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SEGREGATED EARLY WEANING STRESS HAS ACUTE AND LONG-LASTING EFFECTS ON GROWING PIGS

By

Yan Yuan

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ABSTRACT

SEGREGATED EARLY WEANING STRESS HAS ACUTE AND LONG-LASTING EFFECTS ON GROWING PIGS

By

Yan Yuan

The North American swine industry is rapidly adopting a segregated early weaning (SEW) system with little information about potential welfare implications. In this study, the welfare of SEW pigs was examined by studying the development of abnormal behavior, agonistic interactions, and cortisol concentrations post-weaning. Two trials were conducted to investigate the short- and long-term effects of SEW. Urinary cortisol stayed high in SEW pigs up to three day post-weaning ($P \leq 0.0339$). Other responses included high vocalization rate, an increase in activity levels and the exhibition of belly-nosing. The long-term effects were evidenced by higher level of belly-nosing ($P \leq 0.0142$), higher proportion of fights with no clear outcome ($P = 0.0430$), and longer duration of fights ($P = 0.0075$) in SEW pigs than in conventionally weaned pigs. In summary, the behavioral and physiological measures indicate that the welfare of SEW piglets may be poorer than conventionally weaned piglets.

To the love and support of my parents – *Yuan, Xiaoping* and *Yao, Jinmei*. To the love, friendship and help of my husband – *Qian, Jin*

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 INTRODUCTION

From 1988 to 1997, the number of swine farms in the US has decreased from 326,600 to 138,690. However, swine farms that produced 50,000 or more pigs annually increased their market share from 7% to 37% during this time (1998 Pork Industry Study by National Pork Producer Council). This could be largely attributed to the implementation of multi-production sites by large swine farms in order to manage the larger number of animals per farm. For small swine farms, survival strategies in today's competitive market have included formation of small cooperative operations and participation in production contracts with larger companies. Regardless of the strategy that small swine farms implement, each individual farm has a special function. For example, some farms primarily deal with breeding herds and nursing piglets until they are weaned and sold. Others raise the weaned piglets until they reach a weight of 25 kg and/or raise the animals until they reach market weight. These cooperative alliances or production contracts are very similar to a multi-site operation of a single large farm.

The existing multifunctional cooperative setting makes it easy for the swine industry to adopt the segregated ea

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early weaning (SEW) system. The SEW system is a multi-site production system in which piglets are weaned at a very early age, usually between 10 to 19 days after birth, and moved to a separate site for the initial growth period. This system reduces the pig's exposure to pathogens compared to conventional weaning practices and has been rapidly adopted by the North American swine industry (Harris, 1988; Dritz et al., 1996). In a conventional weaning system, piglets are weaned between 21 to 28 days of age to an adjacent nursery room or building. The nursery site has piglets moving in and out on a regular basis, which makes it difficult to maintain a clean site and prevent pathogens from passing from one group of pigs to another.

Despite the potential benefits of preventing infectious disease which results from the improved cleanliness of the nursery housing in SEW, the welfare implications of SEW have been ignored. Welfare is defined as the state of an individual in regard to its attempts to cope with its environment (Broom and Johnson, 1993). Since stress implies difficulty in coping, the presence of stress is regarded as a threat to the welfare of an animal. Weaning (Hinde, 1966; Würbel and Stauffacher, 1997) and t

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transportation (Parrott and Misson, 1989; Baldock et al., 1990) can be stressful to animals.

Stress has universal and multi-organ effects. Stress, especially chronic stress, can result in sub-optimal immune function, a delay in puberty, and a reduction in brain hippocampal volume among many other malfunctions (Munck et al., 1984; De Jonge et al., 1996; Bremner et al., 1995). With a suppressed immune system, animals are not able to properly respond to vaccines and disease challenges. The onset of puberty is an important indicator of reproductive fitness, and its delay could be an indicator of poor welfare. The atrophy of the hippocampus is associated with behavioral and memory problems (Bremner et al., 1995).

Of all these potential detrimental effects of stress during the precocious weaning age, development of abnormal behavior is the most consistent and long-term problem in a variety of species, including mink (Mason, 1994), rats (Würbel and Stauffacher, 1997), and pigs (Gonyou et al., 1998). "Abnormal" is defined as being statistically rare or different from a chosen population which is living free or in naturalistic conditions in captivity (Fraser and Broom, 1990). The occurrence of behavior abnormalities has been used as an indicator of poor welfare (e.g. Broom and Johnson, 1993). It concerns the public when they witness the exhibition of abnormal behavior. The pacing behavior of zoo animals, such as the red panda, tiger and polar bear, is an example (Poulsen et al., 1996; Lyons et al., 1997).

Currently, the swine industry is harshly criticized on environmental issues such as manure management. As a result, pig producers have spent substantial amounts of money to comply with new laws on manure management. If the pork industry is not prepared, SEW may become an easy target for criticism by welfare groups. An immediate concern in the pork industry is that some abnormal behaviors, such as belly-nosing[1], and ear and tail biting may decrease the carcass value (Hunter et al., 1999). In the future, pork producers may have to change their management practices in order to deal with the SEW welfare concerns.

This research was conducted to examine the welfare of SEW piglets. Behavior and physiological measures in neonatal piglets can provide information on the short- and long-term effects of the SEW stress. The hypothesis is that neonatal piglets will respond behaviorally and physiologically to SEW stress. This stressful experience

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[1] Belly-nosing is defined as a rhythmic up-and-down movement with the snout on the belly or soft tissue between the hind or forelegs of another pig (Dybkjær, 1992).

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will have long lasting effects as measured by increased aggression among piglets, increased behavioral abnormalities, impaired ability to establish social hierarchy, and different physiological responses to an acute stressor at an older age when compared to conventional weaned pigs.

To my knowledge, this work is the first of its kind in swine which integrates the behavioral and physiological measures, and the results may be valuable to the pork producers who are adopting or considering to adopt the SEW system. In addition, the results may provide insight on how neonatal stress may influence behavior and physiology in animals.

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Chapter 2 LITERATURE REVIEW

2.1 SEGREGATED EARLY WEANING SYSTEM

The segregated early weaning (SEW) concept was developed from the early work of Alexander et al. (1980). In their study, sows and their litters were medicated, the piglets were weaned at about five days of age and moved to a strictly isolated site. At the end of the trial, herds raised by this method achieved a high health status and gained more weight than conventionally raised herds. Recent research showed that these health benefits can be maintained when sows are not isolated or medicated, and weaning occurs at 10 to 19 days (Harris, 1988; Dritz et al., 1996). The success of SEW was not widely acknowledged until recently. Reasons for the delayed acceptance of SEW included understanding the nutritional needs of the piglets. Diets had to be developed for immature digestive system of neonatal piglets.

The objective of SEW is to control diseases by reducing vertical and horizontal microbial contamination. Vertical contamination refers to the spread of bacterial and/or viral infections from sows to piglets. One way to reduce the risk of spreading infectious diseases is to move piglets away from the sow at an early age to a separated si

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site. Horizontal contamination is defined as the spread of infection from one group of pigs to another. This can be controlled by using the all-in-all-out settings, where pigs are placed in and removed together from rooms, whole buildings, or complete sites (Alexander et al., 1980; Dritz et al., 1996). SEW is a combination of the following two practices: neonatal weaning to a separated site, and all-in-all-out management.

Since neonatal weaning and transportation are integrated in practical SEW systems, these two factors were not separated in my research. As a result, there are at least four stressors associated with SEW in this work: maternal deprivation; nutritional deprivation; exposure to an unfamiliar environment; and transportation. Among them, maternal deprivation, nutritional deprivation and exposure to an unfamiliar environment that accompany weaning are referred to together as "weaning stress".

Few studies have examined the behavior of piglets weaned at 2 weeks of age or younger. The occurrence of abnormal behaviors, such as belly-nosing, increases as weaning age decreases (Metz and Gonyou, 1990; Gonyou et al., 1998).

In addition, some evidence suggests that the sow may not be ready for weaning at an early stage of lactation.

After piglets were removed at an early age, sows were more active, vocalized at a much higher rate, and returned repeatedly to the piglets resting area (Pajor et al., 1996). They also appeared to encounter some reproductive problems such as a delay in return to estrus and a reduction in subsequent litter size (Koketsu and Dial, 1997).

Due to the large size of SEW herds, there is a concern that infectious diseases can spread very rapidly and affect more animals than in smaller conventional herds.

2.2 STRESS RESPONSE

2.2.1 Stress and Stressor

Physiologists consider stress to be a biological phenomenon. Psychologists argue that stress is a psychosocial pressure being consciously sensed by an individual. In many cases, stress has been defined as either a behavioral or physiological response. In general, stress is considered to be the physical and psychological state of animals in response to environmental, physiological and psychological stimuli. The definition proposed by Broom and Johnson (1993), however, can best describe the stress associated with weaning and transportation of piglet. In their definition, stress is a physical, environmental and/or psychological effect on an individual that overtaxes its control systems and reduces its fitness or appears likely to do so. From this viewpoint, stress is induced by noxious environmental stimuli and its occurrence implies a failure to cope. Since the individual's welfare is poor when it finds coping difficult, stress invariably indicates poor welfare, but welfare can be poor without stress (Broom and Johnson, 1993). On the other hand, the success of coping behavior can be measured by its effectiveness in reducing physiological measures of stress or by its effectiveness in reducing the effect of aversive stimuli and thus restoring fitness (Wechsler, 1995).

Stress responses vary among individuals and depend on the following elements: species; the genetic make up; early life experience; type and severity of the stressor; the ability to cope with the situation; and experience involving the same type of stressor (Plomin et al., 1994; Steinhausen, 1994; Plotsky and Meaney, 1993; Levine, 1985).

Stressors can be physical, environmental, or psychological in nature. Physical stressors include tail-docking, castration and physical restraint. Environmental stressors include extreme temperatures, infections. Psychological stressors include maternal separation and social isolation in social animals. Though the stressors are different, they activate three major biological systems: behavior, autonomic nervous system, and neuroendocrine systems (Moberg, 1985).

2.2.2 Behavioral Response to Stress

When an animal faces challenges, the most apparent change is in its behavior. Acute stressors can induce fear-related, anxiety-related, or frustration-related behaviors. Fear responses in pigs include trembling body, moderately loud vocalization, unnatural huddling together, climbing on other pigs, and increased frequency of defecating and urinating (Brundige, 1998).

The long-term behavioral consequences of stress in animals include the occurrence of destructive, stereotypic, redirected, aggressive, inactive, and escape behavior (Fraser and Broom, 1990). Destructive behaviors are those that cause damage to other animals, such as feather pecking in poultry or tail biting in pigs. Stereotypic behaviors are rhythmic, repetitive, and invariant behaviors without apparent function or goal (Mason, 1991). Examples are head-rolling in caged mink and sham chewing in tethered sows. Redirected behaviors are normal behaviors either in abnormal frequency or directed to a substitute object.

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Examples are increased drinking and massaging of penmates in pigs kept in crowded environments (Dybkjær, 1992). Aggressiveness is related to the environment in which the animals are raised. De Jonge et al. (1996) showed that piglets reared in farrowing crates displayed more aggressive behaviors than piglets reared in outdoor pasture with half-open crates. Inactive behaviors include inactivity, apathy and unresponsiveness. Tethered sows responded much less than group housed sows to environment stimuli (Broom, 1987).

Destructive, stereotypic, redirected and inactive behavior are considered abnormal. In the introduction, I have introduced the definition of "abnormal", which is "being statistically rare or different from a chosen population which is living free or in naturalistic conditions in captivity" (Fraser and Broom, 1990). Behavior abnormalities are the most focused issue in the area of applied animal ethology and animal welfare. These behaviors are widely spread in confined animals and can be induced by aversive situation such as unpredictable and uncontrollable draught (Broom, 1991; Scheepens et al., 1991). It is suggested that the development of abnormal behavior may result from the failure to cope with normal behavior (Wechsler, 1995). Some studies indicate that abnormal behaviors are associated with a reduction in physiological measures of stress and can thus be regarded as successful coping behavior (Wiepkema et al., 1987, Mittleman et al., 1991). However, the relationship between abnormal behavior and physiological measures of stress is not unequivocal. For example, Terlouw et al. (1991) reported no correlation between the exhibition of post-feeding stereotypy and plasma cortisol levels in sows. Furthermore, in a pain-sensitivity test, the sows with high levels of stereotypy had shorter tail-flick latencies than sows with low levels of stereotypy. This result suggests that the performance of stereotypic behavior does not narcotize the animal. Although the results of different studies on abnormal behavior and physiological measurement are not consistent, most researchers agree that abnormal behavior indicates difficulties in coping, and therefore is an important indicator of welfare (e.g. Wiepkema, 1983; Fraser and Broom, 1990; Barnett and Hemsworth, 1990; Wechsler, 1995).

