DISSERTATION

INVESTIGATING THE IMPACT OF HUSBANDRY AND MANAGEMENT PRACTICES ON THE INTERACTION OF ANIMAL WELL-BEING AND PRODUCT QUALITY IN BEEF, SWINE, AND DAIRY PRODUCTION SYSTEMS.

Submitted by

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ABSTRACT

INVESTIGATING THE IMPACT OF HUSBANDRY AND MANAGEMENT PRACTICES ON THE INTERACTION OF ANIMAL WELL-BEING AND PRODUCT QUALITY IN BEEF, SWINE, AND DAIRY PRODUCTION SYSTEMS.

The impact of husbandry and management practices were investigated in a beef feedlot, a small slaughter facility, and a dairy. In the first experiment, the impact of β-adrenergic agonist supplementation and implant strategy on the physiological, metabolic, and behavioral responses of feedlot steers was evaluated.

Due to the ineffectiveness of head-only electrical stunning of pigs in small slaughter establishments, a two-stage stunning method was proposed where head-only stunning for 3 s was immediately followed by application of the same stunning wand to the cardiac region of the animal for 3 s while lying in lateral recumbancy. A paired-comparison study was conducted on 89 pigs in a small slaughter facility to compare the head-only method applied for 6 s to the head/heart method. Head/heart stunning eliminated rhythmic breathing, natural blinking, eye tracking to moving objects, and righting reflex, which were all observed in head-only stunned pigs. Blood lactate was not different ($P > 0.05$) between stunning methods (head only: 8.8 ± .7 mmol/l, head/heart: 7.8 ± .7 mmol/l). Stun to bleed time did not differ ($P > 0.05$) (head only: 32 ± 1 s, head/heart: 33 ± 1 s). No heartbeat was observed with the head/heart method.

Longissimus thoracis pH, color, and drip loss were not different ($P > 0.05$) between stunning methods. This study determined that the head/heart electrical stunning method reduced the incidence of signs of return to sensibility without significant effects on meat quality, plant operation speed, or blood lactate concentration.

As concern toward the care afforded to animals on U.S. dairy farms increases, benchmark data and means of assessing the welfare status of the dairy industry are necessary to dovetail with existing National Animal Health Monitoring System and National Cattlemens' Beef Association National Non-fed Beef Quality Audits. The 3rd study was developed to explore the framework of a potential study to dovetail with these two programs and identify variables of relevance to such an analysis. A single Northern Colorado Dairy Herd enrolled in the Cooperatives Working Together (CWT) Dairy Herd Retirement program was selected for this pilot study. Overall, data suggested that risk factors for herd health issues exist based on lactation number, days in milk, daily milk production, and previous lactation 305 d milk. Our analysis showed that muscling and finish scores are potentially valuable tools for assessing body condition score in cows post mortem. Body condition score was different between daily production levels (low: 3.08, medium: 2.70, high: 2.51) ($P < 0.05$). The mean percentage of broken tails in the herd was 44.1% and the occurrence of tail breaks increased as cows became more lame ($P < 0.05$) and as lactation number increased ($P < 0.05$). Overall, this study demonstrated the importance of including welfare-relevant variables in assessing on-farm animal welfare that are not strictly restricted to production. The results of these studies indicate the importance of management in maintaining acceptable animal welfare in livestock production and processing facilities.

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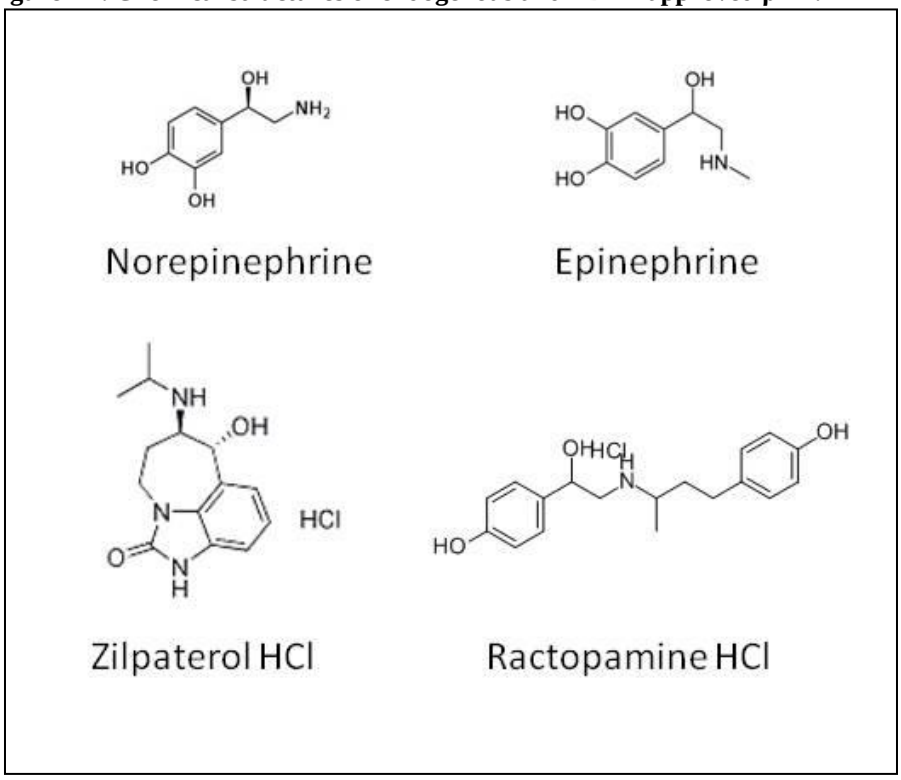
B-ADRENERGIC AGONIST MECHANISMS OF ACTION

Description of β – adrenergic receptor agonists

β – adrenergic receptor agonists (βAA) are a group of organic compounds, known as phenethanolamines, that trigger the activation of signaling pathways and their resultant physiological responses upon binding with β – adrenergic receptors (βAR). The major endogenous βAA are epinephrine and norepinephrine. Two βAA are currently approved by the FDA for use in feedlot cattle to improve growth performance and feed efficiency during the end of the finishing period. All four compounds can be observed in Figure I-1. Ractopamine hydrochloride was approved in June 2003 for feeding cattle in confinement during the final 28 – 42d of the finishing period at a rate of 10.0 – 30.0 ppm in feed on a 100% DM basis (FDA, New Animal Drug Application 141 – 221). Zilpaterol hydrochloride was approved in August 2006 for feeding cattle in confinement during the final 20 – 40d of the finishing period at a rate of 8.3 ppm in feed on a 100% DM basis followed by a minimum of 3d withdrawal before slaughter (FDA, New Animal Drug Application 141-258). Ractopamine HCl is approved for use in cattle and swine in the United States and is marketed by Elanco Animal Health under the trade names Optaflexx and Paylean, respectively. Zilpaterol is approved only for use in cattle and is marketed under the trade name Zilmaxx by Intervet, Inc.

Figure I-1. Chemical structures of endogenous and FDA - approved βAA.

