Chronic stress parameters in pigs

de Jong, Ingrid

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2000

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 1

General Introduction
Modern Pig Husbandry

In the Netherlands, housing conditions of fattening pigs changed considerably in the past decades from extensive systems with large space allowance, substrate and/or outdoor housing, to intensive husbandry systems that have been developed for large scale production of pig meat. In these intensive husbandry systems, fattening pigs are housed with high housing density and without substrate. During their life on the farm, pigs are regularly subjected to potentially stressful management procedures, like castration, tail docking, mixing with unfamiliar pigs and transport.

Intensive husbandry systems largely differ from the natural living conditions of pigs. Wild pigs, that are the ancestors of the domestic pig, live in forest and scrub in dense cover, and spend a large part of the day on feeding and foraging behaviour. Wild pigs have a highly developed social behaviour throughout their entire life, starting within hours after birth (Graves, 1984). Studies of domestic pigs in a semi-natural environment showed that the behaviour of the domestic pig includes most of the behaviour of the wild pig. Apparently, domestication and rearing conditions did not affect the potential of the pig to show a rich repertoire of behaviour (Stolba and Wood-Gush, 1989).

It was shown that intensive husbandry systems cause welfare problems for pigs. The main background of these welfare problems is that the intensive housing conditions did not fulfil the internal need of the pig to perform important species specific behaviours. Pigs housed in barren environments show more abnormal agonistic behaviour, more manipulative social behaviour and have a higher level of aggression than pigs housed in pens with strawbedding, and it was concluded that these behaviours indicate welfare problems (Beattie et al., 1995; De Jonge et al., 1996; Schouten, 1986). Also regular mixing of unfamiliar pigs in intensive husbandry systems has negative effects on health and productivity and causes a higher incidence of aggressive behaviour, and thus has a negative effect on pig welfare (Ekkel, 1996).

A growing public concern for animal welfare led to the establishment of the Dutch Animal Welfare and Health Act (Gezondheids- en Welzijnswet voor Dieren, 1992) and to specific legislation for pig husbandry (Varkensbesluit, 1994), to prevent serious welfare problems. Recently, the existing legislation has been changed into stricter requirements for pig production (Varkensbesluit, 1998). This new legislation has to be applied at new farms since 1-9-1998; existing farms will
Chapter 1

have to apply the new legislation from 1-9-2008. For fattening pigs, the most important requirements are (Varkensbesluit, 1998):

(1) mixing of unfamiliar pigs is only allowed once, within one week after weaning of the pigs;

(2) the minimal surface area per pig has to be increased (e.g. for pigs from 85-110 kg from 0.7 m² to 1.0 m²);

(3) the area of solid floor in a pen has to be increased (e.g. for pigs from 85-110 kg it is advised that 60% of the pen area is solid floor, however, current research has to provide the definite figures);

(4) some substrate should be provided (e.g. straw).

In addition to the improvements of intensive husbandry systems enforced by changing legislation, growing public concern for animal welfare and interest in biological food products caused an increase in the number of other, less intensive husbandry systems (e.g. the biological fattening pig production in the Netherlands increased from 2,000 fattening pigs/year in 1996 to 14,000 fattening pigs/year in 1998 (Platform Biologica, personal communication)). In these husbandry systems pigs are provided with more space, strawbedding or other substrate and an outdoor area. However, the far majority of pigs in the Netherlands is still housed under intensive conditions.

In addition to the development of intensive husbandry systems and improved farm management during the past decades, genetic selection also resulted in a considerable increase of the pig production level. However, negative side effects have become apparent. Animals in a population selected for high production efficiency seem to be at higher risk for behaviourial, physiological and immunological problems (Rauw et al., 1998). In fattening pigs, selection is mainly on high lean tissue growth rate and low feed conversion. High lean tissue growth rate may cause leg weakness, decreased ability to cope with environmental stress (Rauw et al., 1998), and an impaired heart function (Geers et al., 1990; Yang and Lin, 1997) in pigs. It is important to avoid negative side effects of selection, not only because veterinary costs and costs of replacing animals will increase, but also from an ethical point of view (Rauw et al., 1998).