2.2.3 Physiological Response to Stress

Autonomic nervous system and neuroendocrine systems

Besides the behavioral changes, biochemical changes also take place when an animal responds to stress. The autonomic nervous system (ANS) and neuroendocrine systems are major pathways that are activated to regulate these processes in stress responses.

The ANS has three central components, hypothalamus, brain stem, and spinal cord. The peripheral ANS is functionally composed of the sympathetic and parasympathetic systems. During a stressful situation, the sympathetic activity is enhanced leading to an increased heart and respiratory rate, shunting of blood supply from the viscera to the muscles and the brain, and margination of lymphocytes. These prepare the body with the physiological measures needed to handle an emergency situation. The sympathetic activity also stimulates the neuroendocrine system, which releases catecholamines (norepinephrine and epinephrine), and further strengthens the sympathetic activity (Rhoades and Pflanzer, 1996). However, it is difficult to measure hormonal responses associated with sympathetic activity because the concentration of plasma hormones returns to basal levels very quickly. Thus, alternative indicators of stress need to be identified.

The hypothalamic-pituitary-adrenal (HPA) system, the hypothalamic-pituitary-gonadal (HPG) system, and catecholaminergic system are of most interest in stress r

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research (Levine, 1985). The catecholamine responds to conditions that require attention and vigilance. The HPG system is mainly responsive to situations involving social behavior, such as dominance. The HPA response occurs in situations involving novelty and uncertainties, where the animal perceives that it can not control its surroundings (Levine, 1985).

Hypothalamus-pituitary-adrenal axis:

Historically, the HPA axis has received the most attention in stress research. This can be credited largely to Hans Selye, a pioneer and authority in the modern stress research, who interpreted stress as the nonspecific response of the body to any demand made upon it (Selye, 1956). The HPA axis is activated during a variety of situations such as injury, social interaction, and physical restraint (Klemcke and Pond, 1991; Becker et al., 1985). Therefore, Selye advocated that the activation of HPA axis is a component of a nonspecific, stereotyped reaction to stress (Selye, 1973).

Once a stimulus is perceived to be threatening by the central nervous system (CNS), the hypothalamus of the brain releases corticotropin releasing hormone (CRH, also known as CRF). CRH reaches the pituitary gland via the hypophyseal stalk and triggers adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary into the blood (Vale, 1981). When circulating ACTH reaches the adrenal gland, it stimulates the synthesis and release of adrenal glucocorticoids, cortisol and corticosterone.

Glucocorticoids are the final effectors of the HPA axis and participate in the control of whole body homeostasis (Stratakis and Chrousos, 1995). These steroid hormones promote the transformation of non-sugars into sugars, glycogen storage, and protein and lipids decomposition (Rhoades and Pflanzer, 1996). Through these processes, the amount of glucose available to the CNS, heart and skeletal muscles is increased, which enables the animal to handle potential threatening situations. Thus, the activation of HPA axis and the elevation of glucocorticoid concentrations during a stressful event are normal and necessary functions of the body. When in high concentration, glucocorticoids also play a key role in terminating the stress response by exerting negative feedback involving ACTH and CRF at the level of pituitary and hypothalamus (Munck et al., 1984; De Kloet, 1991). In addition to their effects on carbohydrate metabolism and neuropeptide regulation, glucocorticoids suppress inflammation, delay the growth of new tissue, inhibit

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neutrophil migration and production of antibodies by B lymphocytes, inhibit cytokine production and activity, and lower lymphocyte counts (Munck et al., 1984; Burton and Kehrli, 1995, 1996). In general, glucocorticoids suppress normal defense mechanisms.

Chronic release of glucocorticoids results in decreased growth rate, suppressed immune-inflammatory reactions, suppressed reproductive function in both sexes, and progressive neuronal cell loss in the hippocampus (Stratakis and Chrousos, 1995; Sapolsky et al., 1985). In summary, the HPA axis profoundly influences growth, immune function, reproduction, and the thyroid axis (i.e. metabolism), which are important parameters of fitness (Stratakis and Chrousos, 1995).

2.2.4 Cortisol as Physiological Indicator of Stress

Glucocorticoids have frequently been used as indicators of HPA axis activity. Cortisol is the predominant glucocorticoid produced in dogs, cats, pigs, sheep, and horses (reviewed in Stephens, 1980). Cortisol is a small, hydrophobic molecule produced by the adrenal cortex of the adrenal gland, and is detectable in all body fluids including plasma, saliva, and urine. In piglets, the urinary cortisol half-life is long enough (6.0 ± 0.6 hour) so

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so that it can be used as an indicator of stress (Kraan et al., 1986).

Plasma cortisol

Most of the circulating cortisol is bound to plasma proteins. In humans, approximately 80-90% of circulating cortisol is bound to cortisol-binding protein (CBG), 5-10% is loosely bound to albumin, and about 4.0% is unbound (Brody, 1994). In pigs, approximately 9% of cortisol is unbound (Cook et al., 1996). Unbound cortisol is the physiologically active form.

Plasma cortisol concentration provides a reliable tool for the assessment of HPA axis activity and estimates the difficulty for animals to cope with short-term problems. Mice being exposed to three increasingly unfamiliar environments respond correspondingly with elevations in the plasma glucocorticoids level (Hennessy and Levine, 1978). The severity of surgical procedures used in sheep (Shutt et al., 1987) also resulted in increased levels of plasma glucocorticoids.

In pigs, plasma cortisol also increases during stressful events. These events can be maternal separation, social isolation, electric shock, physical restraint, thermal extremes, and transportation (Klemcke et al., 1991; Becker et al., 1985; Hicks et al., 1998).

When blood samples are collected via venipuncture, pigs experience pain and additional stress due to restraint. Therefore blood sampling may artifactually influence the HPA axis response to stress. Consequently, plasma cortisol as a stress marker gives ambiguous results (Bassett and Hinks, 1969; Worsaae and Schmidt, 1980; Moberg, 1987). A noninvasive technique that does not induce an appreciable stress on the animals could eliminate this ambiguity. Most research has focused on whether salivary and urinary cortisol values could reflect plasma cortisol concentration. In my first experiment, urinary cortisol was used since I could not collect enough (about 500 μl) saliva from 10-day old piglets.

Salivary cortisol

Many small organic compounds such as urea, glucose, steroids, and lipids are present in saliva, usually much less than their plasma concentration. The reliability of using saliva to determine the fraction of unbound low molecular weight hormones, such as cortisol, has been well established for humans (Walker, 1989).

Most of the saliva is produced by three major pairs of salivary glands (parotid, submandibular, and sublingual). The numerous small buccal glands, which line the mouth, secrete saliva continuously under local control. Secretion by the major glands is mediated by both sympathetic and parasympathetic pathways in response to physical, chemical and psychological stimuli. In humans, the relative proportion contributed by each of the major glands under resting conditions is typically, submandibular 69%, parotid 26%, sublingual 5% (reviewed in Vining and McGinley, 1986).

Vining and McGinley (1986) proposed several routes that may transport a hormone to saliva: 1) passive intracellular diffusion is for high lipid-soluble molecules like corisol and takes place in the lipid-rich cell membranes of acinar cells or into the cells lining the gland; 2) ultrafiltration, which brings the lipid-insoluble compounds into saliva; and 3) active secretion or transport, an active, energy-consuming process by which many electrolytes and some proteins may be secreted to saliva.

Most of the cortisol present in saliva appears to enter intracellularly by diffusing through the cells of the salivary glands. Saliva flow rates can range from less than 0.05 ml/min (during sleeping) to 10 ml/min (when stimulated

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by acid lemon drops). Fortunately, saliva cortisol levels are not affected by the flow rate because the diffusion rate is high enough to maintain a concentration equilibrium between saliva and plasma (Hubert and De Jong-Meyer, 1989; Cooper et al., 1989). There is a linear correlation between unbound cortisol concentration in plasma and saliva. Correlation coefficients between 0.89 to 0.97 have been reported (reviewed in Vining and McGinley, 1986). Vining et al. (1983) also found the immediate (approximately 1 min) appearance of cortisol in saliva following an intravenous injection of cortisol. In summary, saliva cortisol concentration accurately reflects the current level of unbound cortisol in plasma.

The measurement of cortisol in saliva offers several advantages over the measurement of the hormone in blood: 1) saliva is easily collected and plentiful in supply (Cooper et al., 1989; Parrott and Misson, 1989; Cook et al., 1996); 2) the stress of venipuncture has been shown to increase cortisol levels whereas the non-invasive saliva sampling has not (Kirschbaum and Hellhammer, 1989); and 3) there is no CBG present in saliva. The presence of CBG often complicates the interpretation of plasma cortisol levels because many factors may alter the level of CBG in the blood or affect the binding of cortisol to them.

In pigs, Cook et al. (1996) compared the efficacy of salivary cortisol to serum cortisol for assessment of the HPA response to exogenous ACTH stimulation. These authors found the levels of cortisol in saliva are approximately 9% of the total plasma cortisol. This result is consistent with observation of Parrot et al. (1989). It is well established that salivary cortisol significantly correlates with plasma cortisol in pigs ($r > 0.7$)(Cook et al., 1996; Schönreiter et al., 1999).

Urinary cortisol

Urinary cortisol is also a widely accepted index of circulating cortisol in humans (Beisel et al., 1964; Brooks, 1979). Circulating unbound cortisol distributes along concentration gradients. Unbound plasma cortisol continuously enters the urine pool via glomerular filtration of blood by the kidneys (Beisel et al., 1964; Brooks, 1979). This urine pool accumulates plasma cortisol over several hours. Cortisol concentrations in voided urine represent the average level of unbound cortisol circulating between urinations. Therefore urinary cortisol levels can dampen the temporal variability inherent in instantaneous plasma and saliva values (Miller et al., 1991; Crockett et al., 1993).

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Twenty-four hour urine cortisol is used to evaluate adrenal responses to stressors in varieties of species, such as primates (Crockett et al., 1993), sheep (Berman et al., 1980), and mink (Madej et al., 1992). To collect 24-hour urine samples, animals need to be individually caged. Social isolation is a potent stressor, which increases the activity of HPA axis in social animals such as pigs (Ruis et al., 1997). Thus, this sampling method interferes with the endocrine response it is intended to measure.

To avoid the artifactual effects and the inconvenience of standard 24-hour continuous sampling, the cortisol:creatinine ratio in a single urine sample (UCCR) has been evaluated. The diurnal fluctuation of UCCR is a substitute for the total urinary cortisol secretion in 24 hours in the diagnosis of cortisol excess and deficiency in human (Contreras et al., 1986). A positive linear relationship (r=0.5 to 0.7) between UCCR and plasma cortisol concentrations has been reported in dogs (Jones et al., 1990) and bighorn sheep (Miller et al., 1991).

In the state of elevated plasma cortisol concentration there is an increased percentage of unbound plasma cortisol, an elevated renal clearance for unbound cortisol, and an increased glomerular filtration rate (Jones et al.,

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1990). This may explain why the rise in urinary cortisol is disproportional to that of the plasma cortisol.

2.3 SEGREGATED EARLY WEANING STRESS

2.3.1 Weaning Stress

In a commercial pig production system, weaning includes an abrupt separation of the suckling piglets from the sow when the piglets are still wholly nourished by the sow's milk. Under semi-natural environments, weaning is a gradual process where stronger piglets wean earlier than the weaker pigs in the litter. The natural weaning process is completed between 12 to 17 weeks of age (Newberry and Wood-Gush, 1985; Jensen, 1986; Stolba and Wood-Gush, 1989).

Premature separation from the mother has long been acknowledged as a cause of stress in many species, which is often evidenced by prolonged vocalization, restless activity, HPA axis activation and long-term behavioral and physiological changes (Fraser, 1978; Worsaae and Schmidt, 1980; Blackshaw, 1981; Klemcke and Pond, 1991; Plotsky and Meaney, 1993).

Some studies have examined the behavioral effects of weaning and weaning age in animals. Mason (1994) found that early weaning in mink increased the performance of tail-biting. Würbel and Stauffacher (1997) reported that precociously weaned mice had high levels of stereotypes in adulthood. Fraser (1976) compared the behavior development of suckling and weaned piglets (at three weeks of age) during the first six weeks after birth. He noticed that newly weaned animals had high levels of general activity and aggression. Belly-nosing occurred after weaning and appeared to be stimulated by the sound of the neighboring sow nursing its young. This behavior was never observed in suckling piglets (Fraser, 1976). As piglets' weaning age decreases, more calls/min and higher frequency were recorded post-weaning (Weary et al., 1997). In fact, in many European countries, weaning piglets younger than three weeks of age is considered inhumane and therefore is illegal (The Welfare of Livestock Regulations, 1994).

Long term physiological effects of maternal care on neonates have been examined in rodents. In rats, maternal care during early postnatal age has been demonstrated to have a persistent effect in the offspring's response to stress throughout their life. As adults, offspring who received more maternal care had reduced behavioral fearfulness in response to novelty, and reduced response of HPA axis to acute stress (Caldji et al., 1998; Liu et al., 1997). In addition, Plotsky and Meaney (1993) found that as adults, rats' offspring who were subjected to 3-hour/day mat

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maternal separation during 2-14 days of age had a high level of plasma corticosterone (predominant glucocorticoid in rodents) in response to restraint stress when compared with undisturbed offspring. Würbel and Stauffacher (1997) also reported that precociously weaned mice had elevated plasma corticosterone levels post-weaning.

