To the Graduate Council:
I am submitting herewith a thesis written by Lisa Scroggs entitled "Influence of Split Marketing on the Physiology, Behavior, and Performance of Finishing Swine." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Dr. H. G. Kattesh, Major Professor

We have read this thesis and
recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of the Graduate School

INFLUENCE OF SPLIT MARKETING ON THE PHYSIOLOGY, BEHAVIOR, AND PERFORMANCE OF FINISHING SWINE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Lisa Valeria Scroggs

May 2000

AG-VET-MED.
Thesis
2000
.S27

ACKNOWLEDGMENTS

I would like to express my thanks and appreciation to the following people for their support: Dr. Hank G. Kattesh, my major professor, for his guidance, knowledge of stress physiology, traveling to Crossville weekly with me for an entire summer to collect data and his patience in editing this manuscript; Dr. Julie L. Morrow-Tesch, committee member, whose support, travel, provision of behavior equipment, invaluable expertise in the field of behavior and for fueling my love of animal behavior which will not be forgotten; Dr. Ken J. Stalder, committee member, for giving me the idea to study split marketing, ultrasounding the animals, analyzing the ultrasound data, his expertise in pork production and assistance with the statistics of this project; Dr. Alan G. Mathew, committee member, for his immense support, guidance, friendship, expertise in the field of pork production and allowing me the use of his computer whenever needed; Jeff W. Dailey, who was instrumental in allowing me to survive the analysis of my behavior data by his assistance; John F. Schneider, statistician, for his assistance with the statistical analysis of the data in this thesis; the Crossville Experiment staff, Dan Brendel, Marcus Davis, Walt Hitch, and Kasey Neal, for their excellent care of the animals, assistance with data collection and for being a joy to work with; Glen Conatser, for his assistance with ultrasounding the animals; Russell Gilliam, Melissa Beckmann,

Aggy Spicer, and Danny Sutton, fellow graduate students for their friendship and for taking some of their own precious time to help me collect data in Crossville; Mary Roberts, who not only helped me collect data and analyze the corticosteroid-binding globulin samples but for also extending her knowledge of lab techniques to me; Jeonghoon Heo, for his friendship, assistance with collecting data and for his indispensable help with my total cortisol analysis; Jannette Bartlett, for her help in teaching me the techniques to make whole blood smear slides and how to stain them; Bill DeLeonardis, for delivering countless reference materials to my office from the library; my parents, Charles Scroggs and Brenda Scroggs Adams, for always telling me to do my best and for instilling their principles in me so that this endeavor was even a possibility; Chris Scroggs, for collecting data from my pigs with me so he could gain an understanding of what his older sister did; Kim Scroggs, for believing in me; Jimmy Adams, for his support; Drew Ekman, my fiancee, for his interest in my project, constant love, support, patience and encouragement that made the completion of this degree possible; Samson and Katie, for not only being the best canine companions anyone could ask for as well as sacrificing time spent at the park and with me during the completion of this degree but for remaining ever so affectionate and obedient, and Andy and Sarah, who made many late nights at the computer more enjoyable by keeping my lap warm or by running over my many stacks of organized papers.

ABSTRACT

This study examined the effect of split marketing on selected behavioral, physiological and production parameters in commercial market hogs. One hundred twenty eight-week old high-lean, high-health barrows (20.3 kg BW) were weighed and randomly assigned by weight in a randomized complete block design to one of three treatments: SM (split marketed), 6 pigs/pen (.56 m^2/pig); C (control), 6 pigs/pen (.56 m^2/pig); or MC (modified control), 3 pigs/pen (1.11 m^2/pig). The heaviest (120.3 kg) one half of SM animals were removed one week prior to penmates (116.3 kg) marketing. C (119.2 kg) and MC (121.8 kg) penmates remained grouped together until marketing. Animals were videotaped for the first 72 hours of the study (INITIAL), 72 hours prior to (PRE) and following the removal (POST) of pigs in SM treatment to identify socially dominant, intermediate and submissive pigs. A blood sample was collected from each animal upon completion of INITIAL, PRE, and POST time periods to determine neutrophil:lymphocyte ratio, plasma haptoglobin, total cortisol and corticosteroid-binding globulin levels. Animals were weighed and feed disappearance was calculated every two weeks. Animals also underwent an ultrasound evaluation for tenth rib backfat and loin muscle area at marketing.

Regardless of treatment, animals were more ($P < .0001$) active (feeding, walking/standing and fighting) at INITIAL than at PRE or POST. Neutrophil:lymphocyte ratio, plasma haptoglobin and corticosteroid-binding globulin levels were greater ($P < .0001$) during INITIAL compared to PRE or POST but did not differ between treatments. No treatment or time differences were detected in total plasma cortisol. MC exhibited greater ($P < .0001$) feed efficiency and average daily feed intake compared to C or SM treatments up to split marketing. The remaining penmates in the SM group had higher ($P < .05$) average daily feed intake when compared to C or MC treatments during the last week of the trial. There were no treatment differences in carcass characteristics of backfat or loin eye muscle area although C tended to be leaner ($P = .08$) than MC while SM was not different from C or MC. These results indicate that hogs remaining after SM have greater average daily feed intake when compared to the performance of hogs managed in the same way with respect to group size prior to and after marketing of SM animals. Significant differences in performance between the treatment groups cannot be attributed to any physiological or behavioral measures as reported here.

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CHAPTER I

INTRODUCTION

Many features used in the production of swine can be stressful to the animals. One example is the mixing of animals which elicits increased aggression among penmates until a stable social hierarchy is formed. This increased fighting can lead to injuries and poorer performance particularly in older animals. All-in-all-out production reduces the amount of mixing that occurs between production phases. This production practice allows groups of animals to be moved through each production phase (nursery, growing, finishing) together as they grow. This reduces the mixing of animals and facilitates the cleaning of facilities between groups of animals, hence reducing the possibility of disease transmission.

Pigs of similar age do not grow uniformly and therefore do not reach market weight at the same time. To contend with within pen growth variation, producers implement the practice of split marketing where the heaviest 10-25 percent of the animals from a pen are marketed one to two weeks before the

remaining animals. Thereby, the swine producer will have greater economic success by sending a uniform group of animals to market. There is some concern that this management practice may be stressful to the remaining penmates if split marketing disrupts the already established social hierarchy and induces fighting. Production losses such as injuries, depressed weight gain, poorer feed efficiency and increased production costs may occur as a result of increased fighting.

The objective of this study was to assess the potential stress that split marketing may create on remaining penmates. An examination of the effect of split marketing on selected behavioral, physiological, and production parameters was therefore conducted. Obtaining a better understanding of the effects of split marketing would identify management practices that could contribute to a commercial producer's ability to maximize profit potential from their growing-finishing facilities.

CHAPTER II

LITERATURE REVIEW

A concern of animal agriculture is the development of management practices that contribute to the most efficient husbandry of groups of animals. One way swine producers attain efficiency in raising their animals is to use an all-in-all-out production system. An all-in-all-out production system is where groups of animals are moved into and out of facilities as a single unit to decrease the risk of disease transmission after each stage of production (e.g. farrowing, nursery, growing and finishing) (National Pork Producers Council, 1992). Swine producers have found this advantageous because it eliminates the mixing of animals; thereby reducing fighting among animals as well as facilitating the cleaning and disinfecting of facilities between groups of animals (National Pork Producers Council, 1992). Unfortunately, pigs do not grow uniformly so at the end of the grow-finish phase of production there is usually a weight variation among the animals in each pen. This weight variation restricts producers from marketing the entire pen of animals at one time and thus utilizing an all-in-all-out system to its full extent.

Because of the weight variation, many large swine production operations employ split marketing (SM) of the heaviest hogs. Split marketing is the removal of the top 10-25 percent of a pens' heaviest hogs 7-14 days prior to their penmates (R. Patty, personal communication). Split marketing allows producers to send market-ready hogs to market early so that producers save money in feed costs by not keeping heavier animals an extra one to two weeks. Also, by employing SM, producers can avoid sending underweight or overweight animals to market. The remaining pigs will thus have better access to the feeder due to less competition and will likely grow faster (R. Patty, personal communication).

A potential problem of split marketing is that it might disrupt the already fixed social hierarchy within these pens and induce fighting. There is a concern that the remaining penmates may not gain as well after disruption of their social order. The alteration of a group's social order may serve as a potential stressor to the remaining animals which could alter their physiology and their productivity. In addition, injuries can also be sustained in fighting such as broken legs and abscesses that can cause condemnation of the carcass upon slaughter (Tan and Shackleton, 1990).

The Social Hierarchy

A dominance hierarchy serves to decrease overt aggression by conferring certain individuals priority of access to important resources such as food and mates (Mench et al., 1992). Dominance can be described as the learned relationship between a pair of animals such that one animal consistently dominates another (Mench et al., 1992). This is most evident following the mixing of pigs during the nursery phase which results in vigorous fighting among animals during the first 24 hours with the social hierarchy established within 48 hours (Morrow-Tesch et al., 1994). The social hierarchy is determined by the outcome of these fights. Animals that win the most fights are considered dominants (DOM), those that win and lose about the same amount of fights are intermediates (INT), and those that mostly lose are considered subordinates (SUB) (McGlone et al., 1993).

Social hierarchy in piglets is established soon after birth as a result of vicious encounters among each other to gain access to the preferred anterior teats that produce the most milk and have the least incidence of mastitis (McBride, 1963). McBride and co-investigators (1965) found that the heavier piglets had an advantage of reaching greater weights independent of teat position but did seem to occupy the more anterior teats. The social rank, as established between these piglets while in the nursery, resulted in the more dominant animals being the heaviest at 8 weeks of age, an advantage that was

likely to persist through life and would likely continue its influence on growth (McBride et al., 1965).