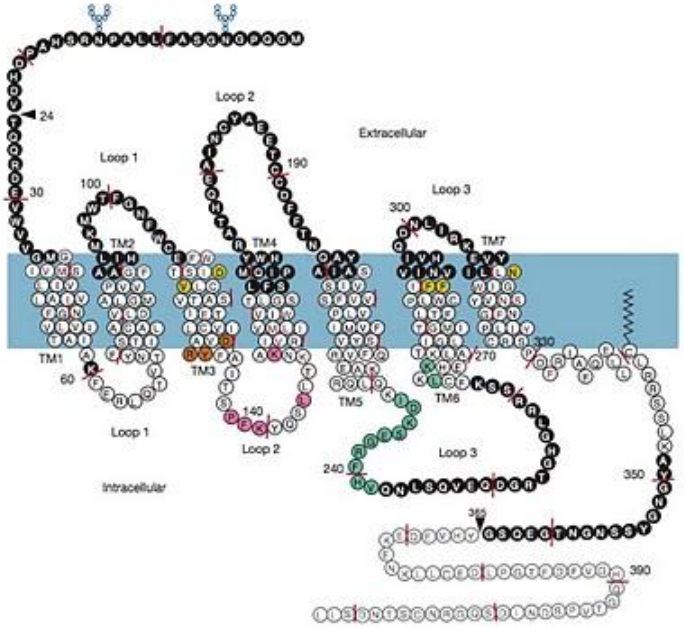


β – adrenergic receptors are located throughout the mammalian body (Mersmann, 1998). Three primary subtypes of βAR, and consequently βAA, have been identified as β_1, β_2, and β_3 (Johnson, 2006; Mersmann, 1998; Mills et al., 2003). Each type of βAR is classically associated with a specific type of tissue in humans (β_1, cardiac tissue; β_2, airway smooth muscle; β_3, adipose tissue), but interspecies differences have been identified (Hollenga et al., 1991; Johnson, 2006; Mills, 2003). Hollenga et al. (1991) concurred that the β_1 - and β_3 - AR played a similar role in rats, but recognized the β_2 - AR as a major affector of skeletal muscle instead of airway smooth muscle in rats. Hoey et al. (1995) recognized the β_2 - AR as the predominant βAR in bovine skeletal muscle. Mills et al. (2003) identified β_1- and β_2 – AR as major affectors of lipolysis in ractopamine supplemented swine. The authors concluded that the β_1 – AR was the primary regulator of βAA induced lipolysis in swine and that 95% of the βAR in swine

adipose tissue are β_1 and β_2. Interestingly, β_1 - and β_2 - AR were identified as the primary β - AR in ovine skeletal muscle (Ekpe et al., 2000). Clearly, prevalence of specific β - AR within tissues of different species is variable. Overall, it appears that a developing consensus regarding the specificity of the β_2 - AR to skeletal and smooth muscle has developed but the role of the β_1 - AR may be more closely associated with adipose tissue, cardiac tissue, skeletal muscle, or a combination of all three tissues, depending on the species in question.

The basic structure of a βAR can be observed in Figure I-2. All βAR are composed of 413 amino acids that form 7 transmembrane domains in α – helix conformation (Johnson, 2006; Rasmussen, 2007). Three intracellular and 3 extracellular loops connect the transmembrane domains (Johnson, 2006; Rasmussen, 2007). Loop 3 of the βAR contains the binding site for the G – protein complex (Lynch and Ryall, 2008). In addition, the βAR contains an extracellular amino–terminus and an intracellular carboxy-terminus (Johnson, 2006; Rasmussen, 2007). N-glycosylation at amino acids 6, 15, and 187 assist in receptor insertion into the plasma membrane (Johnson, 2006).

Figure I-2. Amino acid structure of β - AR (from Rasmussen et al., 2007).



Two primary forms of βAR exist *in vivo*, namely, the activated and inactivated forms (Johnson, 2006; Lynch and Ryall, 2008). The activated form of βAR is associated with the $G_{\alpha}s$ protein and guanosine triphosphate (GTP) (Figure I-3). The inactivated form occurs after the conversion of GTP to guanosine diphosphate (GDP) and the subsequent dissociation of $G_{\alpha}s$ with βAR (Figure I-4). It has been proposed that βAA cause a momentary stabilization of the activated form of βAR which allows dephosphorylation of GTP to occur (Johnson, 2006). Another hypothesis regarding the function of βAR states that β – adrenergic antagonist compounds bind the inactivated βAR because it is conformationally different than the activated form (Johnson, 2006). At first glance, this type of mechanism may appear to be competitive, but the difference in βAR receptor conformation and correlated βAA affinity does not provide the potential for competition between antagonists and agonists (Johnson, 2006). As a result, the affect of

βAA is dependent on βAR concentration within the membrane and the number of activated βAR available within a specific tissue.

Figure I-3. Activated form of βAR (From Lynch and Ryall, 2008).

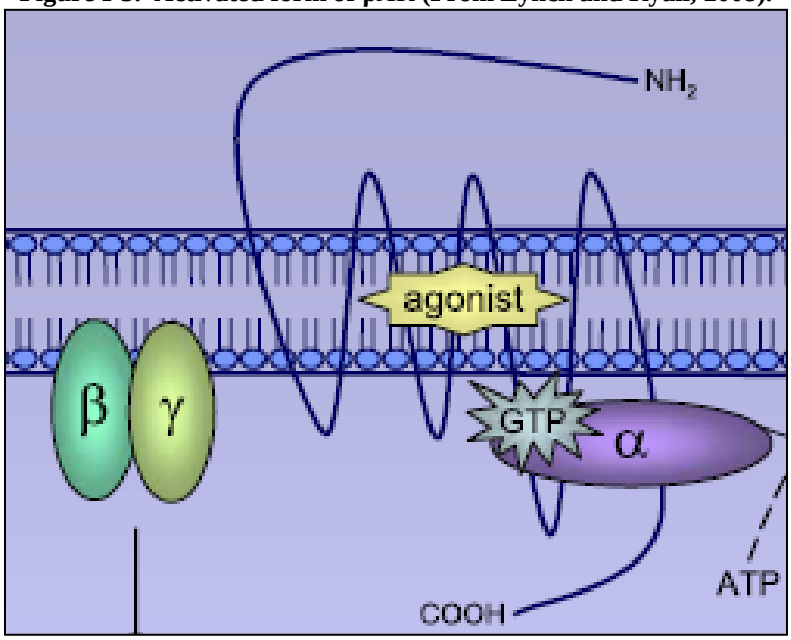
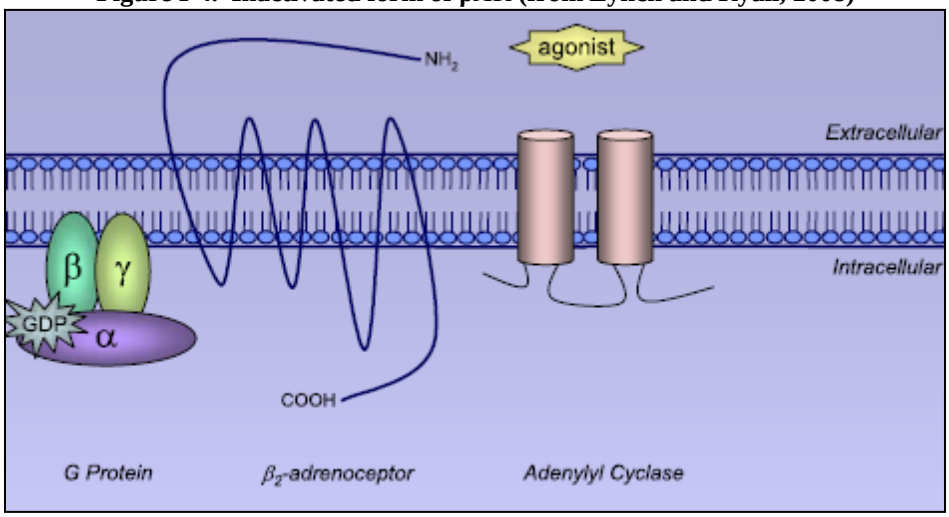