2.3.2 Transportation Stress

A number of reports (Dow, 1976; van Putten and Elshof, 1978; Stephens, 1980) indicate that transport can have a detrimental effect on pigs. Economic loss due to transportation was estimated at more than 43 million dollars in 1994 in the United States (Pork Chain Quality Audit, 1994). The loss resulted from sudden death of pigs, excess bruise of the carcass, and pale, soft, and exudative (PSE) meat. Many environmental conditions contribute to the transportation stress, such as vibration, noise, changes in speed, mixing with unfamiliar animals, and deprivation of food and water (Stephens and Perry, 1990). Stephens et al. (1985) found that pigs can make an operant response to terminate the vibration of their pen and do not habituate to vibration and noise. But the noise alone does not motivate the animals to turn the apparatus off.

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A number of researchers have examined the physiological response to transportation in pigs. Elevated plasma cortisol, reduced body weight, increased numbers of blood neutrophils, and decreased numbers of lymphocytes are reported after a four-hour transportation (McGlone et al., 1993). The transportation of sheep leads to an increase in vocalization, heart rate, and serum and salivary cortisol levels (Fell et al., 1985; Baldock et al., 1990). In horses, transportation leads to immune suppression, high heart rates, hypertonic dehydration, and high serum cortisol concentrations (Owen et al., 1983; Clark et al., 1993; Friend et al., 1998).

2.4 SUMMARY

Maternal care of the offspring seems to have important effects on the stress response of adult offspring. Premature maternal deprivation and transportation are stressful experiences for many animal species. Stress reduces the fitness of an animal as measured by parameters of growth, reproduction, and immune function.

I hypothesize in this thesis that neonatal piglets will respond to SEW stress as measured by urinary cortisol as well as behavior indicators such as vocalization, locomotion, aggression and development of abnormal behavior. I also hypothesize that the stressful SEW experience during neonatal age will be associated with high levels of aggression, behavioral abnormalities, and elevated HPA axis responsiveness in pigs when they are older.

Chapter 3 HYPOTHESES AND OBJECTIVES

The overall hypothesis was that neonatal piglets can respond to segregated early weaning (SEW) stress and that this stressful experience has long lasting effects on aggression, behavioral abnormalities, and HPA axis responsiveness.

The overall objectives were:

1. To investigate the immediate response of neonatal piglets to SEW stress;
2. To investigate the long-term effects of SEW stress in pigs.

EXPERIMENTAL APPROACH

We designed and conducted two experiments. In the first experiment, we hypothesized that neonatal piglets can respond to SEW stress as measured by behavioral indicators and cortisol concentrations. The corresponding objectives were:

- To investigate the effects of SEW stress on the behavior of neonatal piglets shortly after weaning;
- To determine the effects of SEW stress on cortisol concentration in neonatal piglets shortly after weaning.

The specific aims of this experiment were:

- To monitor maintenance, locomotion, aggressive, and abnormal behaviors in neonatal piglets subjected to SEW stress or left undisturbed with the sow;
- To monitor urinary cortisol:creatinine ratio in neonatal piglets subjected to SEW stress or left undisturbed;
- To monitor growth of pigs as a practical indicator of production performance under SEW and left undisturbed.

In the second experiment, the SEW piglets were compared with conventional weaned (CW) piglets that were removed from their sows at a later age than SEW piglets. We tested the hypothesis that stressful SEW experience during neonatal age is associated with high levels of aggression, behavioral abnormalities, compromised social skills and elevated HPA axis responsiveness in pigs when they are older. The objectives of this study were:

- To investigate the development of behavior abnormalities in SEW piglets;
- To determine the HPA axis responsiveness in SEW piglets at a later age;
- To investigate the aggression and the capacity to assess social environment in SEW piglets at a later age.

The specific aims of this experiment were:

- To monitor the development of abnormal behaviors in SEW or CW piglets until 7 weeks of age;
- To monitor baseline AM and PM salivary cortisol concentration in SEW or CW piglets at 5, 6,and 7 weeks of age;
- To monitor salivary cortisol concentrations in response to transportation stress in SEW or CW piglets at 9 weeks of age;
- To monitor the number, duration and outcome of the fights in SEW and CW piglets when they are mixed at 9 weeks of age;
- To monitor piglet growth as a practical indicator of production performance under SEW and CW system until 8 weeks of age.

Chapter 4 IMMEDIATE RESPONSE OF NEONATAL PIGS TO SEGREGATED EARLY WEANING

4.1 INTRODUCTION

Weaning and transportation are stressful to pigs (Fraser, 1978; Lambooij and van Putten, 1993). The segregated early weaning (SEW) system, which subjects piglets to early weaning and transportation has been rapidly adopted by the North American pig industry. However, the potential welfare concerns of exposing the neonatal piglets (as early as 10 days of age) to these two stressors have not been addressed. Pigs raised in this system exhibit high levels of behavior abnormalities, such as belly-nosing, later in their life (Gonyou et al., 1998).

Stress is considered a threat to animal welfare. Cortisol is the hormone most widely used as an indicator of stress (Levine, 1985; Shutt et al., 1987; Klemcke and Pond, 1991). During this experiment, I measured cortisol in urine samples rather than blood samples because urine can be obtained non-invasively. The validity of using urinary cortisol:creatinine ratio (UCCR) in a single urine sample as an indicator of HPA axis activation has been demonstrated in humans, dogs and sheep (Contreras et al., 1986; Jones et al., 1990; Miller et al., 1991). In this study, UCCR was used to evaluate the adrenal responses to SEW stress in piglets.

This study was conducted to address the welfare concerns by investigating the immediate behavior and adrenal response of neonatal piglets to SEW stress. I hypothesized that neonatal piglets can respond to SEW stress as measured by both behavioral indicators and UCCR. The expected results were: 1) SEW piglets would have a reduced growth rate; 2) SEW piglets would have increased activity levels, vocalization rates, aggression and exploratory behavior; and 3) SEW piglets would have elevated urinary cortisol levels post weaning compared with non-weaned animals.

4.2 MATERIALS and METHODS:

4.2.1 Animals and Management

Six Landrace sows were bred using artificial insemination by the same Yorkshire boar. Sows farrowed within five days of each other. Within 24 hours after farrowing, the litter size was standardized to eight piglets, piglets were tail docked, ear notched, teeth clipped and the male piglets were also castrated. Crossfostering was carried out between days 1 and 3 postpartum.

The sows and their litters were housed in farrowing crates. Sows were fed twice a day and water was provided *ad libitum*. A nipple drinker was available for the piglets. Continuous illumination was provided for about 10 hours a day. The room temperature was set to 18°C. Heating lamps were used to provide additional heat.

Twenty-three piglets (due to the loss of one piglet) from three crossfostered litters were weighed, weaned and transported for 5 minutes to a separate site at 1130 h when they were 9 to 13 days old (SEW group). Twenty-four piglets of the other three sows were also weighed, but they were allowed to stay with the sows (control group). Eight days later, their weights were measured again.

The weaned piglets were placed in three 1.18 X 1.19 m pens without regrouping. Artificial lights provided illumination for 10 hours a day. The room temperature was set to 27°C. Heating pads were placed in the pen to provide additional heat. Water was supplied by means of a nipple drinker *ad libitum*. SEW phase I diet (Crude Protein 23.4%, Lysine 1.70%, ME 3429.4 kcal/kg, pellet form) was fed twice a day *ad libitum* in a three-hole feeder. In addition, for the first two days post-weaning, fresh pellets were also given on a pan in the middle of the floor 5 times during the day to acquaint the piglets to the new form of diet.

4.2.2 Behavioral Observations

Piglets were numbered with a livestock marker (LA-CO Industries, Inc., Elk Grove Village, Illinois) the day before weaning. Various behavior categories listed in Table 1 were recorded by two trained observers. Direct observations were carried out on the weaning day (day 0), and on days 1, 2 and 3 post-weaning. Each animal was observed continuously for two minutes in each observation section. The observation was repeated four times between 830 and 1530 h for every animal. Behavior data were logged onto audio tapes for control animals and directly on a computer using a behavior software (The Observer® 3.0, Noldus Information Technology, Wageningen, The Netherlands) for SEW piglets. The audio tape recording was later transferred to the computer using the same behavior software.

4.2.3 Urine Sampling

Urine samples were collected one day before weaning (day -1) and 1, 3 and 5 days post-weaning between 1530 and 1730h. The piglets were placed into individual plastic boxes (57.15 X 39.37 X 31.75 cm) until they urinated. The maximum time that a piglet was kept in the box was 10 minutes. In the SEW group, we were able to collect samples

Table 1: Description of behavior categories in piglets.

Behavior category	**Description**
States[2]	
Lying	Body weight is not supported by the limbs.
Standing	Supporting its body with four limbs upright and idle.
Sitting	Sitting on the posteriors; fore limbs stretched; head free from any support.
Moving	Four limbs upright and not idle.
Rooting	In a standing position, the piglet rubs the snout on the flooring surface in horizontal movements.
Fighting	Two piglets take part in an agonistic encounter involving knocking, biting, parallel and inverse parallel pressing and levering.
Belly-nosing	A rhythmic up-and-down movement with the snout on the belly or soft tissue between the hind and forelegs of another piglet.
Manipulating ear	Having another piglet's ear in mouth, may suck, chew, or bite.
Manipulating tail	Having another piglet's tail in mouth, may suck, chew, or bite.
Manipulating other part of the body	Sucking, chewing, biting or horizontal movement of snout on another piglet's body except ear and tail.
Massaging the sow	A rhythmic up-and-down movement with the snout near the sow's teat, it usually occurs before, between or after suckling.
Sucking	Having sow's teat in the mouth.
Eating	The head is over or in the feed trough.
Drinking	Having the nipple drinker in the mouth.
Bar biting	Chewing or biting the metal bar.
Events[3]	
Grunting	Low tonal vocalization.

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[2] Behaviors of relatively long duration, such as prolonged activities, body postures or proximity measures. The salient feature of states is their **duration** (mean duration, or the proportion of time spent performing the activity) (Martin and Bateson, 1993).

[3] Behaviors of relatively short duration, such as discrete body movements or vocalizations. The salient feature of events is their **frequency** of occurrence (Martin and Bateson, 1993).

Table 1 (Cont'd)

Squealing	Intense high pitch vocalization.
Climbing	Fore limbs on top of another piglet's back, hind limbs stretched and in an upright position.

from 3, 11, 11, 17 piglets on days -1, 1, 3, 5 post-weaning, respectively. In the control group, we obtained 6, 10, 15, 10 samples on day -1, 1, 3, 5 post-weaning, respectively. Urine was collected into 15-ml plastic conical tube and put on ice immediately. Samples were stored at -20°C until analysis. Upon defrosting, urine samples were centrifuged for 15 min (1,300 X g, 4°C) and the supernatant was used for analysis.

4.2.4 Urinary Cortisol and Creatinine Analysis:

Urinary cortisol concentrations were determined using an ^{125}I radioimmunoassay (Coat-A-Count Cortisol Kit, Diagnostic Products Corp, Los Angeles, California) following standard procedures except that samples were not extracted with CH_2Cl_2. Urinary creatinine was analyzed using commercial diagnostic kits (555-A creatinine kit, Sigma Chemical Company, St. Louis, Missouri).

The inter-assay coefficient of variation for quality control (std/mean x 100) on two creatinine assays at 113.15 mmol/l and 256.36 mmol/l was 6.91% and 4.72%, respectively.

The intra-assay coefficient of variation on duplicate samples was 8.14% in the cortisol assay and 3.82% in creatinine assay, and was calculated using the following formula:

$$\text{Intra-assay coefficient} = \left(\frac{\Sigma(d/\mu \times 100)^2}{2N} \right)^{1/2}$$

where;

d = the difference between duplicate estimates

μ = the mean of the duplicate estimates

N = the number of duplicate samples

4.2.5 Statistical Analysis:

Behavior data were expressed as either percentage of observation time (states) or frequency per minute (events and states). Cortisol in urine was expressed as the ratio of urinary cortisol concentration to creatinine concentration (UCCR).

From the viewpoint of data distribution, the data sets obtained from this research can be classified into three groups:

1. Some data were normally distributed, including body weight and % of time spent lying;
1. Some data were not normally distributed. In some of these cases, distributional assumptions were satisfied by using

certain transformations. For the UCCR data, a log-transformation was applied before data analysis;

2. In the other cases where data were not normally distributed, transformations did not satisfy the distributional assumptions. All the rest of the behavior data fit into this category.

