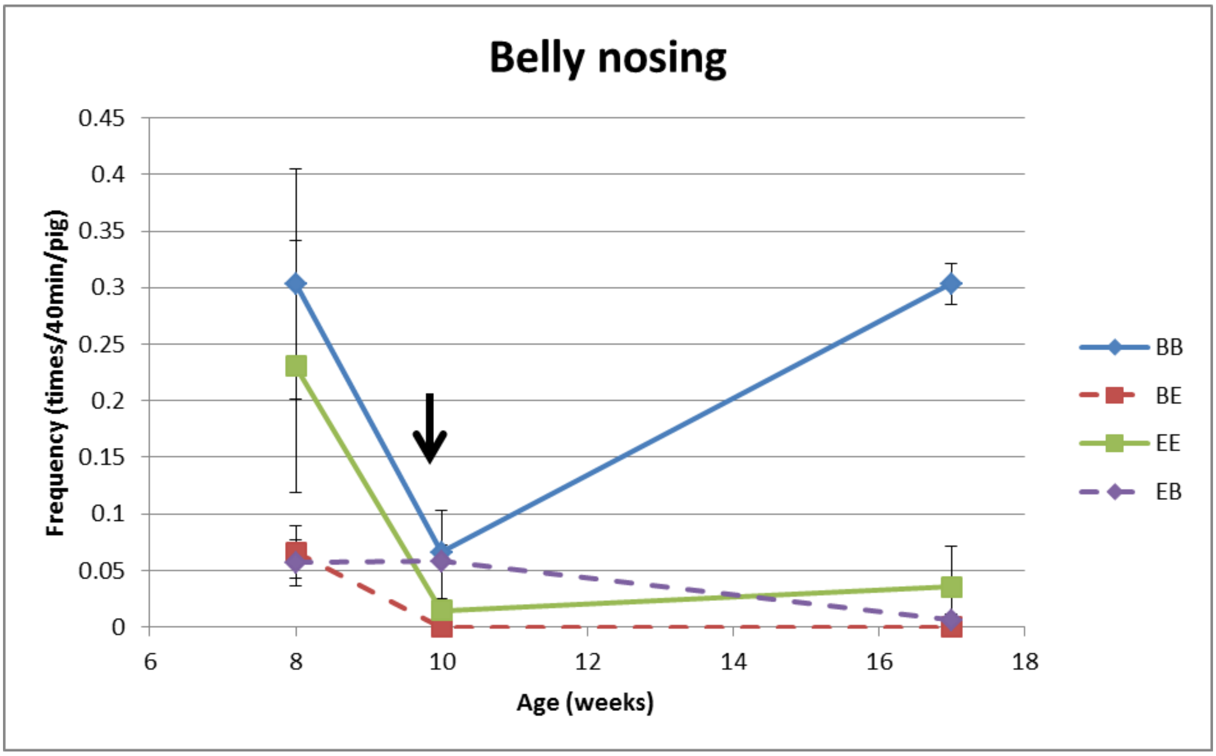

Figure 5. Frequency (times/40min/pig) of belly nosing including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Belly nosing was affected by pre*post switch housing conditions $F(1,28)=6.54$ $p= 0.0162$, week $F(2,56) =20.49$, $p<0.0001$ and their interaction: pre*post*week $F(2,56)=3.5$ $p=0.0371$.
Belly nosing sharply decreased for BB pigs from week 8 to 10 and did not significantly differ between week 10 and 17. With regard to EE pigs, belly nosing decreased from week 8 to 10 with levels staying the same in week 17. Belly nosing of BE and EB pigs did not change over time.
Analysis per week revealed a pre*post $F(1,28)=6.11$, $p=0.0198$ effect in week 8. Post hoc analysis showed that BB pigs showed a higher frequency of the behaviour than EB or BE pigs with EE pigs in between. No differences between the treatment groups were found in week 10. In week 17, pre*post housing conditions again affected the behaviour $F(1,28)=3.72$, $P=0.0640$: BB pigs tended to show more belly nosing than BE pigs in this week.

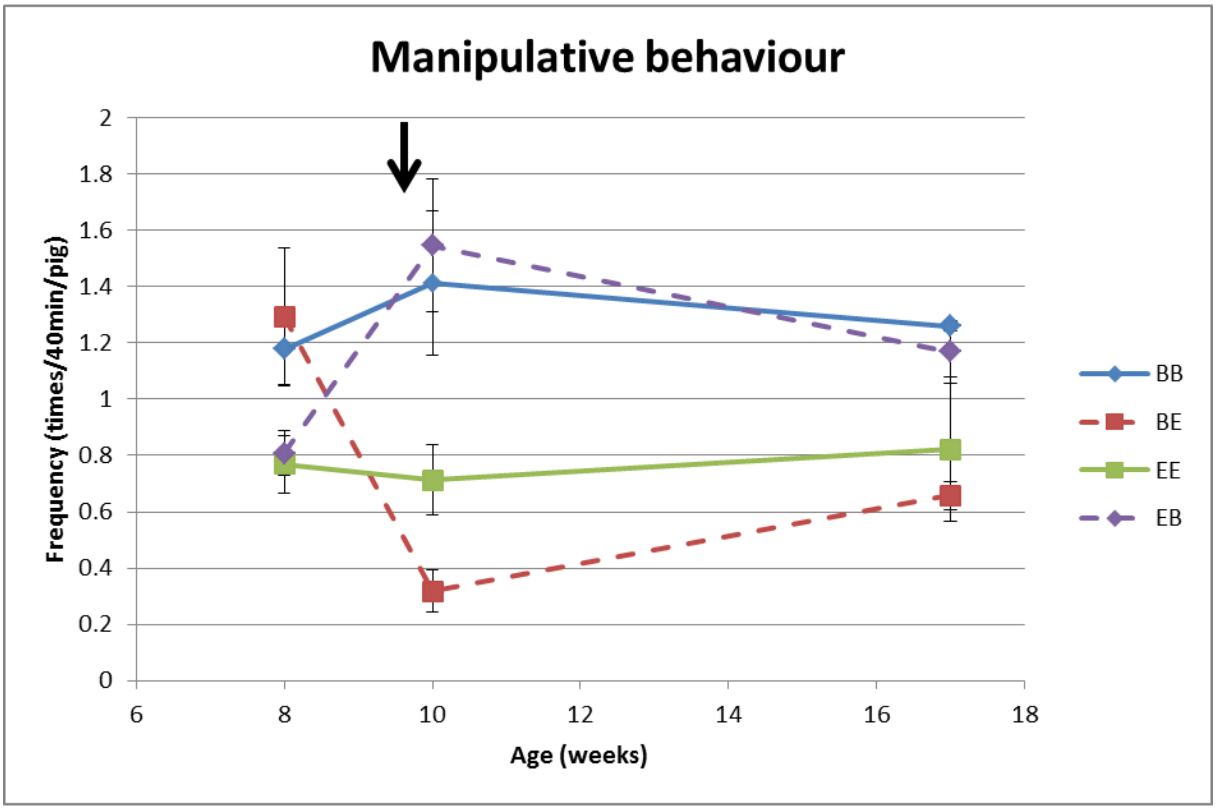

Figure 6. Frequency (times/40min/pig) of manipulative behaviour including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Manipulative behaviour was affected by post switch housing conditions $F(1,28)=30.86$, $p<0.0001$, and interactions between pre*week $F(2,56)=5.56$, $p=0.0063$ and post*week $F(2,56)=9.81$, $p=0.0002$.

The prevalence of manipulative behaviour was mainly determined by the actual housing conditions. Before the switch (week 8), pre housed B (BB and BE) pigs showed more manipulative behaviour compared to pre housed E (EE and EB) pigs. In week 10 and 17 of age, post housed B pigs (BB and EB) showed a higher prevalence of manipulative behaviour compared to post housed E pigs (BE and EE).

The differences in week 10 with regard to the prevalence of this behaviour between the different treatment groups were particularly interesting. Analysis per week showed at 10 weeks of age a trend for a pre effect $(1.28)=4.00$, $p=0.0553$ effect and a significant post $F(1,28)=33.71$, $p<0.0001$ effect. Post hoc analysis showed that pre housed B (BB and BE) pigs tended to show less manipulative behaviour at 10 weeks of age compared to pre housed E (EE and EB) pigs, caused by the fact that BE pigs showed a strong decrease in the frequency of manipulative behaviour after the switch (week 10). However the post effect revealed that post housed B (EB and BB) pigs showed more manipulative behaviour compared to post housed E (EE and BE) pigs. These results indicate that BE pigs showed a steeper decline in manipulative behaviour than EB pigs showed an increase in the behaviour.

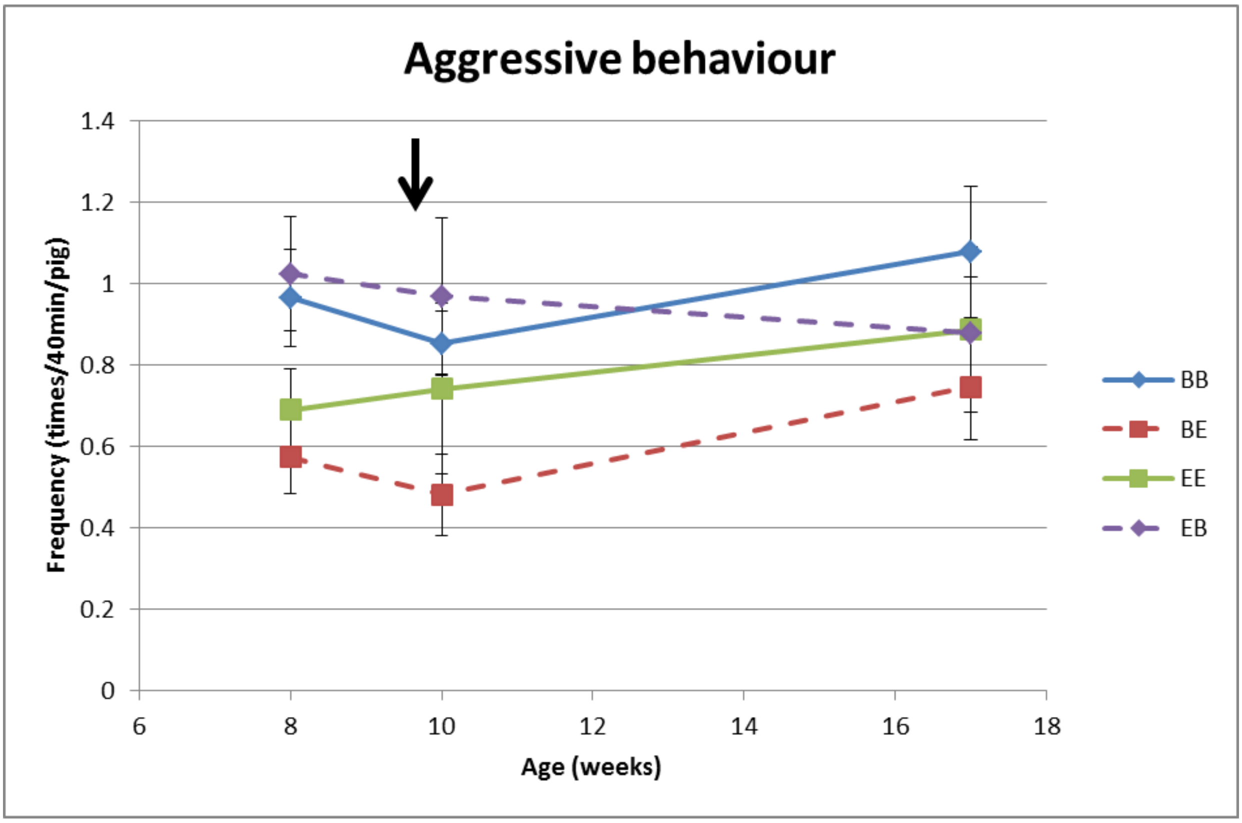

Figure 7. Frequency (times/40min/pig) of aggressive behaviour including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

No overall pre or post effect was found. However, analysis per week revealed a post effect in both week 8 $F(1,28)=9.43$, $p=0.0047$ and 10 $F(1,28)=4.96$, $p=0.0342$. Post housed B (BB and EB) pigs showed more aggressive behaviour compared to post housed E (EE and BE) pigs. No difference between the treatment groups was found in week 17. Also no differences in the prevalence of the behaviour were found over time.

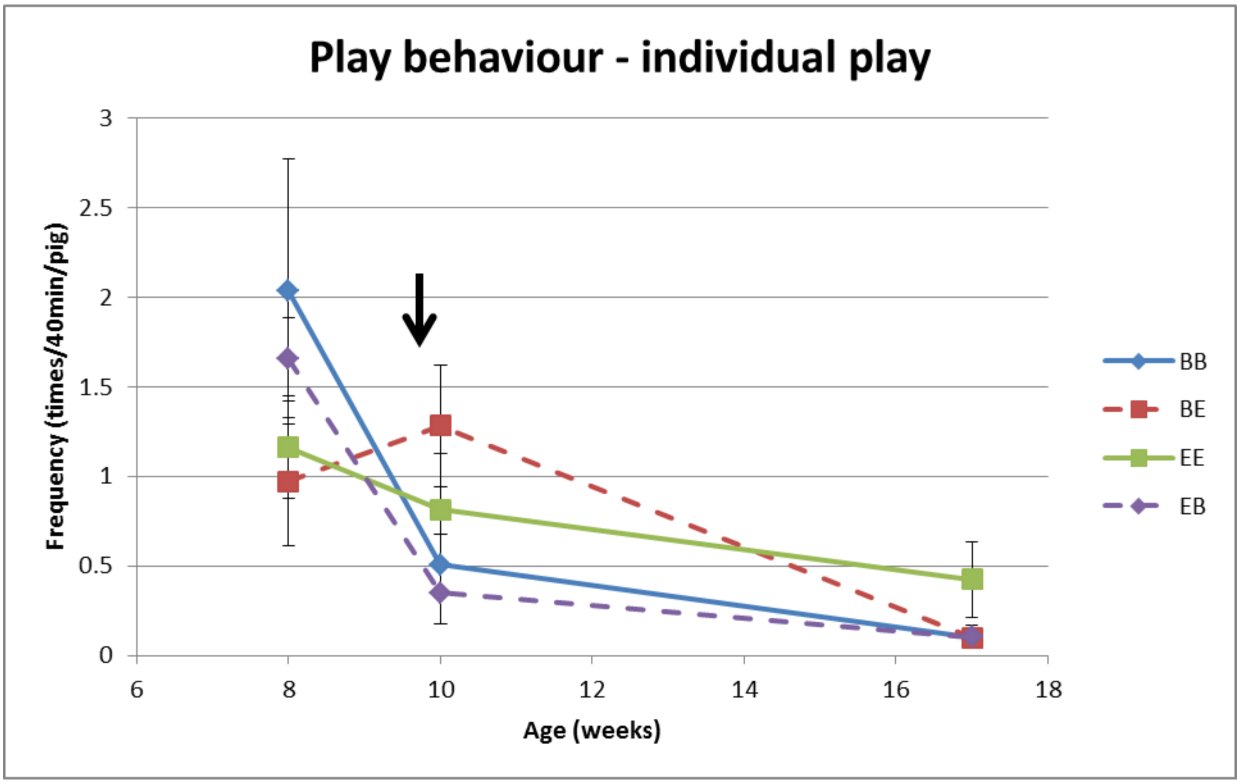

Figure 8. Frequency (times/40min/pig) of individual play behaviour including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Individual play behaviour was significantly affected by week $F(2,56) = 26.79$, $p<0.0001$ and the interaction between post*week $F(2,56)=5.94$, $p= 0.0046$.
Overall, play behaviour decreased from 8 to 17 weeks of age. Post B (BB and EB) pigs showed a trend to display more play behaviour than post E (EE and BE) pigs at 8 weeks of age. With regard to 10 and 17 weeks of age, post B (BB and EB) pigs showed less play behaviour than post E (EE and BE) pigs.