As described above, stress caused by housing and management of pigs may not only affect animal welfare, but also the acceptance of the product by the consumer and the productivity. In addition, stress may also change the product quality (Figure 1). For example, stress caused by mixing of unfamiliar pigs reduced
the growth rate for weeks (Ekkel, 1996), but also impaired meat quality (e.g. Warris and Brown, 1985).

![Diagram of stress, welfare, productivity, product quality, and consumer acceptance relationships]

**Figure 1.** The relationship between stress, caused by housing and management, and welfare, productivity, product quality and acceptance of the animal products by the consumer (after Blokhuis et al., 1998).

**Aims and Scope of the Thesis**

The purpose of this thesis is to identify physiological parameters for monitoring stress in fattening pigs, i.e., to assess stress in fattening pig husbandry. Because stress, caused by housing and management of pigs, may have a negative effect on animal welfare, productivity, product quality and consumer acceptance (see Figure 1), monitoring stress is of importance in pig husbandry. Monitoring stress allows formulation of housing and management requirements of fattening pigs that will benefit animal welfare, health and productivity. In addition, monitoring stress is of importance for certification of animal products. Welfare is clearly compromised in situations of chronic stress (Broom et al., 1996a; Moberg, 1996; Wiepkema and Koolhaas, 1993). Therefore, we emphasise on long-term changes in physiology as indicators of chronic stress.

Various physiological parameters to assess stress are known from literature. However, to study long-term changes in physiology as indicator of chronic stress, parameters should be measured during a long time without disturbing the animal.
Biotelemetry offers the opportunity for long-term stress-free monitoring of body temperature and heart rate. This method has not been used for monitoring chronic stress in pigs yet. Therefore, body temperature and heart rate were studied as possible indicators of chronic stress in fattening pigs. In addition, HPA-axis activity and behaviour were measured as reference indicators of chronic stress. These parameters are frequently measured in response to stressors in various species, and the responses to different stressors are well described. In the experiments described in this thesis, pigs were subjected to situations that were expected to be stressful, and we studied which parameters were sensitive indicators of situations of chronic stress.

**Stress and Animal Welfare**

The concept of stress was first developed by Selye (1932) and Cannon (1914). Selye (1932) and Cannon (1914) stated that environmental stimuli like pain, hunger, thirst or cold resulted in physiological changes in the animal, that were related to the development of a pathological state. Stress referred to the non-specific response of the animal to any demand from the external and internal environment. Nowadays, more broader definitions of stress are used. In this thesis, we applied the definition as described by Terlouw et al. (1997), in which stress is described as the animal's state when it is challenged beyond its behavioural and physiological capacity to adapt to its environment.

Animals try to cope with environmental stressors by using various behavioural and physiological responses. The quantity and quality of the stress response is normally within the range of the coping abilities of an animal. The acute stress response may therefore have an adaptive value for the animal, and animal welfare is not at risk. Moreover, some environmental instability or uncertainty is necessary to avoid boredom and optimise individual vigilance (Wiepkema and Koolhaas, 1993). However, when the stress responses of an animal are thwarted, or when the animal can not restore its homeostasis, symptoms of chronic stress occur and the animal may reach a prepathological state. During this state of chronic stress, undesirable effects on health, reproduction, growth and behaviour may occur, and animal welfare clearly is at risk (Moberg, 1996). Both the occurrence of one single major life event and a long-lasting situation in which the animal can not restore its homeostasis may lead to chronic stress symptoms with a pathological character (Wiepkema and Koolhaas, 1993).
Many definitions of animal welfare have been used in literature. One of the most frequently used definitions of animal welfare, that refers to the coping of the animal with the environment, was stated by Broom (1996a): 'animal welfare is its state as regards its attempts to cope with its environment'. In this definition, there is a negative relationship between the welfare of an animal and the effort it is putting into coping with the environmental stressors. Animal welfare not only refers to the biological functioning of the animal, but also to the quality of life, and feelings like happiness, pain, fear, hunger or thirst. Or, as Dawkins (1990) stated: 'animal welfare involves the subjective feelings of animals'. Other definitions of animal welfare refer to this. E.g. Duncan (1996) stated 'that it is the negative emotions of feeling stressed or frightened or in pain that reduce welfare, and it is the positive emotions of feeling excited or thrilled or happy that increase welfare'. Thus, animal welfare can broadly be defined as a state of physical and mental health (Wiepkema and Koolhaas, 1992).