For the first two types of data, a mixed model with repeated measures from SAS® (SAS institute Inc, Cary, North Carolina, U.S.A.) was used. Lsmeans ± standard error of the means (SEM) were reported or back transformed to the original scale wherever necessary to facilitate interpretation and discussion of the results. Differences between treatment means at various trial days were tested for significance (when $P < 0.05$). The experimental unit used in the analysis was the pen.

For the third type of data, a nonparametric Wilcoxon rank sum test was chosen. It tests whether the distribution of a variable has the same location parameter across different groups based on the simple linear rank statistics. The assumption is that the samples were randomly, independently drawn from one population and received two treatments. Mean rank score ± SEM are reported.

Statistical model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \rho_{k(i)} + \delta_l + \alpha\beta_{ij} + e_{ijklm}$$

Where;

Y_{ijklm} = dependent variable (such as body weight, % of time spend lying, and log-transformed UCCR) observed for the m^{th} piglet, in the i^{th} treatment, at the j^{th} day, in the k^{th} pen, and from the l^{th} litter;

μ = overall mean of dependent variable;

α_i = the fixed treatment effect (i = SEW, Control);

β_j = the fixed day effect (j=1,2; or 1,2,3; or 1,2,3,4, depending on Y_{ijklm});

$\rho_{k(i)}$ = the random effect of the k^{th} (k=1,2,3) pen nested within the i^{th} treatment, $\rho_{k(i)} \sim NIID(0, \sigma^2_{\rho(\alpha)})$ for all i and k;

δ_l = the random effect of the l^{th} litter (l=1,…,6), $\delta_l \sim NIID(0, \sigma^2_{\delta})$ for all l;

$\alpha\beta_{ij}$ = the interaction of treatment and day;

e_{ijklm} = random error term, $e_{ijklm} \sim NIID(0, \sigma^2_e)$ for all i, j, k, l, m.

4.3 RESULTS

The weight of the control and SEW groups were not

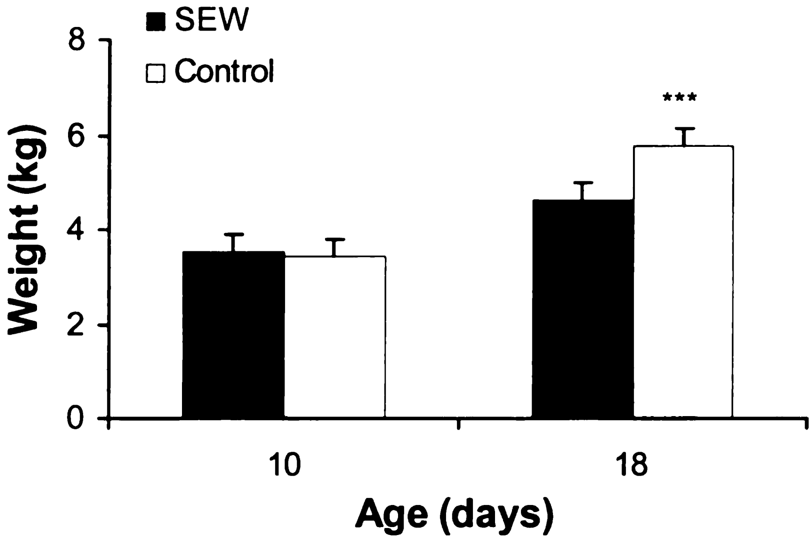


Figure 1: Mean weight of SEW and control piglets before and a week after weaning treatment. *** $P < 0.001$. Comparison was made between SEW and control piglets within the same time period.

different ($P = 0.7741$) before early weaning. The SEW piglets had significantly lower body weight ($P = 0.0002$) than piglets left with sows after one week of weaning (Fig. 1).

Eating and drinking averaged 4% in SEW piglets' time budget. Control piglets spent on average, 15% of observation time sucking and massaging the sow (Table 4 in Appendix).

On days 0 and 1 post-weaning (PW), SEW piglets displayed higher levels of vocalizations ($P \leq 0.0001$), showed higher frequency of climbing ($P \leq 0.037$) and spent

Table 2: Mean Wilcoxon rank sum score (±SEM) in SEW piglets and control piglets (not weaned) for frequency of grunting, climbing, and duration of moving immediately after and in the first day post weaning.

Wilcoxon Rank Sum Score (Mean)*	Day 0 Post Weaning		Day 1 Post Weaning	
	SEW	Control	SEW	Control
Grunting	30.8 ± 1.6^{a}	17.5 ± 1.5^{b}	57.4 ± 4.0^{a}	38.0 ± 3.9^{b}
Climbing	25.6 ± 0.9^{a}	22.5 ± 0.8^{b}	51.2 ± 3.9^{a}	44.0 ± 3.8^{b}
Moving	30.0 ± 2.0^{a}	18.2 ± 1.9^{b}	58.7 ± 5.7^{a}	36.7 ± 5.4^{b}

* Comparison was made between SEW and control piglets within the same time period; different letters indicate a statistical significant difference ($P < 0.05$).

more time moving ($P \leq 0.002$) than undisturbed control piglets (Table 2).

Time spent rooting and frequency of belly-nosing were not different in the two treatment groups on day 0 post-weaning but were consistently higher ($P \leq 0.012$) throughout days 1, 2, and 3 post-weaning in SEW piglets than in control piglets (Table 3). The frequency and time spent fighting did not differ in the two groups at any observation days post-weaning ($P > 0.15$; Table 3).

SEW animals spent less time lying on day 1 ($P = 0.0004$), but more time on day 3 ($P = 0.0057$) post-weaning

Table 3: Mean Wilcoxon rank sum score (±SEM) in SEW piglets and control piglets (not weaned) for frequency of belly-nosing, duration of rooting, and duration and frequency of fighting in days 1, 2 and 3 post weaning.

Wilcoxon Rank Sum Score (Mean) *	Day 1 Post Weaning		Day 2 Post Weaning		Day 3 Post Weaning	
	SEW	Control	SEW	Control	SEW	Control
Belly-nosing	54.8 ± 3.6^{a}	40.5 ± 2.8^{b}	51.7 ± 2.8^{a}	43.5 ± 2.7^{b}	51.4 ± 3.5^{a}	42.8 ± 3.3^{b}
Rooting	54.0 ± 2.1^{a}	41.3 ± 2.0^{b}	52.8 ± 1.9^{a}	42.4 ± 1.8^{b}	52.5 ± 2.0^{a}	42.7 ± 1.9^{b}
Fighting (duration)	49.5 ± 2.0^{a}	45.6 ± 1.9^{a}	49.6 ± 2.1^{a}	45.5 ± 2.0^{a}	48.1 ± 2.1^{a}	46.9 ± 2.0^{a}
Fighting (frequency)	48.7 ± 2.0^{a}	46.3 ± 1.9^{a}	49.3 ± 2.1^{a}	45.7 ± 2.0^{a}	47.5 ± 2.1^{a}	47.5 ± 2.0^{a}

* Comparison was made between SEW and control piglets within the same time period; different letters indicate a statistical significant difference ($P < 0.05$).

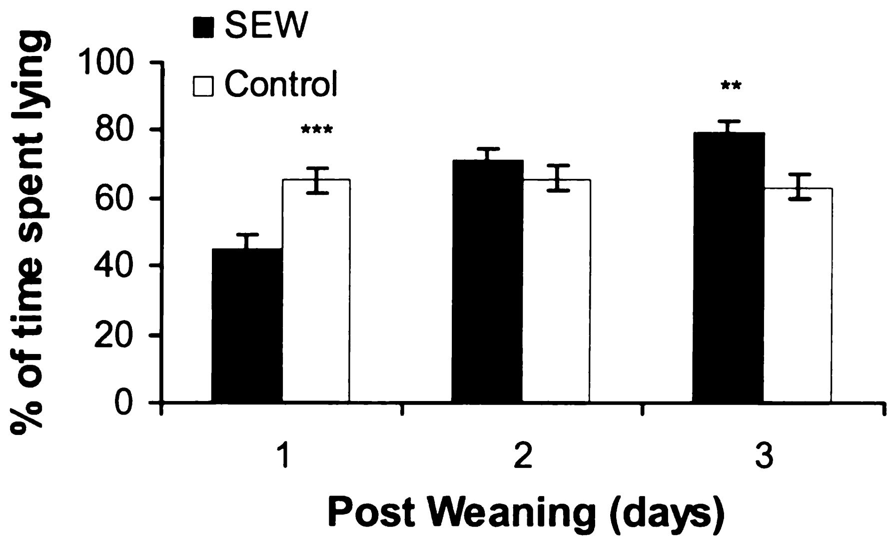


Figure 2: Average percentage of time spent lying from 0830 to 1530 h in SEW piglets over three days post weaning, with reference to control piglets that stayed with the sows. ** $P < 0.01$; *** $P < 0.001$.

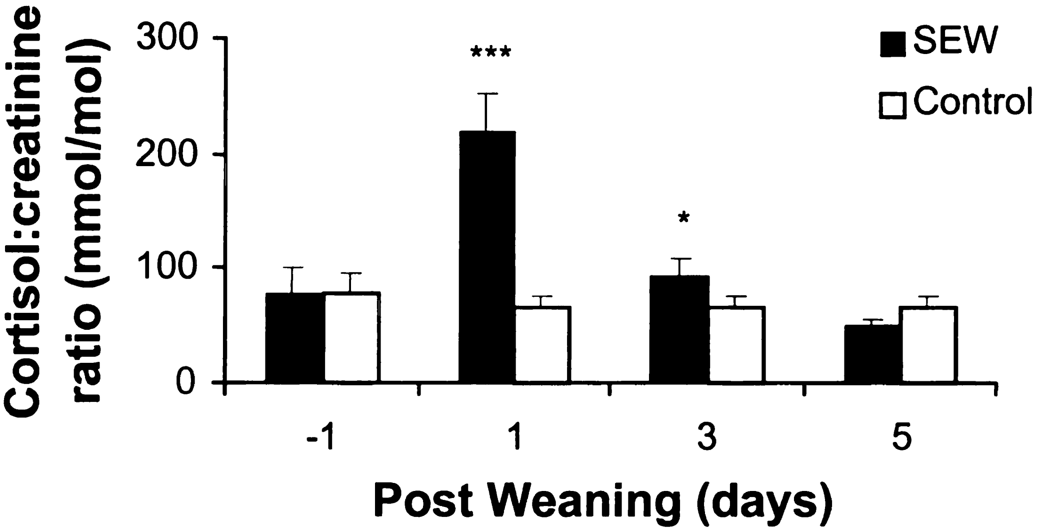


Figure 3: Mean urinary cortisol:creatinine ratio in SEW and control piglets during pre- and post-weaning period. * $P < 0.05$, *** $P < 0.001$.

than control piglets (Figure 2). The morning data contributed to these differences, while the lying time in the afternoon was not different in any given day between the treatments.

The UCCR was elevated in SEW piglets on days 1 ($P \leq 0.0001$) and 3 ($P = 0.0339$) post-weaning compared to control piglets (Fig. 3).

4.4 DISCUSSION

Even when provided with digestible and palatable diets (Dritz et al., 1996), the SEW piglets had a reduced growth rate during the first week after weaning compared with control piglets (Fig. 1). Several elements may contribute to this phenomenon. First, the adaptation to solid feed takes time. Gonyou et al. (1998) reported that the eating behavior in early-weaned piglets (12 days of age) on SEW diets did not develop adequately in the first 36 hours post-weaning. In contrast, piglets weaned at 21 days of age established eating behavior within 24 hours post-weaning. Second, the retardation in growth can be a result of the SEW stress response. The intracerebroventricular administration of corticotrophin releasing hormone (CRH), which triggers the activation of HPA axis during a stressful event, reduces food consumption in rats and mice (Britton et al., 1982; Berridge and Dunn, 1986). The secretion of growth hormone and insulin-like growth factor-I is also suppressed during stress (Diegez et al., 1988). Therefore, increased HPS axis activity in the SEW piglets of this study (Fig. 3) could have contributed to the reduced body weight gains in the first week post-weaning.

Vocalization and climbing on other pigs have been classified as fear-related behavior in response to stress (Brundige, 1998). The SEW piglets vocalized at a high rate up to one day post-weaning (Table 2). Weary et al. (1997) demonstrated that the vocalization of weaned piglets indicates their needs for resources provided by the sow, such as heat and milk. Climbing behavior (Table 2), which was also elevated in SEW piglets on days 0 and 1 post-weaning, could be an expression of their fear towards an unpredictable and uncontrollable environment as suggested by Brundige (1998). I also observed that the newly weaned animals huddled together, which could be interpreted as a cold or fear response. Some weaned piglets kept trying to jump out of the pen immediately post-weaning. Obviously, the jumping was an escape attempt. My results showed that SEW piglets spent more time moving than control pigs on both days 0 and 1 post-weaning (Table 2). Fraser (1978), Metz and Gonyou (1990) concluded that piglets are restless after weaning when the weaning takes place at two or three weeks of age. Restlessness in the initial post-weaning period occurred in the SEW piglets as well, and combined with the fear and cold responses, may have contributed to activation of the HPA axis (Fig. 3).