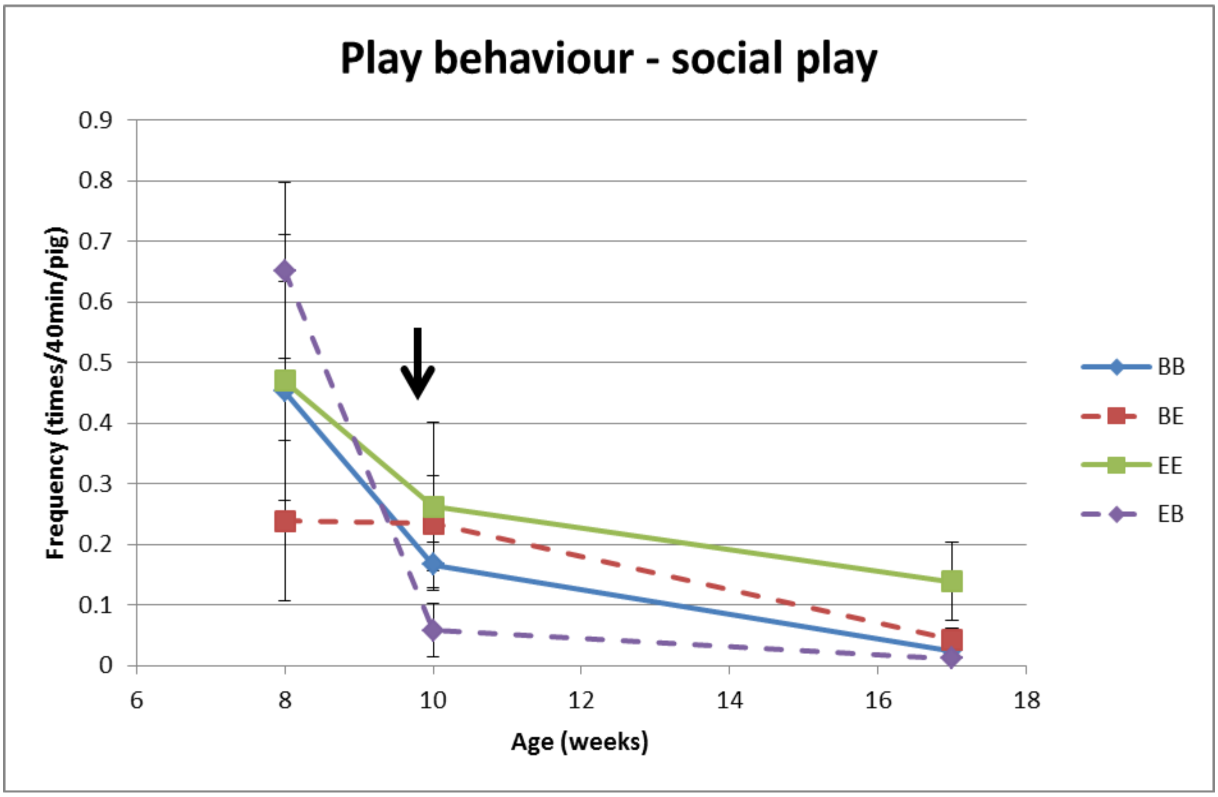

Figure 9. Frequency (times/40min/pig) of social play behaviour including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Social play behaviour, was affected by week $F(2,56)=18.67$, $p<0.0001$) and pre*week $F(2,56)=3.30$, $p=0.0442$ and post*week interactions $F(2,56)=4.20$, $p=0.0200$).
Overall, play behaviour decreased from week 8 till week 17 of age. With regard to the pre*week effect, pre housed B (BB and BE) pigs tended to show less social play behaviour than pre E housed pigs (EE and EB) at 8 weeks of age. However, the post*week interaction showed that at 8 weeks of age, post housed B (BB and EB) pigs showed more social play behaviour compared to post housed E pigs. These contradictory results are caused by the fact that considerable differences in the frequencies of this behaviour were observed between pens of pigs housed in the same housing conditions (both barren and enriched) in the pre housing condition, that were later randomly allocated to different post switch conditions.
At 10 weeks of age, the prevalence of social play behaviour did not differ between the treatment groups. At 17 weeks of age, post housing conditions $F(1,28)=3.91$, $p=0.0579$ tended to affect social play behaviour. Post B (EB and BB) pigs tended to show less social play behaviour than post housed E (EE and BE) pigs.

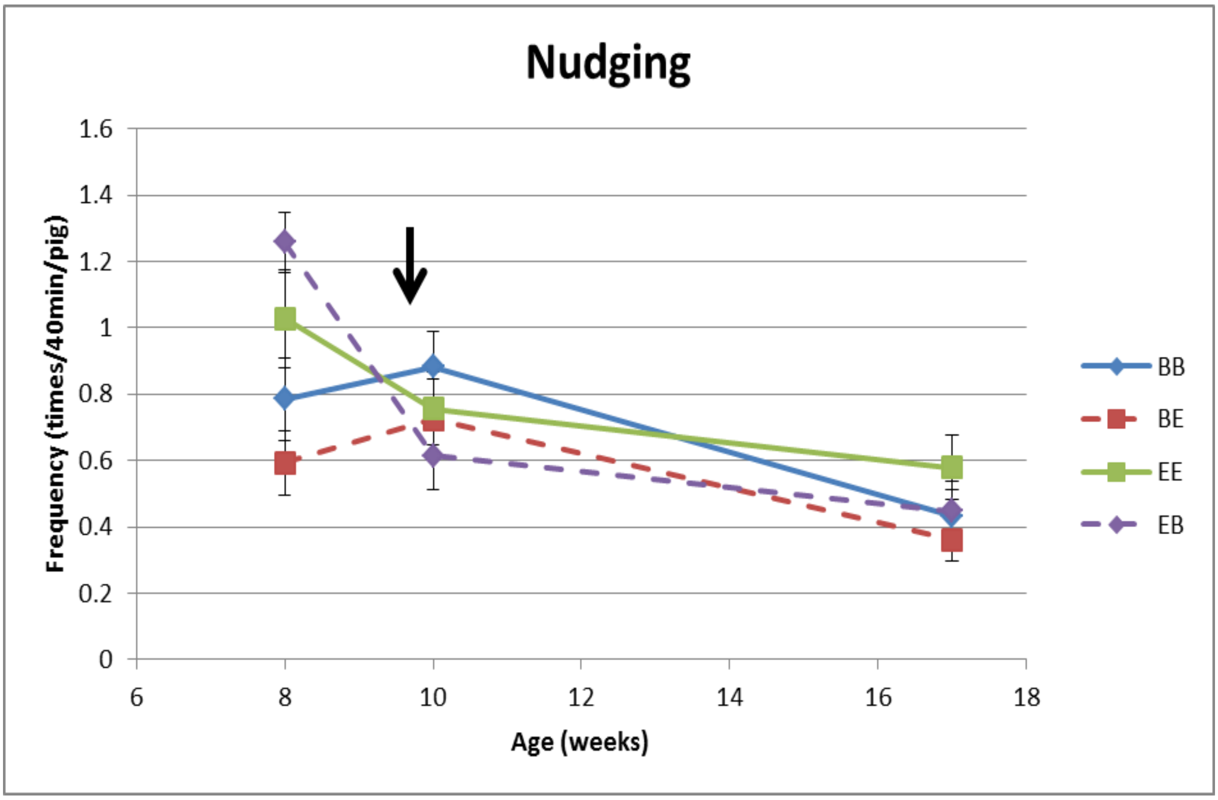

Figure 10. Frequency (times/40min/pig) of nudging behaviour including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Nudging was affected by pre switch housing, $F(1,28)=4.49$, $p=0.0431$, week $F(2,56)=19.44$, $p<0.0001$ and pre*week $F(2,56)=7.02$, $p=0.0019$ effects.
The prevalence of the behaviour overall decreased over time, and did not differ between treatments at week 10 and 17 of age. Only before the switch, at 8 weeks of age, a pre effect $F(1,28)=14.22$, $p=0.0008$ and a trend for a post effect $F(1,28)=3.19$, $p=0.0850$ existed. Pre housed B (BB and BE) pigs showed less nudging behaviour compared to pre housed E (EE and EB) pigs. However, it was also shown that post housed B (BB and EB) pigs showed more nudging behaviour compared to post housed E (EE and BE) pigs. These contradictory results are caused by the fact that different frequencies of this behaviour were observed between pens of pigs housed under the same housing conditions (both barren and enriched) in the pre housing conditions, that were later randomly allocated to different post switch conditions, in addition to the fact that BE animals tended to show *less* and EB animals *more* nudging behaviour compared to the BB and EB groups respectively.

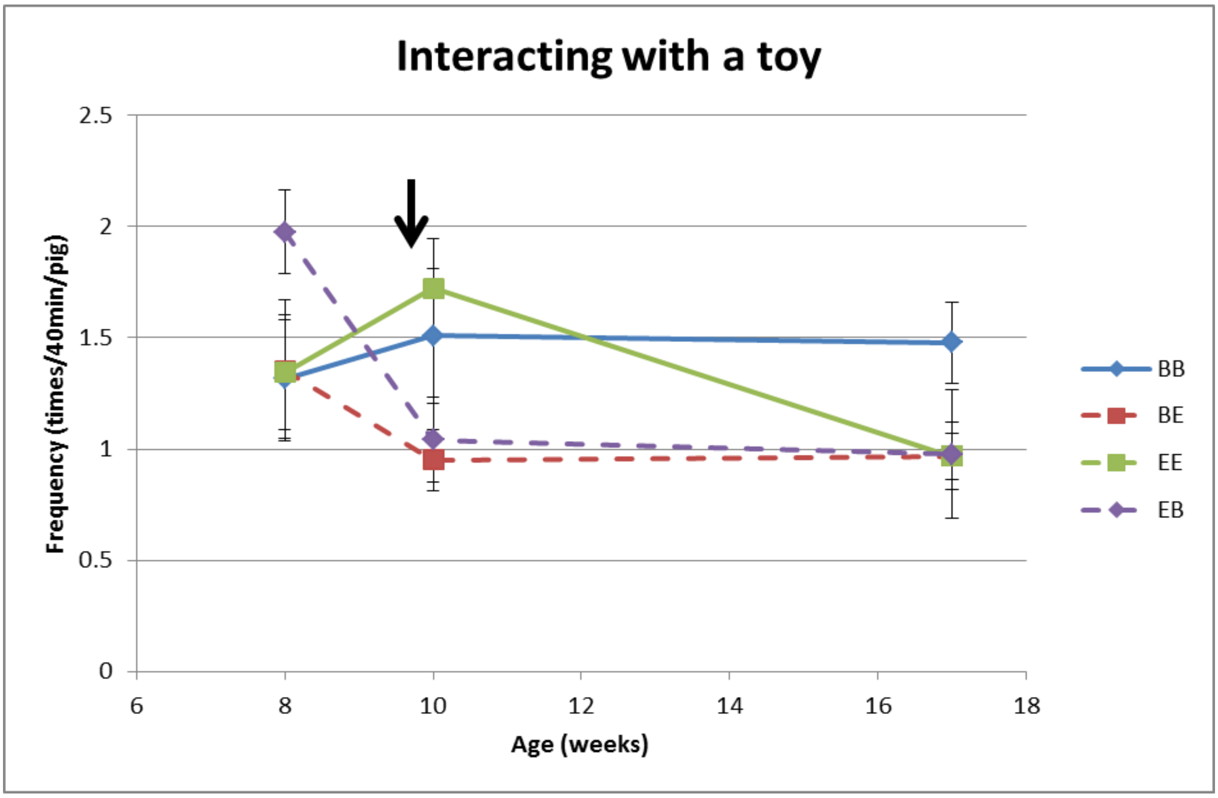

Figure 11. Frequency (times/40min/pig) of interacting with a toy including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB). The arrow indicates when the switch of housing occurred i.e. 2 days before observations in week 10.

Frequencies of interaction with a toy were affected by post switch housing $F(1,28)=4.56$, $p=0.0417$ and week $F(2,56)=5.66$, $p=0.0058$. Pre*week was also significant $F(2,56)=5.36$, $p=0.0074$ as well as a pre*post*week effect $F(2,56)=3.26$, $p=0.0457$.
Overall, more pigs interacted with a toy in week 8 than in week 17. No differences were found between week 8 and 10 nor between week 10 and 17.
Just after the switch in housing conditions, at week 10 of age, more EE pigs interacted with a toy compared to week 17 of age. Analysis based on week showed a pre*post effect in week 10 $F(1,28)=7.75$, $p=0.0095$: BE and EB pigs showed less interaction with a toy compared to EE pigs. At 17 weeks of age, BB pigs interacted more with a toy compared to EE pigs.

8.3 Startle response

Several measurements have been made with regard to the startle response of the pigs. Their startle and freeze response was investigated. Table 6 shows the values of the obtained results.

8.3.1 Latency-to-startle response

With regard to the latency-to-startle response, no effect of treatment was shown. Although the posture shown by the pigs just before the acoustic stimulus tended to influence the latency-to-startle response $F(1,174)=3.59$, $p=0.0598$. Pigs that were not lying before the acoustic stimulus tended to show a shorter latency-to-startle response compared to pigs that were lying.

8.3.2 Freeze response

Several characteristics of the freeze response were investigated. Concerning the duration of the first freeze response, no effect of treatment was found. However, the posture (lying or not lying) shown by the pigs just before the acoustic stimulus was presented affected the duration of the first freeze response $F(1,174)=7.36$, $p=0.0073$ with pigs that were not lying showing a shorter duration of the first freeze response compared to pigs that were lying.

With regard to the total duration of the freeze response, there was no treatment effect on this parameter. However the posture of the pigs shown just before the acoustic stimulus did influence the total duration of the freeze response $F(1,174)=5.02$, $p=0.0264$, with longer freezing responses for pigs that were lying directly before the acoustic stimulus.

Also the total number of freezing bouts was investigated. It was shown that the number of lying bouts was affected by a pre*post effect $F(1,25)=5.47$, $p=0.0276$, with BB pigs showing a higher number of freezing bouts than EB pigs and BE pigs (tendency). The posture of the pigs immediately before the acoustic stimulus influenced the total number of freezing bouts $F(1,174)=5.44$, $p=0.0208$, with pigs that were lying showing a lower number of freezing bouts compared to pigs that were not lying directly before the acoustic stimulus was presented.

No difference between treatments was found with regard to the latency-to-freeze response. However, the posture of the pigs shown directly before the acoustic stimulus did affect the latency-to-freeze response $F(1,174)=5.61$, $p=0.0189$ with pigs that were not lying before the acoustic stimulus showing a longer latency-to-freeze response compared to pigs that were lying.

Table 6. Reaction of pigs to the acoustic stimulus in the startle response test

	Treatment groups				Effects			
Reaction	BB	BE	EE	EB	pre	post	pre*post	posture
Duration first freeze response (s)	16.19 ± 1.90[a]	11.60 ± 1.10[a]	15.54 ± 2.82[a]	16.51 ± 2.10[a]	ns	ns	ns	**
Total duration freeze response (s)	21.29 ± 3.22[a]	15.26 ± 1.96[a]	19.66 ± 3.33[a]	23.12 ± 2.83[a]	ns	ns	ns	*
Total number freezing bouts	2.48 ± 0.32[a]	2.00 ± 0.28[a,b]	2.07 ± 0.40[a,b]	1.58 ± 0.16[b]	ns	ns	*	*
Latency-to-freeze-response (s)	1.92 ± 0.36[a]	2.35 ± 0.35[a]	2.14 ± 0.48[a]	2.06 ± 0.60[a]	ns	ns	ns	*
Latency-to-startle-response (s)	0.28 ± 0.05[a]	0.26 ± 0.06[a]	0.40 ± 0.03[a]	0.33 ± 0.10[a]	ns	ns	ns	+

Significance of effects (means with different letters differ significantly) of pigs housed for the complete experimental period under barren conditions (BB) or on wood shavings (EE), pigs that switched from barren to wood shavings enriched housing at 10 weeks of age (BE) and pigs that switched from wood-shavings enriched to barren housing at 10 weeks of age (EB). ***P < 0.001; **P < 0.01; *P < 0. 05; +P <0.10; ns: non-significant.

8.4 Gain of reward

Both the short (several hours) and longer (3 days) term effect of a gain of reward was investigated.

8.4.1 Short term effect

The short term effect of a gain of reward was investigated at two different ages by observing the behaviour of the pigs in the first two minutes after a hessian bag was provided. Values of the different measurements (i.e. total duration of interaction and total number of interactions with the hessian bag) are given in Table 7.

No differences between the treatment groups existed with regard to the duration of the first interaction with the hessian bag. With regard to the total duration of interaction with the hessian bag, it was shown that a pre $F(1,24)=18.93$, $p=0.0002$, a post $F(1,24)=5.27$, $p=0.0308$ and a trend for a pre*post*week effect $F(1,24)=4.22$, $p=0.0510$ existed. At 8 weeks of age, EE pigs showed a lower total duration of interaction with the hessian bag compared to the other treatment groups. At 12 weeks of age, BB pigs showed a higher total duration compared to the other treatment groups.

EE pigs showed less interaction with a hessian bag at 8 weeks of age compared to 12 weeks of age. The other treatment groups did not differ in the total duration of interaction with the hessian bag at the different weeks.

No differences were found in the number of interactions with the hessian bag in the first two minutes after its provision to the pigs, neither between the treatments nor between the weeks.