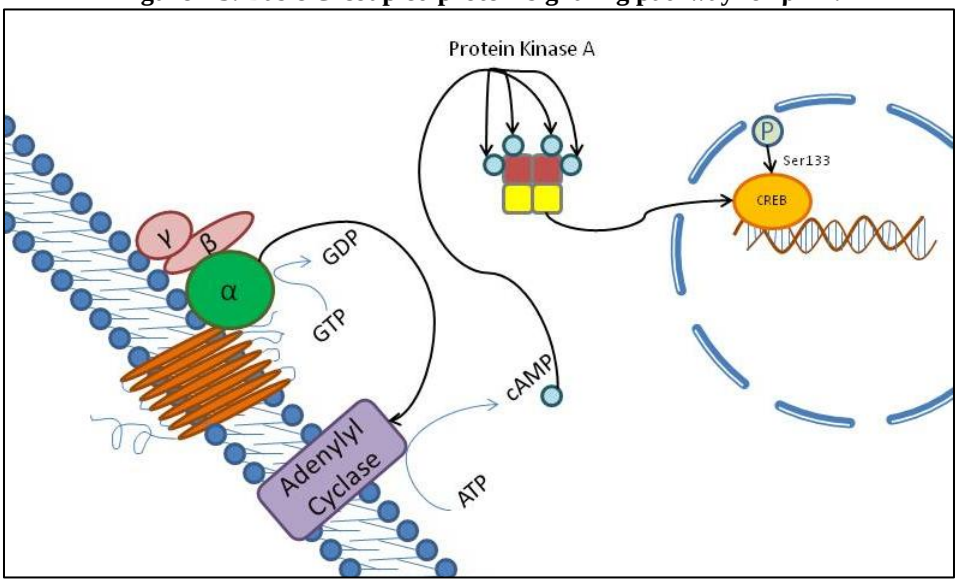
Figure I-4. Inactivated form of βAR (from Lynch and Ryall, 2008)



Modes of action

The predominant mode of βAA action is the G-coupled protein signaling cascade shown in Figure I-5. βAA interact with a binding site that has been well-described by Johnson (2006) and Lynch and Ryall (2008). At approximately 15 Å into the βAR from the amino terminus, amino acid residues 113 (aspartate), 204 (serine), 207 (serine), and 293 (asparagine), which are located on transmembrane domains 3, 5, 5, and 6, respectively, make up the primary binding sites for βAA (Johnson, 2006; Lynch and Ryall, 2008). The β – hydroxy group of the βAA side chain binds asn 293, the two serine residues of the βAR interact with the hydroxyl groups of the phenyl ring of the βAA, and asp 113 forms an ion pair with the the amino N of the βAA (Johnson, 2006). After ligand – receptor binding, dephosphorylation of GTP occurs, which causes the release of the G – protein complex from the βAR (Johnson, 2006; Mersmann, 1998). $G_{\alpha}s$ then activates adenylyl cyclase, which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (Johnson, 2006; Mersmann, 1998). Four cAMP bind the active sites on the regulatory subunits of protein kinase A (PKA). Following cAMP binding to the active sites of PKA, the catalytic subunits disperse and cause the phosphorylation of regulatory proteins within the cell and the phosphorylation of cAMP responsive element binding protein (CREB) (Johnson, 2006, Mersmann, 1998). CREB activates gene transcription in the nucleus of the cell by binding the cAMP response element in the regulatory part of a gene (Mersmann, 1998). Phosphorylation of CREB following binding increases transcriptional activity, which has the greatest impact on skeletal muscle (Lynch and Ryall, 2008; Mersmann, 1998).

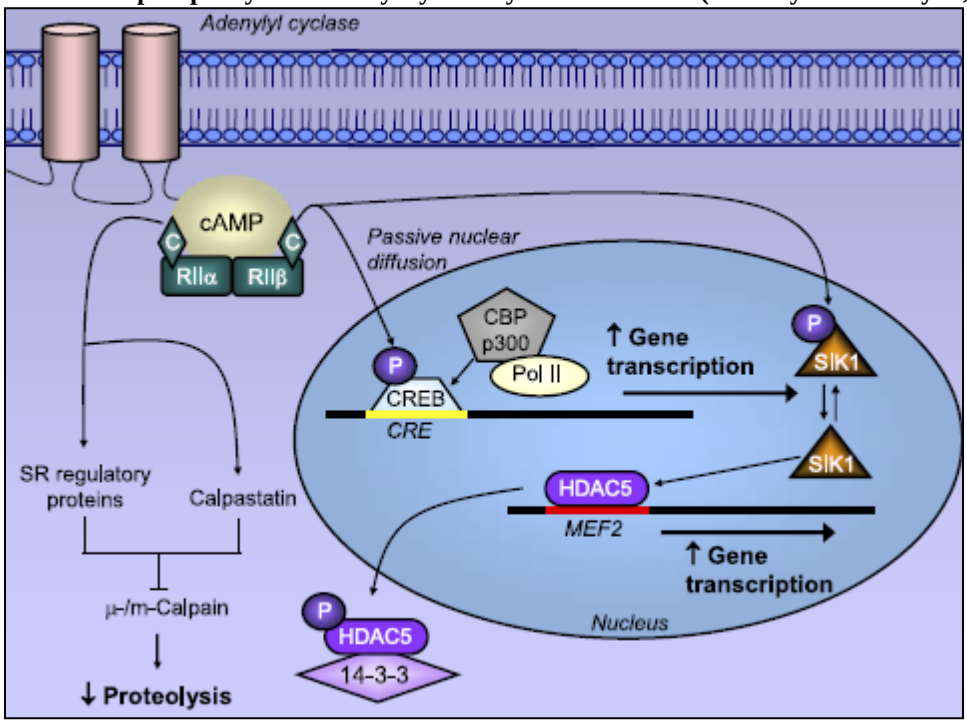
Figure I-5. Basic G-coupled protein signaling pathway for βAR.



Much of the current knowledge regarding the mechanism of action of βAA in skeletal muscle has been derived from work with cardiac muscle (Lynch and Ryall, 2008). Multiple signaling pathways occur in skeletal muscle that impact muscle growth, breakdown and, ultimately, muscle mass. The previously-described pathway involving CREB (Figure I-5) ultimately leads to increased gene transcription (Lynch and Ryall, 2008). A simple description of the gene transcription that occurs following the phosphorylation can be observed in Figure I-6. CBP and p300 are transcriptional adapters that that are activated following the phosphorylation of CREB and promote skeletal muscle myogenesis (Lynch and Ryall, 2008). These adapters also serve as a link between myogenesis and myocyte enhancers such as *MEF2* (Lynch and Ryall, 2008) through the production of salt-inducible kinase 1 (SIK1), which phosphorylates, and activates, HDAC5 (Lynch and Ryall, 2008). It is believed that HDAC5 ultimately activates *MEF2*, which further stimulates myogenesis (Lynch and Ryall, 2008). HDAC5

can be transferred to other nuclei within the myocyte by the chaperone protein 14-3-3 (Lynch and Ryall, 2008).