The physiological and behavioural stress responses, with which the animal tries to cope with the environmental stressors, can be measured. As described above, animal welfare is clearly compromised in situations of chronic stress. Long-term changes in behaviour and physiology of the animal, i.e. chronic activation of the stress responses, may be indicative of a situation of reduced welfare. Therefore, in this thesis we measured long-term changes in physiology as indicator of chronic stress and decreased animal welfare. Stress-parameters that were studied in this thesis as indicator of welfare of fattening pigs are described below.

**Assessment of Stress**

**Physiological Responses**

**HPA-axis Activity.** The secretion of glucocorticoids from the adrenal cortex is frequently measured in response to stressors (Wiepkema and Koolhaas, 1993; Rushen, 1991). In response to an acute stressor Corticotropin-releasing Hormone (CRH), that is released in the hypothalamus, stimulates the pituitary to release Adrenocorticotropic Hormone (ACTH). ACTH stimulates the adrenals to release glucocorticoids. Negative feedback of the glucocorticoids on the brain and pituitary and of ACTH and CRH on the hypothalamus regulate the activity of the Hypothalamus-Pituitary-Adrenal (HPA)-axis.

Glucocorticoids exert many different effects, for example on cardiovascular function, metabolism, muscle function, behaviour and the immune system. In
Chapter 1

general, glucocorticoids rapidly mobilise energy in the body and stimulate behavioural responsiveness. Munck et al. (1984) grouped the effects of glucocorticoids into two categories. The permissive effects of glucocorticoids function to 'permit' other hormones to accomplish their function at a normal level, and are observed primarily in the resting state and may span the resting and stress states. The regulatory effects of glucocorticoids are exerted only by stress-induced levels. Munck et al. (1984) suggested that stress-induced increases in glucocorticoid levels protect the organism against the normal defense reactions that are activated by stress, and prevent the defense reactions from overshooting and thereby threatening homeostasis. Although elevated glucocorticoid levels in the acute phase may be effective, chronic hypersecretion of glucocorticoids may be harmful to the individual. For example, it has been suggested that chronic hypersecretion of glucocorticoids may induce hippocampal neuronal vulnerability (Sapolsky, 1996).

In response to acute stress, glucocorticoids may show a gradual response, dependent on the severity of the stressor (Jensen et al., 1996a; Terlouw et al., 1997). However, during a state of chronic stress the HPA-axis is not in a permanent state of activation, but counterregulatory changes at different levels of the HPA-axis may occur (Jensen et al., 1996a; Rushen, 1991). Exposure to chronic stress may lead to a brief increase in glucocorticoid levels that lasts for several hours or days. However, during situations of chronic stress changes at various levels of the HPA-axis may occur. Production of HPA-axis hormones follows a circadian rhythm; in diurnal animals, like pigs, elevated concentrations of glucocorticoids and ACTH are measured in the morning and low concentrations during the night. Disturbances of the circadian rhythm in cortisol are observed during situations of chronic stress. For example, initial tethering of sows caused an increase in cortisol levels lasting for a few days (Becker et al., 1985), but, after 2 months tethered sows show a flattened circadian rhythm in cortisol, caused by an increased circadian trough (Janssens et al., 1995a). Chronic stress may also change baseline ACTH levels. After 6 days of intermittent foot-shock stress in pigs the baseline levels of ACTH were reduced, whereas cortisol levels remain unchanged (Jensen et al., 1996b). In humans, flattened circadian rhythms in cortisol or ACTH are observed during several psychological disorders, e.g. during certain types of depression (e.g. Deuschle et al., 1997; Souêtre et al., 1989), during chronic fatigue syndrome
(McHale et al., 1998) and during post-traumatic stress disorder (Yehuda et al., 1996).

Chronic stress may also result in a hyper-reactivity of the adrenal cortex, so that the cortisol response to an acute stressor or to ACTH is exaggerated. Tethered sows showed an increased cortisol response to ACTH as compared to loose housed sows (Janssens et al., 1994) and showed an increased cortisol response to acute restraint stress (Janssens et al., 1995b). Changes may also occur at the pituitary level, i.e. that CRH stimulation causes an increased ACTH response. Tethered sows show an increased ACTH response to acute stress as compared to loose housed sows (Janssens et al., 1995b).