8.4.1.1 Interaction with a toy

Pre housed B (BB and BE) animals showed less interaction with the other toy that was provided in the pen together with the hessian bag in the two minutes following the provision of the hessian bag compared to pre housed E (EE and EB) animals as shown by the pre $F(1,24)=4.80$, $p=0.0385$ effect.

Also week influenced the behaviour $F(1,24)=7.04$, $p=0.0139$, with pigs interacting more with a toy in the two minutes after the provision of the hessian bag at 8 weeks of age compared to 12 weeks of age.

8.4.1.2 Play behaviour

There were no significant differences in the prevalence of play behaviour during the first two minutes after presenting the hessian bag neither between the treatment groups, nor between the different weeks.

Table 7. Reaction of pigs during the first two minutes after provision of the hessian bag

	Age (weeks)														
	8				12				Effects						
Behaviour	BB	BE	EE	EB	BB	BE	EE	EB	pre	post	pre*post	week	pre*week	post*week	pre*post*week
Duration first interaction hessian bag (s)	31.24 ±2.52	29.88 ±7.74	25.94 ±7.56	25.93 ±2.26	28.32 ±3.90	23.45 ±4.85	35.09 ±7.15	33.63 ±10.28	ns	ns	ns	ns	ns	ns	ns
Total duration interaction hessian bag (s)	82.48 ± 5.59	82.03 ± 3.98	48.49 ± 9.17	62.27 ± 3.45	90.51 ± 3.21	71.64 ± 6.66	65.49 ± 4.15	67.14 ± 10.03	***	*	ns	ns	ns	ns	+
Total number of interactions with hessian bag(s)	2.21 ± 0.20	2.17 ± 0.26	1.98 ± 0.38	2.64 ± 0.32	2.54 ± 0.25	2.42 ± 0.20	2.50 ± 0.49	1.80 ± 0.28	ns	ns	ns	ns	ns	ns	ns
Interaction with toy (frequency)	0.63 ± 0.12	0.39 ± 0.21	0.62 ± 0.11	0.84 ± 0.14	0.21 ± 0.09	0.31 ± 0.10	0.46 ± 0.11	0.47 ± 0.17	*	ns	ns	*	ns	ns	ns
Play behaviour (frequency)	0.15 ± 0.07	0.18 ± 0.10	0.27 ± 0.10	0.11 ± 0.04	0.12 ± 0.07	0.20 ± 0.18	0.16 ± 0.12	0.06 ± 0.03	ns	ns	ns	ns	ns	ns	ns

Significance of effects of pigs housed for the complete experimental period under barren conditions (BB) or on wood shavings (EE), pigs that switched from barren to wood shavings enriched housing at 10 weeks of age (BE) and pigs that switched from wood-shavings enriched to barren housing at 10 weeks of age (EB). ***P < 0.001; **P < 0.01; *P < 0. 05; +P <0.10; ns: non-significant.

8.4.2 Longer term effect

The longer term effect of a gain of reward was investigated by scoring, at eight different time points over three consecutive days, how many pigs in a pen were interacting with the hessian bag.

A pre $F(1,25)=4.82$, $p=0.0376$, a week $F(1,28)=13.56$, $p=0.0010$, a post*week $F(1,28)=5.90$, $p=0.0218$ and a pre*post*week $F(1,28)=4.85$, $p=0.0361$ effect was found.

At 8 weeks of age, BE animals interacted more with the hessian bag compared to the other treatment groups. At 12 weeks of age, BB pigs interacted more with the hessian bag compared to the other treatment groups.

BE animals showed more interaction with the hessian bag at 8 weeks of age compared to 12 weeks of age.

8.5 Relative contrast test

The relative contrast test has been analysed in three different ways: an overall analysis of the 24 trials was conducted, as well as an analysis based on phase and an analysis based on phase with trial included in the model. Due to some fluctuations between the different trials for which the reason is unknown it appeared that the analysis based on phase was most useful. The results of the overall analysis and the analysis based on phase with trial included in the model will therefore not be used. A part of these results can be found in Appendix II.
In addition to the description of the results based on phase, some trials are also analysed and described individually. The very first trial of the relative contrast test is individually analysed for all behaviours scored for both the anticipation and test time. For the test time, each first trial of phase 2 and 3 was analysed individually. With regard to the anticipation time in phase 2 and 3, the second trial of each phase was analysed individually i.e. when the pigs knew the reward had changed.

All the different behaviour patterns for the anticipatory part are presented first, followed by all the different behaviour patterns for the test part of the relative contrast test. These are followed by the results of the 25th trial, which was conducted six weeks after the relative contrast test. This trial consisted of the anticipation part only. At the end of this section, a summary of the key results is given.

8.5.1 Relative contrast test results anticipatory and test part

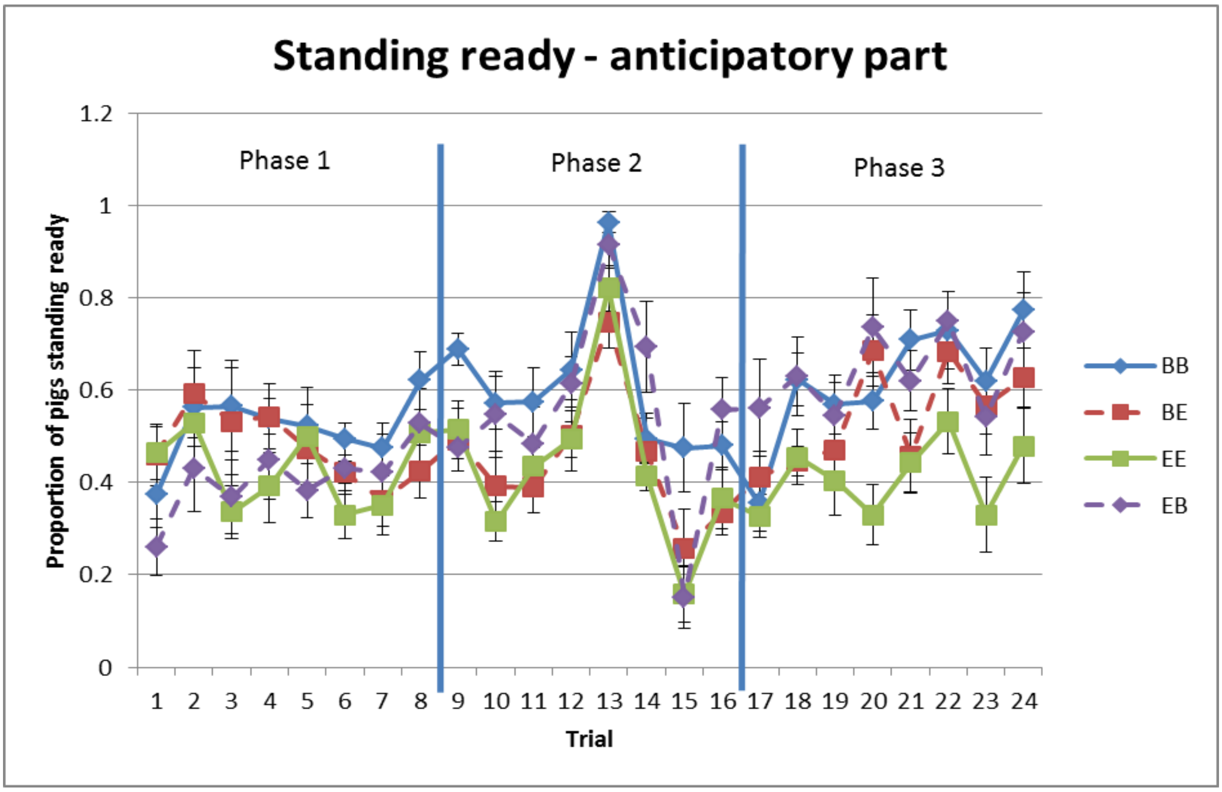

Figure 12. Proportion of pigs per pen standing ready in the anticipatory part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

The proportion of pigs standing ready during anticipation of the food rewards was affected by the pre switch housing condition $F(1,28)=11.87$, $p=0.0018$, the post switch housing condition $F(1,28)=43.47$, $p<0.0001$, phase $F(2,56)=12.64$, $p<0.0001$, post*phase $F(2,56)=5.19$, $p=0.0085$ and tended to be affected by a pre*post*phase effect $F(2,56)=2.66$, $p=0.0786$.
In phase 2 less standing ready behaviour was shown compared to phase 3 for all the groups. This is, however, difficult to see from the graph because of the strong increase during trial 13.
In post housed B (BB and EB) pigs, the proportion of pigs standing ready was lower in phase 1 than in phase 2, whereas in post E housing (BE and EE), there was no difference between phases 1 and 2.
In phase 2, a higher proportion of BB pigs showed standing ready behaviour compared to BE and EE pigs with EB in between.
In phase 3, a higher proportion of BB and EB pigs showed standing ready behaviour compared to EE pigs. In this phase, BE pigs tended to stand ready in a higher amount than EE pigs.

No differences between the treatment groups were found for trial 1 nor 2. Trial 10 and 18 showed a post effect $F(1,28)=8.70$, 0.0064 and $F(1,28)=8.09$, $p=0.0082$: in both trials, post housed B (BB and EB) animals showed more standing ready behaviour compared to post housed E (EE and BE) pigs.

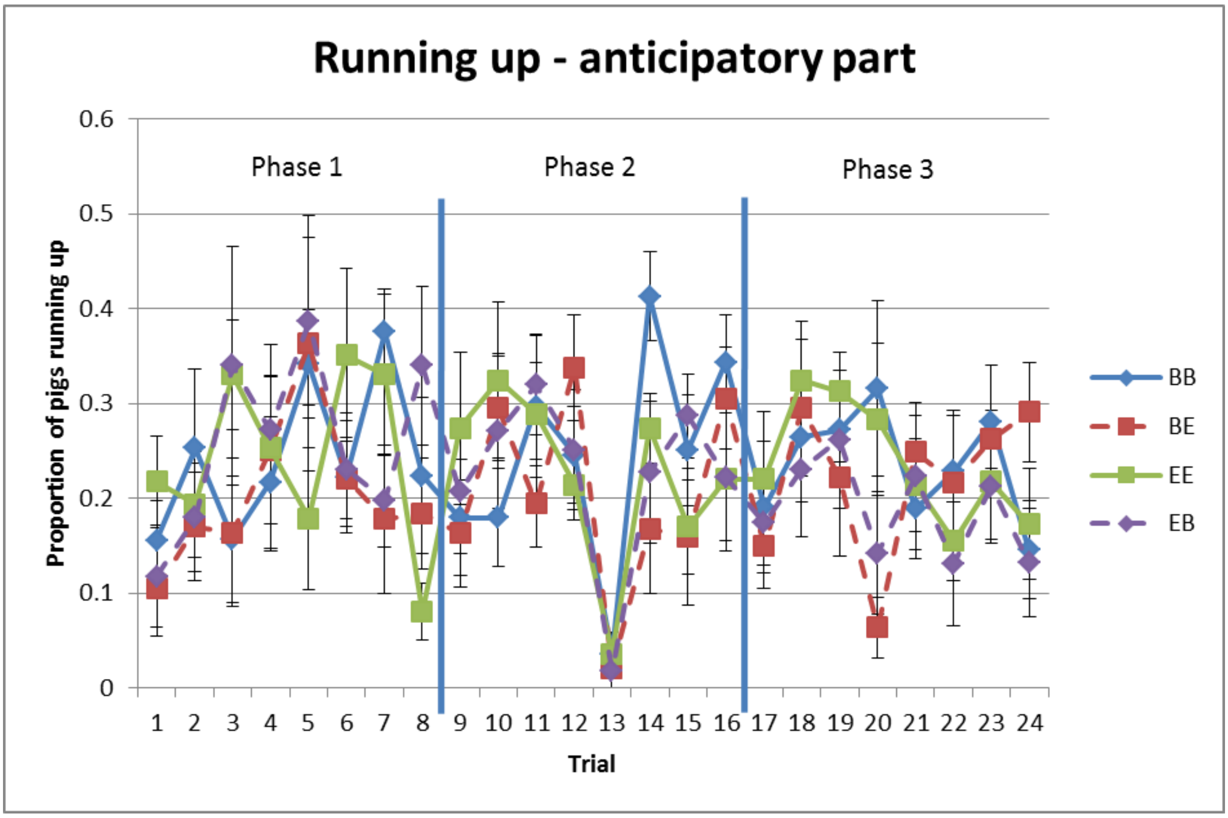

Figure 13. Proportion of pigs per pen running up in the anticipatory part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

From the analysis based on phase, no differences were found with regard to the display of standing ready behaviour between the phases.

Also no differences were found for the separate analyses of trial 1, 2 10 and 18.

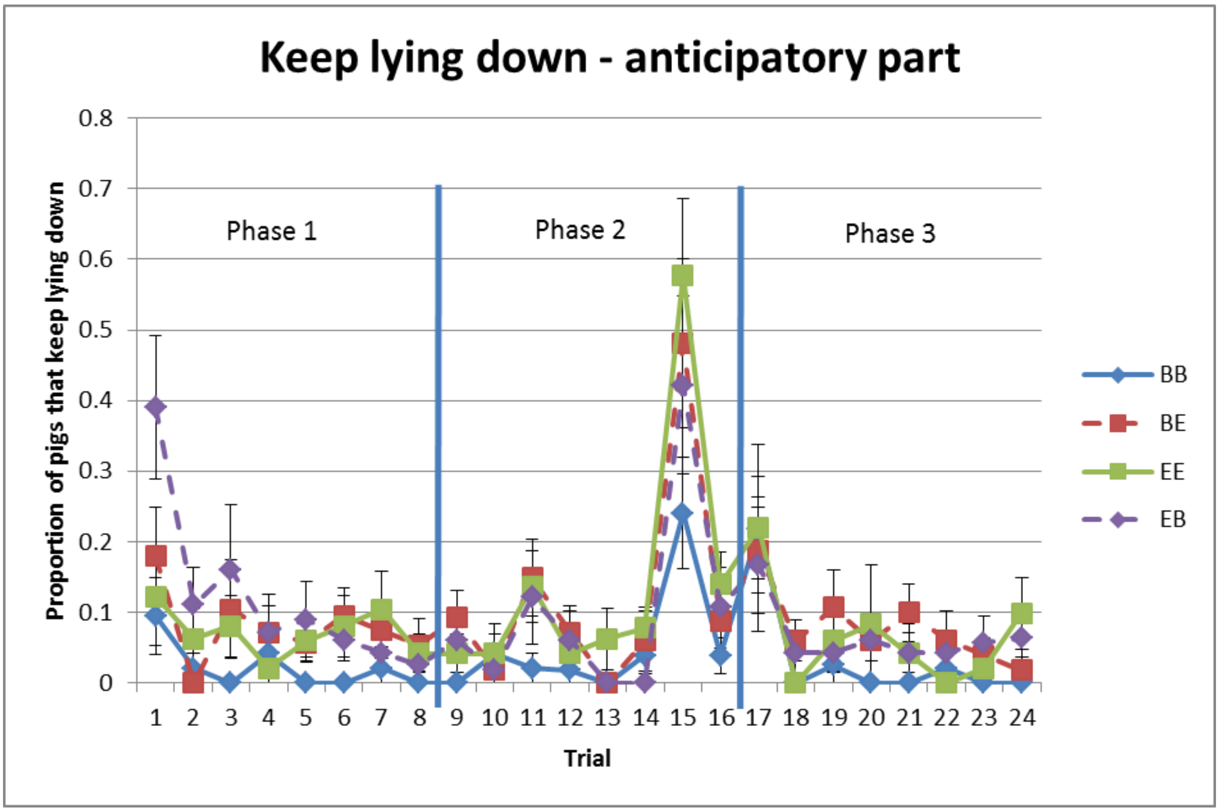

Figure 14. Proportion of pigs per pen that kept lying down in the anticipatory part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

A pre $F(1,28)=6.45$, $p=0.0169$, a post $F(1,28)=13.65$, $p=0.0009$, a pre*post effect $F(1,28)=7.74$, $p=0.0096$ and a phase effect $F(2,56)=12.88$, $p<0.0001$ were found with regard to the behaviour to keep lying down in the anticipatory part of the relative contrast test.
Pigs kept lying down more in phase 1 than in phase 2. This is however difficult to see from Figure 14, because of the strong increase during trial 15 (phase 2). In phase 2, more pigs kept lying down than in phase 3. The pre*post effect revealed that a lower proportion of BB animals kept lying down during the anticipation time than the rest of the treatment groups (BE, EE and EB).