Belly-nosing may be a redirected behavior, which results from the deprivation of the appropriate environmental cues for sucking and massaging behavior. Blackshaw (1981) and Metz and Gonyou (1990) suggested that a time lag exist before redirected nosing started in weaned piglets. In contrast, belly-nosing was recorded on day 1 post-weaning in the current group of SEW piglets and the frequency of belly-nosing was significantly higher from day 1 post-weaning in SEW than in control piglets (Table 3). The differences across studies might be due to the fact that piglets were weaned at two to three weeks of age in the previous studies (Blackshaw, 1981; Metz and Gonyou, 1990) versus day 10 of age in the current study. As expected, SEW piglets spent more time showing exploratory behavior (rooting) than undisturbed piglets on days 1, 2 and 3 post-weaning (Table 3). Contrary to my expectation, however, the time spent and frequency of fighting was not different between the two treatment groups (Table 3). One explanation could be that all litters were initially crossfostered. Fighting occurs more often in crossfostered litters than in natural litters (Robert, 1997). Therefore, a disturbed basal aggression level in the control piglets may have made it difficult to detect differences in aggression between the SEW and control piglets in this study.

Lying time on the day 1 post-weaning was much shorter in SEW than in control animals. However, lying time rose sharply on the following days so that the percentage of time spend lying was much longer on day 3 post-weaning in SEW group (Fig. 2). It is unknown what caused this dramatic increase in lying time, but a depletion of energy and/or acquaintance to the new environment may be the factor(s). The piglets spent more time lying in the afternoons than in the mornings. Surprisingly, the lying time remained constant in the afternoons, regardless the treatment groups. It seems that the SEW stress did not affect activity levels in the afternoon.

As indicated previously, the cortisol concentration as a parameter of physiological stress in pigs was examined (Klemcke and Pond, 1991; Becker et al., 1985; Hicks et al., 1998). The UCCR in SEW animals was 328% and 143% of the control animals on days 1 and 3 post weaning, respectively

(Fig. 3). This demonstrates a sustained activation of the HPA axis. When male piglets were castrated at two to four weeks of age, a 291% increase in unbounded plasma cortisol was observed after an hour of castration. Cortisol concentration returns to basal levels within 24 hours (Schönreiter et al., 1999). Therefore, the magnitudes of cortisol elevation were comparable between castration and SEW. Since 50% of administrated ^{14}C cortisol excreted through urine within 6.0 ± 0.6 hours in piglets (Kraan et al., 1986), SEW stress could be more chronic than castration stress as indicated by the duration of cortisol elevation. Cortisol could affect carbohydrate metabolism, providing necessary energy and participating in thermal regulation, which may be important to the newly weaned animals. However, high levels of cortisol also have unwanted effects such as the suppression of immune response (Owen et al., 1983; Munck et al., 1984; Burton and Kehrli, 1995, 1996). Hence, the sustained high levels of cortisol in SEW piglets may increase their susceptibility to infection. If true, it would be very important to reduce pathogen loads in piglets rearing facilities to a minimum as well as supplement piglets with appropriate dietary vitamins and minerals that are known to enhance immunocompetence. SEW pigs are usually regarded as healthier than conventionally raised animals. However, healthier does not necessarily mean a better immune function. The improved health status of SEW animals may partially due to the cleanness of improved housing. More research is needed to reach a conclusion on this aspect.

4.5 CONCLUSION

The short-term responses to SEW include reduced growth rate, altered behavior patterns, and elevated cortisol levels. These evidences confirmed my hypothesis that the neonatal piglets could respond to SEW stress. All of the responses are indicators of an inadequate environment and difficulties in coping, therefore the welfare of SEW piglets is probably poor for the first few days post-weaning. More research is needed to determined whether SEW risks piglet's welfare in the long term.

Chapter 5 LONG-TERM EFFECTS OF SEGREGATED EARLY WEANING STRESS ON PIGS

5.1 INTRODUCTION

Premature maternal deprivation imposes profound changes in behavioral patterns in several animal species. Examples have been demonstrated in horses, pigs, mink, mice, monkeys and polar bear (Heleski et al. 1999; Worobec et al., 1999; Mason, 1994; Würbel and Stauffacher, 1997; Hinde et al., 1966; Poulsen et al., 1996). At six weeks of age, piglets that were weaned at 6 and 7 days of age displayed more belly-nosing, and gained less weight than piglets weaned at 14 or 28 days (Orgeur et al., 1998; Worobec et al., 1999).

Maternal care during early post-natal age also affects long term physiological responses to stress in the offspring. Rat puppies that received higher levels of maternal care had a reduced corticosterone response to stress in adulthood (Liu et al., 1997). In contrast, rat puppies subjected to 3-hour/day maternal separation during early age (2 to 14 days post-natal) had an increased corticosterone response to restraint stress compared with undisturbed offspring (Plotsky and Meaney, 1993).

These evidence lead to the hypothesis that premature maternal deprivation has long-term effects on the welfare of animals. As a result, the welfare of animals subjected to early maternal deprivation may at risk in the long-term.

In the previous experiment (chapter 4), I showed that neonatal piglets can respond behaviorally and physiologically to SEW stress in the short term. For the current experiment, conventionally weaned (CW) piglets were used as a control group to compare welfare indicators of SEW piglets later in life. I hypothesized that the stressful SEW experience during neonatal age is associated with high levels of aggression, behavioral abnormalities, compromised ability to establish a stable hierarchy, a loss in circadian pattern of cortisol, and elevated HPA axis responsiveness in pigs when they are older.

5.2 MATERIAL AND METHODS

5.2.1 Animals and Management

Six Landrace sows were bred using artificial insemination by the same Yorkshire boar. Sows farrowed within four days of each other. Each litter consisted of 8 to 12 piglets. The piglets were tail docked, ear notched, teeth clipped within 24 hours after birth, and the male piglets were also castrated.

Four piglets from each litter were randomly selected, weighed, weaned and transported 5 min to a separate site when they were 9 to 12 days old (SEW group). The remaining piglets were weaned at 20 to 23 days of age. Four piglets from each litter were randomly selected, weighed and transported to the same building where the SEW group was housed (CW group). The animals were then weighed weekly after weaning until week 8 (except for weeks 4 and 7).

The weaned piglets were placed in twelve 1.17 X 0.98 m pens without regrouping. The layout of the pen is pictured in Figure 4. Piglets were exposed to artificial lights from 830 to 1830 h. The room temperature was maintained at 27°C for newly weaned animals and gradually decreased to 21°C by 9 weeks of age. Heating pads were used to provide additional heating until the piglets reached the age of 5 weeks. Water was supplied by means of a nipple drinker *ad libitum.* During the first week after conventional weaning, one piglet in the CW group was removed due to severe diarrhea.

SEW phase I diet (Crude Protein 23.4%, Lysine 1.70%, ME 3429.4 kcal/kg) in pellet form was provided fresh twice a day *ad libitum* in a feeding trough. For the first four days after SEW and first two days after CW, the pellets were also given five times a day on a pan placed in

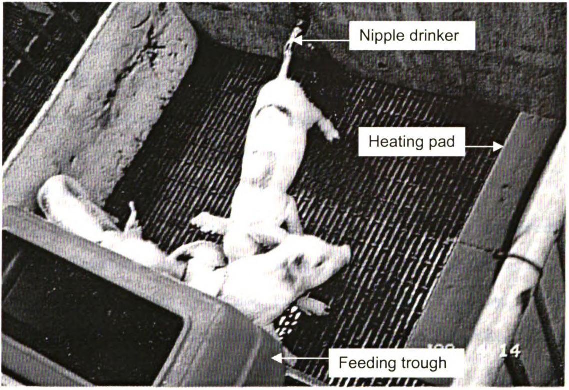


Fig. 4: Size and design of a nursery pen.

the middle of the floor to acquaint the newly weaned piglets to the new form of diet. SEW piglets were fed phase I diet for 2 weeks, phase II (Crude Protein 21.48%, Lysine 1.45%, ME 3409.09 kcal/kg) for 2 weeks, and phase III (Crude Protein 19.64%, Lysine 1.25%, ME 3396.4 kcal/kg) until weeks 9 of age. The CW piglets were fed phase I diet for four days, phase II for 2 weeks, and phase III until weeks 9 of age.

At 9 weeks of age, piglets were mixed, loaded and transported to a growing unit. Littermates housed in the same pen after weaning were separated into four groups according to their body weight (i.e. the heaviest to group 1, the lightest to group 4). After the regrouping, each pen consisted of 12 pigs (one pen had only 11 pigs), six of them were SEW pigs, the other six were CW pigs.

5.2.2 Behavior Observations

Immediately after weaning, the piglets were numbered with black hair dye (Clairol Inc., Stamford, Connecticut). Piglets were video recorded from 830 to 1800 h daily for a week post-weaning, then weekly up to 7 weeks of age. Nine o'clock was arbitrarily picked as the start point for the video decoding. The videotapes were then observed continuously for 5-min. This 5-min observation was repeated every 30 min of recording time. Frequency of eight behaviors, including belly-nosing, bar biting, fighting, manipulating pen-mates (ears, tails and other body parts), drinking, lying were collected for all piglets using behavior software (The Observer® 3.0, Noldus Information Technology, Wageningen, The Netherlands).

After the pigs were regrouped and transported to the growing unit, they were video taped for 12 hour/day for three consecutive days. All agonistic interactions were monitored. Data were obtained considering the initiator pig, the opponent pig, the duration of the fight, and whether a clear outcome resulted. An outcome was clear when a winner could be identified at the end of the fight.

5.2.3 Saliva Sampling

Pig saliva was collected using cotton dental roll (diameter: 1 cm, length: 4 cm, TIDI Products, Inc., Troy, Michigan). To retrieve the dental roll from the pig's mouth and allow the pig to move freely, approximately 1 m of dental floss (Johnson & Johnson, Skilman, New Jersey) was tied at the center of the cotton roll.

During the fifth week of age, piglets were trained to chew on cotton rolls in three different days. They were allowed to chew for about a minute. Morning (7:00) and afternoon (15:00) saliva samples were taken using this method on Fridays in weeks 6, 7 and 8. At 8 weeks of age, each pig was placed into a wood box by itself and isolated for 30 minutes. Saliva samples were obtained before and after the isolation. Pre- and post-transportation saliva samples were also collected for the transportation at 9 weeks of age.

Cotton rolls containing saliva were placed into 15 ml conical tubes, sealed and placed on ice immediately after collection. These samples were centrifuged within two hours. A 15-min centrifugation protocol (1,000 X g, 4°C) was used to retrieve the saliva from the cotton rolls into the conical tubes. Samples were frozen at -20°C, thawed and centrifuged again (15-min at 1,300 X g, 4°C) to separate saliva from particulate matter. The supernatant was stored for subsequent assay.

5.2.4 Salivary Cortisol Analysis

Salivary cortisol concentrations were determined using an ^{125}I radioimmunoassay (Coat-A-Count Cortisol Kit, Diagnostic Products Corp, Los Angeles, California, U.S.A.) following the modified procedure (Determinations in Saliva, September, 1992) provided by the company.

Gemus (1998) has validated the use of cotton rolls for the assay of salivary cortisol. Her results showed that the cotton rolls retained 1.4% of the fluid, and there is no non-specific binding of cortisol in the cotton rolls.

5.2.5 Statistical Analysis:

Behavior data was expressed in frequency per hour. Two types of data distribution were identified in this research:

1. Some data were normally distributed, such as body weight;
2. In the other cases data were not normally distributed, but distributional assumptions were satisfied by using

certain transformations. The data on salivary cortisol and duration of fights was log-transformed before data analysis. For the behavior data, a square-root transformation was applied before data analysis;

A mixed model with repeated measures from SAS® software (SAS institute Inc, Cary, North Carolina, U.S.A.) was used (see below). Lsmeans ± standard error of the means (SEM) were reported or back transformed to the original scale wherever necessary to facilitate interpretation and discussion of the results. Differences between SEW and CW treatment means at different time periods were tested for significance (when $P < 0.05$). The experiment unit used in this analysis was the pen.

The data on outcome of agonistic interactions consisted of frequencies in discrete categories. A chi-square test was used to examine the significance of differences among three types of possible encounters (two CW pigs fight, two SEW pigs fight, and a SEW pig fights with a CW pig).

Statistical model:

$$Y_{ijlm} = \mu + \alpha_i + \beta_j + \delta_l + \alpha\beta_{ij} + e_{ijlm}$$

Where;

Y_{ijlm} = dependent variable (weight, log-transformed salivary cortisol and duration of fight, and square root transformed behavior data) observed for the m^{th} pig, in the i^{th} treatment, at the j^{th} age, and from the l^{th} litter;

μ = overall mean of dependent variable;

α_i = the fixed effect of treatment (i=SEW, CW) or possible encounter (i=SS, SC, CC) depending on Y_{ijlm};

β_j = the fixed age or day effect (j=1,2,3; or 1,2 depending on Y_{ijlm});

δ_l = the random and blocking effect of the l^{th} litter (l=1,…,6) or the random pen effect (l=1,2,3,4) depending on Y_{ijlm}, $\delta_l \sim \text{NIID}(0, \sigma^2_\delta)$ for all l;

$\alpha\beta_{ij}$ = the interaction term;

e_{ijlm} = the random error term of the observation, $e_{ijlm} \sim \text{NIID}(0, \sigma^2_e)$ for all i, j, l, m.