The time course and type of changes in the HPA-axis differ between different types of stressors. Therefore, the strength and features of the stressor seem to determine the speed of the process of changes in the HPA-axis during chronic stress (Jensen et al., 1996a).

Thus, for the assessment of a situation of chronic stress, long-term measurements of HPA-axis (re)activity are necessary. When secreted from the adrenal gland, glucocorticoids bind with high affinity to glucocorticoid binding globulins. The free, i.e. unbound glucocorticoid is the biologically active form. In pigs, cortisol is the main glucocorticoid secreted, but low amounts of corticosterone are also secreted (Bottoms et al., 1972). Free cortisol in the blood plasma diffuses to the saliva, and the concentration in the saliva is a good representation of the concentration of free cortisol in plasma (Kirschbaum and Hellhammer, 1989; Parrott and Misson, 1989). Sampling saliva is an easy non-invasive method, and therefore very useful to measure long-term changes in baseline cortisol levels and/or the circadian rhythm in cortisol as possible indicators of chronic stress in pigs.

Heart Rate. Heart rate frequency is often measured in response to stressors, to get an impression of the activation of the autonomic nervous system (e.g. in pigs: Geverink et al., 1998; Hessing et al., 1994; Marchant et al., 1995; Otten et al., 1998). Stressors may either increase (tachycardia) or decrease (bradycardia) the heart rate. Changes in heart rate, however, can be caused by physical as well as psychological factors. E.g. during agonistic encounters in pigs, locomotor activity is increased and psychological stress is present, and it is very difficult to separately determine the effects of physical and psychological changes. However, it has been
shown that psychological stress in itself may cause a change in heart rate. During a situation of threat in sows the heart rate increased, whereas no increased physical activity was present (Marchant et al., 1995).

In addition to measurements of heart rate frequency, measuring heart rate variability may give information about the balance between the sympathetic and the parasympathetic branch of the autonomic nervous system. In general, there is tonic activity in both divisions of the autonomic nervous system and the net effect on heart rate represents the balance between the two antagonistic effects. Under resting conditions the parasympathetic activity is dominant, whereas with increasing levels of exercise the sympathetic activity dominates. Psychological stress may alter the balance between the sympathetic and the parasympathetic branch of the autonomic nervous system; different stressors may shift the balance in a different direction (Sgoifo et al., 1997; Stein et al., 1994). During restraint stress in rats the autonomic balance shifts towards a more parasympathetic prevalence, whereas during social stress the autonomic balance shifts towards a more sympathetic prevalence (Sgoifo et al., 1997). In general, a higher heart rate variability represents a shift of the autonomic balance towards a more parasympathetic prevalence, whereas a lower heart rate variability represents a shift of the autonomic balance towards a more sympathetic prevalence (Sgoifo et al., 1997; Stein et al., 1994). Increased sympathetic activity of the heart has been shown to increase the vulnerability to cardiac arrhythmias, that are risk markers for sudden cardiac death (Verrier and Lown, 1984; Sgoifo et al., 1997). During social stress in rats a high occurrence of ventricular arrhythmias was observed that was related to a high sympathetic activation of the heart (Sgoifo et al., 1997).

Thus, in addition to measurements of heart rate frequency, measurements of heart rate variability may give information about changes in the balance between the parasympathetic and the sympathetic activity as a result of (chronic) stress. Moreover, measurements of heart rate variability and of the occurrence of vulnerability to cardiac arrhythmias can be used to study the relationship between chronic stress and the occurrence of cardiovascular pathologies.

In pigs, heart rate frequency is often measured in response to stressors by using external heart rate monitors (e.g. Geverink et al., 1998; Marchant et al., 1995). However, the attachment of heart rate monitors itself may cause a heart rate response, and may therefore affect the stress response. In addition, external heart rate monitors are not very useful for long-term measurements of heart rate. In this
thesis, we used implanted biotelemetric transmitters for long-term stress-free measurements of heart rate in pigs.