In the first trial, a pre*post effect was found $F(1,28)=6.00$, $p=0.0208$: EB pigs tended to keep lying down more than pigs in the other treatment groups (BB, EE and BE). In trial 2, a pre housing effect was found $F(1,28)=5.04$, $p=0.0329$: pre housed B (BB and BE) pigs kept lying down less compared to pre housed E (EE and EB) pigs. With regard to trial 10, no significant effects of housing condition were found. Trial 18 showed a pre*post effect $F(1,28)=6.42$, $p=0.0172$, with fewer BB and EE pigs that kept lying down compared to BE animals.

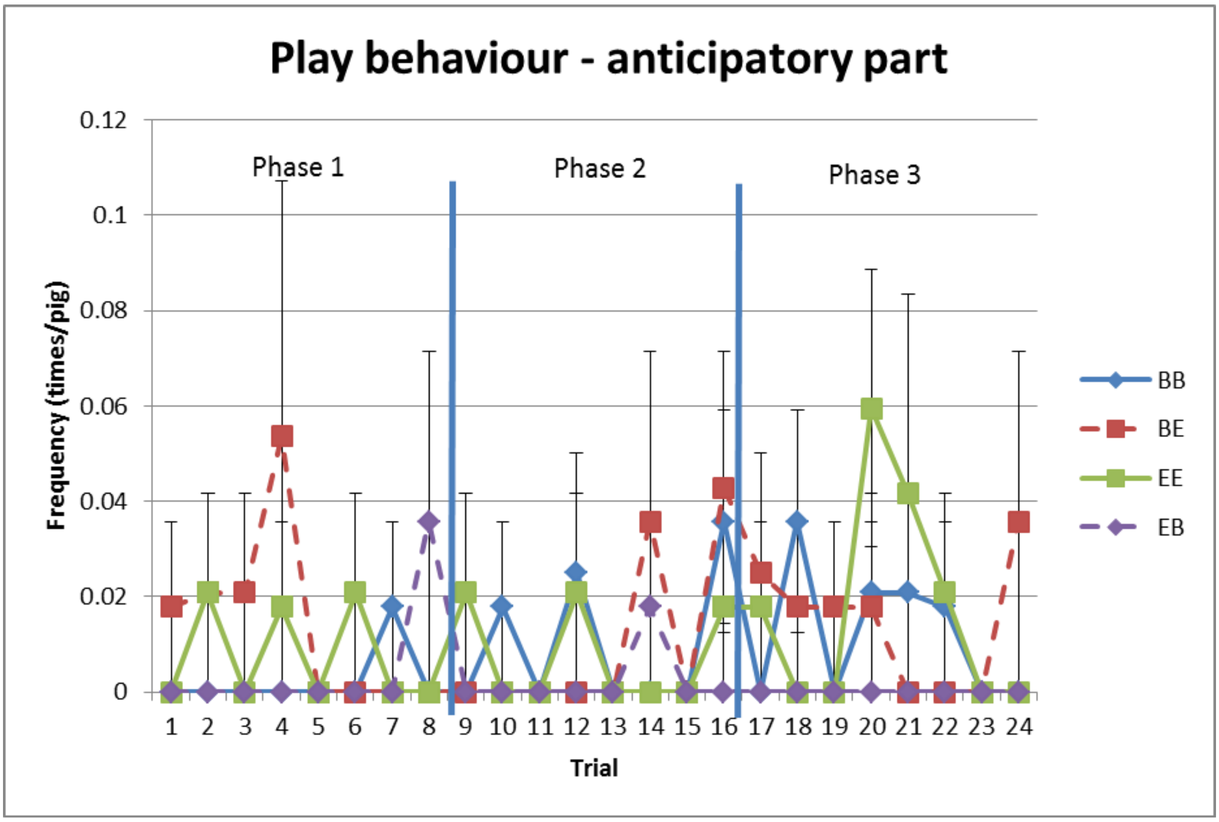

Figure 15. Frequency (times/pig) of play behaviour per pen observed in the anticipatory part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

Analysis based on phase did not reveal any significant differences between the phases or the treatment groups in play behaviour during anticipation of the food reward.

Also no differences existed in the first, second or 10th trial. Play behaviour only showed a trend for a pre housing effect in trial 18 $F(1,28)=3.32$, $p=0.0793$: pre housed B (BB and BE) animals showed more play behaviour compared to pre housed E (EE and EB) animals.

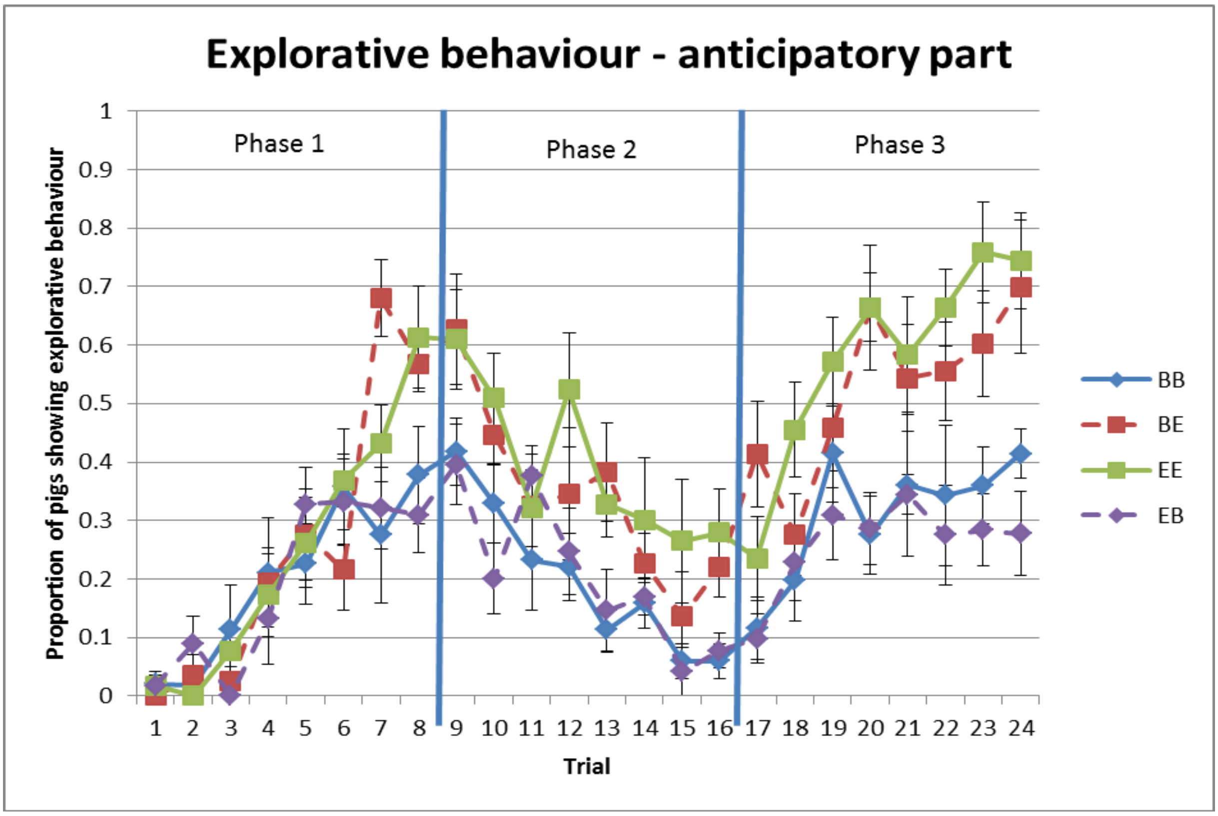

Figure 16. Proportion of pigs per pen that showed explorative behaviour in the anticipation part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

Explorative behaviour during the anticipation time was affected by post switch housing conditions, $F(1,28)=82.57$, $p<0.0001$, tended to be affected by the pre*post interaction $F(1,28)=3.21$, $p=0.0839$ and a phase effect was found $F(2,56)=54.39$, $p<0.0001$ as well as a post*phase effect $F(2,56)=12.12$, $p<0.0001$.
Overall, it was shown by the pre*post effect that a lower proportion of BB pigs showed exploratory behaviour than BE and EE. A lower and respectively higher proportion of BE pigs showed explorative behaviour compared to EE and EB pigs.
The post*phase effect showed that in phase 2 and 3, a lower proportion of post housed B (BB and EB) pigs showed exploratory behaviour compared to post E (EE and BE) housed animals. It was also shown that in phase 2 less post housed B (BB and EB) pigs showed explorative behaviour compared to phase 3. This was also true for post housed E (EE and BE) pigs.

No difference existed for the first trial with regard to the display of explorative behaviour. In trial 2, EB animals tended to show more explorative behaviour compared to EE animals pre*post effect $F(1,28)=3.07$, $p=0.0905$. In trial 10 $F(1,28)=11.03$, $p=0.0025$ and trial 18 $F(1,28)=4.52$, $p=0.0424$ there was a significant post effect: post housed B (BB and EB) pigs showed less explorative behaviour in these trials compared to post housed E (EE and BE) pigs.

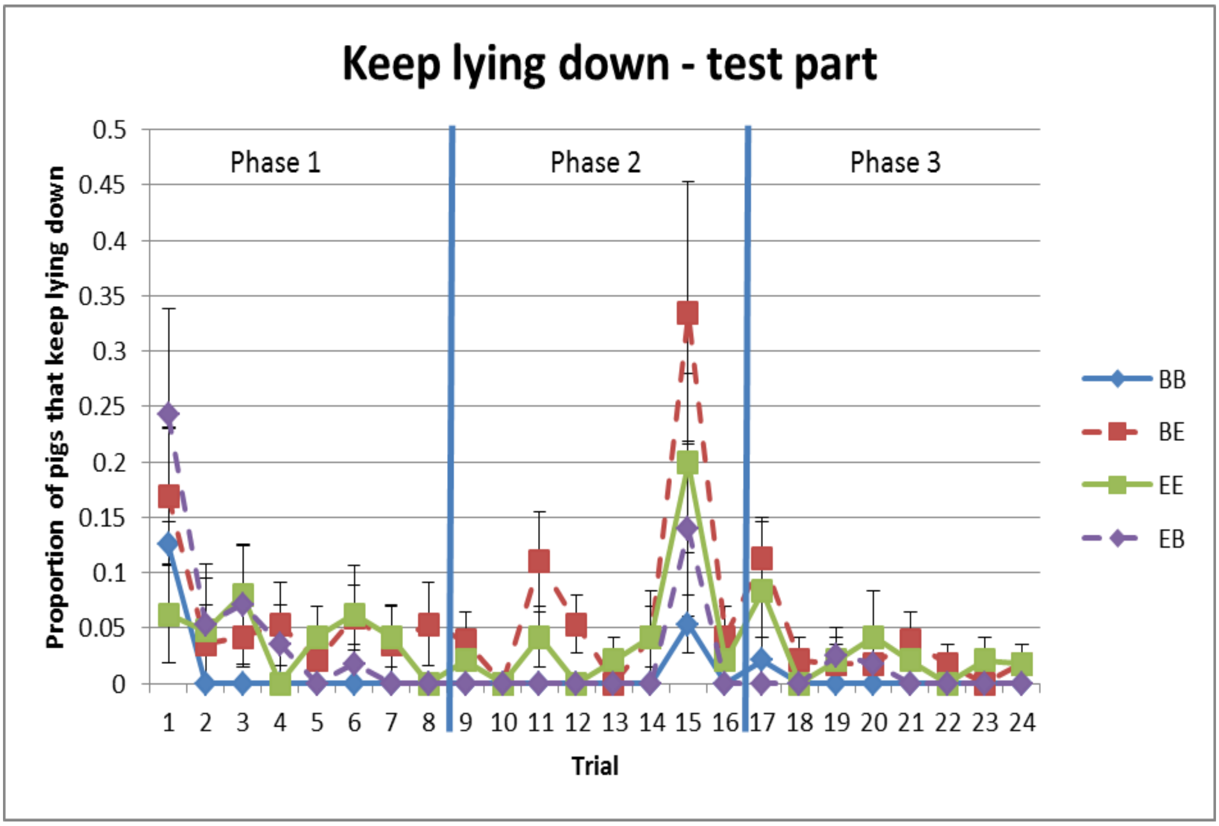

Figure 17. Proportion of pigs per pen that kept lying down in the test part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

A post effect F(1,28)=31.16, p<0.0001, a pre*post effect F(1,28)=4.94, p=0.0346 and a phase effect F(2,56)=6.13, p=0.0039 were found with regard to keep lying down behaviour during the test part of the relative contrast test.
The proportion of pigs that kept lying down in the test part did not differ between phase 1 and 2. However, a higher proportion of pigs kept lying down in phase 2 compared to phase 3. The pre*post effect revealed that BB animals kept lying down less than BE and EE pigs. Also, BE pigs kept lying down more than EB pigs.

Trial 1 showed a trend for a pre*post effect, F(1,28)=2.98, p=0.0951. Post hoc analysis however did not reveal any significant pair wise difference.
In trials 9 and 17, post housed B (BB and EB) pigs tended to keep lying down less than post housed E (EE and BE) animals (post effect trial 9 F(1,28)=3.30, p=0.0800, trial 17 F(1,28)=6.71, p=0.0150).

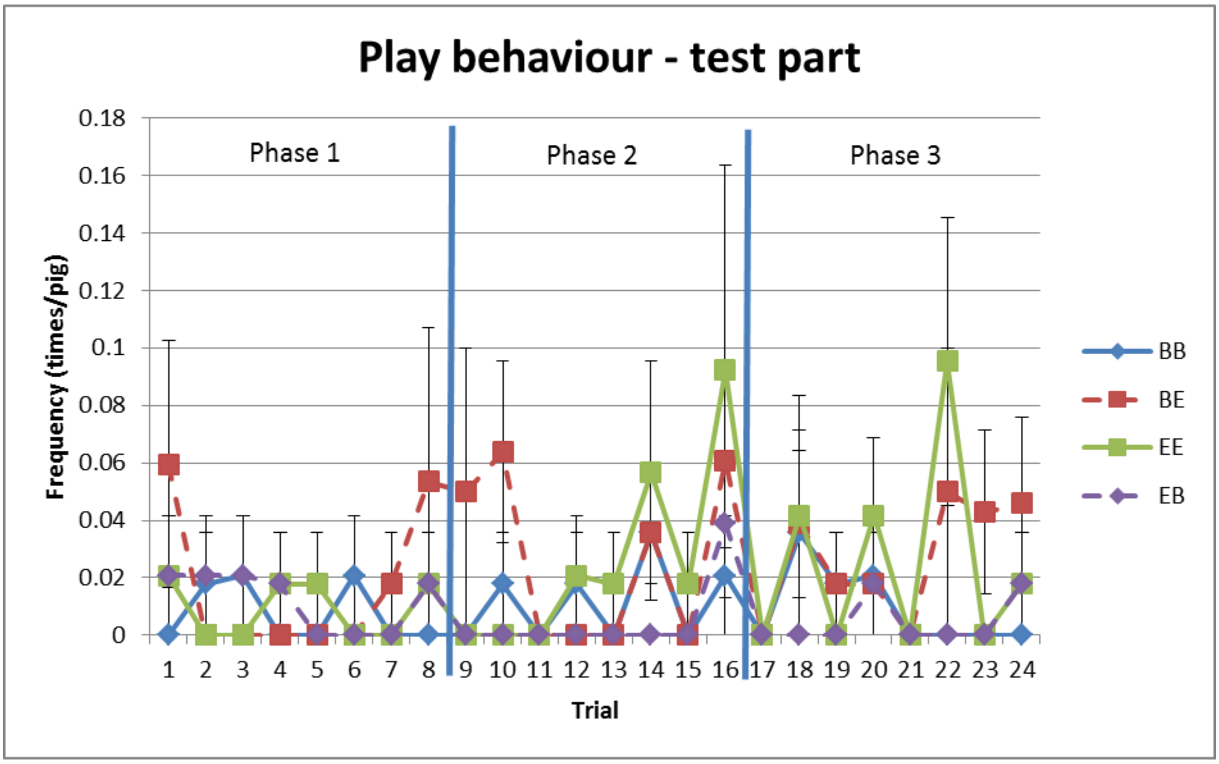

Figure 18. Frequency (times/pig) of play behaviour per pen observed in the test part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

With regard to the analysis based on phase, a post $F(1,28)=9.00$, $p=0.0056$ and a phase $F(2,56)=2.84$, 0.0668 effect were found for play behaviour. Post housed B (BB and EB) pigs showed less play behaviour in the test phase than post housed E (EE and BE) pigs. With regard to phase, a trend existed which showed that pigs tended to display less play behaviour in phase 1 compared to phase 2 and 3.

In trial 1, 9 and 17 no significant differences were found in the amount of play behaviour.