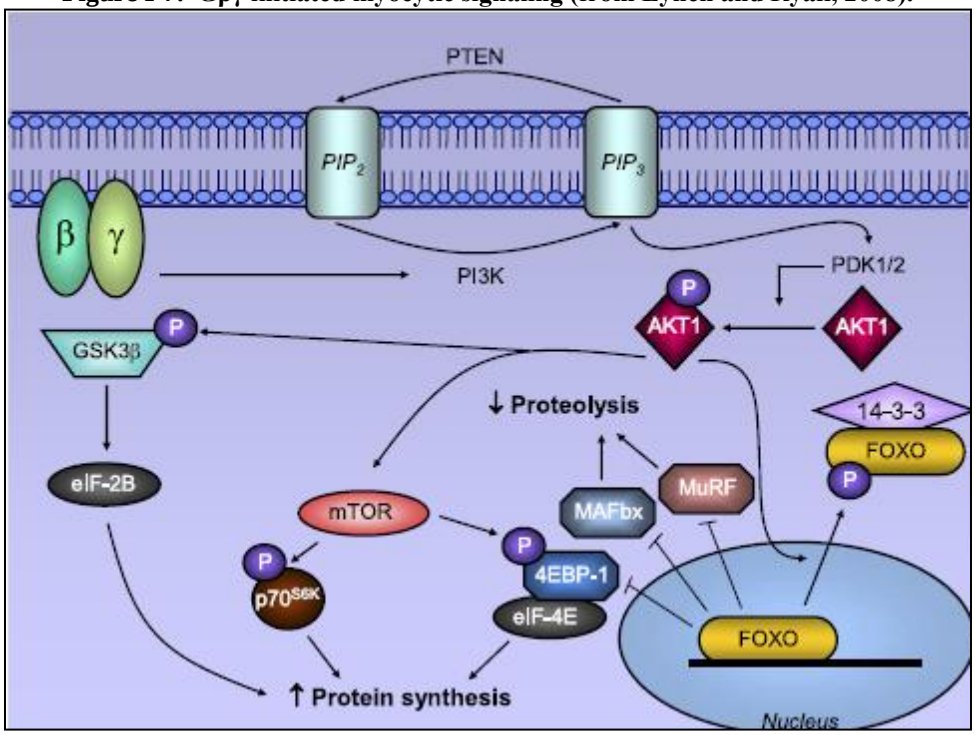
Figure I-6. cAMP phosphorylation of myocytic enzymes and CREB (from Lynch and Ryall, 2008).



In addition to the $G_{\alpha}s$ signaling cascade, the $G_{\beta\gamma}$ subunit also is responsible for myocyte signaling through the PI3K-AKT signaling pathway (Figure I-7) (Lynch and Ryall, 2008). Following activation of PI3K by $G_{\beta\gamma}$, PI3K phosphorylates PIP_2. PIP_2 serves as a binding site for lipids at the myocyte membrane (Lynch and Ryall, 2008). The formation of PIP_3 activates PDK, which in turn phosphorylates AKT1 (Lynch and Ryall, 2008). AKT1 is responsible for the phosphorylation of several signaling proteins, two of which are mTOR and FOXO (Lynch and Ryall, 2008). mTOR phosphorylates and inactivates 4EBP-1, which typically has an inhibitory impact on protein synthesis.

The translation and elongation of ribosomal proteins is controlled by $p70^{s6K}$, which is upregulated following phosphorylation by mTOR (Lynch and Ryall, 2008). AKT1 also phosphorlyates glycogen synthase kinase 3β (GSK3β), which inhibits eIF-2B, a transcription initiation factor, if it is not phosphorylated (Lynch and Ryall, 2008). FOXO is responsible for the production of MAFbx and muRF1, two proteins that signal for the conjugation of ubiquitin to muscle proteins and subsequent muscular atrophy (Lynch and Ryall, 2008). Phosphorylation of FOXO leads to the removal of FOXO from the nucleus by 14-3-3 and a decrease in proteolysis (Lynch and Ryall, 2008). It has also been shown that increased Ca release occurs following the activation of the $G_{\beta\gamma}$ subunit. Ultimately, the $G_{\beta\gamma}$ subunit is responsible for the facilitation of lipid uptake by myocytes as well as the decrease in proteolysis and increase in protein synthesis associated with βAA.

Figure I-7. Gβγ initiated myocytic signaling (from Lynch and Ryall, 2008).



Several enzymes are impacted by the activation of βAR (Mersmann, 1998), and the enzymes that are activated are tissue-dependent (Mills et al, 2003). In adipose tissue, the catalytic subunit of PKA is responsible for the phosphorylation of hormone sensitive lipase (Mersmann, 1998). Hormone sensitive lipase stimulates the degradation of stored triacylglycerol in the adiposite. In addition, PKA facilitates the phosphorylation of acetyl-Coenzyme A carboxylase, which is the rate-limiting enzyme for long-chain fatty acid synthesis (Mersmann, 1998). In summary, βAA appear to affect adipose tissue through at least two mechanisms: the stimulation of lipolysis and the inhibition of lipogenesis. In skeletal muscle tissue (Figure I-6), βAA trigger the phosphorylation of calpastatin, which prompts the inhibition of μ- and m- calpains, which are responsible for Ca – dependent proteolysis of the Z-disc of skeletal muscle (Lynch and Ryall, 2008).

The overall impact of βAA supplementation on skeletal muscle and adipose tissue, the two major tissues of economic concern in food animal species has now been characterized. In skeletal muscle, the ultimate result of βAA supplementation is muscle hypertrophy, or increase in size without myocyte proliferation. Increased protein synthesis as well as decreased proteolysis ultimately leads to a net increase in muscle size. In adipose tissue, βAA have an inverse affect as they inhibit lipogenesis, which is the production of new adipose tissue, and stimulate lipolysis, which is the breakdown of existing adipose stores to supply energy to other tissues, such as skeletal muscle. Research colleagues in the Center for Meat Safety & Quality have reported observational evidence of increased purge loss during the cooking of steaks from βAA treated beef cattle. Further research is warranted to determine the exact mechanism of the observed

purge production, but it is possible that the purge could be an indicator of the production of metabolic water as a byproduct of β-oxidation of lipid in the muscle cell.

B – ADRENERGIC AGONIST IMPACT ON BEHAVIOR

The current pool of literature regarding the behavior of livestock that have been supplemented with βAA is limited, with fewer studies focused on cattle than swine. Of these studies, all documented the impact of ractopamine supplementation on swine or bovine behavior and none investigated the impact of zilpaterol supplementation on bovine behavior. It is likely that more examinations of the impact of βAA on livestock behavior will occur soon as livestock behavior and welfare has become an increasingly popular area of research.

The impact of ractopamine supplementation on the behavior of feedlot cattle of British, Continental, and Brahman genetic influence was investigated by Baszczak et al. (2006). In the study, the authors compared the impact of ractopamine supplementation at a rate of 200 $mg \cdot steer^{-1} \cdot d^{-1}$ for the final 28 d of finishing before slaughter to unsupplemented cattle. Subjective scores of chute entry force, chute entry speed (1 = walk, 2 = trot, 3 = run), chute behavior score, and chute exit speed (1 = walk, 2 = trot, 3 = run) were collected on the final weight collection day before the cattle were shipped for slaughter. No impact of ractopamine supplementation was recognized on these measures of behavior with the exception of chute entry speed score, which was 1.4 ± 0.1 and 1.5 ± 0.1 for control and supplemented cattle, respectively. However, the authors concluded that such a difference, although statistically significant, was not practically significant. In the same study, the authors found that chute entry force score was greatest in the cattle of predominant Continental influence (2.8 ± 0.2) and not different between British (2.4 ± 0.2) and Brahman influence (2.1 ± 0.2) cattle. Additionally, Brahman influence cattle and Continental influence cattle displayed greater chute exit speed scores (2.1 ± 0.2 and

1.9 ± 0.2, respectively) than British cattle (1.5 ± 0.2) (Baszczak et al., 2006). The authors concluded that chute exit speed appeared to be an effective means of assessing the temperament of feedlot cattle.

More extensive work regarding the interaction of ractopamine supplementation and animal behavior has been performed in swine. Schaefer et al. (1992) observed that pigs supplemented with 10 to 20 ppm ractopamine for 5 to 6 weeks before behavioral assessment lied down in groups more frequently and walked around in their pens less frequently than unsupplemented pigs. The authors did not observe any abnormal, stereotypical, agonistic, or aggressive behavior in the pigs in the study, and they concluded that ractopamine did not significantly impact pig behavior.