Social rank among groups of pigs has been shown to be positively correlated with productivity. McBride and James (1964) found that pigs that were heavier at weaning had a higher social rank and tended to gain faster when fed ad libitum. Morrow-Tesch and coworkers (1994) also showed that upon mixing swine of different weights, the heaviest animals were destined to become the dominant animals. Differences in body weight between the DOM and SUB pigs increased as time progressed. Hicks and co-workers (1998) similarly found that socially dominant pigs had a faster rate of gain than intermediate or subordinate pigs. The dominant animals were the heaviest animals at the beginning of the study and maintained their dominance and their heavier weight throughout the experiment. Bates and Newcomb (1997) demonstrated that when the heaviest 50 percent of a pen was marketed two weeks earlier than their penmates there was significant weight gain and increased feed intake in the remaining animals. However, feed efficiency did not change. They also investigated loin eye muscle area and backfat at the tenth rib and found no differences between treatments, except in pigs allocated to medium density pens (.74 m^2/pig). The animals remaining after SM were fatter than those in pens that remained intact.

Virtually no literature exists regarding what effect the removal of penmates has on altering social order. Ewbank and Meese (1971) reported that the removal of one pig from a pen of eight animals did not increase aggression among the remaining penmates leading them to conclude that social hierarchy was not changed. However, in a subsequent study, they found that reversals of social status did occur spontaneously especially among INT and SUB animals while the DOM animals rarely lost their social status (Meese and Ewbank, 1972). Therefore, significant fighting between the lower ranking animals may occur after removal of the dominant individuals especially since Fraser (1974) found that subordinate animals challenge the more dominant animals with increasing familiarity of group members.

The Stress Response

Subordinate pigs have been found to have higher cortisol levels after fighting but both the DOM and SUB pigs have elevated catecholamines (Fernandez et al., 1994). These changes are suggestive of a typical stress response. Cannon (1929) first described stress as a variety of stimuli that causes the autonomic nervous system (ANS) to become activated. Cannon referred to this response to stress as the "fight or flight" syndrome. The autonomic nervous system (ANS), which is activated by the central nervous

system, controls this initial response (Moberg, 1985). Activation of the sympathetic portion of the ANS allows overall body arousal, stimulating the effector tissues needed in Cannon's "fight or flight" response and inhibiting those such as the GI tract and the kidneys that are not required for rapid motor activity (Moberg, 1985; Guyton and Hall, 1996). The effects of ANS stimulation in response to a stress are rapid due to it being directed via neural transmission. The ANS effects are not long-lasting because of the rapid degradation and re-uptake of the neurotransmitter substance.

Selye (1950) termed stress as the nonspecific result of any demand upon the body. He further divided stress into "distress," a damaging or unpleasant stress, or "eustress," a pleasant stress (Selye, 1974). The term stress generally refers to distress. Stressors are defined as stressful stimuli or situations, and an individual's reaction to a stressor is the stress response. Selye (1980) described the three phases of the body's response to stress as the general adaption syndrome (GAS). The initial phase of the GAS is termed the alarm reaction. This is also known as the sympathetic activation or the "fight or flight" response as previously described by Cannon (1929). The adrenal medulla is activated by sympathetic stimulation resulting in the release of the catecholamine epinephrine into the blood stream. Epinephrine intensifies the general adrenergic activity of the sympathetic nervous system.

This can result in peripheral vasoconstriction and an increase in heart rate, blood pressure, respiratory rate and blood glucose levels (Ewbank, 1985).

The second phase of the GAS, known as the stage of resistance, corresponds to the activation of the neuroendocrine system. The neuroendocrine system involves the hypothalamus, pituitary and adrenal glands which provide the connection between the integration centers of the central nervous system and the endocrine system to maintain an arousal state for longer periods of time (Asterita, 1985). The hypothalamic-pituitary-adrenal (HPA) axis involves perception of an external threat in the brain with release mainly of hypothalamic corticotropin-releasing factor (CRF) which stimulates the corticotrophic cells in the anterior portion of the pituitary gland to secrete adrenocorticotropic hormone (ACTH). Circulating ACTH stimulates the cortical region of the adrenal gland to produce glucocorticoids (Dohms and Metz, 1991). Cortisol, the major glucocorticoid in swine, can affect metabolism by promoting gluconeogenesis by the liver. Cortisol causes mobilization of energy via gluconeogenesis after proteolysis and lipolysis occurs (Guyton and Hall, 1996). Corticosteroids can modulate reproduction, metabolism and growth as well as having the ability to influence either directly or indirectly the even more diverse biological systems such as immunity and behavior (Moberg, 1985). Activation of the HPA axis accounts for the most prolonged and chronic phase

of the stress response.

The last phase of the GAS is termed the stage of exhaustion. Elevated plasma cortisol levels produce pronounced effects on the circulatory, digestive, immune and other systems of the body. Moberg (1985) introduced the term pre-pathological state which he defined as a state of vulnerability before a pathology develops. A stressor, or even a summation of sub-threshold stressors, can result in a biological cost that can lead to the development of a pre-pathological state conducive to the development of a pathology. A pre-pathological state may be seen as a decrease in growth rate or reproductive performance. The longer the stress period persists, the greater likelihood the animal will develop a pathology. Therefore, evidence of a pre-pathological state may serve as a better indicator of stress since it is evident that the animal is responding to a significant threat and not merely reacting to a minor stimulus (Moberg, 1985).

Behavioral and Physiological Measurements of Stress

Measurement of plasma cortisol concentrations has been typically used to document the biological response of swine to stress (Becker et al., 1985). Social status has an effect on cortisol, but the relationship is not clear. Within a single pen, DOM and SUB sows were found to have lower plasma cortisol

concentrations than INT sows (Zanella et al., 1998). Zanella and coworkers defined DOM as sows who won more competitions than lost, INT as sows that fought actively in competition but were not very successful, and SUB sows as passive avoiders. They concluded that INT, having no success in competitive interactions, were chronically stressed. Hicks and coworkers (1998) found that in pens of three piglets of similar sex, SUB had higher plasma cortisol concentrations after experiencing heat, cold or shipping stress than INT, while DOM piglets displayed intermediate cortisol levels. These researchers defined DOM piglets as those that defeated the other two penmates, INT defeated one penmate and SUB did not win any fights. The mixing of animals has also been shown to alter plasma cortisol concentrations. Raab and co-workers (1986) found that SUB rats have elevated cortisol levels compared to DOM after fighting and coexisting for 10 days. Arnone and Dantzer (1980) found that cortisol levels in both DOM and SUB three month old pigs increased significantly after a food competition test and subsequent initial fighting. After a few sessions of paired food competition tests, the animals had a stable social hierarchy and their cortisol levels were lower and not effected by subsequent fights.

As much as 75% of plasma cortisol in swine is tightly bound to a specific binding protein, known as corticosteroid-binding globulin (CBG), making

cortisol biologically unavailable (Kattesh et al., 1990, Behrens et al., 1993). CBG-bound cortisol acts as a circulating reservoir that keeps cortisol available to the tissues and, in addition, can limit the amount of biologically available cortisol in the circulation. Cortisol can also be bound to albumin (approximately 20-25%), however, since it is such a loose bond it is generally considered biologically available (Englebienne, 1984). Chronic elevation of cortisol in swine will result in a relative decrease in CBG-bound cortisol (Behrens et al., 1993). The resultant increase in unbound cortisol is subject to rapid clearance from the circulation through the urinary and digestive systems. This may serve as a physiological means by which the animal adapts to a chronic stress and thus re-establishes homeostasis.

An increase in unbound or biologically available cortisol within the circulation can result from an increase in the production of cortisol and/or decrease in the concentration of CBG (Kattesh et al., 1995). Kattesh and coworkers (1980) suggested that measurement of CBG might serve as a more precise indicator of a chronic stress response, especially when infrequent blood samplings are employed. Characterization of changes in CBG associated with social hierarchy has been demonstrated most recently in horses. Three to four days following the introduction of new horses to a group of dominant residents resulted in a decrease in CBG binding capacity while

free cortisol concentrations had risen from pre-stress values (Alexander and Irvine, 1998). The authors found total plasma cortisol levels had not changed leading them to conclude that evidence of a stress response could not be detected when only total cortisol was measured.

Haptoglobin is an acute phase protein that increases in the circulation of humans within six hours following inflammation or infection, increasing 10 to 1000 fold within 2 or 3 days (Eurell et al., 1992). Haptoglobin has therefore been indicated as a potential measure of stress (Francisco et al., 1996a). Serum haptoglobin levels have been found to increase in rats after cortisone administration due to an increase in haptoglobin's rate of synthesis (Krauss, 1968). During stress, protein synthesis is either decreased so that glucose can be attained by proteolysis or increased for the synthesis of acute phase proteins (Klasing, 1985). Many of the changes in protein metabolism resulting from inflammation may in part be a result of elevated gluconeogenesis, but may also be mediated by a cytokine, or a hormone like factor produced by the immune system called interleukin-1 (IL-1). Interleukin-1 is released from many cells including monocytes stimulated by phagocytosis, endotoxin or antigen-antibody complexes (Klasing, 1985). Once released, circulating IL-1 can directly effect protein metabolism in the liver and increase the synthesis of the acute phase proteins including haptoglobin (Klasing, 1985). The influence of

IL-1 on the production of acute phase proteins in the liver is dependent on the presence of glucocorticoids and is identical to the changes that occur in liver protein metabolism that result from inflammatory stress (Thompson et al., 1976). Elevation of serum haptoglobin levels is generally nonspecific in regard to the nature of the inciting agent (stress, mixing, disease, age and weight) and therefore can be used to gauge the extent of activity of many different pathological processes (Eurell et al., 1992; Francisco et al., 1996b).