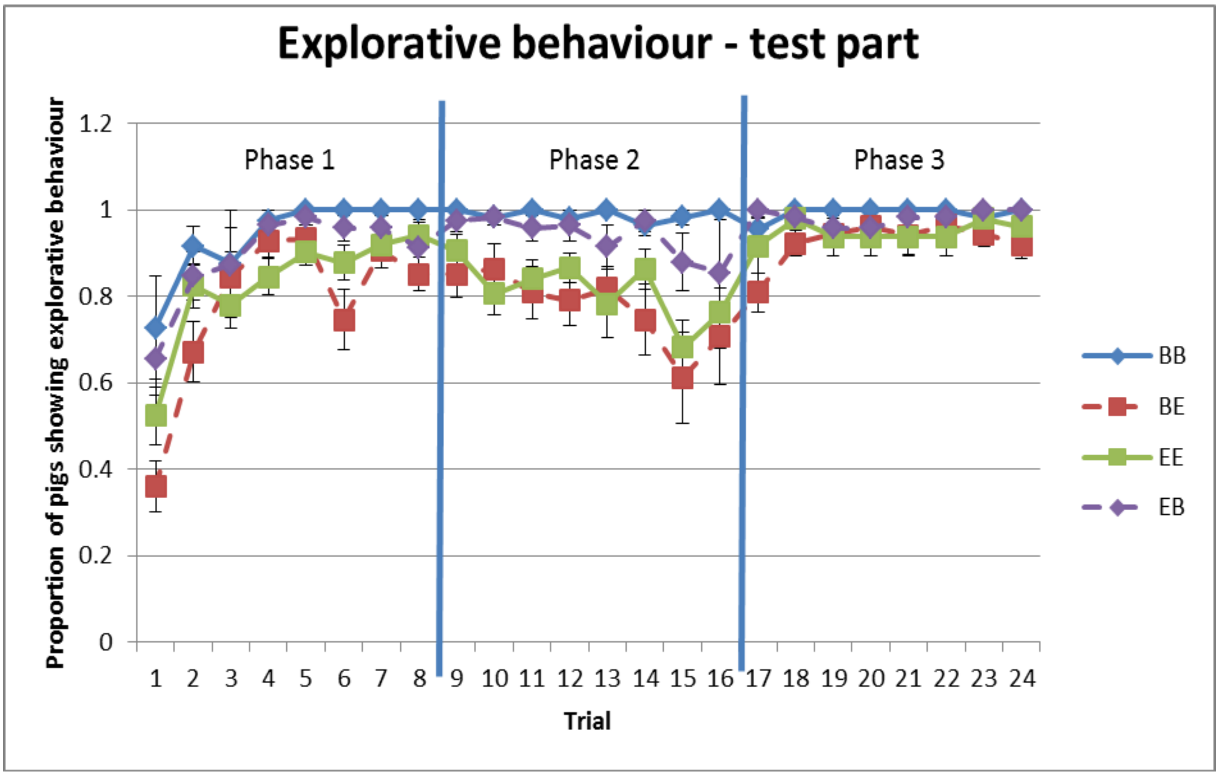

Figure 19. Proportion of pigs per pen that showed explorative behaviour in the test part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

With regard to the analysis based on phase, a post effect was found $F(1,28)=102.11$, $p<0.0001$ as well as a pre*post effect $F(1,28)=9.35$, $p=0.0049$, a phase effect $F(2,56)=31.46$, $p<0.0001$ and a post*phase effect $F(2,56)=15.01$, $p<0.0001$.

The pre*post effect revealed that BB pigs showed more exploratory behaviour than BE and EE pigs and a trend existed for them to show more exploratory behaviour than EB pigs. Also, BE pigs showed less exploratory behaviour than EB pigs, but more exploratory behaviour than EE pigs.

The post*phase effect showed that post housed B (BB and EB) pigs showed more explorative behaviour compared to post housed E (EE and BE) pigs in phase 1 and 2. It was also revealed that post housed E pigs (EE and BE) showed more explorative behaviour in phase 1 and 3 compared to phase 2. No difference between the phases existed for post housed B (BB and EB) pigs.

In trial 1 $F(1,28)=8.53$, $p=0.0068$, 9 $F(1,28)=9.96$, $p=0.0038$ and 17 $F(1,28)=7.72$, $p=0.0096$ housed B (BB and EB) animals showed more explorative behaviour compared to post housed E (EE and BE) animals.

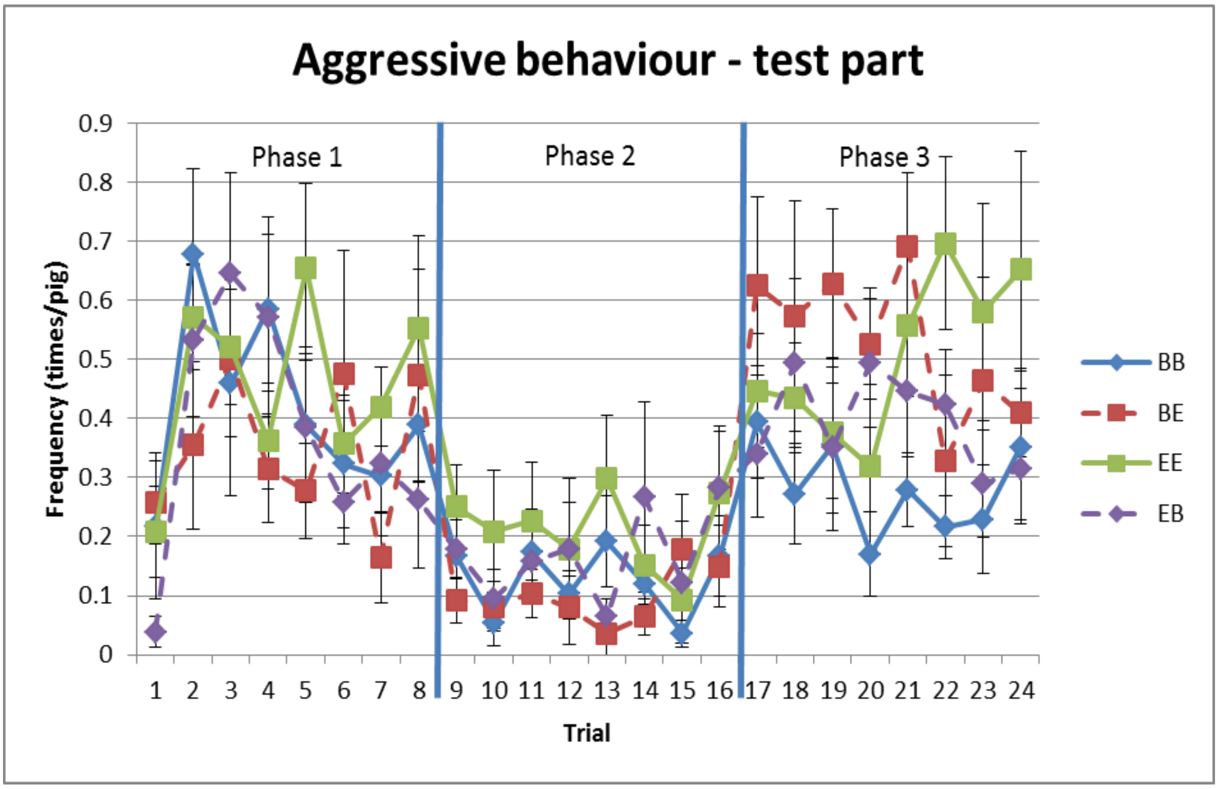

Figure 20. Frequency (times/pig) of play behaviour per pen observed in the test part of the relative contrast test including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

With regard to the analysis based on phase, a pre effect was present $F(1,28)=8.14$, $p=0.0081$ as well as a post effect $F(1,28)=6.18$, $p=0.0192$. Also a very clear phase effect was present $F(2,56)=77.81$, $p<0.0001$ as well as a post*phase $F(2,56)=4.62$, $p=0.0139$ and pre*post*phase $F(2,56)=4.96$, $p=0.0101$.
With regard to the pre*post*phase effect, it was shown that BB pigs showed less aggressive behaviour in phase 3 compared to EE and BE pigs with EB in between. Overall, in phase 2 less aggressive behaviour was shown compared to the other phases. However, this effect did not exist for BB pigs: the amount of aggressive behaviour of BB pigs did not differ between the phases.

In trial 1, post housed B (BB and EB) pigs tended to show less aggressive behaviours compared to post housed E (EE and BE), $F(1,28)=3.24$, $p=0.0826$.

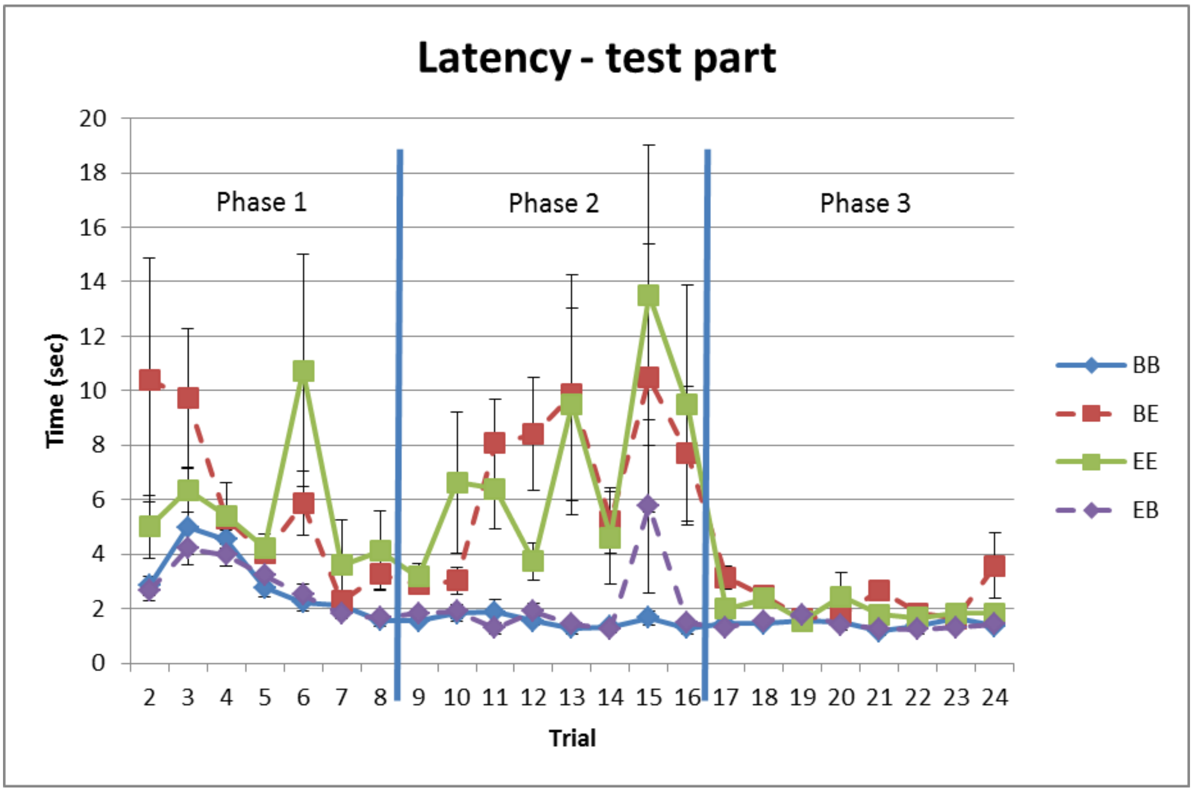

Figure 21. Latency of the first pig per pen to ingest the reward including standard error of the mean of barren housed pigs (BB), pigs that switched from barren to wood shavings-enriched housing at 10 weeks of age (arrow, i.e. 2 days before observations in week 10) (BE), wood shavings-enriched pens (EE) and pigs that switched from wood shavings-enriched to barren housing at 10 weeks of age (EB).

A post effect $F(1,28)=209.00$, $p<0.0001$, a phase $F(2,56)=87.76$, $p<0.0001$ and a post*phase effect $F(2,56)=32.01$, $p<0.0001$ were found on the latency of the first pig within a pen to ingest the food reward. In post housed E pigs (BE and EE), the latency to obtain a reward strongly decreased in phase 3 as compared with phase 2, whereas in B (BB and EB) pigs, latencies were similar in phases 2 and 3.

With regard to trial 2, a post effect was present $F(1,26)=8.30$, $p=0.0078$ showing that post housed B (BB and EB) animals showed a lower latency compared to post housed E (EE and BE) animals. This was also true for trial 10 $F(1,28)=7.79$, $p=0.0093$ and trial 18 $F(1,28)=16.50$, $p=0.0004$.

8.5.2 Long term effects relative contrast test

With regard to the anticipatory behaviour in trial 25, the only long term effect was a significant effect was found with regard to explorative behaviour. A post effect $F(1,28)=5.45$, $p=0.0269$ showed that post housed B (BB and EB) pigs showed less exploratory behaviour compared to post housed E (EE and BE) pigs. No other differences were found in this trial.

8.5.3 Summary results relative contrast test

It was observed that some behaviour patterns showed a clear difference between the treatment groups and between the different phases.

Overall, barren housed pigs were more reactive to the presence of the observers. A higher proportion of barren housed pigs were standing ready, BB animals kept lying down less in both the anticipation and test phase and both groups of barren housed pigs (BB and EB) showed more explorative behaviour during the test time. The enriched housed pigs showed more explorative behaviour in the anticipation time and more play behaviour in the test part compared to barren housed pigs.

With regard to the different phases, most differences were observed between phase 2 and 3. In the anticipation phase of the relative contrast test, pigs showed less standing ready, explorative and were less likely to keep lying down in phase 2 compared to phase 3. In the test part, pigs tended to display less play behaviour in phase 1 than in phase 2 and 3. Pigs kept lying down more in phase 2 compared to phase 3. They also showed less aggressive and explorative behaviour in phase 2 (when the normal pellet feed was provided) compared to the other two phases in which chocolate/yoghurt raisins were provided.

Pre*post effects and pre*post*phase effects, indicative of the importance of experience of housing, were mainly found in the last two phases of the relative contrast test for both during the anticipation and the test time. Indications of the influence of both a negative and a positive was observed. A lower proportion of BB animals kept lying down in the anticipation phase compared to the other groups. A lower proportion of BE pigs showed exploratory behaviour in the anticipation time compared to EE pigs. With regard to the test time, more BE animals showed explorative behaviour compared to EE pigs. With regard to aggression in the test time, BB animals showed less aggressive interactions compared to the other groups.

8.6 Mortality rate

Mortality rate before $F(1,207)=1.66$, $p=0.1985$ or after $F(1,205)=0.11$, $p=0.7392$ the switch in housing did not differ between the treatment groups. The mortality rate for the whole experimental period was 5.66% for BB, EB and BE pigs, the EE pigs group had a mortality rate of 3.77%.

9. Discussion

In this study, the importance of the experience of housing conditions on the welfare of gilts was investigated. The welfare of the pigs was assessed in different ways, using welfare indicators from the animal body, nature and mind approach.
First, the experiments and results of the welfare assessment taken from the different perspectives (i.e. body, nature and mind) are discussed. This will lead to three conclusions of the importance of experience of certain housing conditions for the welfare of pigs, since every different perspective will lead to its own conclusion of animal welfare. This will form the basis for a discussion about animal welfare assessment and the importance of the three approaches. Thereafter, several practical issues are discussed which could have influenced the results.