Body Temperature. Body temperature is seldom used as indicator of stress in pigs, although previous research showed that body temperature may be a very useful indicator of (chronic) stress. Psychological stressors induces a rise in core body temperature in various species. This so-called stress-induced hyperthermia, lasting for 1-2 h, has been observed after examination stress in humans (Briese, 1995), restraint stress (Terlouw et al., 1996), social stress (Tornatzky and Miczek, 1993) and open-field stress (Kluger et al., 1987) in rats and handling stress in mice (Zethof et al., 1994). In pigs, this stress-induced hyperthermia has been observed after restraint stress (Parrott and Lloyd, 1995).

Stress-induced hyperthermia is due to a shift in body temperature set-point. It can be blocked by antipyretic drugs, like sodium salicylate (Singer et al., 1986) or indomethacin (Parrot and Lloyd, 1995). It was suggested that stress-induced hyperthermia is at least partially mediated by prostaglandins released by the central nervous system, and thus involves the same mechanisms as the febrile response (Kluger et al., 1987; Parrott and Lloyd, 1995).

In contrast to the stress-induced hyperthermia, a decrease in core temperature can also be observed after psychological stress. Chen and Herbert (1995) observed a decrease in body temperature of ± 0.5°C after restraint stress in rats. In pigs, a hypothermic response was observed in response to transport stress (Parrott et al., 1998). It was suggested that vasopressin release may have caused the hypothermia in rats in response to restraint (Chen and Herbert, 1995) as well as the hypothermic response to transport in pigs (Parrott et al., 1998).

In rats, long-term changes in body temperature can be observed during a situation of chronic stress. The circadian rhythm in body temperature has been disturbed for days after repeated foot shock stress (Kant et al., 1991) or a repeated social defeat (Tornatzky and Miczek, 1993). This was mainly due to an increased body temperature during the light period (resting period). Also after a single social defeat in rats, a model that is suggested to resemble certain human psychopathologies like anxiety or depression (Koolhaas et al., 1997; Ruis et al., 1997), the circadian rhythm in body temperature has been changed for days (Meerlo et al., 1996). In pigs, peripheral body temperature was increased after mixing of unfamiliar animals, and a circadian rhythm longer than 24 hours was
observed during the first weeks after mixing (Ekkel, 1996). Effects of stress on the circadian rhythm in core body temperature, comparable to those in rats, have not been reported in pigs yet.

Thus, changes in body temperature level or changes in the circadian rhythm in body temperature may be sensitive indicators of chronic stress. Long-term changes in body temperature or the circadian body temperature rhythm can be measured stress-free by using biotelemetric transmitters. In this thesis, we used biotelemetry to determine if core body temperature is a sensitive indicator of chronic stress in pigs.

**Behavioural Responses**

Behavioural observations can provide cues about the preferences, needs and internal states of the animals. Knowledge of normal species-specific behaviour is necessary for using behaviour as indicator of stress and animal welfare. A part of the behavioural response to stress may be cessation of normal behaviour. Therefore, the delay before normal behaviour is resumed can be a useful measure of stress (Broom, 1996b; Mench and Mason, 1997).

Stressful events may evoke uncertainty and conflict behaviour. The occurrence of conflict behaviour is normal and even desirable in certain situations, as it can help the organism to solve the conflict and thus falls within the adaptive range of the animal. However, when the situation is such that the animal can not solve the conflict, because retreat or escape from the situation is impossible or the normal routines cannot be performed, behavioural changes may indicate chronic stress and decreased animal welfare (Mench and Mason, 1997; Wiepkema and Koolhaas, 1993). The occurrence of abnormal behaviour like apathy, redirected behaviour, unresponsiveness, injurious and stereotypic behaviour is a typical symptom of a situation of chronic stress (Broom, 1996b; Wiepkema and Koolhaas, 1993).

Behaviour in a novel environment, for example exploration or locomotion, is often associated with emotions of the animal like fear or excitement (Hessing et al., 1994; Lawrence et al., 1991). Fear in a novel environment test may be indicative for the quality of the home situation, and can thus be related to animal welfare. The behavioural tests can be validated for measuring emotions, e.g. fear, by using pharmaca (e.g. anxiolytica) (in pigs: Andersen et al., 1999).
Thus, in addition to physiological measurements, changes in normal behaviour, occurrence of abnormal behaviour and performance in specific behavioural tests may be useful indicators of a situation of chronic stress and decreased animal welfare.