White blood cells (leukocytes) include neutrophils, eosinophils, basophils, monocytes, lymphocytes and plasma cells. Neutrophils can usually phagocytize 5 to 20 bacteria and/or viruses before becoming inactivated. Monocytes maturing into macrophages after leaving the blood and entering the tissues, can phagocytize as many as 100 bacteria and viruses as well as larger particles (Guyton and Hall, 1996). Eosinophils comprise about 2 percent of the total white blood cells and are not important in combating bacterial or viral infections. They increase in number during parasitic infections and allergic reactions. Basophils release histamine, bradykinin, serotonin, prostaglandin and leukotrienes (inflammatory mediators) especially during allergic reactions. Lymphocytes attack foreign proteins (antigens) by producing antibodies especially to viruses and bacteria (Guyton and Hall, 1996).

Neutrophil:lymphocyte (N:L) ratio has also been proposed as an indicator of a stress response. Gilts injected once with cortisol at 7 to 9 weeks of age had increased N:L ratio within one hour of injection compared to controls (Salak-Johnson et al., 1996). N:L ratio was increased due to increased neutrophil and decreased lymphocyte numbers. Widowski and co-workers (1989) found within one day of feeding different concentrations of cortisol to weanling pigs, N:L ratio increased relative to the amount of cortisol fed. Basophils, eosinophils and monocytes, did not vary.

Fauci and Dale (1975) attempted to explain how corticosteroids influence lymphocyte numbers. They found corticosteroids redirect the intravascular recirculating pool of lymphocytes into extravascular compartments and thereby decrease the number of lymphocytes in the circulation. On the other hand, neutrophil numbers increase in the circulation under glucocorticoid influence due to increased neutrophil mobilization from bone marrow stores and diapedasis from blood vessel walls resulting in neutrophilia (Athens et al., 1961).

CHAPTER III

MATERIALS AND METHODS

Animals

One hundred and twenty eight-week old barrows (Premier Swine Breeding Systems, Michigantown, IN, T-MAX Boar [½ Hampshire x ½ Irish Large White] x P-100 Sow [¼ Yorkshire x ¼ Landrace x ½ Large White]) having high lean growth potential were allotted into 4 blocks of 30 pigs each. Each block of animals was delivered over four consecutive weeks. All pigs were ear-tagged prior to arrival. Immediately after arrival, all pigs were weighed and randomly assigned by weight to either a split-marketed group consisting of 6 pigs/pen with a density of .56 m^2/pig (SM), a control group consisting of 6 pigs/pen at a density of .56 m^2/pig (C) or a modified control consisting of 3 pigs/pen at a density of 1.1 m^2/pig (MC). SM had a 50% removal rate of the heaviest barrows 1 week prior to penmates. C and MC had all pigs remaining in the pen throughout the experiment. The animals were housed in a curtain-sided animal facility. Each pen was partially slatted and contained a two-hole feeder placed at the front of the pen on the solid portion of the floor. Animals were fed ad libitum a grower diet (3410 kcal/kg ME, 15.97

% CP, 1.82 % Lysine) until 20 weeks of age (92.2 kg BW) and a finishing diet (3452 kcal/kg ME, 14.03 % CP, 1.68 % Lysine) until marketed (Table 1). These diets met or exceeded NRC (1998) requirements for all nutrients. Animals were provided water ad libitum via one nipple waterer per pen.

Behavior Data Collection

All animals were videotaped for the first 72 hours of the study (INITIAL), 72 hours prior to (PRE) and following the removal (POST) of pigs in the SM treatment. Prior to videotaping, all animals were individually marked (Fig. 1) with farm animal crayon markers (All Weather Paintstik, La-Co Industries, Inc., Elk Grove Village, IL) so the animals could be distinguished on the videotapes for later analysis. The animal facility was illuminated continuously. Behaviors were recorded continuously by 72-hour (1 frame/1.6 sec) time lapse video cassette recorders (Panasonic AG-6040). Video cameras (Panasonic WV CP412) hung from the ceiling (2.3 m above the floor) allowed for filming of two pens with one camera. The following maintenance behaviors were observed by scan sampling, each animal was viewed in one frame of videotape every 15 minutes:

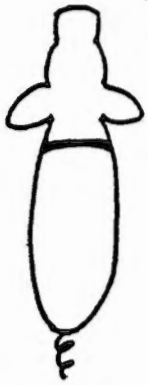
Stand/walking - the pig was up on all four feet or moving while up on all four feet

Table 1. Composition of grower and finishing diets.

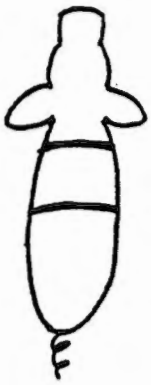
	GROWER DIET	FINISHING DIET
Corn	74.86	80.44
Soybean Meal	20.70	15.50
Liquid Energy	2.00	2.00
Dicalcium Phosphate	0.94	0.54
Limestone	0.80	0.82
Salt	0.35	0.35
L-lysine-HCl	0.10	0.10
Vitamin Premix[a]	0.25	0.25
	100.0 %	100.0 %
	Calculated Analysis	
ME (kcal/kg)	3410	3452
Crude Protein (%)	16.0	14.0
Lysine (%)	1.8	1.7

[a] Nutrients of AH&N Swine GF 5 VTM Premix exceeded the minimum NRC requirements.

Figure 1. Pig identification system during each videotaping period.



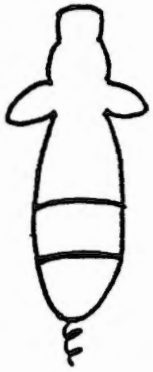
PIG # 1



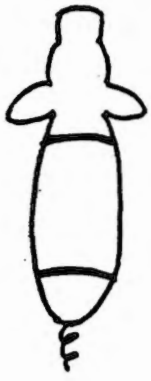
PIG # 2



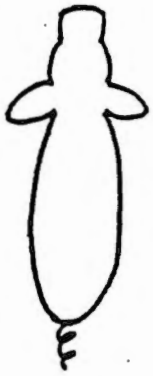
PIG # 3



PIG # 4



PIG # 5



PIG # 6

Eating - the pig's head was in the feeder

Lying - the pig was lying down (pig was not standing/walking on all four feet) or sitting

Drinking - the pig's mouth/snout was touching the waterer

Not in Pen - the pig was missing from designated pen

Too Dark - the pig was in the designated pen but there was not enough light to distinguish between the pigs or in if a power outage was experienced

Fight data was also collected. Fights were defined as an aggressive social interaction involving interchange of forceful or potentially harmful actions generally by some means of physical contact (Hurnick et al., 1995). Length of fight, pigs involved and winner were recorded for each observed fight. The following designations were used to describe some fights that were unclear:

NCW - no clear winner (could not observe one of the two pigs involved in the fight as the clear winner due to no submissive behavior)

AFS - another fight started (two pigs were fighting when a third pig became involved with one of the first two fighting pigs and one of the first two pigs dropped out of the fight)

The dominance hierarchy was divided into 3 ranks. DOM animals won

more fights than the other animals. INT won more fights than SUB but less than DOM. SUB animals lost more fights than the INT and DOM. When a fight was observed between two pigs in continuous mode, the video was slowed so exact time data could be documented for length of fight. Winners were defined as the pig that pursued the second pig or caused the second pig to turn away from it. Based on the fight data, animals were assigned to a rank by use of a dominance matrix where the number of fights between each pair of pigs in a pen were listed and who the winner was in each fight. For pens of six pigs, 2 animals were assigned as DOM, 2 animals were INT, and 2 were SUB. For pens having three animals, only one animal was designated in each social rank.

Blood Sample Collection

A blood sample (10 mL) was collected from each animal via the external jugular vein at the end of each 72-hour videotaping. Sampling began at approximately 0830 hour and each sample was obtained within two minutes of restraint. The animals removed one week prior to their penmates from the SM pens did not have a third blood sample collected. The blood samples were placed immediately into EDTA vacutainer tubes and gently mixed. Drops of whole blood from each sample were smeared onto duplicate slides and allowed to dry. Plasma was collected from each sample following centrifugation at 800

x *g* for 20 minutes. The plasma samples were aliquoted into four 1-mL aliquots and frozen in cryogenic vials at -20°C until analyzed for corticosteroid-binding globulin (CBG), total cortisol and haptoglobin concentration.

Neutrophil:lymphocyte Ratio

Whole blood slides were stained using Hema-Stat 3 stain set (Protocol, Biochemical Sciences Inc., Swedesboro, NJ) for determination of differential white blood cell numbers. One hundred white blood cells were counted using a light microscope at 100x using oil immersion. Leukocytes were characterized and identified as monocytes, lymphocytes, neutrophils, eosinophils or basophils. The neutrophil to lymphocyte ratio (N:L) was calculated for each sample.

Corticosteroid-binding Globulin

Plasma samples were was analyzed for corticosteroid-binding globulin concentrations in duplicate using a direct ELISA method (Kattesh and Roberts, 1993) as outlined in appendix A. Inter-assay and intra-assay coefficient of variation was 11.34 % and 8.65 %, respectively.

Total Cortisol

Total cortisol concentration was determined using a commercial

radioimmunoassay kit (Coat-A-Count, [^{125}I], Diagnostic Products Corporation, Los Angeles, CA). Samples were analyzed in duplicate and counted for 1 minute using a gamma counter (Cobra II Auto-gamma counter, Packard Instrument Co., Model D5005). Cortisol concentration was expressed as mg/mL. Intra-assay and inter-assay coefficient of variation was 6.91 % and 8.91 %, respectively.

