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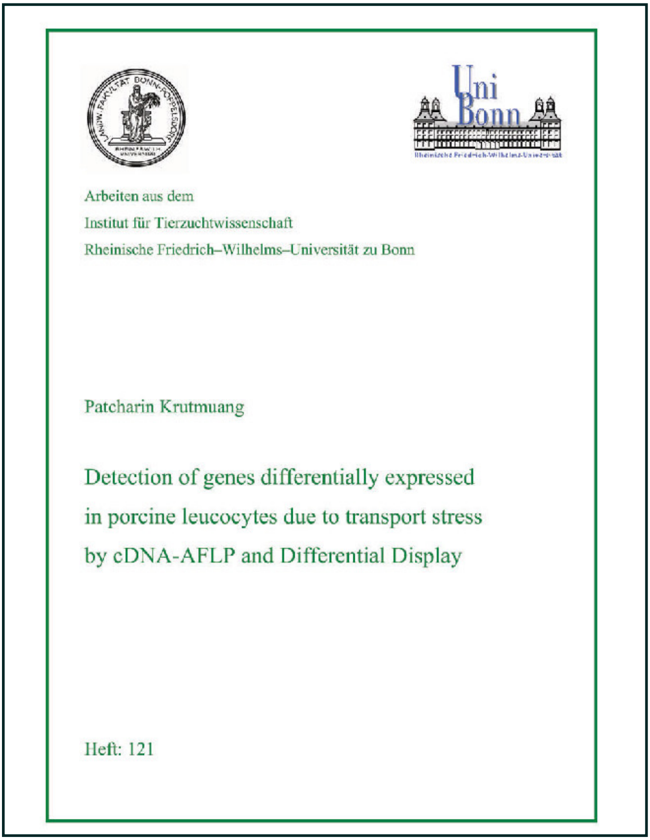
Detection of genes differentially expressed in porcine leucocytes due to transport stress by using cDNA-AFLP and Differential Display



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1 Introduction

Transport stress is one of the problems in pig production. The process of pig management often includes the transport of piglets from several producers to fattening units and then to slaughterhouses. Stress is the reaction of the body to stimulate which disturb the normal homeostasis, physiology and biochemical activities of an animal. Stress response likely leads to adverse effects on growth, behaviour, reproductive performance and disease resistance (Johnson et al. 1992). The pigs respond to stress by becoming overly excited and developing reddish blotches on their skin and show signs of nervousness. This and other responses of the animal have adverse effects on the quality of products, specially of pork. Hence, controlling the stress problem would be a major step forward in overall improvement of pork quality. The solution to this problem can be searched first by understanding the cause and genetic factors associated to it. If this is clearly understood and identified, testing and screening animals against the trait (stress susceptibility) would be possible by raising (keeping) tolerant animals in the herd. Second approach to reduce stress in the herd is by improving animal handling which is directly associated with a well-being and productivity of animals.

Currently a number of techniques are used to identify genes expressed at particular physiological and/or developmental stages, of which Differential Display Reverse Transcription Polymerase Chain Reaction (DDRT-PCR) is the one, that has been developed first by Liang and Pardee (1992). Investigating on the molecular genetic mechanisms involved in the process of stress can contribute to understand genetic control of these factors. For this, DDRT-PCR is a powerful method that has been successfully used to detect and characterize gene expression in closely related cell lines or tissue type which is extremely important in molecular biology. Another method named cDNA-AFLP (amplified fragment length polymorphism) is a novel RNA fingerprinting technique to display differentially expressed genes with high reproducibility. These can help to elucidate molecular mechanisms associated with disease, condition of stress or particular cell tissue development state. The objective of this study are:

(1) to identify candidate genes which are differentially expressed under transport stress based on day of transport by using cDNA-AFLP technique.

(2) to identify candidate genes which are differentially expressed under transport stress based on cortisol response levels by using differential display technique.

(3) to localize candidate genes which are differentially expressed under transport stress on porcine chromosomes.

(4) to confirm the differentially expressed genes by quantitative real time PCR.