5.3 RESULTS

Unfortunately, all the A.M. and P.M. saliva samples from 47 piglets at 6 and 7 weeks of age, and half of the samples from the social isolation experiment were mistakenly discarded by laboratory personnel. Due to the loss of 236 samples, neither the circadian of cortisol, nor

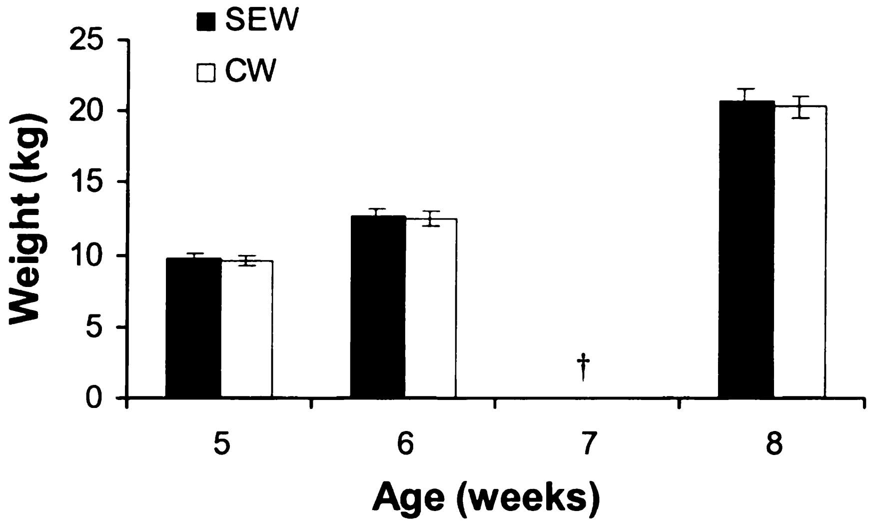


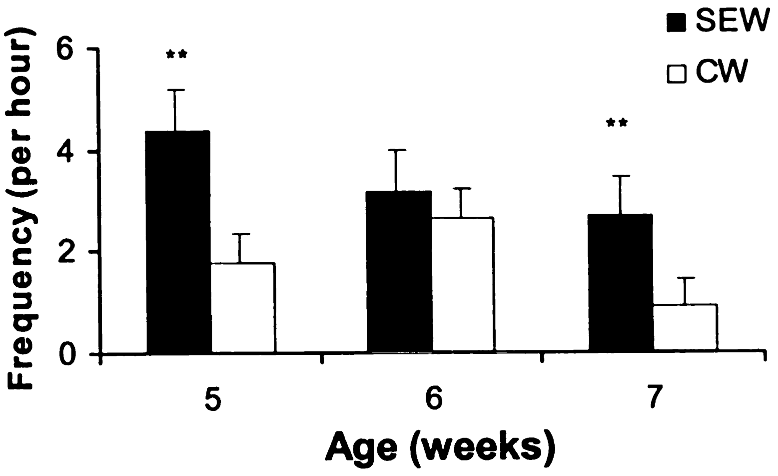
Fig. 5: Average body weight of SEW and CW piglets at weeks 5, 6 and 8 of age. † Weight on weeks 7 was not measured.

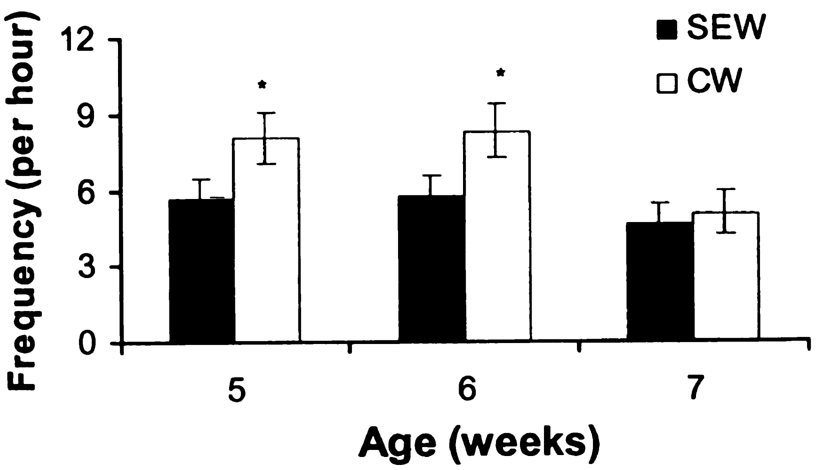
the HPA axis response to the social isolation stressor could be investigated. For this reason, a considerable amount of physiological data from the SEW and CW animal models was missing.

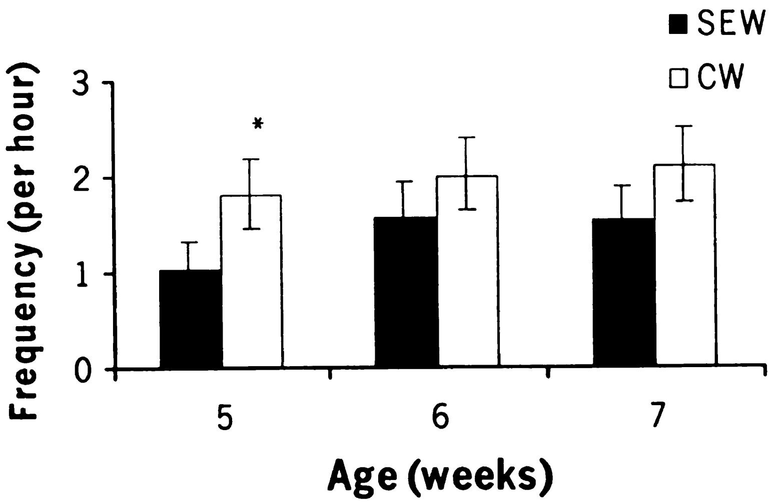
There were no differences in the weight gains between treatment groups across different ages post weaning (P = 0.7085, Fig. 5).

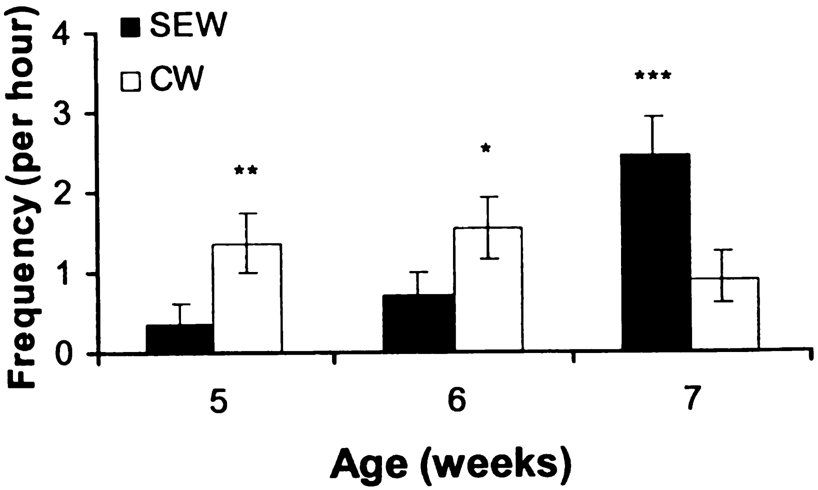
The behavior characteristics of the SEW and CW piglets are summarized in Figure 6, which consists of four individual graphs. The frequency of belly-nosing behavior is presented in Figure 6a. A higher incidence of belly-nosing ($P \leq 0.0142$) was observed in SEW piglets than in CW piglets at both weeks 5 and 7 of age. There was no age (P =

Fig. 6: Average frequency of (a) belly-nosing, (b) manipulating, (c) fighting, and (d) bar biting from 0900 to 1800 h in SEW and CW piglets at weeks 5, 6 and 7 of age. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.


a) Belly-nosing


b) Manipulating pen-mates


c) Fighting


d) Bar-biting

0.1190) or age by treatment interaction (*P* = 0.1125) effects on the belly-nosing behavior.

Conventional weaned piglets had a higher frequency of manipulating other piglets than SEW piglets ($P \leq 0.017$) in weeks 5 and 6 (Fig. 6b). The frequency of this behavior was affected by age in CW piglets (*P* = 0.0019), but not in SEW piglets (*P* = 0.4219).

Figure 6c shows that CW piglets fought more often (*P* = 0.0366) than SEW piglets in week 5. There was no age (*P* = 0.2178) or treatment by age (*P* = 0.7255) effect on the fighting behavior.

The result of bar biting behavior is summarized in Figure 6d. Frequency of this behavior increased as SEW piglets were getting older (*P* = 0.0001), while the performance of this behavior did not change in CW piglets over the three weeks observation (*P* = 0.2327). CW piglets showed more bar biting than SEW piglets in both weeks 5 and 6 ($P \leq 0.0166$). In week 7, however, a higher frequency of bar biting was recorded in the SEW piglets than in the CW piglets (*P* = 0.0002).

A twenty-minute transportation experiment at week 9 of age increased salivary cortisol levels by 834% and 646% in SEW and CW piglets, respectively. The percentage of increase was not significantly different (*P* = 0.45) between

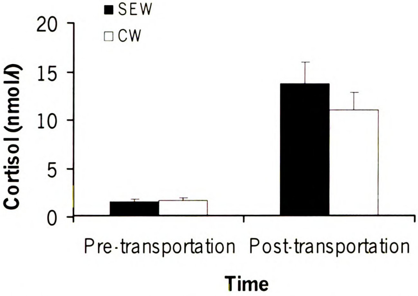
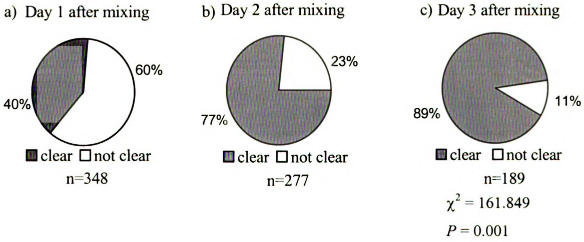


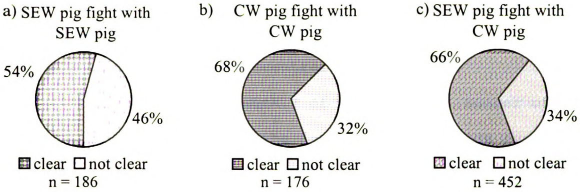
Fig. 7: Mean salivary cortisol concentration before and after a 20-minute transportation in SEW and CW pigs at 9 weeks of age.


a) Day 1 after mixing

b) Day 2 after mixing

c) Day 3 after mixing

Fig. 8: The distribution of clear and unclear outcome of fights in three days after regrouping when SEW pigs were mixed with CW pigs at 9 weeks of age.



a) SEW pig fight with SEW pig

b) CW pig fight with CW pig

c) SEW pig fight with CW pig

$\chi^2 = 10.034$

$P = 0.007$

Fig. 9: The distribution of clear and unclear outcome of fights in three types of encounters when SEW pigs were mixed with CW pigs at 9 weeks of age.

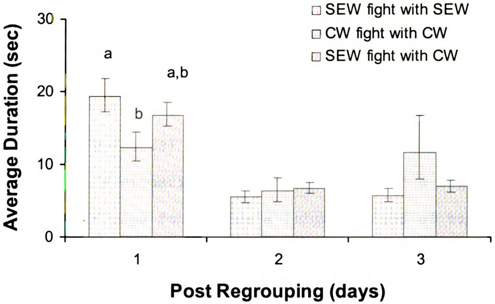


Fig. 10: Average duration of fights when outcomes were not clear in days 1, 2 and 3 after the mixing of SEW and CW pigs at 9 weeks of age. $^{a, b}$ Means without a common superscript differ ($P < 0.05$).

treatments. There were no significant differences ($P > 0.18$) between treatments in either pre or post-transportation cortisol concentrations (Fig. 7).

After regrouping at week 9 of age, a total number of 821 fights was observed in the four pens of 12 pigs over a three-day period. Winners were identified in 40.23% of the agonistic interactions in the first day. The percentage of clear outcomes increased ($P < 0.0001$) in the second and third day (Fig. 8). Of all the interactions monitored, fights within SEW pigs (Fig. 9a) were more likely to have an unclear outcome ($P < 0.007$) than those within CW pigs (Fig. 9b), and between CW and SEW pigs (Fig. 9c). The number of fights was not different between these three types of encounters ($\chi^2 = 0.0007$, $P > 0.99$). When the fight had a clear outcome, the duration of the fights was not different ($P = 0.58$) among the three types of encounters. However, in a situation where the outcome of a fight was unclear, SEW pigs fought for a longer period of time ($P = 0.0075$) than CW pigs in the first day after mixing (Fig. 10). Regardless of the outcomes and types of fights, the average duration of a fight was significantly longer in the first day than that of the second and third day after regrouping (16.61 vs. 7.02 and 7.98 seconds respectively, $P = 0.0001$).