Haptoglobin

Haptoglobin was determined by a single radial immunodiffusion (SRID) commercial kit (Porcine Haptoglobin Measurement Kit, Cardiotech Services, Inc., Louisville, KY) incubated at 32° C for 24 hours and the diameter of the precipitation ring was measured. Plasma samples or standards were placed in gelatin wells. The gelatin contained porcine anti-haptoglobin antibodies. Thus the diameter of the ring was inversely related to the amount of plasma haptoglobin. Results were evaluated using a logarithmic regression ($Y = -577.18 + 167.79$, $r^2 = .99$) to determine haptoglobin plasma concentration in each sample using known standards. Intra-assay coefficient of variation of duplicate estimates was 5.1 %.

Production Data

Each animal was weighed at the beginning of the experiment, every two

weeks and prior to marketing. Feed was added as needed and feed disappearance was measured every two weeks to obtain feed intake. Feed disappearance was calculated as the total amount of feed added minus the amount of feed remaining at the time feeders were weighed. Average daily gain, average daily feed intake and feed efficiency were calculated from the weights obtained from the pigs and their feed intake every two weeks. Daily high and low temperatures were recorded on each end of the animal facility by maximum-minimum self-registering temperature gauges (Taylor, model no. 5458, Fletcher, NC).

Ultrasound Data

All animals underwent an ultrasounic evaluation with a real-time ultrasonic machine (ALOKA 500V, Corometrics Medical Systems, Wallingford, CT) equipped with 12.5 cm, 3.5 MHz linear array transducer at the time of SM. Pigs were immobilized in the weigh crate and vegetable oil was used as a sound-conducting material for transmission of sound waves between the transducer and the skin. A cross-sectional image of the loin muscle area (LMA) and overlying subcutaneous fat (backfat) on the right side of the pig was obtained using an ultrasound stand-off guide (Superflab, Mick Radio Nuclear Instruments, Bronx, NY) mounted on the linear probe and conforming to the curvature of the pig's back. Recorded images were digitized and interpreted

using the AUSkey program (Animal Ultrasound Services, Ithaca, NY). The following equation was used to predict body composition: ((3.950 + (.308 x live weight of animal) - (16.440 x tenth rib backfat depth) + (4.693 x tenth rib LMA) ÷ (live weight ÷ .74) x 100). (National Swine Improvement Federation, 1997).

Statistics

Data was analyzed as a randomized complete block design using PROC MIXED procedure of SAS (SAS, 1999). Block and replicate were included in the analysis model as random effects. The fixed effects of treatment (C, MC, or SM), time period (INITIAL, PRE, or POST), and treatment x time period interaction were used to analyze the physiological measures of N:L ratio, corticosteroid-binding globulin, haptoglobin, and total cortisol concentrations as well as the behavioral observations of all fight data (frequency or duration of fights) and maintenance behaviors (drinking, eating, lying, or stand/walking). Due to non-normally distributed data for corticosteroid-binding globulin, duration of fight per pen and per pig, a log transformation was implemented. The production traits of backfat, LMA, percent lean, ADG, ADFI and feed efficiency had the fixed effect of treatment included in the model. Least square means and standard errors of the means were calculated for all variables. Significant mean differences were separated by pairwise contrasts (Saxton, 1998).

CHAPTER IV

RESULTS

Of the 120 animals that began this study, four were removed due to injury or sickness. The number of pigs removed from each treatment were: 2/48 (C), 2/48 (SM) and 0/24 (MC). One removal from C occurred early in the study whereas the other 3 removals occurred late in the study before split marketing.

Behavior

Frequency of drinking, eating, lying, and stand/walking (maintenance behaviors) during each videotaping period is represented in Table 2. Maintenance behaviors were not different as a result of treatment (Table 3). During the first three days of the study (INITIAL) all animals, regardless of treatment, spent more ($P < .0001$) time eating and stand/walking than during the three days prior to (PRE) or following (POST) split marketing. Subsequently, pigs spent less ($P < .0001$) time lying during INITIAL than PRE or POST. Drinking was greatest ($P < .01$) during PRE and POST than INITIAL. A time by treatment interaction was noted for drinking where C spent less ($P <$

Table 2. Least square means (± SEM) for frequency of drinking, eating, lying and stand/walking (maintenance behaviors) during first three days of experiment (INITIAL), three days before (PRE) and after (POST) split marketing.

Time period	n	Drinking	Eating	Lying	Stand/walking
INITIAL	24	.66 ± .06[a]	7.82 ± .21[a]	82.35 ± .54[a]	8.65 ± .41[a]
PRE	24	.90 ± .06[b]	5.66 ± .21[b]	88.26 ± .54[b]	5.18 ± .41[b]
POST	24	.88 ± .06[b]	6.11 ± .21[c]	87.12 ± .54[c]	5.88 ± .41[c]
P value		P < .01	P < .0001	P < .0001	P < .0001

[ab]Within a column, means without a common superscript letter differ.

Table 3. Least square means (± SEM) for percentage of time spent drinking, eating, lying and stand/walking (maintenance behaviors) between control (C), modified control (MC) and split marketed (SM) treatments.

Treatment	n	Drinking	Eating	Lying	Stand/walking
C	24	.80 ± .08	6.28 ± .30	86.20 ± .86	6.63 ± .65
MC	24	.89 ± .08	6.58 ± .30	85.67 ± .86	6.68 ± .65
SM	24	.74 ± .08	6.74 ± .30	85.87 ± .86	6.40 ± .65
P value		P = .43	P = .55	P = .91	P = .95

.005) time drinking during INITIAL compared to MC during POST. Lying and stand/walking behavior also had a time by treatment interaction. SM animals spent more ($P < .05$) time lying during PRE than in POST. Consequently, SM animals spent less ($P < .05$) time stand/walking during PRE than during POST.

Frequency and duration of fighting within a pen and per pig were greater ($P < .0001$) during INITIAL than PRE or POST (Table 4). Regardless of observation period, animals in MC fought less ($P < .0005$) frequently and for shorter ($P < .005$) periods than SM or C (Table 5).

There was a significant ($P < .0005$) treatment by time interaction for frequency of fights. Animals in SM and C fought more ($P < .0005$) than MC animals during INITIAL. During PRE, SM animals fought more ($P < .0005$) than MC, and during POST, MC fought less ($P < .0005$) than C while SM was not different from MC or C.

Production

MC exhibited greater ($P < .0001$) feed efficiency and greater ($P < .0001$) average daily feed intake compared to C or SM treatments from the beginning of the study until split marketing (Figure 2 and 3). Two weeks prior to split marketing, MC consumed more feed ($P < .0001$) and had superior ($P < .01$)

Table 4. Least square means (± SEM) of the frequency (number per taping period) and duration of time (seconds) of fights between pigs during the first three days of the experiment (INITIAL), the three days before split marketing (PRE), and the three days after split marketing (POST).

TIME	n	Frequency of fights per pen	Duration of fight per pen	n	Frequency of fights per pig	Duration of fight per pig
INITIAL	24	19.37 ± 1.09[a]	1324.1 ± 266.4[a]	120	3.73 ± .20[a]	419.67 ± 105.8[a]
PRE	24	4.06 ± 1.09[b]	43.1 ± 266.4[b]	116	.73 ± .20[b]	7.76 ± 105.8[c]
POST	24	4.21 ± 1.09[b]	59.0 ± 266.4[b]	93	.98 ± .20[b]	15.38 ± 105.8[b]

[abc]Within a column, means without a common superscript letter differ ($P < .0001$).

Table 5. Least square means (± SEM) of the frequency (number per taping period) and duration (seconds) of fights between pigs for control (C), modified control (MC), and split marketed (SM) treatments.

TRT	n	Frequency of fights per pen	Duration of fight per pen	n	Frequency of fights per pig	Duration of fight per pig
C	24	11.85 ± 1.28^{b}	734.6 ± 256.80^{a}	48	$1.98 \pm .24^{ab}$	122.44 ± 102.2^{ab}
MC	24	3.83 ± 1.36^{a}	111.6 ± 272.33^{b}	24	$1.28 \pm .25^{a}$	37.19 ± 108.4^{a}
SM	24	11.95 ± 1.46^{b}	580.0 ± 291.13^{a}	48	$2.19 \pm .27^{b}$	283.17 ± 115.88^{b}
P value		$P < .0005$	$P < .005$		$P < .05$	$P = .09$

[ab]Within a column, means without a common superscript letter differ.