2 Literature review

2.1 Stress

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis (Khansari et al. 1990, Hicks et al. 1998). Stress is defined as a condition in an animal that results from the action of one or more stressors that may be of either external or internal origin. The effort of the body to maintain a stable internal environment to challenges from widely variable environments was referred to as homeostasis (Von Borell 2000). Stressors can be physical, environmental or psychological in nature. Physical stressors include taildocking, castration and physical restraint. Environmental stressors include extreme temperature, infection and transport. Psychological stressors include maternal separation and social isolation in social animals. Moreover, stressors can be divided into two groups, acute stress and chronic stress. Acute stress is short-term stress that results from a sudden environmental change of short duration. Various environmental effects are defined as acute stressor in farm animals such as heat stress, cold stress, relocation, isolation, mixing, restraint, weaning, noise, hunger and transportation (De Jong et al. 2000, Geverink et al. 2002, Hicks et al. 1998, Ruis et al. 2001). Chronic stress on the other hand results from long-term, continued influence of a stressor and thought adaptative mechanisms (Odio and Brodish 1990). Environmental effects of chronic stressors in farm animals have been defined as a long time tethered animal in housing (Janssens et al. 1995, Loijens et al. 2002). The effect of stress directly influences homeostasis of animals and triggers biological defence or stress response in animals that consists of the behavioural response, the autonomic nervous system response, the neuroendocrine response and the immune response (Figure 1). These effects impact on animal well-being, performance and animal product quality.

2.1.1 Transportation stress in pigs

In Europe, millions of piglets and pigs are transported by road each year (Perremans et al. 2001). Transport stress is a major global problem leading to economic losses and is

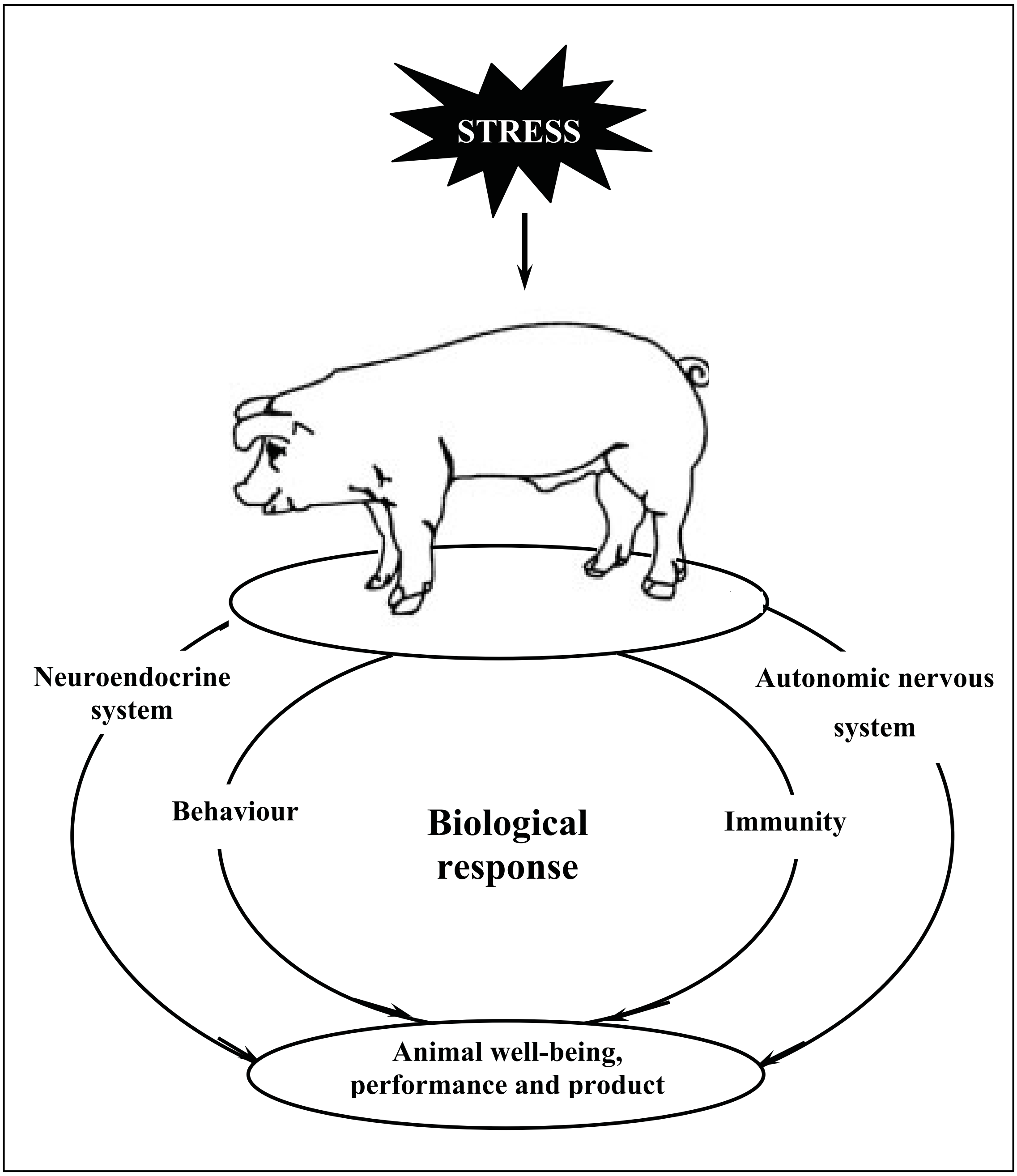

Figure 1: Biological response of animals to stress (adapted from Moberg 2000)