On the contrary, Marchant-Forde et al. (2003) reported that pigs supplemented with 10 ppm ractopamine over the final 4 weeks of finishing took 136% longer to remove from their home pens, 83% longer to load onto a weighing scale, and required 52% more pats, slaps, and pushes from the handlers to enter the scale than unsupplemented contol pigs. In addition, 51% fewer ractopamine supplemented pigs would voluntarily leave their home pen in comparison to unsupplemented control pigs (Marchant-Forde et al., 2003). The authors also cited greater amounts of time spent active, alert, and lying in sternal recumbancy by the supplemented pigs than the unsupplemented pigs during the first two weeks of supplementation. During the first week of the study, it was also observed that ractopamine supplemented pigs took longer to lie down after a behavioral disturbance, such as a fight, than unsupplemented pigs (Marchant-Forde et al., 2003). This result may indicate that ractopamine supplementation increases the amount of time

that is necessary for a pig to settle down after a behavioral disturbance, but only until the animal becomes acclimated to the βAA.

Marchant-Forde et al. (2003) included a logically-sound explanation for the disparity with Schaefer et al. (1992) wherein they explained that the length of βAA supplementation prior to behavioral assessment likely played a role the difference in results. It has been shown that βAR downregulation occurs with increasing length of βAA supplementation (Lynch and Ryall, 2008). Marchant-Forde et al. (2003) observed the pigs once per week during the supplementation period and reported that the significant differences in time budgeting between supplemented and control pigs had disappeared by the fourth week of their study. Since Schaefer et al. (1992) only observed their pigs on the 5^{th} week of their study, they likely missed the behavioral responses to βAA supplementation as there was ample time for βAR downregulation to occur before observations were made.

Polletto and others (2009, 2010a, 2010b) have performed the most comprehensive peer-reviewed series of studies of the impact of ractopamine supplementation on the behavior of finishing pigs to date. The first study (Poletto et al., 2009) investigated the impact of ractopamine feeding in a "step up" program (5mg/kg for 2 wk followed by 10mg/kg for 2 wk), sex, and social rank on growth performance, hoof lesions, and enteric bacteria shedding on finishing pigs [(US Duroc × Hampshire) × (US York × Landrace)]. In this particular study, the impact of ractopamine feeding, sex, and social rank were reported individually, and the interaction of ractopamine feeding and social rank on average daily gain (ADG), body weight (BW), and gain : feed (G:F) were not reported. With regard to social rank, dominant pigs (0.915 ± 0.02 kg/d) displayed less ADG ($P <$

0.05) than intermediate (0.998 ± 0.02 kg/d) and subordinate pigs (0.973 ± 0.02 kg/d), which were not different ($P > 0.05$) (Poletto et al., 2009). Dominant pigs also developed more hoof lesions than subordinate pigs [front hoof cracks and erosions: 2.19 ± 0.24% vs. 1.44 ± 0.24%; rear hoof splits: 1.94 ± 0.24% vs. 1.19 ± 0.24%; total rear hoof lesions: 5.94 ± 0.37% vs. 4.81 ± 0.37%), which is indicative of differences in hoof horn growth and potential differences in the frequency and amplitude of aggressive movements that may cause hoof damage. The authors concluded that dominant pigs were more likely to engage in agnostic behaviors, such as fighting, which are energy-expensive. In addition, it is likely that intermediate and subordinate pigs moved less throughout the pen to avoid potential interactions with dominant animals. The decreased movement would result in less energy expenditure and the potential for more energy availability for growth as well as less risk of hoof injury.

Poletto et al. (2010a) studied the same group of pigs used by Poletto et al. (2009) with the objective of ascertaining the effects of ractopamine supplementation (5mg/kg for 2 wk followed by 10mg/kg for 2 wk), social rank, and sex on agnostic interactions (biting, head knocks, and pursuits), time-budget behavioral activity, and peripheral concentrations of norepinephrine, epinephrine, dopamine, serotonin, and tryptophan in finishing pigs (Poletto et al., 2010a). The peripheral concentrations of amines will be discussed in the catecholamine section of this literature review. The authors used 10-min instantaneous scan sampling on every 3rd day of the study to assess time budgeting in the top dominant and bottom subordinate pigs. Pigs that were supplemented with ractopamine spent a lesser percentage of their time inactive (73.9 ± 1.8%) than unsupplemented pigs (77.8 ± 1.8%) ($P < 0.05$) (Poletto et al., 2010a). Ractopamine

supplemented pigs spent more time alert (4.4 ± 0.6% vs. 2.2 ± 0.6%), more time biting the bars of the pen (0.5 ± 0.0% vs. 0.3 ± 0.0%), more time sham chewing (2.7 ± 0.2% vs. 2.0 ± 0.2%), and more time feeding (6.2 ± 0.3% vs. 5.3 ± 0.3%) than unsupplemented pigs, respectively ($P < 0.05$) (Poletto et al., 2010a). No difference ($P > 0.05$) was observed in the percentage of time pigs spent in a standing posture, but ractopamine supplemented pigs spent more time sitting (2.4 ± 0.4% vs. 0.9 ± 0.4%) and less time lying (81.7 ± 0.9% vs. 84.0 ± 0.9%) than unsupplemented controls ($P < 0.05$), respectively (Poletto et al., 2010a). In summary, pigs that were supplemented with ractopamine were more active, showed more incidence of stereotypical behavior (bar biting and sham chewing, spent more time feeding, more time sitting, and less time lying down than unsupplemented controls. In their earlier study, Poletto et al. (2009) reported lighter body weights and less ADG in ractopamine supplemented pigs than unsupplemented controls. The results of Poletto et al. (2010a) suggest that those results were at least in part a consequence of increased activity to the degree that the observed increase in feeding time in ractopamine supplemented pigs could not offset the increased activity level.

The third study conducted by Poletto et al. (2010b) was an investigation of the effects of ractopamine supplementation (5mg/kg for 2 wk followed by 10mg/kg for 2 wk), sex, and social rank on the aggressiveness of pigs assessed by the resident – intruder test. The authors also assessed the concentrations of catecholamines, dopaminergic metabolites, and serotonin and its metabolite in the amygdala, frontal cortex, raphe nuclei, and hypothalamus of the pigs. The pigs in this study were the same as used previously by Poletto et al. (2009 and 2010a). Using the resident – intruder test, a

behavioral test of aggression where an unfamiliar pig (the "intruder") is introduced to a section of the home pen of another pig (the "resident") and the amount of time until an attack occurs is recorded, it was determined that resident, ractopamine supplemented, dominant and subordinate gilts performed more attacks (biting the intruder pig) at the 30 s timepoint than any other subgroup combination of supplementation, sex, and social rank (Poletto et al., 2010b). Ractopamine supplemented, subordinate gilts also showed the greatest percentages of attacks to intruder gilts at the 90, 180, and 300 s time points (Poletto et al., 2010b). The results of the resident-intruder tests performed by Poletto et al. (2010b) can be observed in Table I-1.

Table I-1: Cumulative percentage of resident pig attacks (i.e., bites) and nonattacks during the 300 s of resident-intruder tests (from Poletto et al., 2010b).