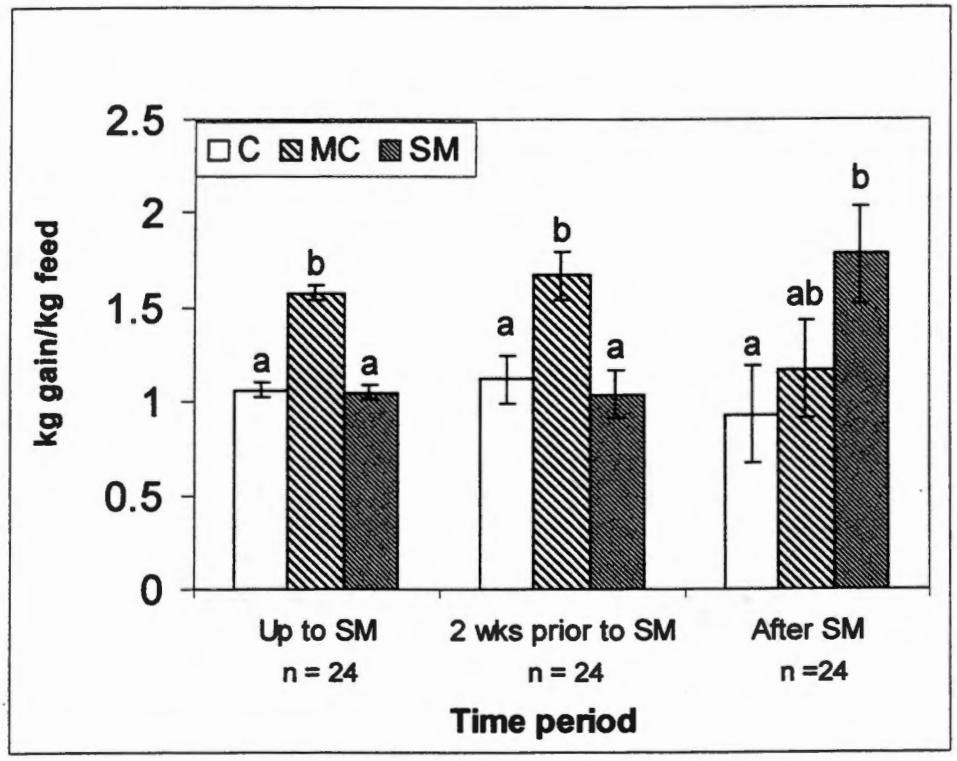


Figure 2. Feed efficiency for each treatment during three different time periods of the experiment. Treatments are represented by control (C), modified control (MC) and split marketed (SM). Time periods were from day zero of study until split marketing (Up to SM), two weeks prior to split marketing (2 weeks prior to SM) and one week following split marketing (After SM). Bars represent least square means $\pm$ SEM. Bars with different letters within a time period differ ($P < .0001$).

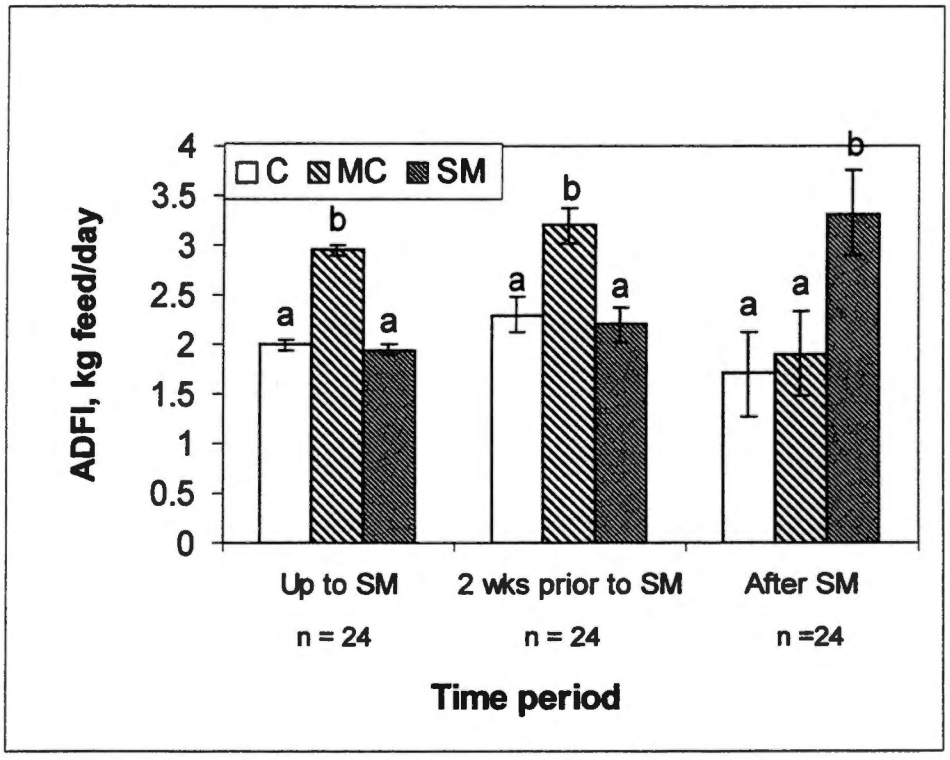


Figure 3. Average daily feed intake for each treatment during three different periods of experiment. Treatments are represented by control (C), modified control (MC) and split marketed (SM). Time periods were from day zero of study until split marketing (Up to SM), two weeks prior to split marketing (2 weeks prior to SM) and one week following split marketing (After SM). Bars represent least square means $\pm$ SEM. Bars with different letters within a time period differ ($P < .0001$).

feed efficiency compared to C or SM. The remaining penmates in the SM group exhibited greater ($P < .05$) average daily feed intake when compared to C or MC treatments after split marketing and tended ($P = .09$) to have better feed efficiency. There were no treatment differences in the live ultrasound analysis for the carcass characteristics of backfat or loin eye muscle area although C tended to be leaner ($P = .08$) than MC while SM was not different from C or MC (Table 6). Temperatures in the animal facility throughout the experiment are represented in Figure 4.

Physiological Measures

Neutrophil:lymphocyte ratio, plasma haptoglobin and CBG concentrations were greater ($P < .0001$) during INITIAL compared to PRE or POST (Table 7). Total plasma cortisol concentrations were not different as a result of treatment or time of sampling. CBG concentration was elevated ($P < .05$) over all time periods in MC and SM treatments compared to C (Figure 5).

Table 6. Least square means (± SEM) of the live carcass measurement as determined by ultrasound at the tenth rib for backfat, loin-eye muscle area (LMA), and percent lean between control (C), modified control (MC) and split marketed (SM) treatments.

Production measure	C (n = 47)	MC (n = 22)	SM (n = 45)	P value
Backfat (mm)	23.15 ± .81	25.06 ± 1.03	23.41 ± .82	.21
LMA (mm^2)	44.19 ± 1.2	43.35 ± 1.35	43.23 ± 1.23	.55
Percent Lean (%)	52.75 ± .57[a]	51.49 ± .65[b]	52.20 ± .57[ab]	.08

[ab]Within a row, means without a common superscript letter differ (P < .10).

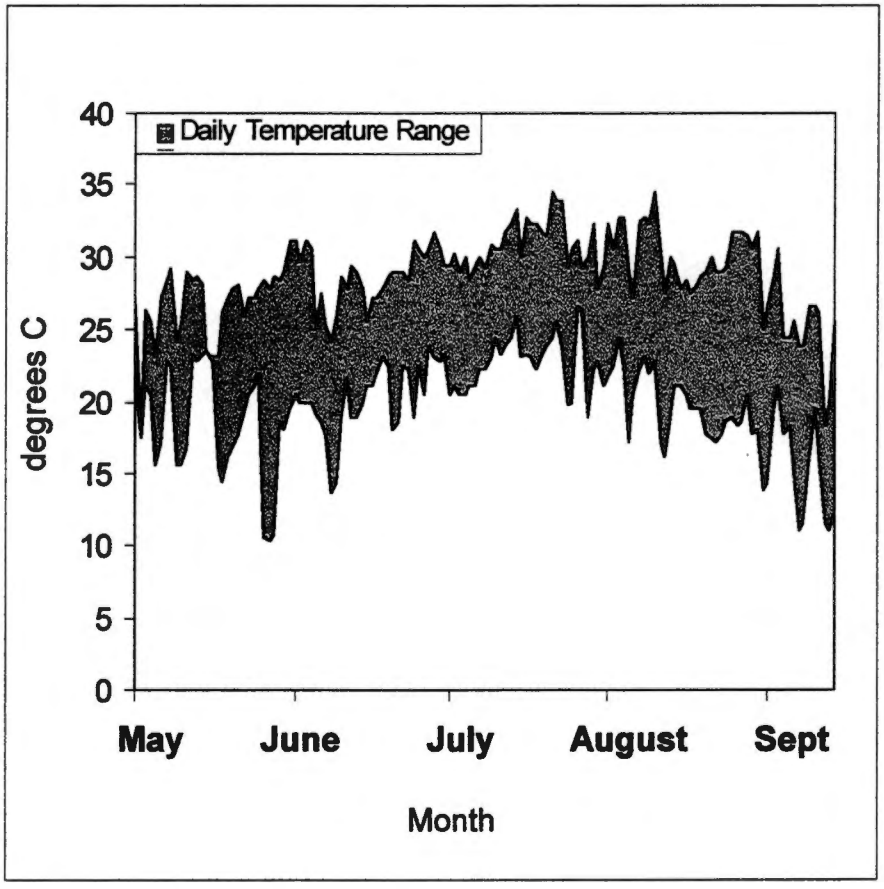


Figure 4. Average daily high and low air temperature in the animal facility over the entire experiment. Range during the first three days of the experiment (INITIAL) was 18° - 29° C, and 25° - 34° C during the three days before (PRE) and after (POST) split marketing.

Table 7. Least square means (± SEM) for neutrophil:lymphocyte (N:L) ratio, plasma concentrations of haptoglobin, corticosteroid-binding globulin (CBG), and total cortisol after the first three days of experiment (INITIAL), the three days before split marketing (PRE), and the three days after split marketing (POST).

Physiological Measure	INITIAL	PRE	POST
N:L ratio	55.3 ± 2.1[a] (n = 120)	34.4 ± 2.1[b] (n = 113)	35.5 ± 2.3[b] (n = 91)
Haptoglobin (mg/mL)	978.81 ± 50.38[a] (n = 120)	732.00 ± 51.62[b] (n = 96)	769.63 ± 54.60[b] (n = 88)
CBG (mg/mL)	11.28 ± .52[a] (n = 109)	15.48 ± .56[b] (n = 109)	14.88 ± .60[b] (n = 88)
Total cortisol (ng/mL) P = .36	48.52 ± 3.64 (n = 116)	52.36 ± 3.67 (n = 111)	49.19 ± 3.85 (n = 83)

[ab]Within a row, means without a common superscript letter differ (P < .0001).