9.1 Body approach: stomach health

As predicted, barren housing and especially barren housing after a period of enriched housing on a deep layer of wood shavings resulted in pigs having higher stomach scores and more stomach lesions, possibly reflecting a lower physical welfare. According to Ayles et al. (1996) the prevalence of lesions in the pars oesophagus of slaughter pigs ranges from 32-65% which is in line with the percentages found in this study. The aetiology of ulceration of the pars oesophagus is multifactorial (Robertson, 2002). Feeding practices, fineness of feed particles, feed nutrient content, disruptions in feed delivery, weather, *Helicobacter*-like organisms and stress might all influence gastric health (Robertson et al., 2002; Friendship et al., 2004; Barbosa et al., 1995; Hessing, 1992). The provision of wood shavings positively influenced gastric health in the current study. Other studies (Bolhuis et al., 2006; Bolhuis et al., 2007; Guy et al., 2002; Nielsen and Ingvartsen, 2000a) showed that the provision of straw resulted in a lower amount of gastric lesions compared to pigs kept under barren conditions, although Day et al. (2002b) did not observe an effect of the daily provision of half a bucket of unchopped straw on stomach health.
The effect of housing can either relate to the (beneficial) effects of consuming wood shavings or to stress related to (the experience of) housing systems. With regard to the first, it is known that ulcers of the pars oesophagus in pigs are acid dependent (Barbosa et al., 1995). Enzymes and bile protect the pars oesophagus from damage caused by gastric acid while the stomach is full (Friendship, 2004). Also, a higher firmness of the stomach content is related to a low score of gastric lesions (Nielsen and Ingvartsen, 2000b). Therefore, if pigs housed on wood shavings consumed wood shavings on a regular basis this would have reduced the risk of gastric lesions and ulceration. A few (approx. 5) pigs were found to have (some) wood shavings in their stomach. However, it is unknown if they indeed consumed wood shavings on a regular basis.
It is also possible that pigs housed in barren pens suffered more (chronic) stress. Barren housed pigs were unable to perform rooting behaviour, a behaviour which pigs are highly motivated to perform (Studnitz et al., 2007). Indeed, more signs of chronic stress have been found in barren housed pigs as compared with pigs kept in straw-bedded pens (e.g. Beattie et al. 2000). Since a wood shavings bedding has also been shown to be very important to pigs (Ladewig and Matthews, 1996), it is likely that wood shavings have similar, beneficial, effect on the pigs' welfare as straw bedding. Since (chronic) stress is one of the risk factors for ulceration of the pars oesophagus in pigs (Hessing et al., 1992), the lower level of (chronic) stress to which enriched housed pigs were exposed might also explain the differences in stomach lesions. The latter likely accounts for the major part of the difference in stomach health. Both EB and BB pigs were housed under barren conditions after the switch in housing. Overall, EB pigs showed the lowest stomach health. Since *both* EB and BB animals were

housed under barren conditions, stress from the environment was likely the main factor that caused stomach damage. This can be explained by the presumption that EB pigs suffered more stress compared to BB pigs, since these pigs had already had time to cope in the best way with their barren environment and did not have the experience of a higher quality environment. This is supported by the study of Day et al. (2002) who showed that prior experience of straw may reduce welfare of pigs when straw is not provided, compared to pigs with no prior experience with straw. Bolhuis et al. (2006) showed that pigs that were switched from straw-enriched to barren housing experienced a lower welfare (indicated by an increased inactivity) compared to pigs that were always housed barren.

It would be interesting in a next experiment to correlate the stomach score of the individual pig to its behaviour (e.g. positive and negative behaviour). This will allow to control for more factors and to draw stronger conclusions of whether stress related to (the experience of) housing systems was indeed the (main) cause for the development of the stomach ulcers.

The way in which the stomachs were scored (i.e. at the abattoir, at the end of the experimental period) might also (partially) have accounted for the fact that not all predictions were obtained. Ulcers may develop relatively quickly (within 12 h) and also heal relatively quickly (Friendship, 2004). Since an abattoir survey can be done only once, only one indication of stomach health was obtained. If problems did occur several weeks or months before slaughter, these could not be traced back. It would have been very interesting to investigate the development of ulcers using endoscopic examination. This way, the effect of the switch could have been investigated more directly and more thoroughly. However, this practice was not feasible in this experiment. Also, endoscopic examination has its own limitations. The stomachs should be empty, which will increase the risk of gastric ulcer development (Friendship, 2004).

Overall, the body approach would suggest that enriched housed pigs had a higher welfare compared to pigs housed under barren conditions. An indication existed with regard to a longer term effect of experience, as pigs that switched from enriched to barren housing tended to have higher stomach scores and a higher proportion of these pigs showed stomach lesions compared to pigs that were always housed enriched. The body approach therefore indicated that experience of housing was important in the long term, only with regard to a negative switch.

9.2 Nature approach: behavioural observations

Based on the behavioural observations, actual housing conditions would seem most important with regard to pig welfare, although also some indications existed for the importance of the experience of other housing conditions in the past. As hypothesised, pigs housed under barren conditions showed more manipulative and less play behaviour compared to pigs housed on wood shavings. This is in line with studies comparing barren housed pigs with pigs housed on straw bedding (e.g. Fraser et al. 1991; Lyons et al., 1995; Bolhuis et al., 2006; Peeters et al., 2006). Housing on wood shavings seems therefore beneficial for pig welfare.

Pigs that changed from barren to enriched housing conditions (BE pigs) showed a strong decrease in manipulative behaviour and an increase in play behaviour. Pigs that received a switch in housing conditions the other way around, showed the opposite trends. Most of the effects were caused by pre*week and post*week interactions in week 8. Therefore, these effects were only temporarily and not very straightforward.

After the switch in housing conditions, the behaviour of BE and EE pigs (i.e. both enriched housed pigs) and EB and BB pigs (i.e. both barren housed pigs) was more or less similar. This indicates that actual housing was the main determinant of the behaviour of the pigs. Bolhuis et al. (2006) showed a similar result as they also did not find a sustained effect of rearing conditions on these behaviours. With regard to manipulative behaviour, several studies

indicated that actual housing was the main determinant for the prevalence of this behaviour (e.g. Schouten, 1986) whereas other studies showed an influence of rearing conditions on the prevalence of some manipulative behaviours (Day et al., 2002a; Ruiterkamp, 1985). Bolhuis et al. (2006) suggested that these differences could partially be explained by their finding that the impact of rearing history on manipulative behaviour later in life depended on coping style.
It should be noted, though, that several behaviours that were scored in the current study showed a (tendency for a) post effect already *before* the switch. This should of course not have occurred, since it showed that animals housed under the same housing conditions differed or tended to differ in the prevalence of their behaviour, which could not be explained by a difference in experience of these pigs. For each behaviour in which this was found, there are different possible explanations. Belly nosing is highly pig-specific and seems to be 'contagious' (Fraser, 1978). Therefore, prevalence's might have differed to a large extent between pens. The study of Camerlink et al. (2012) supports this. However, it is still worth to mention that pigs never housed on wood shavings (i.e. BB pigs) overall showed more belly nosing compared to the other treatment groups, of which pigs had been housed on wood shavings for at least a part of their lives. A study conducted by van Putten and Dammers (1976), showed that the incidence of belly nosing can be reduced by the provision of straw. This observation is supported by Dybkjaer (1992) who found that less belly nosing was shown by pigs weaned at 4 weeks of age in the presence of straw. Therefore, it might be suggested that the presence of wood shavings also has the ability to reduce the prevalence of this behaviour.
Play fighting is a form of social play which is commonly reported in the young of many mammals (Pellis and Pellis, 1998). It is reported that play fighting is sometimes difficult to distinguish from real fighting (Hinde, 1970 in: Blackshaw et al., 1997), since play easily merges into aggression and vice versa (Schouten, 1986). The difficulty in observing these behaviours might have been the cause for the fact that post effects were already observed in these behaviours before the switch in housing conditions.
A similar explanation can be applied to nudging and manipulative behaviour. Nudging, sometimes referred to as social nosing, is a gentle snout contact in which pigs explore their pen mates. This behaviour might therefore be connected to manipulative behaviour, which is also a way of exploration. Since Beattie et al. (2005) showed a positive correlation between social nosing and tail biting, it might be the case that nudging and manipulative behaviour sometimes develop into each other, making observation at times unreliable. On the other hand, Carmerlink and Turner (2013) showed that social nosing in pigs was largely unrelated to the prevalence of harmful behaviours. However, pigs in their study were housed on straw bedding in which the prevalence of manipulative behaviours is known to be much lower compared to pigs housed in barren pens (Fraser, 1991; Lyons et al., 1995). The relation between harmful behaviours and social nosing might be different in pigs housed in barren pens. This is supported by the fact that pigs in the study conducted by Beattie et al. (2005) were housed in barren pens.
The last behaviour that was observed was another explorative behaviour (interacting with a toy), in which no clear effects of housing were obtained. It is possible that the time when the toys were changed influenced this behaviour. For some observation days, toys were changed during the day by the animal caretakers, thereby providing some pigs with new toys just before they were observed.
An interesting observation has been made with regard to play behaviour. Play behaviour is known to decrease with increasing age of the animal. In the study of Newberry et al. (1988), the frequency of play behaviour of pigs decreased over time but showed a peak in frequency between 2-6 weeks of age. In the current study, this decreasing trend of play behaviour

seemed to be delayed for pigs housed under enriched housing conditions, and especially for pigs that switched from barren to enriched housing.
By means of behavioural observations, it was shown that from the nature point of view, actual housing seemed to be the most important determinant of the welfare of pigs with pigs housed on wood shavings experiencing a higher welfare compared to barren housed pigs. Also a short term effect of the experience of housing was found for both a positive and negative switch. However, no longer term effect of experience was found.

9.3 Mind approach: startle response, gain of reward and relative contrast test

With regard to the animal mind approach, several tests have been conducted. A startle response test, a gain of reward and a relative contrast test were all conducted to assess the mental welfare of the pigs.
It was hypothesised that enriched housed pigs would have a more positive emotional state compared to barren housed pigs. Overall, this hypothesis seemed to be accepted. It was also hypothesised that pigs receiving a switch from barren to enriched housing would have the most positive emotional state and pigs switching housing conditions the other way around were predicted to have the most negative emotional state. This hypothesis could not be confirmed but indications of the importance of experience were however present for both a positive and negative switch in housing.
Each individual test will be discussed as well as its results. Also, implications for further research are stated with regard to these tests, since they have not yet been conducted (on pigs) much.

9.3.1 Startle response test

Only a minor effect of treatment was found with regard to the startle response test. BB pigs tended to show a higher number of freezing bouts compared to EB and BE pigs. Although this might imply that these pigs were in the most negative emotional state, the hypotheses with regard to the mental welfare of the pigs could not be confirmed by this test. No startle response test that investigate the emotional state of pigs after a switch in housing conditions are known. Therefore, no direct comparison with results of other startle test responses can be made in this regard. However, a comparison of this startle response test and other startle response tests in pigs can be made with the aim to increase the knowledge of startle response tests conducted on pigs. By this means, recommendations of how best to conduct startle response tests on pigs can be made.
Blackshaw et al. (1998) showed that pigs housed with three other conspecifics showed a first freeze response of 1-12 sec when subjected to a startle response test. The duration of the first freeze responses obtained in this study was on average 14.90 sec and ranged from 0 – 56.55 sec. The reason for this difference with the study of Blackshaw and coworkers is unknown, but might be caused by the difference in age between the pigs in both studies. Pigs in the current study were subjected to the startle response test at an age of 12 weeks, whereas in the study of Blackshaw et al. (1998), pigs were only 5-6 weeks old. Blaszczyk and Tajchert (1996) showed a developmental effect of the acoustic startle response (ASR) of rats: a positive correlation between body weight and ASR amplitude was found. It might therefore be the case that a similar age or weight effect caused the relatively long freeze response in this study as compared with Blackshaw et al. (1998). It is also possible that the difference was caused by the fact that different amounts of pigs were kept in the pens, i.e. in the study of Blackshaw et al. (1998) 3 and in the current study 6 or 7 pigs were housed in a pen. It is possible that the reaction of the pigs partially depends on group size. Another factor which might have (partially) accounted for the difference was difference in pen size between the study of Blackshaw et al. (1998) and this study (1.44m2 vs 8.4 m2 respectively). Even though

the piglets in the first study were younger and were therefore also smaller, this might also have influenced the results since a difference in density was present..

It might of course have been the case that the emotional state of the pigs did not differ at the moment of the startle response test. However, another and more likely possibility of why the startle test did not reveal a clear housing and/or experience effect might be linked to one or several factors which will be explained below.

First of all, it is likely that most parameters were not measured in the best way. In rodents, a startle response test can be measured in a startle apparatus in which the latency to startle and reflex amplitude can be assessed in a much more standardized way as shown by Swerdlow et al. (1986). This method allows for a more precise registration of the startle reflex. The startle reflex of pigs can also be investigated by measuring the eye blink reflex using electromyograpic (EMG) recording electrodes (Blumenthal et al., 2005). Both methods allow a higher accuracy in the assessment of the startle response. However, it was not possible in the current experimental setting to measure the startle response of the pigs in a similar way.

Secondly, the posture of the pigs shown directly before the acoustic stimulus was presented influenced some aspects of their response. Pigs that were not lying when the acoustic stimulus was presented, tended to show a shorter latency-to-startle. These pigs might have been in a more alert state, probably in response to the presence of the persons entering several minutes before. Brown et al. (1991) showed that in humans, the startle response is also influenced by posture. A shorter latency to the onset of reflex EMG activity following auditory stimulation was observed in persons that were standing compared to persons that were sitting. This indicates that the position in which people were positioned probably influenced their level of alertness. This might also have been the case in the current study. Pigs that were not lying were also more reactive in their response: they showed a shorter duration of the first and total freeze response, a greater number of freezing bouts and a longer latency-to-freeze, i.e. they showed a higher amplitude in their response compared to pigs that were lying. This might indeed indicate a higher level of alertness. Pigs that were lying might have shown a little less intense startle response because their posture did not easily allow them to show an intense reaction. However, this does of course not necessarily imply that the latter were in a more *positive* emotional state. Therefore, the influence of the posture of the pigs should be taken into account when conducting a similar startle response test, pigs should preferably have the same posture when a startle test is conducted. However, to accomplish this in group housing is difficult of course.

With regard to the practical issues of the startle response test, the persons who exploded the balloons were all standing in front of two pens in the middle of the unit. Therefore, it might be possible that all these 8 pens (4 units*2 pens) were influenced by the presence of the persons in a slightly different way compared to the other groups. Also, the decibel level at the end of the unit was 94.1. This is similar to decibel levels used in the study of Blackshaw et al. (1998). Even though this is quite a loud noise, it is likely that this value was higher in the middle of the unit which might have had an influence on the startle response of the pigs. Also, pigs of adjacent pens might have influenced each other. If this was true, pigs housed in pens at the end of each unit were likely to be the least influenced, since they were only connected to one adjacent pen. Since the four units were exact replicates of each other, this implies that some treatments were always more influenced by certain of the previously mentioned factors than other treatments.

The startle response test was conducted in four units at the same time and a slight difference (ms) was observed in the time when the balloons were exploded. This might have accounted for the unit effect, which was observed in some behaviours.

Blackshaw et al. (1998) showed that pigs show a clear startle-freeze response in reaction to a novel acoustic stimulus. Other studies (Lang et al., 1998; Vrana and Lang, 1990) show that a startle response test can indeed indicate the emotional state of an animal. Therefore, the further development of the current paradigm is highly recommended.

9.3.2 Gain of reward test

With regard to both the short and long term effect of a gain of reward (a hessian bag), post housing effects were already observed before the switch in housing. Therefore, the gain of reward test did also not seem to generate very reliable results. Hence, no conclusions could be drawn based on the gain of reward test and the hypotheses as previously stated could not be confirmed.

It might have been that an insufficient number of different time points were observed to obtain reliable results. Another possibility is that the hessian bag was not perceived by the pigs as a *gain* of reward, but was valued by them approximately the same as any other toy. They might not have interacted with the hessian bag enough which made analysis prone to random effects, resulting in the observation of pre*post effects already before the switch in housing conditions. The study of Van de Weerd et al. (2003) showed that pigs perceived a hanging hessian bag at day one of provision as a relatively popular (according to relative object interaction) toy (i.e. rank 18 out of 74 toys tested). However, after 5 days the hessian bag no longer belonged to the top 25 of popular toys. This indicates that pigs might perceive the hessian bag as a gain of reward in the short, but not in the longer term.

It is recommended that in a next experiment, a reward should have (scientific) evidence that it is perceived by the pigs as a gain of reward (i.e. something above average nice). For example, lavender straw with whole peanuts in a box (most popular toy in the study of Van de Weerd et al., 2003) or a food reward such as chocolate raisins can be chosen. Chocolate raisins have successfully been used in a number of studies as a reward for pigs (Arts et al., 2009; this study). Arts et al. (2009) reported that feed deprivation was not needed to motivate pigs to search for the chocolate raisins, which was similar to the observations made in this study (see section 9.3.3).

9.3.3 Relative contrast test

The relative contrast test indicated, again, that actual housing would seem most important with enriched housed (EE and BE) pigs showing a more optimistic reaction, indicative of a more positive emotional state, compared to barren housed (BB and EB) pigs. However, indications of the importance of experience were also present. Pigs that were housed in barren pens after a period of enriched housing seemed to be in the most negative emotional state compared to the other treatment groups.

Overall, pigs of all treatment groups showed more or less the same response towards the *change* in rewards over the different phases. However, *between* the treatment groups, a clear difference was observed in their behaviour in the test.