difficult to avoid in modern animal production. It is considered as a strong stressor or acute stressor in pigs and the other farm animals during transit (McGlone et al. 1993, Grandin 1997). Stress due to transportation is a complex of factors that consists of ambient temperature and velocity, loading density, mixing with unfamiliar pigs, withdrawal of feed and water and motion as well as vibration (Perremans et al. 2001). These factors can greatly disturb behaviour and homeostasis system of animals in a very short time. The pigs have elevated plasma cortisol concentration, heart rate and blood pressure immediately after transportation (Geers et al. 1994, Perremans et al. 2001). On observation of the pig behaviour during transportation, the animals were found to have decreased standing behaviour but increased lying behaviour (Hicks et al. 1998).

Moreover, transport stress influenced loss of live-weight in piglets (Hicks et al. 1998, McGlone et al. 1993) and pigs which are transited to slaughterhouse as well as the carcass weight (Broom 1996). It is the result of withdrawn feed and water for a long time, dehydration and mobilization of fat and muscle glycogen. Additionally, stress causes increasing mortality rate of pigs during transport that is associated with increased ambient temperature (Warriss and Brown 1997) as well as genetic defects of the halothane or ryanodine receptor gene particularly in european breeds. Meat and carcass quality are impaired by stress transport known as pale, soft, exudative (PSE) or dark, firm, dry (DFD) traits (Warriss 2003). This is a result of increasing pH or acidification in muscle due to alteration of physiological and biochemical response and is also related to genetic factors such as genetic defects of the ryanodine receptor gene in pigs. Additionally, mixing of unfamiliar pigs during transportation increases skin damage and leads to decreased meat quality. The transport stress effects have been found to alter several immune parameters in pigs (Edfors-Lilja et al. 2000, Dalin et al. 1993, McGlone et al. 1993) and other farm animals such as goats (Kannan et al. 2000) and cattle (Blecha et al. 1984, Kegley et al. 1997).

2.1.2 Stress response mechanisms in animals

Stress response of animals for adaptation to environmental challenges are involved in behavioural and neuroendocrine systems which is the interacting network between the central nervous system (CNS) and endocrine glands (Désautes et al. 1997, Matteri et al. 2000). The neuroendocrine system consists in part of the sympathoadrenal (SA) system and the hypothalamic-pituitary-adrenocortical (HPA) system and plays a major role in preparing the animal to meet emergencies and adaptive response during exposure to stressors (Desautes et al. 1997).

The sympathoadrenal system or hypothalamic-adrenal medullary stress-response system involves the hypothalamus, pituitary gland (neurohypophysis), the sympathetic neural pathways to the adrenal medulla, and the release of catecholamine hormones (epinephrine and norepinephrine) by the adrenal gland (Von Borell 2000). Both, epinephrine (adrenaline) and norepinephrine (noradrenaline) hormones, effect increased metabolic rate, increased heart rate and blood flow. Moreover, epinephrine initiates conversion of liver and muscle glycogen to glucose to generate more readily available energy from protein and fat. This short acting stress-response is referred to as the fight-flight syndrome.

On the other hand, the HPA system is referred to as the general adaptation syndrome. The hypothalamus is stimulated and responds by releasing corticotropin-releasing hormone (CRH) as in the SA system. The CRH stimulates the anterior hypophysis (anterior pituitary gland) to release adrenocorticotropin hormone (ACTH) and β-endorphine. The ACTH hormone activates the adrenal cortex to produce glucocorticoids hormone (cortisol, corticosterone) and mineralcorticoid (aldosterone). The glucocorticoids play a major role in glucose metabolism, inhibit protein synthesis, initiate proteolysis while, the mineralcorticoids play a major role in homeostasis of mineral concentration (Knowles and Warriss 2000).

2.2 Stress indicators in pig

Many criteria were used to indicate the stress response of pigs including, behaviour, biochemical, physiological and immunological parameters as well as genetic markers (Table 1). Behavioural response to stressors or environmental challenges of pigs have been reported (Lawrence et al. 1991). Although, behaviour of animals in response to stress is complex and difficult to interpret (Rushen 2000). Several studies have shown the behavioural activity of pigs which are challenged with novel environment (e.g. locomotion, exploration, vocalization and defecation) and were also used as indicators for stress response (Désautes et al. 1997, Désautes et al. 1999, Désautes et al. 2000). Whereas, biochemical and physiological paramenters were widely used as stress indicators in mammals.