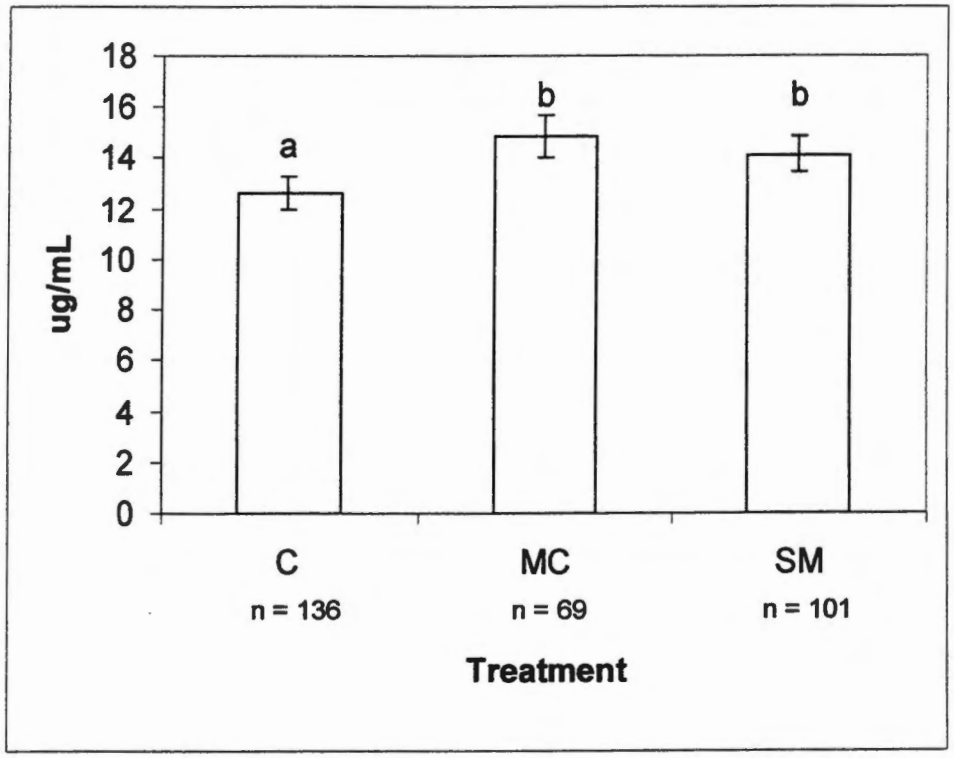


Figure 5. Plasma corticosteroid-binding globulin concentration for each treatment regardless of time. Treatments are represented by control (C), modified control (MC) and split marketed (SM). Bars represent least square means ± SEM. Bars with different letters differ ($P < .05$).

CHAPTER V

DISCUSSION

In this study, barrows were more active during the first three days of the experiment (INITIAL) than during the three days prior to (PRE) or after (POST) split marketing as determined by the amount of time spent stand/walking, fighting and eating. An increased activity level is normal when first placing pigs in a pen since animals investigate their new environment and penmates. They also establish a social hierarchy by fighting vigorously within the first 24 hours of mixing and usually have a stable social hierarchy within 48 hours (Morrow-Tesch et al., 1994). The greater frequency and duration of fighting observed during INITIAL may have contributed to the INITIAL stand/walking behavior. Maintenance behaviors were analyzed irrespective of fighting and all fights occurred while stand/walking. Therefore, increased fighting causes increased stand/walking activity. Increased eating during INITIAL could be age related since pigs eat more meals when younger but as they grow larger, they eat larger meals less frequently (Bigelow and Houpt, 1988). Also, feed consumption is inversely related to environmental temperature. During the INITIAL time period, the average high temperature in the animal facility was 27°

C compared to the average 30° C during PRE and POST. Ideally during growing, the floor temperature should be 21° C and 18° C during finishing for optimal growth and feed consumption (Premier Swine Production Manual, 1998).

During PRE and POST time periods, pigs were less active as represented by increased lying behavior. Pigs also fought less indicative of a fixed social hierarchy with fights being replaced with threats. Ewbank and Meese (1971) found no increased fighting after removal of one pig from a pen of eight that had a stable social hierarchy. They also found that if a dominant animal was removed, the second highest ranking animal moved into the dominant position. Overall in the present study, C and SM treatments fought more often and for longer periods of time compared to MC. The greater density among C and SM could have contributed to the increased fighting. As group size increases, it is common for the dominance hierarchy to become more complex lending itself to increased fighting in order for the hierarchy to become stable (Craig, 1986).

Drinking behavior was also increased during the PRE and POST observation periods. Higher temperatures cause an animal to consume or at least have more contact with water which could explain why the animals spent

more time drinking during PRE and POST. Also, it is questionable how accurately we detected drinking behavior since it has a high day to day variation (Bigelow and Houpt, 1988). Other researchers have found that pigs do not always consume water when their mouths are touching the waterer (McBride and James, 1964; Bryant and Ewbank, 1974). The pigs may have been playing with the waterer after being forced away from the feeder as a form of displacement behavior contributing to the day to day variation of drinking behavior observed in this study (McBride and James, 1964) or as a means of evaporative cooling.

In the present study, MC exhibited lower feed efficiency and greater average daily feed intake compared to C or SM without substantial weight gains. Brumm and coworkers (1996) found that barrows in lower density pens usually consume more food without a significant increase in body weight resulting in poorer feed efficiency. This could be due to animals with increased space being more active and thereby consuming more feed. Brumm and coworkers (1996) also found that barrows up to 136 kg body weight had no improvement in productivity if provided more than 1 m^2/pig. Growing-finishing swine provided up to 1 m^2/pig have increased daily gain and feed disappearance with less of an effect on feed efficiency (Kornegay and Notter, 1984). Over the duration of the present study, MC had two feeder holes per

three pigs while C and SM had two feeder holes per six pigs until split marketing when SM had space equal to the MC group. The discrepancy in feeder space probably did not account for the increased feed intake by the MC pigs. Increasing or decreasing feeder space beyond what is typically provided for nursery pigs does not improve or depress daily gain, daily feed intake or feed efficiency (Lindemann et al., 1987).

The animals remaining in SM pens during POST had greater ADFI without an increase in average daily gain. There was a trend for feed efficiency to be improved for the remaining penmates. Bates and Newcomb (1997) demonstrated that when the heaviest 50 percent of the animals within a pen were marketed two weeks earlier than their penmates, there were significant weight gains and increased feed intake in the remaining animals. However, feed efficiency in their study was not improved. Based on the results of Lindemann and coworkers (1987), the increased average daily feed intake for SM pigs during POST is probably more a function of increased space per pig than increased feeder space. Bryant and Ewbank (1974) found that decreasing pen density increased feed intake while number of feeder holes remained the same between treatments. However, in their study, there were no significant differences in feed conversion or average daily gain. They suggested that an increased stocking density reduces an individual's ability to

move about its living space and, as the pigs grow and further reduce their area for maneuvering, visits to the feeder and/or waterer may become more difficult.

Backfat, loin eye muscle area, and percent lean were not significantly different among treatments. Production measures are usually only affected during a chronic stress and the physiological measures in this study did not reflect that the pigs were experiencing a chronic stress. However, density can contribute to productivity in pigs. When pigs are given either .62 m^2/pig, .72 m^2/pig, or .93 m^2/pig and marketed at 107 kg, the leanest carcasses with the smallest backfat depth occur at the greatest density of .62 m^2/pig (Bates and Newcomb, 1997). Limited data suggest there is slight improvement in carcass backfat depth as space is restricted due to decreased daily feed intake (PIC, 1997). The present study found no such improvements in productivity even though the treatments in this study could be considered crowded after the pigs reached 68 kg since the National Pork Producers Council (1992) recommends that pigs from 68 kg to marketing should have .74 m^2/pig.

Total cortisol concentration was not different between animals regardless of treatment or time. Only one blood sample was collected in the morning during each sampling time which other studies have shown may not be sufficient to detect differences unless multiple samples are collected over the

course of a day. Differences in cortisol levels due to circadian rhythms or stress can be detected when collecting more than one sample, especially by collecting morning and afternoon samples (Kattesh et al., 1995; Zanella et al., 1998). Similar to the present study, Alexander and Irvine (1998) also found no changes in cortisol in socially stressed horses by taking one morning sample.

Elevation of corticosteroid-binding globulin (CBG) concentration during INITIAL compared to PRE or POST may be an age related phenomena. The CBG plasma concentrations during INITIAL are comparable to that in pigs of similar age that were not stressed (J. Heo, personal communication). However, age related differences in CBG concentration have been found with levels greater at 40 days of age (J. Heo, personal communication) compared to pigs 125 days of age (H.G. Kattesh, unpublished data). Overall, CBG levels for C were lower than MC and SM treated animals. CBG concentration has been reported to decrease in stressed animals (Kattesh et al., 1995; Alexander and Irvine, 1998) which would suggest that animals in the C treatment were stressed. The reason for this is not clear except that the C animals were maintained at the greatest density for the entire trial whereas MC was maintained at half the density of the C treatment for the entire trial. SM pigs had the same density as C until the last week of the trial when they had the equivalent density of the MC treatment but there was no time by treatment

interaction for CBG.

N:L ratio was greatest during INITIAL than PRE or POST. Stull and co-investigators (1999) found that both 28 and 56-day old pigs had greater N:L ratio that decreased up to 168 days of age. The authors concluded the increased N:L ratio at 28 and 56 days of age may be attributed to the stress of mixing litters or partial influence of glucocorticoids instead of age. Although N:L ratios in the present study were greatest during INITIAL, other studies have found even higher N:L ratios with other stressors such as transport, heat or cold stress (McGlone et al., 1993; Hicks et al., 1998).