Item[1]	Resident pig	Social rank	Cumulative attacks by resident pigs, %			
			30 s	90 s	180 s	300 s[2]
Treatment						
CTL	Barrow	Dominant (n = 4)	8.3^{bc}	33.3^{b}	62.5^{a}	66.7^{ab}
CTL	Barrow	Subordinate (n = 4)	16.7^{b}	25.0^{b}	45.8^{b}	54.2^{b}
CTL	Gilt	Dominant (n = 4)	20.8^{b}	50.0^{ab}	79.2^{a}	91.7^{a}
CTL	Gilt	Subordinate (n = 4)	0.0^{c}	8.3^{c}	37.5^{b}	45.8^{b}
RAC	Barrow	Dominant (n = 4)	16.7^{b}	45.8^{ab}	62.5^{a}	79.2^{a}
RAC	Barrow	Subordinate (n = 4)	0.0^{c}	16.7^{bc}	37.5^{b}	45.8^{b}
RAC	Gilt	Dominant (n = 4)	37.5^{a}	54.2^{ab}	62.5^{a}	75.0^{ab}
RAC	Gilt	Subordinate (n = 4)	41.7^{a}	70.8^{a}	75.0^{a}	79.2^{a}
Pooled SEM, %			5.5	6.9	5.4	6.2

$^{a–c}$Mean percentages within columns with unlike superscript letters are statistically different ($P < 0.05$); dietary treatment x sex x social rank, $P < 0.05$.
[1]Dietary treatment: CTL = control; RAC = ractopamine-fed. The diet with RAC (ractopamine hydrochloride, Paylean, Elanco Animal Health, Greenfield, IN) added was delivered as 5 mg/kg for 2 wk, followed by 10 mg/kg for an additional 2 wk.
[2]Resident attacks during resident-intruder tests 2 and 3 did not differ ($P > 0.05$). The percentage of nonattacks for each subgroup can be calculated by taking the difference of the cumulative percentage of attacks at 300 s from 100%.

Interestingly, Poletto et al. (2010a) recognized a 64.5% decrease in agonistic interactions between ractopamine supplemented gilts, but an increase of 334.6% in

pursuits and an increase of 95.7% in bites per agonistic interaction in comparison to pre-trial baseline interactions. In essence, ractopamine supplemented gilts fought less often over time, but they became more aggressive during the fights. In Poletto et al. (2010b), the authors explained that much of increased aggression in gilts was perhaps linked to a significant decrease in serotonin concentration in the amygdala of the brain, a brain region that has been identified as important in the control of aggression. In addition, the authors found that ractopamine supplemented gilts had greater dopamine turnover rates and lesser 5-hydroxyindoleacetic acid (a metabolite of serotonin) concentrations in the amygdala (Poletto et al., 2010b), which was an important part of the aggression circuit in the brain (Nelson and Trainor, 2007).

In conclusion, it appears that ractopamine causes increased aggressive behavior in female swine, as well as increased alertness, and increased activity in pigs. In cattle, little affect from ractopamine on behavior during handling has been recognized. However, the current body of literature is limited. As more investigations regarding the impact of β - AA supplementation on livestock behavior come into publication, we will gain a firmer understanding of the implications of including synthetic β - AA in the diets of domestic livestock.

BIOLOGICAL OVERLOAD IN LIVESTOCK

Until recently, the focus of genetic selection and management in livestock has been to achieve maximal efficiency in production of economically relevant tissues. Due to the major adaptive capacity of domestic livestock animals, meat, milk, and egg yields have responded favorably to this selection goal. Unfortunately, the capacity of livestock to produce meat, milk, and eggs is limited by the physiological capacity of the animal to

consume and process feedstuffs into these tissues while maintaining normal physiological and metabolic functions. 'Biological system overload' describes a condition characterized by the inability of an animal to maintain normal biological functions as a result of energy partitioning to production. Biological system overload has been recognized as a result of genetic selection of poultry and swine for lean meat yield and rapid growth. It appears that the heavy emphasis on production that is a hallmark of the U.S. dairy industry may have placed the U.S. dairy herd on a path toward biological system overload. In addition, the use of aggressive growth promotant strategies and high-performance feedstuffs in feedlot cattle may contribute to the occurrence of biological system overload in cattle during feeding for slaughter. The following sections serve to explore the evidence for biological system overload in modern dairy, poultry, and swine production systems.

DAIRY

Over the past 30 years, individual dairy cow production has increased substantially. During the period from 1980 to 2000, average U.S. milk production per cow increased 53.3% (1980: 5397.7 kg/yr, 2000: 8274.5 kg/yr) (NASS, 1981; NASS, 2000). An additional increase of 12.8% per cow was recognized between 2000 and 2010 (2010: 9,333.1 kg/yr) (NASS, 2010). The production efficiency that have been gained through genetic selection, improved nutrition, and changes in management practices has come largely through advances in scientific knowledge of animal breeding, physiology, metabolism, and nutrition. The goal of this research was primarily focused on maximizing immediate productivity. Although the goal of maximal production was malicious, actually quite the contrary, it appears that modern production levels may be taxing the metabolic and physiologic capacities of multiple food animal species. As a

result, some food animals may be producing at levels that are detrimental to maintaining animal health and longevity without stringent management practices.

Increasing occurrence of metabolic and conformational disorders in dairy cattle:

Over the 10 year period between National Animal Health Monitoring System (NAHMS) dairy industry surveys, increased incidence of mastitis, reproductive problems, lameness, and cow mortality were detected (NAHMS, 1996; NAHMS, 2007). Other studies have identified genetic selection, management, nutrition, and environment as influential factors in the incidence of health and welfare issues in modern, high-producing dairy cattle. Although factors have been identified that influence specific issues in dairy cattle health and welfare, the need exists to integrate the current body of knowledge related to dairy cattle health and welfare to identify common contributors to the incidence of the most prevalent dairy cattle health and welfare issues. Identification of the common factors in these issues will assist in directing the focus of future research and management decisions relative to dairy cattle welfare.

The most common producer-identified cow health problems in the 1996 NAHMS survey were clinical mastitis (13.4 ± 0.3%), reproductive problems (11.6 ± 0.3%), and lameness (10.5 ± 0.3%) (NAHMS, 1996). In the 2006 NAHMS survey, the three most common producer-identified dairy health problems were clinical mastitis (16.5 ± 0.5%), lameness (14.0 ± 0.4%), and infertility problems (12.9 ± 0.3%) (NAHMS, 2007). Due to the common occurrence of these health conditions, udder or mastitis problems, lameness or injury, and reproductive problems accounted for 23.0 ± 0.6, 16.0 ± 0.4, and 26.3 ± 0.4 percent of culled dairy cattle during 2006, respectively (NAHMS, 2007). Within the population of cows that were sold for slaughter in 1995, the most common reasons for

culling were reproductive problems (26.7 ± 0.8%) and udder or mastitis problems (26.5 ± 0.5%), followed by poor production not related to disease, mastitis, or lameness and injury (22.4 ± 0.8%), and lameness or injury (15.0 ± 0.6%) (NAHMS, 1996). The most common specific producer-attributed causes of death were calving problems (18.3 ± 0.7%), other known causes that were unspecified (17.0 ± 0.9%), mastitis (16.3 ± 0.8%), unknown causes (14.8 ± 0.8%), and euthanasia due to lameness and injury (12.7 ± 0.7%) (NAHMS, 1996). In 1995, 3.8 ± 0.1% of dairy cows died and 24.0 ± 0.4% were culled. In 2006, 13.0 ± 0.9 percent of dairy cows died on-farm and 23.6 ± 0.4 percent were culled. A survey of 153 U.S. dairies reported 34 ± 1% of cows were removed from their respective herds in 2004 (Caraviello et al., 2006).

In the 2006 NAHMS survey, the causes of death were in line with the most common health problems, including lameness or injury, mastitis, and calving problems. These conditions accounted for 20.0 ± 0.8, 16.5 ± 0.7, and 15.2 ± 0.7 percent of cow deaths, respectively (NAHMS, 2007).

The underlying factors in causing lameness, mastitis, infertility and mortality vary widely, but a growing consensus has developed regarding the importance of management, environment, and genetic predisposition in the occurrence of such conditions (Cook and Nordlund, 2009; Dechow and Goodling, 2008; Dobson et al., 2008; Evans et al., 2006, Green et al., 2008; Hadley et al., 2006; Hansen, 2000). The conclusions of the previously listed studies consistently identified not one, but multiple, underlying factors that culminated in the one common health problem under investigation. Little consensus has been established regarding the respective roles of management, environment, and genetic predisposition in the causation of these specific

health disorders in dairy cattle. More research is necessary to isolate the role of intensive genetic selection in the development of biological system overload in modern dairy cows, but the experience of domestic food animal species with shorter generation intervals can serve as a guide to prevent similar detriment to animal health and consumer perception for the dairy industry.