Barren housed pigs showed a stronger reaction towards the presence of the observers, i.e. they were more aroused: a lower proportion of pigs kept lying down, a higher proportion was standing ready and these pigs showed a shorter latency to obtain the reward compared to enriched housed pigs. Stolba and Wood-Gush (1980) suggested that in barren environments, arousal is generally increased. They observed that the more barren the environment, the stronger the reaction of the pigs towards a stimulus (i.e. a tyre in their study). Pearce and Paterson (1992) reported that pigs reared in an environment which was supplied with toys showed a lower reactivity towards a novel object and a stationary human compared to pigs which were not provided with toys. De Jong et al. (1998) reported that barren housed pigs showed a higher behavioural reactivity to novel situations compared to enriched housed pigs when transported to the abattoir. A study conducted on mink by Meagher and Mason (2012) showed that mink from non-enriched cages showed a heightened investigation of stimuli when presented. They also showed that mink from non-enriched cages had a shorter latency to contact the stimuli.

It therefore seems that animals housed in barren environments experience a certain level of boredom. Boredom indicates a negative state and is caused by under-stimulation. Therefore, boredom should increase the interest in all kinds of stimuli (Meagher and Mason, 2012) which was also observed in the current study. Barren housed pigs seemed to respond more positively (i.e. higher reactivity) towards the gain and even the loss of reward. Even though this might indicate that barren housed pigs are in a more positive emotional state compared to enriched housed pigs, it is more likely that barren housed pigs showed a higher level of reactivity in the test since they experienced a higher level of boredom. A higher reactivity should not be confused with a more positive response. Barren housed pigs also showed less play behaviour compared to enriched housed pigs which supports this. However, the frequency of play behaviour shown during the test was very low, so these results should be handled with caution.

Aggressive behaviour was shown more by enriched housed pigs during delivery of the reward. Since feeding behaviour is known to induce aggression in pigs (Baxter, 1983), this heightened aggression arising from the motivation to obtain the rewards was likely to be higher in enriched housed pigs, who had more difficulty in obtaining the food rewards among the wood shavings. This is supported by the fact that in phase 2, when the normal pellet feed was provided, less aggression was shown by the pigs (i.e. their normal pellet feed was not worth fighting for).

Overall, wood shavings seemed to induce a more positive emotional state in the pigs, indicative of a higher welfare. These pigs seemed to experience a lower level of boredom compared to barren housed pigs.

In barren housing conditions, the effect of a *switch* of housing conditions was also present. Overall, BB pigs showed a higher behavioural reactivity compared to EB pigs, i.e. they kept lying down to a lesser extent and also tended to stand ready to a higher extent. Since barren housed pigs seemed to be more bored and EB pigs showed less interest in the stimuli than BB pigs, the possibility exists that EB pigs suffered from a certain degree of apathy. Apathy is a decreased motivation to obtain or interact with any stimuli (Meagher and Mason, 2012). This is supported by the observation made in the very first trial, in which EB pigs tended to keep lying down in a higher proportion than the other treatment groups. This is interesting since it seems that EB pigs were less responsive to the first presence of the observers in the test setting, indicative of a more negative view of what was going to happen (i.e. it was not worth standing up for). The amount of aggressive behaviour of BB pigs was also lower than that shown by EB pigs. Therefore, it seems that BB pigs were in a less negative emotional state compared to EB pigs. Pigs that had experience of a barren pen and were subsequently housed on wood shavings (BE pigs), showed a less clear difference from EE pigs. This might be explained by the fact that humans and therefore possibly also animals, seem to be more sensitive to reward loss (i.e. moving from enriched to barren housing) compared to a reward gain (i.e. moving from barren to enriched housing) (Dreher, 2007).

Explorative behaviour will be described separately since it gave some contradictory and inconsistent results with regard to the different treatment groups, the different phases and the anticipation and test part.

Barren housed pigs showed less explorative behaviour in the anticipation phase compared to pigs housed on wood shavings. This is likely due to the differences in flooring of these two groups. It was more difficult for enriched housed pigs to find the treats, since the treats were prone to sink into the wood shavings bedding. Therefore, they could not see the rewards in an easy way and they started searching for them already before the treats were provided to them, i.e. in the anticipation phase. Barren housed pigs could see the rewards much clearer on the

barren floor which is possibly why most of them did not start searching before the treats were thrown into the pen.
In the test phase however, barren housed pigs showed in phase 1 and 2 more explorative behaviour compared to enriched housed pigs. This might be explained by their higher level of reactiveness since they likely experienced a higher level of boredom. Barren housed pigs kept displaying explorative behaviour to a greater extent compared to enriched housed pigs in phase 2 (when the pellet feed was provided). Again, the result of the current study might have been due to the differences in flooring, but it might also indicate a difference in emotional state of the animals. Burman et al. (2008) showed that rats from unenriched housing conditions showed a more negative response towards a reduction of reward, i.e. a longer latency to obtain the reward. This is contrary to the result obtained in this experiment in which barren housed pigs seemed to be less sensitive to reward loss, with regard to latency (i.e. barren housed pigs showed a shorter latency) and explorative behaviour.
In both the experiment of Burman et al. (2008) and the current experiment, a relative contrast was induced. In the design of the current study, it is possible that exploratory behaviour did not give a valid indication of the emotional state of the pigs. Flaherty (1996) stated that the response to a reward reduction involves also searching for the lost reward (i.e. in this case the mix of chocolate and yoghurt raisins). This can be perceived as a non-emotional process (it has been shown to be insensitive to anxiolytics) and is a parameter that is likely to be less responsive to differences in background affect. This might explain why a higher proportion of barren pigs kept showing exploratory behaviour in phase two since the normal pellet feed was provided at that time while they were expecting the raisins. It might be that barren housed pigs reacted more negatively to the loss of reward (i.e. they kept searching instead of accepting the situation). On the other hand, the observation that a higher proportion of barren housed pigs kept displaying explorative behaviour and the fact that they showed a shorter latency to obtain the reward might also be explained by the probability that barren housed pigs were less sensitive to a loss of reward, indicative of a more positive emotional state. However, it should be noted that rats that shifted from a preferred to a less preferred reward showed a decrease in goal-directed behaviour and an increase in exploratory behaviour (Crespi, 1942, 1944; Elliott, 1928). An early aspect of the reaction towards reward loss is therefore searching (Elliott, 1928; Flaherty, 1996), the emotional components of the reward loss have been reported to occur later. Based on this, it would have been expected that pigs initially showed an increase in explorative behaviour and after a few trials, the frustration due to the loss of reward would occur. This only seemed to happen in the enriched housed pigs and would mean that enriched housed pigs are therefore more sensitive to a loss of reward, implying that they are in a more negative emotional state. However, this is not in line with the results of the other behaviours and might be explained by the observation that exploration reduces boredom (Studnitz et al., 2007). Since barren housed pigs likely experienced a higher level of boredom, this was a confounding factor.
A disappointment reaction (i.e. a higher latency to reach the reward) is easier found in experiments which use a runway maze, as is done for example in the study of Burman et al. (2008) and Crespi (1942). In these experiments, the only parameter tested is the animals' latency to reach the reward. In the current study, the *amount* of pigs exploring the ground was scored, thereby preventing a similar, highly sensitive way of scoring the disappointment reaction to the loss of reward.
It is therefore recommended that in a next study, not only the number of pigs showing explorative behaviours is scored, but also the duration that each pig shows this behaviour. It should be mentioned that a strong advantage of conducting a relative contrast test in this way compared to the usual speed of running test, is the fact that there is unlikely to be a ceiling effect as pigs could show as much play or aggressive behaviour as they wanted (Flaherty,

1996). It is highly recommended that when a similar study is conducted, the effect of different flooring is eliminated by means of conducting the test in another room. This might make the effects of experience and actual housing on the mental welfare of pigs more clear.
The chocolate/yoghurt raisins and the normal pellet feed used as rewards in the relative contrast test did seem to be perceived as a gain and loss of reward respectively. Overall, pigs showed less response in the second phase of the test compared to the other two phases (i.e. a higher proportion kept lying down, a lower amount of pigs were standing ready, less explorative behaviour was shown). Also, overall, less aggression was shown in the test part of phase 2, which indicates that normal pellet feed is valued lower compared to the raisins for which the pigs were willing to 'fight for'.
The test was analyzed by phase since some trials did give some inconsistent results which could not be explained. Especially trial 13, 14 and 15 differed strongly from the other trials for some of the behaviours. Trial 13 and 14 were conducted on a Sunday, trial 15 was conducted on Monday morning. It might have been that during these days, less people were present at the farm causing the pigs to react in a different way during the test.
If the higher reactivity of barren housed pigs was indeed caused by a higher level of boredom it can be concluded that pigs housed on wood shavings indeed experienced a higher level of welfare compared to barren housed pigs. The relative contrast test therefore also seemed to indicate that actual housing was most important. This observation is in line with the hypotheses. However, it has also been predicted that pigs that switched from barren to enriched housing would be in the most positive emotional state and pigs that switched housing conditions the other way around were hypothesised to be in the most negative emotional state. Some indications existed that pigs that switched from enriched to barren housing conditions were in a more negative emotional state compared to the rest of the treatment groups. However, no clear indications were obtained with regard to the hypothesis that pigs that switched from barren to enriched housing conditions were in a more positive emotional state.

Comparing the three different welfare indicators

The different welfare indicators all gave other 'pieces of information' with regard to animal welfare. The physical welfare indicator of the animal body approach (i.e. stomach health) indicated that it was mainly a negative switch in housing that affected the welfare of pigs. If animal welfare was assessed based on this physical welfare indicator alone, valuable information would have been missed, since the welfare indicator of the animal nature approach also indicated that the effect of a positive switch was important, even though the effects were not very strong and only a short term effect seemed to be present. The animal mind approach, in addition, gave the best indication that also a longer term effect of experience was present. The different welfare indicators can thus be regarded as complementary towards each other. Therefore, in order to give a valid and complete conclusion about animal welfare it is necessary to assess the welfare of an animal with use of all three welfare indicators.

9.4 General discussion

Welfare indicators of the body, nature and mind approach all seemed to indicate that barren housed pigs experienced a lower (physical and mental) welfare compared to pigs housed on wood shavings. With regard to the switch in housing conditions, it seemed that pigs that had changed from barren to enriched housing conditions experienced the highest level of welfare compared to the other treatment groups. This effect only seemed to be temporarily. However, pigs that changed housing conditions the other way around (i.e. from enriched to barren) seemed to experience the lowest level of welfare (after the switch) of all treatment groups.

Instead of being temporarily, the effect of the change of this group seemed to be a longer term effect.
Animal welfare assessed by physical and/or behavioural means only might therefore lead to wrong conclusions. An example of this comes from McGlone (1993). He stated that an animals' welfare is poor only if its physiological systems are disturbed to the point that survival or reproduction are impaired. Based on this, he concluded that crated sows, because of their superior reproductive success as compared to sows in pastures or with neck tethers, do not experience chronic stress and therefore their welfare is not impaired. However, it is known that sows housed in this way develop stereotypies like chain rooting, chain chewing and sham actions (Sambraus, 1981; Stolba et al., 1983; Cronin and Wiepkema, 1984 in: Cronin et al., 1986). As stated previously, stereotypies develop over time and are indicative of longer term welfare impairment. Therefore, crated sows do seem to suffer even though their physiology does not show signs of suffering.
In a study about castration in piglets, McGlone and Hellman (1988) found that castrated piglets reduced their feeding behaviour and increased their time spent lying. However, according to McGlone (1993), this behavioural change does not necessarily predict poor welfare. This is of course disputable, however, McGlone (1993) also stated that *only* if immunity was suppressed would the welfare of the piglets due to the castration be poor. He stated that only when the animal reaches a prepathological state, can its welfare be concluded to be poor (Moberg, 1985). Therefore, in another study on castrated piglets, natural killer (NK) cytotoxicity was measured. A reduced NK cytotoxicity would suggest that the castrated piglets experienced poor welfare. No significant differences in NK cytotoxicity were found between castrated and uncastrated males. Based on this, McGlone (1993) concluded that only for *some* piglets castration is painful since in some cases suppressed NK activity was found and in others not. However, it is very unlikely that some piglets, when castrated, did not experience poor welfare. Evidence that painful procedures lead to a negative emotional state indicative of impaired welfare comes from Neave et al. (2013). They showed that calves exhibited a negative judgement bias for at least 22 hours, indicative of a negative emotional state, due to post-operative pain following hot-iron disbudding. It is very likely that the piglets used in the study of McGlone (1993) would also be in a more negative emotional state, due to the pain caused by castration. This was however not shown by the physical and, according to McGlone (1993), also not by the behavioural welfare indicators that were used. This again stresses the importance to use all *three* welfare indicators to assess animal welfare.
Of course more measures should be taken for all three welfare parameters to obtain a more valid conclusion regarding animal welfare assessment than used in this study. However, based on the results of this thesis it is clear that all three welfare indicators are needed in order to assess animal welfare in a valid way. It is recommended that further research is done with regard to the importance of the three welfare indicators.
Since physical and mental welfare do influence each other, pigs might have been (unconsciously) selected not only on their physical performance but also based on their mental state. Since the adoption of intensive animal husbandry practices, the welfare of an animal is not necessarily linked to its productivity anymore and a healthy physical state of animals does not necessarily mean that their mental welfare is good (Pond, 2012). This is another reason of why it is very important not to base complete welfare assessments on physical welfare indicators solely. Since pigs (and other intensively kept animals) have to cope with intense physical and mental challenges, animals that were best able to cope with these mental challenges might also have been in a better physical state and were therefore selected to produce offspring. This by no means implies that the current pigs do not suffer in barren environments, they still have the strong need to perform natural behaviour, but it might be possible that there is a certain heritability in mental state. In humans, it is shown that

approximately 40-50% of the risk for a depression is genetic, which makes this mental disease a highly heritable trait (Sanders et al., 1999; Fava and Kendles, 2000 in: Nestler et al., 2002). It is possible that the same principle also applies to other mammals.

Housing conditions could also have influenced the reaction of the pigs to the switch in housing conditions in a different way than stated before (i.e. importance of the experience of a worse or better housing environment). Even though the environment of the pigs was the same for the first four weeks of their life, the environments in which the pigs were housed from four weeks of age (i.e. the beginning of the experiment) until the switch in housing conditions at 10 weeks of age might have influenced several characteristics of the pigs' brains. Experience can influence brain plasticity (i.e. the ability of the brain to change structure and function) and can cause multiple, dissociable changes in the brain (such as increases in dendritic length, increased glial activity). These anatomical changes influence the behaviour of the animal concerned (Kolb and Whishow, 1998). The rearing of animals under deprived or abnormal (i.e. barren housing) conditions has severe implications for the growth and survival of dendrites, axons, synapses, interneurons, neurons and glia (Joseph, 1999). This is closely connected to the development and therefore the working of the limbic system, which is important for good social and emotional capacities. The developing limbic system is experience dependent, therefore, pigs reared in barren environments might develop a limbic system that resembles limbic systems that have suffered destruction or amputation (Joseph, 1999). As a consequence, pigs reared under enriched environments might have been better in coping with emotionally challenging conditions, because their brain, and especially their limbic system in this regard, has been able to develop in a better way compared to pigs raised in barren environments. This implies that pigs switching from an enriched to a barren environment might have been better able to cope with this switch due to their better developed limbic system. This would have mitigated the effect of the negative switch. If this is true, this will have influenced not only the welfare indicators of the animal mind approach, but also the other welfare indicators, since mind and body are connected.

Rearing conditions and their effect on brain development are also linked to behavioural flexibility which is defined as the ability of an individual to directly respond and adjust its behaviour to environmental stimuli (Coppens et al., 2010). It has been shown in rodents that relatively barren rearing environments may reduce behavioural flexibility (Morgan, 1973). Therefore, pigs that were reared under enriched conditions but moved to barren housing, might have been better able to cope with the (negative) change, because of their greater behavioural flexibility. This might also have accounted for the fact that not much differences existed between post housed barren (BB and BE) pigs. However, this does not apply to enriched housed pigs, where, based on this way of reasoning, more clear differences would be expected. However, it seems that negative contrast effects are easier to detect (Flaherty, 1996) which might explain this result. It should be mentioned that differences in housing in the current study were not that large to expect major effects on brain development and subsequent working. Further investigation of the importance of rearing on the mental welfare of pigs is therefore needed.

9.5 Practical issues

Some practical issues which could have influenced the results should be addressed. Treatments, i.e. the combinations of pre- and post-switch housing conditions, were evenly distributed over the four units, however, within each unit the pens were placed in the same order (i.e. units were exact replicates of each other). The first pens of each unit were pens containing wood shavings. Animals housed in pens closest to the door, were therefore most disturbed by people opening the door of the unit. They were likely to be the most aroused each day compared to the other pens. This might have influenced their behaviour and might have caused them to experience more stress, thereby possibly also influencing their (stomach) health and welfare. The last pens always were barren pens which were housed under the air cleaning machine. It appeared that pigs of these pens suffered more from coughing compared to pigs in other pens, which was tried to be counteracted by changing the working of the air cleaner. However, it appeared that the pens at the back of each unit suffered from the most draught. This also might have influenced their (stomach) health and welfare.