Haptoglobin was also elevated during INTIAL compared to PRE or POST. This may be due to an age related effect as Eurell and coworkers (1992) found that haptoglobin values peaked at six to eight weeks of age and decreased up to 13 weeks of age. The peak values Eurell and coworkers (1992) found where much lower than the haptoglobin levels found in the present study for similar aged animals suggesting that the INITIAL animals were experiencing a stress response. However, it must be considered that during INITIAL, the animals were fighting vigorously which has been shown to cause tissue damage (Tan and Shackleton, 1990). Increased tissue damage can cause an inflammatory response thereby also increasing plasma

haptoglobin levels (Eurell et al., 1992).

CHAPTER VI

IMPLICATIONS

Split marketing apparently has no detrimental effects on the remaining penmates' behavior, physiology or production parameters as measured in this study. The removal of the heaviest animals from a given pen did not induce fighting among the remaining penmates indicating that the social hierarchy was not disrupted. Only average daily feed intake was affected by split marketing during the last week of the trial. The only differences found in the physiological measures examined in this study that would suggest that the animals experienced stress was during the first three days of the experiment. Therefore, marketing the heaviest 50 percent of a pens' hogs one week prior to their penmates does not appear to be detrimental to the remaining penmates as evidenced by a lack of change in behavioral, physiological or production measures employed in out study. Split marketing earlier than one week prior to marketing penmates may have beneficial effects for a commercial producer by improvements in average daily gain in the remaining animals.

Although this study at its maximum density had only 6 pigs per pen, the

pigs in this study compared well with what commercial producers achieve in pens that contain more pigs (approximately 25 to 30 pigs per pen) at densities similar to this study's. Therefore, the results of this study should compare well with what happens in a commercial setting. But, in pens containing more animals per pen, it is possible for sub-groups to develop that could add a new dynamic to the effects of split marketing. Unfortunately, previous studies in this area of research have had limited numbers of animals per pen.

Unfortunately, it is difficult to measure stress physiologically without extraneous factors such as circadian rhythms or age causing possible misinterpretation. Until better physiological measures of stress are found, a battery of tests are recommended to help elucidate the possible stress an animal is experiencing.

LITERATURE CITED

Alexander, S.L., and C.H.G. Irvine. 1998. The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. J. Endocrinol. 157:425-432.

Arnone, M., and R. Dantzer. 1980. Does frustration induce aggression in pigs? Appl. Anim. Ethol. 6:351-362.

Asterita, M.F. 1985. The Physiology of Stress. Human Sciences Press, Inc., New York.

Athens, J.W., O.P. Haab, A.M. Mauer, H. Ashenbrucker, G.E. Cartwright, and M.M. Wintrobe. 1961. Leukokinetic studies. IV. The total blood circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. J. Clin. Invest. 40:989-995.

Bates, R.O., and M.D. Newcomb. 1997. Removal of market ready pen mates improved growth rate of remaining pigs. J. Anim. Sci. 75(Suppl. 1):247 (Abstr.)

Becker, B.A., J.A. Nienaber, R.K. Christenson, R.C. Manak, J.A. DeShazer, and G.L. Hahn. 1985. Peripheral concentrations of cortisol as an indicator of stress in the pig. Am. J. Vet. Res. 46:1034-1038.

Behrens, M.J., H.G. Kattesh, and G.A. Baumbach. 1993. Plasma and uterine cortisol, progesterone and protein changes in pseudopregnant gilts treated with hydrocortisone acetate. Theriogenology 40:1231-1241.

Bigelow, J.A., and T.R. Houpt. 1988. Feeding and drinking patterns in young pigs. Phys. Behav. 43:99-109.

Brumm, M.C., and NCR-89 Committee on Management of Swine. 1996. Effect of space allowance on barrow performance to 136 kilograms body weight. J. Anim. Sci. 74:745-749.

Bryant, M.J., and R. Ewbank. 1974. Effects of stocking rate upon the performance, general activity and ingestive behaviour of groups of growing pigs. Br. Vet. J. 130:139-149.

Cannon, W.B. 1929. Bodily Changes in Pain, Hunger, Fear and Rage. D. Appleton-Century Company, New York.

Craig, J.V. 1986. Measuring social behavior: social dominance. J. Anim. Sci.

62:1120-1129.

Dohms, J.E., and A. Metz. 1991. Stress - mechanisms of immunosuppression. Vet. Immunol. Immunopath. 30:89-109.

Englebienne, P. 1984. The serum steroid transport proteins: biochemistry and clinical significance. Mol. Aspects Med. 7:313-396.

Eurell, T.E., D.P. Bane, W.F. Hall, and D.J. Schaeffer. 1992. Serum haptoglobin concentration as an indicator of weight gain in pigs. Can. J. Vet. Res. 56:6-9.

Ewbank, R., and G.B. Meese. 1971. Aggressive behavior in groups of domesticated pigs on removal and return of individuals. Anim. Prod. 13:685-693.

Ewbank, R. 1985. Behavioral response to stress in farm animals. In: Animal Stress. Ed. G.P. Moberg. American Physiological Society. Bethesda, Maryland. pp. 71-79.

Fauci, A.S., and D.C. Dale. 1975. The effect of hydrocortisone on the kinetics of normal human lymphocytes. Blood. 46:235-243.

Fernandez, X., M.C. Meunier-Salaun, and P. Ecolan. 1994. Glycogen depletion according to muscle and fibre types in response to dyadic encounters in pigs (*Sus scrofa domesticus*) - relationships with plasma epinephrine and aggressive behavior. Comp. Biochem. Physiol. 109:869-879.

Francisco, C.J., D.P. Bane, R.M. Weigel, and L.Unverzagt. 1996a. The influence of pen density, weaning age, and feeder space on serum haptoglobin concentration in young swine. Swine Health and Production. 4:67-71.

Francisco, C.J., D.P. Bane, and L. Unverzagt. 1996b. The effects of enrofloxacin and tiamulin on serum haptoglobin and alpha-1-acid glycoprotein concentrations in modified medicated early weaned pigs. Swine Health Prod. 4:113-117.

Fraser, D. 1974. The behaviour of growing pigs during experimental social encounters. J. Agric. Sci., Camb. 82:147-163.

Guyton, A.C., and J.E. Hall. 1996. Textbook of Medical Physiology. W.B. Saunders Company, Philadelphia, Pennsylvania.

Hicks, T.A., J.J. McGlone, C.S. Whisnant, H.G. Kattesh, and R.L. Norman. 1998. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J. Anim. Sci. 76:474-483.

Hurnick, J.F., A.B. Webster, and P.B. Siegel. 1995. Dictionary of Farm Animal Behavior (2nd Ed.). Iowa State University Press, Ames.

Kattesh, H.G., E.T. Kornegay, J.W. Knight, F.G. Gwazdauskas, H.R. Thomas, and D.R. Notter. 1980. Glucocorticoid concentrations, corticosteroid binding globulin protein characteristics and reproduction performance of sows and gilts subjected to applied stress during mid-gestation. J. Anim. Sci. 50:897-905.

Kattesh, H.G., S.F. Charles, G.A. Baumbach, and B.E. Gillespie. 1990. Plasma cortisol distribution in the pig from birth to six weeks of age. Biology of the Neonate. 58:220-226.

Kattesh, H.G., and M.P. Roberts. 1993. Age related changes in plasma concentrations of porcine corticosteroid binding globulin (pCBG) as determined by an enzyme -linked immunosorbent assay (ELISA). Proceedings of the 85th annual meeting of the American Society of Animal Science. p 234 (Abstr.)

Kattesh, H.G., M.E. Brown-Crutchfield, J.L. Wolf II, and J.F. Schneider. 1995. Effect of restricted movement on the physiology and behavior of swine. Tennessee Agri. Science. 176:31-35.

Klasing, K.C. 1985. Influence of stress on protein metabolism. In: Animal Stress. Ed. G.P. Moberg. American Physiological Society. Bethesda, Maryland. pp 269-280.

Kornegay, E.T., and D.R. Notter. 1984. Effects of floor space and number of pigs per pen on performance. Pig News Info. 5:23-33.

Krauss, S. 1968. Effect of hormones on serum haptoglobin biosynthesis. Fed. Proc. 27:54. (Abstr.)

Lindemann, M.D., E.T. Kornegay, J.B. Meldrum, G. Schurig, and F.C. Gwazdauskas. 1987. The effect of feeder space allowance on weaned

pig performance. J. Anim. Sci. 64:8-14.

McBride, G. 1963. The 'teat order' and communication in young pigs. Anim. Behav. 11:53-56.

McBride, G., and J.W. James. 1964. Social behavior of domestic animals. IV. Growing pigs. Anim. Prod. 6:129-139.

McBride, G., J.W. James, and G.S. Wyeth. 1965. Social behavior of domestic animals. VII. Growing pigs. Anim. Prod. 7:67-74.

McGlone, J.J., J.L. Salak, E.A. Lumpkin, R.L. Nicholson, M. Gibson, and R.L. Norman. 1993. Shipping stress and social status effects on pig performance, plasma cortisol, natural killer cell activity, and leukocyte numbers. J. Anim. Sci. 71:888-896.

Meese, G.B., and R. Ewbank. 1972. A note on the instability of the dominance hierarchy and variations in level of aggression within groups of fattening pigs. Anim. Prod. 14:359-362.

Mench, J.A., W.R. Stricklin, and D. Purcell. 1992. Social and Spacing Behavior. The Well Being of Agricultural Animals in Biomedical Research. pp 69-73.

Moberg, G.P. 1985. Biological response to stress: key to assessment of animal well-being? In: Animal Stress. Ed. G.P. Moberg. American Physiological Society. Bethesda, Maryland. pp 27-49.