5.4 DISCUSSION

In Chapter 4 Experiment 1, I demonstrated that SEW piglets had a reduced growth rate in the first week after weaning when compared with suckling piglets. However, no weight differences were observed between SEW and CW piglets following weaning at weeks 5, 6 and 8 of age (Fig. 5). It is likely due to that CW piglets also experienced a period of growth decline immediately after weaning while the SEW piglets had an opportunity to catch up in weight development (Table 6 in Appendix). Worobec et al. (1999) showed that at 6 weeks of age, piglets weaned at 7 days of age were lighter than those weaned at 14 and 28 days. One possible explanation for the differences in results could be that the nutrient requirements of 7-day piglets are different from those of 10-day old piglets. Alternatively, the younger piglets (7 days old) may have a stronger bond with the sow than my 10 days old piglets, making weaning more difficult to cope with. Piglets under semi-natural environment start to rest outside their own nest site only after 8 days of age (Newberry and Wood-Gush, 1988). In addition, by 8 days of age, piglets play an active role in the reinforcement of sow-piglet relationship (Blackshaw and Hagelsø, 1990).

Previous studies in pigs have shown that the earlier the weaning took place, the higher the incidence of belly-nosing (Gonyou et al., 1998; Worobec et al., 1999). Corroborating these observations, piglets weaned at 10 days of age in the current study displayed a significantly higher frequency of belly-nosing in weeks 5 and 7 than CW piglets (Fig. 6a). Gonyou et al. (1998) also reported that the highest level of belly-nosing occurred during 2 to 3 weeks post-weaning. My results showed that at 6 weeks of age when CW piglets have been weaned for 3 weeks (SEW piglets have been weaned for 4.5 weeks), the frequency of belly-nosing behavior was not different between SEW and CW piglets. Early-weaned animals displayed continued elevated levels of belly-nosing and chewing on other pigs throughout the growing/finishing phase (Gonyou et al., 1998). It appears as though a higher baseline of belly-nosing behavior may be developed as a result of early maternal deprivation.

Contrary to my initial hypothesis, the frequency of manipulating pen-mates was higher in CW piglets than SEW piglets at weeks 5 and 6 of age (Fig. 6b). Previously it was reported that if weaned piglets were stressed by crowding, they were more likely to engage in the manipulation of other animals (Dybkjær, 1992). In my study, behavior was compared at the same age but at different post weaning times (CW piglets were weaned for 2 or 3 weeks and SEW piglets were weaned for 3.5 or 4.5 weeks). However, in the study by Dybkjær (1992), behavior of piglets was compared at the same ages and post weaning time. This may be responsible for the discrepancies in the results.

The incidence of fighting remained constant over three weeks in both groups, indicating that a stable social relationship was established before week 5. Surprisingly, the overall agonistic interactions were higher in the CW piglets. The CW piglets fought more often at 5 weeks of age than the SEW piglets (Fig. 6c). However, Worobec et al. (1999) reported no differences in the aggression level in weeks 4 and 6 between animals weaned at 7, 14 and 28 days. In their study, instantaneous behavioral samples were obtained every 5-min from 48 h videotapes. Aggression included pushing, head thrusting, biting and chasing. I monitored fighting according to the definition in Table 1 (Chapter 4). Since pushing, head thrusting, and biting do not necessarily result in fighting, and chasing does not necessarily result from fighting, Worobec et al.'s (1999) results and my results are not necessarily conflicting.

Besides belly-nosing, another long-term change in the behavior of SEW was the development of bar biting. While the occurrence of bar biting in CW piglets remained the same, it increased in SEW piglets as they grew (Fig. 6d). Dybkjær (1992) concluded that this type of redirected oral behavior pattern was one indicator of stress in weaned piglets. As well as belly-nosing, manipulating penmate and bar biting are both considered as abnormal behavior. Animals in both treatment groups displayed these three types of behavior abnormalities, indicating coping is difficult in the current intensive housing system regardless the weaning age. Hence I conclude that the welfare of pigs in both groups is poorer than free-range animals. The SEW piglets showed a consistent high frequency of belly-nosing and an increase in frequency of bar biting, suggesting that the welfare of SEW animals is poorer than CW animals. But CW animals directed more oral activities toward their penmates than SEW animals, suggesting that the welfare is poorer in CW piglets than in SEW piglets.

As expected, mixing, followed by a 20-minute transportation, induced a significant increase in salivary cortisol concentration in 9 week old pigs (Fig. 7). This observation was consistent with that of Hicks et al. (1998). However, the pre- and post-transportation salivary cortisol concentrations, and the percentage of increases in salivary cortisol were not different between SEW and CW pigs (Fig. 7). Apparently, the SEW stress and the weaning age of piglets did not alter the response of the HPA axis to transportation stress later in life. This result conflicted with the literature on rodents (Plotsky and Meaney, 1993; Liu et al., 1997). Physiological and behavioral differences between rodents and pigs may account for the conflicting results. Firstly, the maternal behavior of rats or mice is different from sows confined in farrowing crates. Maternal behavior in rodents includes licking and grooming of the young (Liu et al., 1997). Sows display sniffing, grunting, and deliberate nose contact with the piglets (Blackshaw and Hagelsø, 1990). Secondly, the rats have a stress hyporesponsive period approximately from day 4 to 14 after birth (Schapiro et al., 1962) not seen in pigs (Klemcke and Pond, 1991). Thirdly, transportation can be a very stressful experience for pigs, which may maximize the response of HPA axis. It may mask the differences in the HPA axis responsiveness between SEW and CW pigs.

Every agonistic interaction that resulted in an unclear outcome denied a piglet from either being advantaged (if it was declared the winner) or disadvantaged (if it was declared the loser). According to the results, pigs from the SEW group were subjected to the most agonistic interactions that resulted in unclear outcomes (Fig. 9). This information suggests that SEW pigs were more unassertive when in combat. When the outcome was not clear, a longer average duration of fights was also found on the first day after mixing in SEW pigs than that of CW pigs (Fig. 10). It requires more energy and may cause more body damage for an animal to fight longer. This result suggests that SEW pigs were more aggressive. All the information imply that the social skills of SEW pigs may be impaired. It may be due to the fact that they were given a shorter period of time to interact with dams than CW pigs. Interestingly, when a SEW pig fought with a CW pig, the distribution of the outcomes was similar to that of the fights among CW pigs (Fig. 9). The average duration of agonistic interactions did not differ from the other two types of encounters (Fig. 10). Two pieces of information suggests that a dominant hierarchy became stable over the three-day period. One was that the percentage of unclear outcome decreased from 59.77% in the first day to 10.58% in the third day (Fig. 8). The other was that the average duration of a fight decreased from 17 seconds in the first day to 8 seconds in the third day.

5.5 CONCLUSION

There were no advantages or disadvantages of SEW over CW in the production performance indicator as measured by their body weights. The basal cortisol concentration and the HPA axis response to stress at nine weeks of age was unchanged comparing SEW practice with CW practice. On the other hand, the long-term effects of SEW seemed to be reflected in the differences in behavioral patterns between SEW and CW piglets. The SEW piglets showed higher frequency of belly-nosing and lower frequency of manipulating pen-mates than the CW piglets. The incidence of bar biting increased with time in the SEW piglets but remained constant in the CW piglets. In a stable group, the overall fighting levels were low in the SEW piglets. But when the environment was disturbed, the SEW pigs were less assertive and had more difficulties in establishing a stable hierarchy. These results add to the complexity of comparing the welfare status of SEW and CW pigs. As my results indicated, SEW pigs in some aspects may have worse welfare, while in other cases they may have better welfare than CW pigs. Overall, it seems that welfare is poorer in SEW pigs than in CW pigs. I feel the evidence suggest this conclusion outweigh the rest.

Chapter 6 GENERAL DISCUSSION

6.1 BEHAVIOR

Maternal behavior is vital to the survival of young animals, especially in mammals. Domesticated animals are usually cared for by humans. As a result, it is possible for the offspring to be nutritionally independent from their dams earlier than wild animals. Young animals, however, also learn relevant behaviors from their parent(s). For example, foals hand-raised by humans do not know how to interact with other horses (Shelle, personal communication). Therefore, a sudden deprivation of maternal care during early development may influence the offspring's behavioral pattern and possibly their coping strategies. My research work found that there were short and long term changes in the behavioral patterns of SEW animals, most notably in aggressive behavior and the development of belly-nosing.

6.1.1 Belly-nosing

Belly-nosing closely resembles a principal component of normal nursing behavior and its occurrence is clearly associated with weaning age (Fraser, 1978; Gonyou et al., 1998, Worobec et al., 1999). Fraser (1980) has described the nursing behavior in detail. Nursing behavior starts with the piglets vigorously nosing the udder of the sow. This causes the sow to grunt rhythmically, and the grunts notify any absent piglets that nursing is in progress. As the grunting rate increases and oxytocin is released, most piglets start sucking on the teat. The milk ejection begins 20 s after the release of oxytocin and lasts 15 to 25 s. After the milk ejection ceases, some piglets continue to suck, some may change quickly to a different teat, others resume nosing the udder. It is suggested that vigorous nosing of the udder before the milk ejection may increase the flow of oxytocin-rich blood through the mammary gland, thereby increasing the milk yield (Fraser, 1980). In addition, the piglets start nosing and pushing any vertical surface they encounter within the first 20 minutes after birth (Hartsock and Graves, 1976). These facts lead to the hypothesis that nosing the udder constitutes a significant part of suckling behavior and may be critical to piglets' growth.

The nosing directed to the sow's udder is probably some element of appetitive feeding in piglets. A redirected behavior of nosing the udder, known as belly-nosing, is developed in some piglets after the appropriate environmental cue (sow) is removed. Therefore, it may be important for piglets to be able to perform this behavior. Furthermore, the "nursing posture[4]" can sometimes be elicited in very young pigs by rubbing the belly (Fraser, 1976). This behavior response in pigs may facilitate the development of belly-nosing.

Gardner et al., (1999) showed that the establishment of feeding and the presence of milk in the diet had no effect on the performance of belly-nosing in piglets weaned between 14-18 days of age. It seems that belly-nosing is connected to the sucking motivation.

Over the first 6 weeks postpartum, piglets increase their activity and feeding behavior. Piglets' interest in solid food becomes apparent at about three weeks of age (Fraser, 1978). Piglets develop and express more complex behavioral patterns as they grow (Newberry and Wood-Gush, 1988). This might account for the negative relationship between the exhibition of belly-nosing and the weaning age.

6.1.2 Aggression

Pigs have a complex social structure. They live in groups and have hierarchies, which are established through agonistic interactions. Pigs displayed aggressive behaviour

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[4] Sow lies on one side, exposes both rows of teats and often thrust its front legs back (Fraser, 1984).

throughout their growth period. Aggressive interactions start with the formation of teat order, which peaks at about the second hour after birth (Hartsock and Graves, 1976). Once a teat order is established, the piglets settle into an orderly sequence during suckling with little fighting at the udder.

Previous studies have showed that, in commercial settings, aggression level is high at weaning. Producers commonly sort pigs at several production stages to create homogenous groups. In general, the first time that pigs are sorted is at weaning. Fraser (1978) found that artificial weaning increases the levels of aggression in littermate piglets. In addition, grouping of strange pigs causes fierce fighting (Meese and Ewbank, 1973). These studies strongly suggest that weaning combined with regrouping may lead to intense aggression. Social stability within a newly formed group may be observed within 24 h after mixing (McGlone, 1986). Once the hierarchy is formed, fighting is often replaced by non-contact threats (Ewbank and Meese, 1971).