**Individual Differences**

Studies in many species showed that the behavioural response to stress shows large inter-individual variation. These behavioural differences were correlated with differences in physiological responses, and appeared to be consistent over time (e.g. Benus et al., 1987; Hessing et al., 1994; Henry and Stephens, 1977; Koolhaas et al., 1999).

Many studies of individual variation in behaviour and physiology have been carried out in rodents in relation to aggressive behaviour. High- and low-aggressive male mice and rats differed in their response to the social and non-social environment. These responses seemed to reflect two different coping strategies. The coping strategy of aggressive animals is aimed at the removal of themselves from the source of stress or at removal of the stress source itself ('active' or 'proactive' strategy). The coping strategy of non-aggressive rodents seems to aim at reduction of the emotional impact of stress ('passive' or 'reactive' strategy). These different behavioural responses were correlated with specific physiological responses. In short, the proactive animals seemed to be predominated by the sympathetic nervous system, whereas the reactive animals seemed to be predominated by the parasympathetic nervous system (Benus et al., 1987; Bohus et al., 1987; Fokkema et al., 1988). Important is that the proactive and the reactive strategy are successful under different environmental conditions (Koolhaas et al., 1999).

Also in pigs individual differences in behavioural and physiological responses to stress have been reported, although it is unclear if two distinct coping strategies can be distinguished (Ehrhard et al., 1999a, 1999b; Hessing et al., 1994; Mendl et al., 1992; Ruis et al., 2000). Different behavioural strategies can be distinguished at an early age by using the so-called 'backtest', that measures resistance behaviour of piglets in a non-social situation (Hessing et al., 1994; Ruis et al., 1999). Also a so-called 'tonic immobility test' has been used to distinguish different behavioural strategies in piglets (Ehrhard et al., 1999a, 1999b).
Chapter 1

In conclusion, when measuring stress responses in pigs, it should be taken into account that individuals may differ in the response to a particular stressor because they may apply a different coping strategy.

Outline of the Thesis

The main scientific question of this thesis is to identify physiological indicators of chronic stress in growing pigs. Behavioural studies showed that barren housing conditions subject pigs to a situation of chronic stress as compared to more enriched housing conditions (e.g. Beattie et al., 1995; De Jonge et al., 1996; Schouten, 1986). This could therefore be used as an experimental paradigm to identify physiological indicators of chronic stress. First, we obtained further evidence that barren versus enriched environmental conditions can be used as a paradigm to study indicators of chronic stress (chapter 2). Because chronic stress may change the physiological responses to acute stressors (e.g. Janssens et al., 1995b), physiological (heart rate, body temperature and cortisol) responses to acute stressors were measured in pigs housed under barren conditions and in pigs housed in a more enriched environment (chapter 2). In addition, we studied the home-pen behaviour of enriched and barren housed pigs (chapter 2). As it turned out in chapter 2 that enriched and barren housed pigs differed in HPA-axis activity, the circadian rhythm in cortisol and the relation between baseline circulating cortisol concentrations and behaviour were studied as possible indicators of chronic stress in pigs (chapter 3). In chapter 4, the behavioural and cortisol responses of enriched and barren housed pigs to management-related stressors (pre-slaughter handling, mixing and transport to the slaughterhouse) were studied.

Social stress caused by mixing of unfamiliar pigs is a common phenomenon in pig husbandry that has long-term effects on productivity and health (Ekkel, 1996). In chapter 5, heart rate frequency, heart rate variability and the occurrence of cardiac arrhythmias were studied as indicators of social stress in fattening pigs. In chapter 6, long-term responses of body temperature, heart rate and behaviour were measured after mixing of unfamiliar pigs.

Because deep body temperature depends on metabolic processes, we hypothesised in chapter 7 that the nutritional level of pigs may affect the level of body temperature and the circadian rhythm in body temperature. In addition, we measured the effect of the nutritional level on heart rate, behaviour and salivary cortisol levels.
In Chapter 8, the major findings of chapter 2-7 are summarised and discussed.

REFERENCES


Chapter 1


Chapter 1