Morrow-Tesch, J.L., J.J. McGlone, and J.L. Salak-Johnson. 1994. Heat and social stress effects on pig immune measures. J. Anim. Sci. 72:2599-2609.

National Pork Producers Council (NPPC). 1992. Swine Care Handbook. NPPC, Des Moines, Iowa.

National Swine Improvement Federation (NSIF). 1997. Guidelines for Uniform Swine Improvement Programs (Revised Edn.). United States Department of Agriculture Program AID 1157, Washington, DC.

NRC. 1998. Nutrient Requirements of Swine (10th Ed).). National Academy Press. Washington, DC.

PIC. 1997. PIC USA Production Tech. Update. Vol. 2, No. 1. Available at http://www.pic.com/usa

Premier Swine Production Manual. 1998. Premier Swine Production Systems, Michigantown, IN.

Raab, A., R. Dantzer, B. Michaud, P. Mormede, K. Taghzouti, H. Simon, and M. Le Moal. 1986. Behavioural, physiological and immunological consequences of social status and aggression in chronically coexisting resident-intruder dyads of male rats. Phys. Behav. 36:223-228.

Salak-Johnson, J.L., J.J. McGlone, and R.L. Norman. 1996. In vivo glucocorticoid effects on porcine natural killer cell activity and circulating leukocytes. J. Anim. Sci. 74:584-592.

SAS. 1999. SAS OnlineDoc® Version 8. SAS Inst. Inc., Cary. NC.

Saxton, A. 1998. A macro for converting mean separation output to letter groupings in PROC MIXED. Proceedings of the Twenty-Third SAS Users Group International Conference, Cary, NC: SAS Institute Inc., pp. 1243-1246.

Selye, H. 1950. The Physiology and Pathology of Exposure to Stress. Acta, Inc. Montreal, Canada.

Selye, H. 1974. Stress Without Distress. J.B. Lippincott Company., New York.

Selye, H. 1980. Selye's Guide to Stress Research. Van Nostrand Reinhold Company, New York.

Stull, C.L., C.J. Kachulis, J.L. Farley, and G.J. Koenig. 1999. The effect of age and teat order on a1-acid glycoprotein, neutrohil-to-lymphocyte ratio, cortisol, and average daily gain in commercial growing pigs. J. Anim. Sci. 77:70-74.

Tan, S.S.L., and D.M. Shackleton. 1990. Effects of mixing unfamiliar individuals and of azaperone on the social behavior of finishing pigs. Appl. Anim. Behav. Sci. 26:157-168.

Thompson, W.L., F.B. Abeles, F.A. Beall, R.E. Dinterman, and R.W. Wannemacher. 1976. Influence of the adrenal glucocorticoids on the stimulation of synthesis of hepatic ribonucleic acid and plasma acute-

phase globulins by leucocytic endogenous mediator. Biochem. J. 156:25-32.

Widowski, T.M., S.E. Curtis, and C.N. Graves. 1989. The neutrophil:lymphocyte ratio in pigs fed cortisol. Can. J. Anim. Sci. 69:501-504.

Zanella, A.J., P. Brunner, J. Unshelm, M.T. Mendl, and D.M. Broom. 1998. The relationship between housing and social rank on cortisol, b-endorphin and dynorphin (1-13) secretion in sows. Appl. Anim. Behav. Sci. 59:1-10.

APPENDIX

APPENDIX A

PROCEDURE FOR ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

I. Preparation of Reagents

A. 10x Phosphate buffered saline (PBS) solution

80 g NaCl, 12.07 g Na_2HPO_4, 20 g KH_2PO_4,

2.0 g KCL, 2.0 g Sodium azide

1. Bring to near 1,000 mL with deionized water.
2. Adjust pH to 6.8.
3. Bring to 1,000 mL with deionized water.
4. Store at 4° C.

B. 10% Bovine serum albumin (BSA) solution

25 g bovine serum albumin (Fisher Biotech, NJ),

25 mL 10x PBS

1. Bring to 250 mL with deionized water.
2. Stir until dissolved at 4° C.
3. Sterilize with 0.2 nm filter.
4. Store at 4° C.

C. 10x Tris-buffered saline (TBS) solution

350.64 g NaCl, 96.88 g Tris, 8.0 g Sodium azide

1. Bring to near 4,000 mL with deionized water.
2. Adjust pH to 7.4.
3. Bring to 4,000 mL with deionized water.
4. Store at room temperature.

D. 10% Tween 20

50 mL Tween 20

1. Bring to 500 mL with deionized water.
2. Throughly rinse tween container to wash out total 50 mL.
3. Store at 4° C.

E. Glassware treatment solution

10 mL 10% BSA, 90 mL 10x PBS

1. Bring to 1,000 mL with deionized water.
2. Store at 4° C.

F. Wash buffer

400 mL 10x TBS, 20 mL 10% Tween 20

1. Bring to 4,000 mL with deionized water.
2. Store at room temperature.

G. 1x Diluent solution

100 mL 10% BSA, 90 mL 10x PBS, 5 mL 10% Tween 20, 5 mg Bromophenol blue

1. Bring to 1,000 mL with deionized water.
2. Store at 4° C.

H. 2x Diluent solution

200 mL 10% BSA, 180 mL 10x PBS, 10 mL 10% Tween 20, 300 mL 0.2 N Heps

1. Bring to just 1,000 mL with deionized water.
2. Adjust pH to 7.4.
3. Store at 4° C.

I. Tris coating buffer solution

2.4422 g Tris, 5.84 g NaCl, 0.2 g Sodium azide

1. Bring to 1,000 mL with deionized water.
2. Adjust pH to 8.5.
3. Store at 4° C.

J. 5% Na_2EDTA solution

25 g Na_2EDTA

1. Bring to 500 mL with deionized water.
2. Store at 4° C.

K. Standard solution

1. For standards, porcine CBG from pig serum pool was isolated and purified by affinity chromatography and HPLC-DEAE anion exchange technique and adjusted to 12.152 ng/μL by dilution with 1x PBS.
2. Standard stock solution was prepared in bulk and aliquoted with Williams' Medium E.
3. Standard concentrations are:

ng/well	ng/μL	pCBG stock (μL)	Williams' Medium (μL)
160	3.2	2,632.88	7,367.17
50	1.0	822.77	9,177.23
20	0.4	329.11	9,670.89
8	0.16	131.64	9,868.36
2.5	0.05	41.14	9,958.86
1.0	0.02	16.46	9,983.54
0.3	0.006	4.94	9,995.06

L. CBG antibody stock solution

1. Rabbit anti-pCBG was from a rabbit injected with CBG purified from porcine plasma.

2. A 1/1,000 dilution of antiserum was prepared with 1x Diluent solution.

3. Store in 250 μL aliquots in screw cap vial and frozen at -70° C.

M. CBG coating solution for 50 plates

8,8227.74 μL pCBG stock (12.154 ng/μL) solution,

491,772.26 μL Tris coating buffer.

1. Prepare immediately before use.

N. Substrate solution

1. For every 5 mLs of substrate, mix 1 mL diethanolamine buffer (5x), 4 mL deionized water, and 1 tablet of p-nitrophenyl-phostpate (Kirkegard and Perry Lab., MD).

2. Agitate gently to dissolve the tablet.

3. Prepare the buffer just before use.

II. Procedure

A. Coat plate (Corning 96 well ELISA plate, NY) with purified porcine CBG (20 ng/well) by incubating at 4° C for 48 hours.

B. Concurrently, in a separate previously blocked plate, incubate

samples (40 μL/well), reference porcine serum (5 μL/well), 2x Diluent control (50 μL/well) or standard porcine CBG (0.3 to 160 ng/well) with rabbit anti-pCBG serum at final dilution of 1/500,000 at 4° C for 48 hours.

C. Wash the pCBG coated plate with wash buffer using a Dynatech Ultrawash II Microplate Washer (Chantilly, VA), 8-4-1-4 cycle and tap dry on paper towels.

D. Transfer the sample-antibody mixture (100 μL/well) to the corresponding wells of the coated plate and incubate in a humidified chamber at 30° C for 2 hours.

E. Wash the plate using 8-4-1-4 cycle on the plate washer and tap dry.

F. Add 100 μL/well of anti-rabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co., MO) in a 1/12,000 dilution with 1x Diluent and incubate in a humidified chamber at 30° C for 2 hours.

G. Wash the plate using 8-4-1-4 cycle on the plate washer and tap dry.

H. Add the phosphate substrate (100 μL/well) and incubate in a humidified chamber at 30° C for 1 hour.

I. Stop the reaction with an equal volume of 5% Na_2EDTA solution.

J. Read the plate at 405 nm on a BioTek Automated Microplate

Reader (Winooski, VT).

K. Calculate the amount of CBG in the sample using a four parameter fit, kineticalc software (Bio-Tek Instruments, Inc., VT).

VITA

Lisa Valeria Scroggs was born in Framingham, Massachusetts and after moving to North Carolina and California, attended Johnson High School in Gainesville, Georgia where she graduated in 1993. In September of 1993, she attended Gainesville College and transferred in September of 1995 to the University of Georgia where she later graduated with a Bachelor of Science in Agriculture degree in June of 1997 majoring in Biological Science. She then worked at the University of Tennessee College of Veterinary Medicine as a research assistant until she enrolled in the Department of Animal Science at the University of Tennessee, Knoxville in pursuit of a Master of Science degree in August of 1998. In May of 2000 she received her Master of Science Degree from The University of Tennessee, Knoxville. She will now attend the University of Georgia College of Veterinary Medicine in August of 2000 to attain a Doctor of Veterinary Medicine degree and speciality board certification in animal behavior.

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