The behavior sequence of fight is shown in Fig. 11 (Adapted from McGlone, 1985). Normally, fighting begins with mutual nosing and sniffing, but quickly escalates into

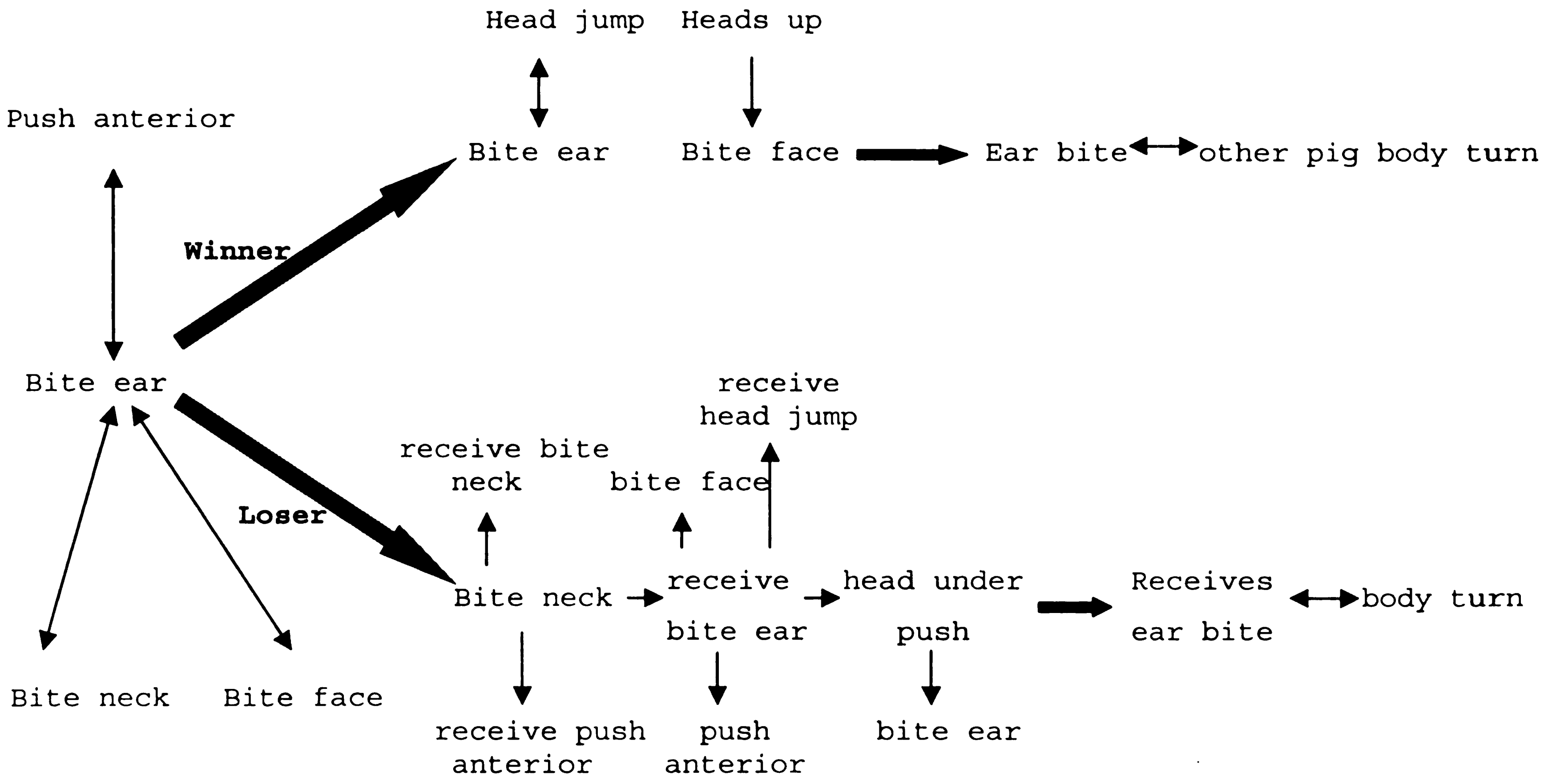

Fig. 11. Behavioral sequences of fighting from the first bite to the first sign of submission for young pigs. Body-turning was always preceded by ear bites (Adapted from McGlone, 1985).

a contest in which two pigs face each other with their shoulders pressed together. As the fight progresses, more damaging behaviors occur. Submissive behavior begins with a 180-degree body turning after receiving ear biting. Then the winner chases and tries to bite the rump and ear of the loser. The submissive behavior can last several seconds to a few minutes (McGlone, 1985).

The ability to establish hierarchy is important for the stability of a group. Maintaining social stability is important for pig welfare. McGlone (1985) suggested that the physical damage from aggressive behavior might be reduced if all fights have a clear outcome and the test of strength does not have to be repeated. When an unclear outcome (McGlone, 1985 refers that as "undecided fights") occurs, a hierarchy between two animals is undecided. My result showed that the percentage of unclear outcomes decreased from 59.77% to 10.58% during the first three days after mixing, suggesting that a stable dominance hierarchy was under development. Since longer duration and a higher percentage of unclear outcome occurred when two SEW piglets' fought, mixing of SEW piglets should be avoided.

6.2 RESPONSIVENESS OF HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The HPA axis is activated when homeostasis is disturbed or threatened. It is hypothesized that the stress-induced HPA axis activation protects the body by preventing physiological reactions from overshooting, thereby helping to restore the homeostasis (Munck et al., 1984). Thus it is critical for an animal to have a HPA axis that can respond properly. The responsiveness of HPA axis can be altered by maternal care (e.g. Liu et al., 1997). An altered HPA axis may imply a time difference in re-achieving homeostasis and vulnerability to stress-related diseases (Seckl and Meaney, 1993).

The development of HPA axis has a distinct pattern in rats. Corticosterone levels in neonatal rats are high immediately after birth, then decreases dramatically in two days and remains low until around 14 days. This period from 4 to 14 days postpartum is known as stress hyporesponsive period (SHRP)(Levine, 1994). During SHRP, the HPA axis does not respond to stressors or the administration of ACTH. However, SHRP is critical for the development of the HPA axis. Offspring who received more maternal care during this period demonstrated a reduced response of the HPA axis to stressors (Liu et al., 1997). A similar period does not exist in pigs (Klemcke and Pond, 1991). This implies that the development stage of the HPA axis differs during postnatal age between piglets and rats. Hence, maternal care or early experience of piglets may not be able to alter the HPA axis responsiveness in their adult life. My results support this hypothesis by showing that the response of HPA axis to transportation is similar between SEW and CW pigs.

6.3 BEHAVIOR AND HORMONES

A causal relationship between corticotropin releasing hormone (CRH) and certain behaviors has been suggested. CRH is a 41 amino acid peptide that is mainly released from the hypothalamus. CRH triggers the activation of the HPA axis and also acts as a neurotransmitter within the central nervous system (CNS) (Owens and Nemeroff, 1991). Previous studies on CRH strongly suggest that CRH integrates not only the endocrine but also the autonomic, immunological and behavioral responses of mammalians to stress (Owens and Nemeroff, 1991). For example, CRH increases locomotion in familiar environments and suppresses locomotion and vocalization in novel, stressful environments (Owens and Nemeroff, 1991; McInturf and Hennessy, 1996). CRH reduces food consumption in both familiar and novel environments (Britton et al., 1982; Berridge and Dunn, 1986). Sexual behavior is also potently inhibited by central administration of CRH in both males and females (Owens and Nemeroff, 1991). In addition, research data supported that CRH may mediate many of the fear-related behaviors of stress (Britton et al., 1982; Swerdlow et al., 1989).

Results on the relationship between glucocorticoids and stereotypies are conflicting. Glucocorticoids are the end products of the HPA axis. Stereotypies are abnormal behaviors characterized by a repetitive, invariant sequence of behavior without obvious function. Stereotypies are often observed in farm and zoo animals housed in a confined environment. Zanella and Mason (1997) reported a lower concentration of plasma cortisol in mink with high stereotypy than mink with low stereotypy. Von Borell and Hurnik (1991) found that sows showing stereotypies had a higher cortisol response to ACTH challenge than sows without stereotypies. However, Terlouw et al. (1991) reported no correlation between the exhibition of stereotypies and plasma cortisol level in sows. Due to the loss of more than 200 saliva samples, I have limited information on the activity of HPA axis in SEW versus CW pigs to assess its relationship with abnormal behavior, including belly-nosing, manipulating, and bar biting.

6.4 ANIMAL WELFARE AND SEGREGATED EARLY WEANING

Human-animal relationship changes as society evolves. Currently, social attitudes have been challenged by the claims that "the interests of non-human animals are being neglected, and that we have a moral obligation to consider them more than we do at present" (Broom and Johnson, 1993). Since there is a high public demand to address these concerns, philosophers and biologists promote the study of animal welfare. Important welfare indicators include growth rate, behavior patterns, endocrine profile, susceptibility to disease, body damage, mortality, and reproduction performance (Blackshaw, 1986; Broom and Johnson, 1993; Wechsler, 1995). As I mentioned before, welfare is defined as the state of an individual in regard to its attempts to cope with its environment (Broom and Johnson, 1993). Since welfare of an individual depends on the efforts to cope and the outcome of the coping attempts, this definition implies that welfare could vary from very poor to very good. For example, death, failure to grow, or failure to reproduce are evidences of failure to cope, where welfare is considered very poor. Sometimes, animal may succeed in coping but with great difficulty. In this case, the animal welfare is still poor, but better than if it fails to cope.

This research focused on the welfare of SEW piglets. The results of the welfare study were as follows:

1. SEW caused vocalization, climbing, belly-nosing and an increase in activity levels within the first three days post-weaning, and a reduced growth rate in the first week post-weaning. Taken together, these data suggested that the welfare of SEW piglets was poor during the initial post-weaning period.
2. The urinary cortisol concentration continued to be elevated for three days post weaning. This result also indicates that welfare problems may exist for at least three days post weaning in SEW piglets.
3. SEW animals displayed elevated levels of belly-nosing throughout the two-month experiment period. Belly-nosing is an abnormal behavior and can lead to skin lesions in the piglets who receive it (Worobec, 1999). Thus, welfare is reduced in animals that are exhibiting or receiving belly-nosing. As discussed before, nosing warm and soft surfaces is probably important to piglets. To minimize the occurrence of belly-nosing, one solution is to wean the piglets older than 3 weeks of age because of a negative correlation between weaning age and belly-nosing. Though no research has been done yet, another solution may be to provide appropriate environmental

enrichment devices, which have warm and soft surfaces, so that the occurrence of nosing pen-mates' bellies could be reduced.

4. The impaired ability to establish a stable hierarchy (as indicated by the duration of fight and the proportion of unclear outcome after mixing) also suggests a poor welfare of SEW pigs, because impaired social skills implies that coping is more difficult for these pigs in new social environments. To solve this problem, more information is needed on the fundamentals of aggressive behavior, the maternal behavior of sow and its influence on piglets.

In summary, the results suggest that the welfare of the SEW piglets may be poor. Therefore the SEW practice needs to be re-evaluated and modified to accommodate the nature and biological needs of the animals.

APPENDIX

APPENDIX

Table 4: Summary of basic statistics on behavioral and physiological data in Experiment 1:

Dependent variable	*Unit*	*Geometric mean*	*Standard deviation*	*Minimum*	*Maximum*
Lying	%	66.30	31.00	0	100
Standing	%	7.25	9.71	0	45.88
Sitting	%	1.58	4.35	0	57.38
Moving	%	7.70	13.10	0	80.50
Rooting	%	1.36	4.38	0	39.30
Fighting	min^{-1}	0.08	0.19	0	1.25
Belly-nosing	min^{-1}	0.06	0.27	0	2.75
Manipulating ear	min^{-1}	0.05	0.13	0	0.75
Manipulating tail	min^{-1}	0.02	0.07	0	0.5
Manipulating other part of the body	min^{-1}	0.13	0.27	0	1.75
Massaging the sow	%	4.00	8.20	0	42.90
Sucking at the sow	%	11.28	17.61	0	75.71
Eating	%	3.50	7.90	0	48.70
Drinking	%	0.6	2.00	0	14.30
Bar biting	min^{-1}	0.02	0.11	0	1.25
Grunting	min^{-1}	0.17	0.79	0	7.25
Squealing	min^{-1}	0.03	0.26	0	3.75
Climbing	min^{-1}	0.03	0.10	0	0.75
Jumping	min^{-1}	0.03	0.18	0	1.75
Cortisol	mol/l	0.25	0.30	0.03	1.69
Creatinine	mol/l	2.71	2.54	0.41	12.41
UCCR	mmol/mol	94.23	70.37	23.74	405.87

Table 5: Summary of basic statistics on behavioral and cortisol data in Experiment 2:

Dependent variable	*Unit*	*Geometric mean*	*Standard deviation*	*Minimum*	*Maximum*
Belly-nosing	h^{-1}	2.61	3.39	0	19.33
Manipulating ear	h^{-1}	1.85	1.96	0	12
Manipulating tail	h^{-1}	0.95	1.15	0	8
Manipulating other part of the body	h^{-1}	3.90	2.54	0	13.33
Bar biting	h^{-1}	1.51	1.73	0	8
Fighting	h^{-1}	1.97	1.57	0	6
Drinking	h^{-1}	3.45	2.23	0	12.67
Cortisol	mmol/l	7.59	7.78	0.38	28.94
Fight after mixing	s	19.63	47.14	1	1047

Table 6: Summary of body weight of SEW and CW piglets in Experiment 2:

Weight (kg)	Day 10	Day 18	Day 21	Day 40	Day 46	Day 61
SEW	3.23± 0.16	4.18± 0.21		9.88± 0.51	12.71± 0.65	20.83± 1.07
CW			4.91± 0.25	9.12± 0.47	11.95± 0.61	19.71± 1.01

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