POULTRY

Broiler chickens and turkeys have been intensively selected for increased growth rate and breast muscle size (Velleman et al., 2003). Such selection has been highly successful in its objective as bird growth rate has quadrupuled since 1970 (Emmans and Kyriazakis, 2000). Unfortunately, single trait selection in turkeys and broiler chickens has yielded multiple meat quality defects that are resultant of biological system overload. Some of these conditions include focal myopathy and pale, soft and exudative (PSE) muscle. Focal myopathy occurs as a result of limited space within the muscle fascia during rapid muscle growth in poultry (Martindale et al., 1979). The spatial limitation limits blood flow to the muscle capillaries, resulting in ischemia. This condition is exacerbated by exercise and results in the development of necrosis within the muscle tissue (Julian, 2005; Martindale et al., 1979; Sosnicki et al., 1991). Due to the rapid rate of hypertrophy in the pectoral muscle of broiler chickens and turkeys, focal myopathy is typically observed in breast meat (Martindale et al., 1979). PSE breast meat has been commonly observed in both broilers and turkeys, but the mechanism that causes the condition is not well known (Barbut et al., 2008; Solomon et al., 1997). There is consensus in the literature that focal myopathy and PSE are directly related to increased

selection for growth rate and body weight in commercial poultry (Julian, 2005; Solomon et al., 1997; Velleman et al., 2003).

Skeletal deformities also have been recognized as a result of selection for rapid growth and muscle deposition in poultry. Julian (2005) provided a useful guide in the identification of several skeletal conditions associated with rapid growth in poultry. Spondylolisthesis, a dislocation of the vertebral column commonly observed at the 4th thoracic vertebra, is a condition that results from the interaction of rapid growth and genetic susceptibility for the condition. It has been found in 11.4% of commercial broiler flocks (McNamee et al., 1998). Tibial dyschondroplasia also appears to have a genetic etiology that is exacerbated by rapid growth (Fanatico et al., 2008; Julian, 2005). In this condition, growth plate cartilage fails to mature at a rapid enough rate to support bone formation during skeletal growth. As a result, a cartilaginous mass develops at the growth plate and bones become prone to malformation and breakage due to the heavy musculature of the growing bird (Julian, 2005). It is estimated that 30 to 50% of male poultry raised for meat production develop dyschondroplasic lesions (Julian, 2005).

Fortunately, the influence of genetic selection in poultry is recognized rapidly due to the relative brevity of generation intervals when compared with other domestic species. Selection for rapid growth has been very successful in poultry species, but continued improvement in growth rate without regard for animal health will inevitably lead to continued biological system overload to the point of system failure. The broiler and turkey industries must continue to improve the capacity of birds to adapt to rapid growth and not just rapid growth alone. In order for this to occur, the goal of poultry

breeders must shift from creating genetic lines that are capable of maximal production to lines that achieve optimal production that balances bird welfare and production level.

SWINE

Similar to poultry, genetic selection for increased lean muscle deposition in swine has been highly successful. Drastic increases in muscle mass are typically associated with an increase in the proportion of fast-contracting glycolytic muscle fibers in lean tissue (Solomon et al., 1998). Heavily muscled pigs are typically more susceptible to the influence of handling, transport, and pre-slaughter stress than their lighter-muscled counterparts (Fábrega et al., 2002). As a result, very rapid post-mortem metabolic rates have been observed in stressed, heavily-muscled pigs (Webb and Casey, 2010). Such rapid post-mortem metabolism generally yields pale, soft, and exudative (PSE) pork (Sosnicki et al., 1998; Webb and Casey, 2010). PSE pork is not a favorable raw material for further processing due to its inability to retain moisture (Band et al., 2005; Leach et al., 1996). As a result, intensive selection for muscling without regard for meat quality and structural soundness is counterproductive due to lapses in meat quality and animal welfare (Band et al., 2005; Fábrega et al., 2002).

Fortunately, the pork industry has made major advances in the reduction of PSE pork in recent years (Barbut et al., 2008). The driving force behind the acknowledgement of meat quality as a relevant trait in pig selection has been consumer demand for meat quality (Barbut et al., 2008). Additionally, the focus of swine genetics companies on elimination of the RN^- and HAL-1843^{TM} genes has virtually eliminated PSS from commercial pork production strains (Barbut et al., 2008). The elimination of these genes has improved the biological well-being of commercial swine by greatly reducing the

negative impact of handling and transport stress on physiological well-being. Through evaluation of thousands of pigs each year as an animal welfare auditor, the second author has observed improvements in pig conformation and mobility over the past decade. The rapid advances in pork quality and reduction of biological system overload in commercial swine have been achieved largely in part by the relatively short generation interval of swine, which make them highly receptive to rapid genetic change.

IMPLICATIONS OF BIOLOGICAL SYSTEM OVERLOAD

Over the past half-century, the U.S. swine and poultry industries have gone through periods of intensive genetic selection for heavily-muscled, lean, high-yielding animals and selection to correct overselection for economically relevant traits, typically associated with yield, as consumer demand shifted toward improved product quality. The level of genetic improvement relative to lean muscle yield and growth rate that has been displayed by the pork and poultry industries is astounding and very well intentioned. However, the negative impact of single-trait selection for growth and yield on the capability of the biological system of the animal to compensate for the increased metabolic and physiologic loads associated with such change has been recognized. This point was well characterized by Emmans and Kyriazakis, 2000:

> "Where there has been selection for the yield of one part, such as breast meat in turkeys…, it follows in a logical, but not always appreciated, way that there will necessarily be a reduction in one, or more, other parts at a given weight. While it is possible to predict that, in the long run, the results of such selection, if

effective, will be disastrous, it is always difficult to predict when the long run will arrive, and whether the disaster will be gradual or abrupt."

The dairy industry appears to be following a similar path to that of the pork and poultry industries. Over the past decade, per-cow milk production has continued to increase along with the occurrence of metabolic and reproductive disorders and mastitis. Additionally, cow mortality has increased significantly over the past decade. Just as the pork and poultry industries have begun to acknowledge, the dairy industry must recognize that the production potential of the modern dairy cow may exceed her ability to sustainably withstand the stress of such high production. As a result, the occurrence of reproductive inefficiency, metabolic disease, and mortality are likely to continue in dairy cows unless the single–trait focus for milk production continues to be reduced and selection for traits that are relevant to the sustainability of the animal becomes a common part of producer selection practices.

Chapter II : Assessment of implant strategy and β-adrenergic agonist supplementation effects on the growth performance, physiological, biochemical, and behavioral responses of feedlot steers.