It was difficult to trace back any possible influence of these issues with regard to the experiments and observation sessions conducted. This is because pigs that did not switch housing conditions were also moved to another pen to control for a possible effect of a new environment. This way, after the switch, pigs of other treatments were exposed to the before mentioned factors. On the other hand, this practice did mitigate the influence of being more aroused in the first pens and suffering from draught in the last pens since in this way more than two treatments were exposed to these factors in each unit.

This division of units as mentioned before has been chosen based on considerations of the experiment that investigated Osteochondrosis (OC) development in the gilts. One of the main considerations was that barren pens would pose more risk to the pigs of slipping when they would be startled by the opening of the unit door. Pigs housed on wood-shavings would have more adhesion because of the floor and therefore they were exposed to less risk of slipping on the floor. This way, OC scores would less be influenced by pigs falling on slippery floors.

It is possible that some diseases influenced the behaviour of the pigs in the observation sessions and it might also have influenced their reaction in the experiments. Especially during the month of August, infections with *Actinobacillus Pleuropneumoniae* (App) were present, which caused the death of three pigs.

The influence of OC development is less likely to have been influential to the health and behaviour of the pigs since no major differences were obtained between pigs in different housing conditions with regard to OC scores.

The last point to address is that coping styles have not been taken into account in this study. Bolhuis et al. (2006) showed that the influence of rearing history and actual housing on behaviour and gastric lesions depended on a certain extend on coping style. It might therefore be the case that, if coping styles were taken into account, (slightly) different results would have been obtained.

10. Conclusions

From the previous, several conclusions can be drawn:

First of all, pigs housed under barren housing conditions have a lower (physical and mental) welfare compared to pigs housed on wood shavings. This was shown by welfare indicators of all three approaches toward animal welfare (i.e. animal body, nature and mind). Secondly, experience of housing systems matters with regard to pig welfare.

With regard to the importance of experience, it was shown by the body approach (stomachs) that a negative switch in housing affects pig welfare. The nature (behaviour) and mind (relative contrast test) approach showed that both a positive and negative switch in housing influences pig welfare although current housing conditions seem most important.

All three welfare indicators (body, nature and mind) give overall consistent results: experience of housing systems matters. However, they each provide different, valuable information regarding the welfare of pigs. Therefore, for complete welfare assessment, all three welfare indicators are needed.

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Appendices

Appendix I

Pilot study relative contrast test
The main concern for the group wise relative contrast test to generate reliable results was that all the pigs in a pen had to be able to simultaneously participate in the test. A reward with high incentive value (a mix of chocolate and yoghurt raisins) was provided to the pigs for nine times (three days for three times a day) after which a reward with lower value (their normal pellet feed) was provided for six times (two days for three times a day). Thereafter, the chocolate raisins were provided once again.
The gilts used in this study had already been used in another study which was on emotional contagion, conducted by Ir. I. Reimert. They were housed in groups of four in pens with different dimensions than the pigs used in this experiment. The provision of the rewards was done in a specific setting. Two observers stood in front of the pen of which observer 1 scored all the behaviours and observer 2 scored the vocalizations and threw the reward in the pen. A video camera was placed in front of the pen too. Each trial consisted of approximately one minute (several different lengths of time were investigated). At the start of the minute observer 1 shouted 'ja' after which observer 2 put a white basket on the top of the fence of the pen. After 5-10 seconds (anticipatory phase) observer 1 again shouted: 'ja' and observer 2 threw the reward into the pen. This was the start of the 'test phase' which lasted for 50-60 seconds. The end of the minute was indicated by observer 1 shouting: 'stop'!
The pilot study showed that the pigs needed approximately four trials to make a good association between the cue (white basket) and the reward. To counteract a possible olfactory effect, both food rewards (i.e. the mixture of chocolate/yoghurt raisins and pellet feed) were placed in the bucket.
Based on Reimert et al. (2013), the vocalizations of the pigs were investigated. Play and aggressive behaviour were scored shortly before (5 and 10 seconds), during and after the provision of the food rewards (50 and 60 seconds) to investigate their anticipatory response to the reward as well as their response to (the loss of) reward and their possible recovery from the disappointment over the different trials. The latency to approach the reward was also investigated. Exact measurements and timings were determined during this pilot study and led to the final relative contrast test as described.

Appendix II

Analysis relative contrast test per phase, trial included in the model

Anticipation part

Standing ready

Analysis per phase showed that a pre housing effect existed $F(1,28)=5.11$, $p=0.0318$.
Pre B housed animals (BB and BE) appeared to stand ready in a higher amount than pre E housed animals (EE and EB) in the first phase.
With regard to the second phase, a post housing effect was present $F(1,28)=32.27$, $p<0.0001$. It appeared that post housed B (BB and EB) animals showed a higher amount of standing ready behaviour compared to post housed E (EE and BE) animals.
In phase 2 also a trial effect was present $F(6,168)=30.50$, $p<0.0001$ as well as a pre*trial effect. All pigs showed less standing ready behaviour in trial 12 compared to trial 13 and showed more standing ready behaviour in trial 13 compared to trial 14.
With regard to trial 15, pre housed B (BB and BE) pigs showed more standing ready behaviour compared to pre housed E (EE and EB) pigs. Pre housed E (EE and EB) pigs showed more standing ready behaviour in trial 14 than 15. They also showed more standing ready behaviour in trials 16 than trial 15.
With regard to phase 3, a pre effect $F(1,28)=5.72$, $p=0.0238$ and a post effect $F(1,28)=27.85$, $P<0.0001$ existed. As well as a pre*post effect $F(1,28)=4.65$, $p=0.0399$ and a trial $F(6,168)=3.66$, $p=0.0019$ existed. Also a trend with regard to pre*post*trial was present.
BB pigs showed more standing ready behaviour compared to EE and BE pigs. BE pigs showed more standing ready behaviour than EE pigs. EB pigs showed more standing ready behaviour than EE pigs. Also, EB pigs showed more standing ready behaviour compared to BE pigs.

Running up

With regard to the anticipatory part of running up, it was shown that in phase 1, a pre*post*trial effect was present in a trend $F(6,168)=2.13$, $p=0.0522$. Post hoc analysis however did not reveal a significant effect.
With regard to phase 2, a trial effect existed $F(6,168)=10.21$, $p<0.0001$. Post hoc analysis revealed that in trial 12 more running up was displayed compared to trial 13. In trial 13, less running up was displayed compared to trial 14.
With regard to phase 3, no significant effects were found.

Keep lying down

In phase 1, no differences were found with regard to keep lying down. In phase 2 a post $F(1,28)=9.96$, $p=0.0038$ and a trial $(6,168)=28.40$, $p<0.0001$ effect were found. With regard to the post effect, it appeared that post housed B (BB and EB) pigs showed less keep lying down behaviour compared to post housed E (EE and BE) pigs. With regard to the trial effect, trial 14 had a lower amount of animals that kept lying down than trial 15. Also, trial 15 was higher in keeping lying down than trial 16.
With regard to phase 3, a pre*post effect existed $F(1,28)=7.86$, $p=0.0091$. Post hoc analysis revealed that BB animals showed less keep lying down than BE animals. A trend existed with regard to BB animals keep lying down less than EB animals.

Play

Analysis per phase (trial included in the model) showed that no differences between the treatment groups existed in phase 1 and 3 with regard to the display of play behaviour. Only in phase 2 a trend for a trial effect existed F(6,168)=2.26, p=0.0401. Post hoc analysis revealed that pigs tended to show less play behaviour in trial 15 compared to trial 16.

Explorative behaviour

With regard to phase 1, a trend for post effect F(1,28)=4.08, p=0.0529, a significant effect of trial F(6,168)=21.34, p<0.0001, post*trial F(6,168)=3.72, p=0.0017 and pre*post*trial effect F(6,168)=2.28, p=0.0387 existed.
With regard to the post effect, post B (BB and EB) animals appeared to explore less than post E (EE and BE) animals, a trend existed.
With regard to trial, trial 3 tended to be less than trial 4 with regard to exploratory behaviour.
With regard to post*trial, post housed B (BB and EB) pigs tended to show less explorative behaviour in trial 7 and 8 than post housed E (EE and BE) pigs. In trial 7, post housed E (EE and BE) pigs tended to show more exploratory behaviour compared to trial 6.
With regard to the pre*post*trial effect, BB in trial 7 was lower than BE in trial 7. In trial 6, BE was lower than in trial 7.
With regard to phase 2, a significant post F(1,28)=21.91, p<0.0001 effect existed as well as a trial effect F(6,168)=5.69, p<0.0001.
With regard to the post effect, post housed B (EB and BB) pigs showed less explorative behaviour compared to post housed E (EE and BE) pigs. With regard to the trial effect, no interesting significant differences were found post hoc.
In phase 3, a post F(1,28)=70.77, p<0.0001, a pre*post F1.28)=4.74, p=0.0380 and a trial effect F(6,168)=3.52, p=0.0027 were found.
With regard to the post effect, it appeared that post housed B (BB and EB) animals showed less exploratory behaviour compared to post housed E (EE and BE) pigs.
With regard to the pre*post effect, post hoc analysis revealed that BB animals showed less exploratory behaviour compared to EE and BE animals. Also, BE animals showed more than EB and EB less than EE.
With regard to the trial effect, no interesting significant differences were found post hoc.

<u>Test part:</u>

Keep lying down

With regard to phase 1, a post effect was found F(1,28)=4.54, p=0.0421 which showed that post housed B (BB and EB) animals kept less lying down behaviour to post housed E (EE and BE) animals.
With regard to phase 2, a post effect F(1,28)=14.78, p=0.0006, a trial effect F(6,168)=15.30, p<0.0001 and a post*trial effect F(6,168)=2.63, p=0.0182 existed.
With regard to the post effect, it appeared that post housed B (BB and EB) animals kept less lying down compared to post housed E (EE and BE) pigs. With regard to the trial effect, it was shown that pigs kept lying down in trial 14 less than in trial 15 and also kept lying down in trial 15 more than in 16.
With regard to the post*trial effect, a trend existed which showed that post B (BB and EB) housed animals kept lying down in trial 14 less than in trial 15 and more in trial 15 compared to trial 16. It was shown that in trial 15 post B (BB and EB) animals kept lying down less than post housed E (EE and BE) animals. With regard to trial 14, post housed E (EE and BE)

animals kept lying down less than in trial 15. They kept lying more in trial 15 compared to trial 16.
With regard to phase 3, a post effect existed F(1,28)=4.91, p=0.0350 which showed that post housed B (BB and EB) pigs kept less lying down compared to post housed E (EE and BE) pigs.

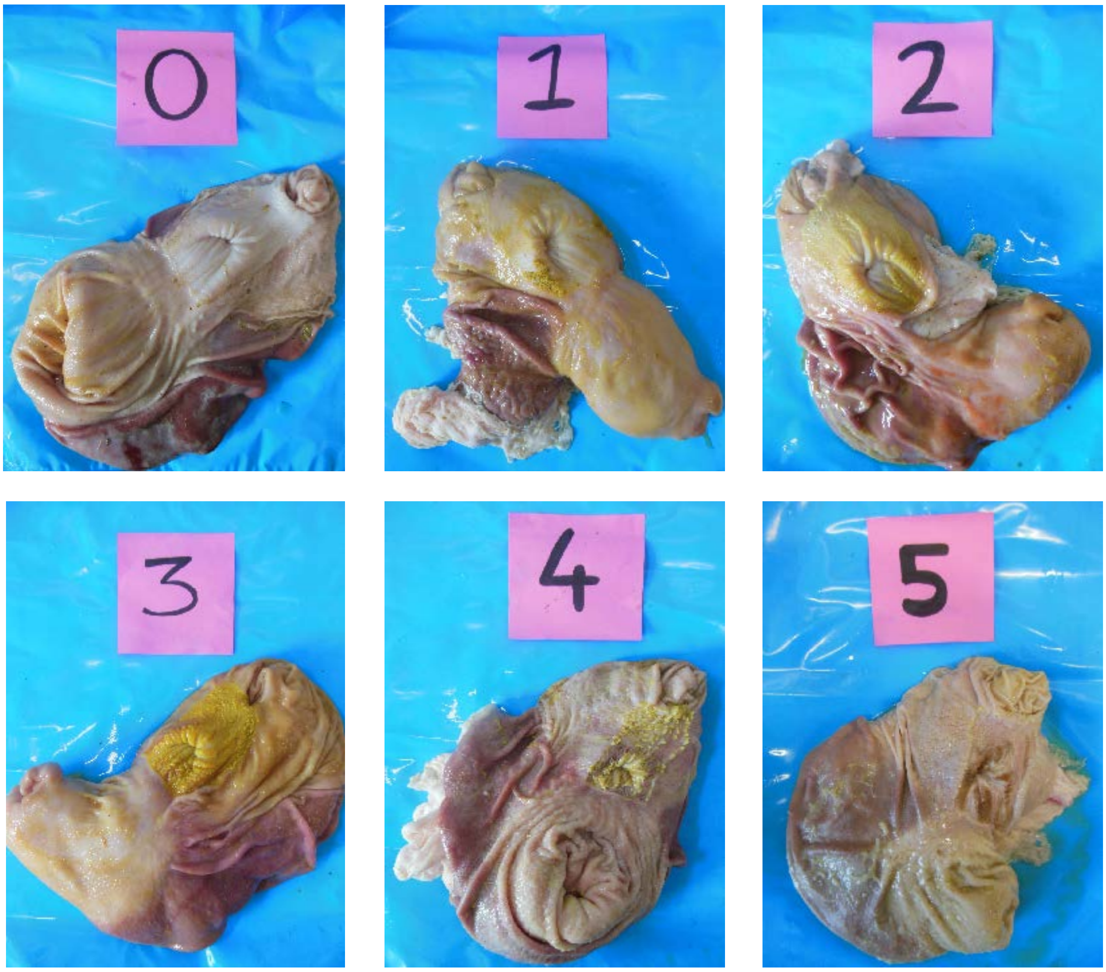
Play
With regard to phase 1, no significant effects were found. In phase 2, an effect of post housing conditions was found F(1,28)=4.86, p=0.0359. Also an effect of trial was found F(6,168)=3.30, p=0.0043. With regard to the post housing effect, it was shown that post housed B (BB and EB) pigs showed less play behaviour compared to post housed E (BE and EE) pigs. With regard to the trial effect, it was shown post hoc that less play behaviour was displayed in trial 15 than in trial 16. In phase 3, a post effect was found F(1,28)=5.20, p=0.0304. It was shown post hoc that post housed B (BB and EB) pigs showed less behaviour compared to post housed E (EE and BE) pigs.

Explorative behaviour
In phase 1, a post effect F(1,28)=19.27, p=0.0001 existed as well as a trial effect F(6,168)=4.50, p=0.0003. With regard to the post effect, it was shown that post B housed pigs (BB and EB) showed more explorative behaviour than post housed E (EE and BE) pigs. The trial effect showed no interesting significant results.
With regard to phase 2, a post effect F(1,28)=38.97, p<0.0001 existed as well as a trend for a trial effect F(6,168)=2.14, p=0.0514. With regard to the post effect, it was shown that post B housed pigs (BB and EB) showed more explorative behaviour than post housed E (EE and BE) pigs. With regard to the trial effect, it was shown that in trial 14, a trend existed with regard to more explorative behaviour was present than in trial 15.
In phase 3 a post effect was found F91.28)=7.76, p=0.0095 which showed that post housed B (BB and EB) pigs showed more exploratory behaviour compared to post E housed pigs (EE and BE).

Aggressive behaviour
In phase 1, a trend for a pre*post effect was present F(1,28)=3.69, p=0.0649. However, post hoc analysis did not reveal any significant difference between the 4 treatment groups.
With regard to phase 2, no significant effect existed.
With regard to phase 3, a post effect was present F(1,28)=9.45, p=0.0047. Post hoc analysis revealed that post housed B (BB and EB) pigs showed less aggressive behaviour than post housed E (EE and BE) pigs.

Appendix III

Pictures of stomachs with accompanying scores



Scores:

0 = Normal pars oesophagus

1 = Minor hyperkeratosis (< 50% of the surface)

2 = Severe hyperkeratosis (> 50% of the surface)

3 = Small lesions, < 5 and < 2.5 cm

4 = > 4 lesions or lesion(s) > 2.5 cm and < 5 cm

5 = > 10 lesions or lesion(s) > 5 cm or ulcer

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