INTRODUCTION

Substantial literature has evolved describing the beneficial gains in feedlot cattle efficiency and productivity when growth promoting technologies, such as β adrenergic agonists (βAA) are included in feed supplements (Bryant et al., 2010; Delmore et al., 2010; Montgomery et al., 2009; Parr et al., 2011; Scramlin et al., 2010; Woerner et al., 2011). Two βAA are currently approved by the FDA for use in feedlot cattle during the finishing period. Ractopamine hydrochloride was approved in June 2003 for feeding cattle in confinement during the final 28 – 42d of the finishing period at a rate of 10.0 – 30.0 ppm in feed on a 100% DM basis (FDA, New Animal Drug Application 141 – 221). Zilpaterol hydrochloride was approved in August 2006 for feeding cattle in confinement during the final 20 – 40d of the finishing period at a rate of 8.3 ppm in feed on a 100% DM basis followed by a minimum of 3d withdrawal before slaughter (FDA, New Animal Drug Application 141-258). The use of βAA supplements is still in its infancy in North American cattle feeding operations and, as a result, the body of literature regarding the function and implications of using these products is not fully understood. Multiple studies have recognized decreased tenderness in fresh beef as a side effect of βAA supplementation (Delmore et al., 2010; Woerner et al., 2011), but increases in feed efficiency and lean meat yield are conducive to trading off some product quality. Up to this point in time, limited research data have been accumulated to identify physiological, metabolic, and/or behavioral gains or losses that may accompany the use of βAA supplements. Due to the commonality of growth promoting implants in commercial cattle feeding, an investigation of the potential interaction effects of feeding ractopamine hydrochloride or zilpaterol hydrochloride to cattle that have been treated with growth

promoting implants also deserves research attention. Therefore, the current study was developed to investigate the physiological, metabolic, and behavioral responses of feedlot steers to ractopamine hydrochloride or zilpaterol hydrochloride supplementation with or without an aggressive use of terminal implants.

MATERIALS AND METHODS

Animal use protocol

All procedures involving live animals in this study were approved by the Colorado State University Animal Care and Use Committee. The approved protocol number was 10-1788A.

Description of animals

In April 2010, 478 yearling steers (BW = 382.6 ± 2.7 kg) of predominant British phenotypic influence were received at a Colorado feedlot from two sources. All steers were provided *ad libitum* access to long-stem grass hay and water upon arrival. Two to 3 d later, all cattle were treated for internal parasites (Noromectin®, Noorboork Laboratories Ltd, Northern Ireland; Panacur®, Intervet / Schering Plough Animal Health, Inc., Millsboro, DE) and vaccinated for IBR-BVD (Express 3, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) following label dosage indications. Within each source group of cattle, a long-acting terminal implant containing 200 mg of trenbolone acetate and 40 mg of estradiol (Revalor-XS, Intervet / Schering-Plough Animal Health, Inc., Millsboro, DE) was administered to the left ear of every other group of 20 steers that were processed. No existing implants were detected during palpation of both ears of each steer. Steers that did not receive the terminal implant were not sham implanted.

Following initial processing, all steers remained separated by implant treatment. In mid - May 2010, all steers were moved to the Southeast Colorado Research Center (SECRC) feedlot, where they were sorted into 10-hd pens. All steers remained separated by implant treatment for the remaining time on feed at SECRC.

Treatment description

A 2 × 3 factorial arrangement in a randomized complete block design was utilized to determine the effects of 2 growth-promoting implant strategies and 3 types/levels of β-adrenergic agonist supplementation. Growth-management treatments included: 1) **No IMP – No βAA** – steers received no βAA or growth promoting implants; 2) **IMP – No βAA** – steers received an aggressive terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS) but no βAA; 3) **No IMP – ZIL** – steers were not treated with a terminal implant but were supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3 d withdrawal; 4) **IMP – ZIL** – steers received an aggressive terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS) and were supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 days during the final 23 d of finishing with a 3 d withdrawal; 5) **No IMP – RAC** – steers were not treated with a terminal implant but were supplemented daily with ractopamine hydrochloride (200 mg/animal/d) during the final 28 d of finishing; and 6) **IMP – RAC** - steers received an aggressive terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS) and

were supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for 20 d during the final 23 d of finishing with a 3 d withdrawal prior to slaughter.

Treatment allocation

In early August 2010, all steers were individually weighed and breed type data were collected for all steers. All animals were then returned to their home pens. All steers that weighed greater than or less than 2 SD of the mean or displayed *bos indicus* breed characteristics were automatically excluded from the study. Of the remaining steers, 432 were blocked by repetition (8 repetitions were assigned to account for body weight variation and slaughter day variation) and assigned to 48 - 9 animal pens with coat color stratified within each pen. Twenty four of the pens contained implanted cattle and 24 pens contained non-implanted cattle. The 6 treatment levels of the 2 X 3 factorial design were applied to the 48-pens so that 8 pens would represent each treatment combination (n = 8).

A day later, the steers selected to remain in the study were reweighed, and an ear tag and electronic ID were placed in the right ear of each steer. The steers were then sorted into their respective pens and allowed to acclimate to the new environment for 20d before the commencement of β-adrenergic agonist supplementation.

Diets

During the final 28d of finishing, all pens of cattle received one of three diets (Table II-1): Control (no added β – adrenergic agonist supplement); RAC (diet formulated to deliver 200 $mg \cdot hd^{-1} \cdot d^{-1}$ based on 9.55 kg of dry matter intake per animal per day); ZIL (diet formulated to deliver 75 $mg \cdot hd^{-1} \cdot d^{-1}$ based on 9.55 kg of dry matter intake per animal per day). The Control diet was fed twice daily (60% delivery on the

first daily feeding, 40% delivery on second daily feeding) to all pens of cattle on the study prior to the commencement of ractopamine and zilpaterol supplementation. Pens that were assigned to the Control diet remained on the control diet during the final 28d of the finishing period. The RAC diet was fed twice daily (60% delivery on first daily feeding, 40% delivery on second daily feeding) to all pens in the RAC treatment group for the final 28d of the finishing period prior to slaughter. The ZIL diet was fed twice daily (60% delivery on first daily feeding, 40% delivery on second daily feeding) to all pens in the ZIL treatment group for the first 20d of the final 23d of the finishing period prior to slaughter. A 3d withdrawal from zilpaterol supplementation was administered prior to slaughter. All finishing diets contained monensin and tylosin at concentrations of 33 mg/kg DM and 11 mg/kg DM, respectively.

Weight collection

During the final 28d of the finishing period, cattle were weighed weekly on either an individual or pen basis on a schedule that resulted in the use of the same weight collection method every other week. Individual weights (scale readability: ± 0.45 kg) were collected four times over the final 28d of the finishing period (28d prior to slaughter, 14d prior to slaughter, 2d prior to slaughter, and 1d prior to slaughter). The average of the final 2 weights was calculated to determine the final body weight of each animal at the end of the finishing period. The average of the 2 consecutive final weights was used to increase the accuracy of the final body weights for dressing percentage calculation. On d 21 and d 7 prior to slaughter, pen weights were collected (scale readability: ± 2.27 kg). Within 1 h before weight collection commencement, scale calibration was checked with the use of 454.5 kg of certified weights in the case of the

individual animal scale and the crosscheck of the weight of a skid steer loader between the pen scale and a platform scale. All weights were analyzed on a pen mean basis and a 4% pencil shrink was applied to all body weights before analysis.

Chute side physiology monitoring

On three individual animal weight collection days (28 d, 14 d, and 2 d prior to slaughter), heart rate, rectal temperature, and respiratory rate were measured on 4 steers per pen. The same 4 steers in each pen were assessed during each weight collection day. The same 4 steers also received a rumen temperature bolus. Heart rate was measured by placing a stethoscope in the cardiac region of the animal and counting the number of heart beats in a 15 s period measured by a digital stopwatch. This initial heart rate was multiplied by a factor of 4 to attain a heart rate in beats per min. Rectal temperature was also measured in the same 4 steers. A quick-read oral thermometer (calibrated daily) was used to measure rectal temperature. Respiratory rate was measured on the same 4 steers per pen by counting the number of flank movements in a 30 s period measured using a digital stopwatch. This initial respiratory rate was multiplied by a factor of 2 to attain a respiratory rate in breaths per min.

Chute entry score and exit speed

To identify possible treatment effects on cattle handling efficiency and behavior, the pre- and post-restraint behavior of all steers on each individual weight collection day was assessed by assigning a chute entry score and recording chute exit speed. Each animal received one of the following scores: 1: Entry on its own without provocation; 2: Entry following visual or auditory provocation such as pressure on the flight zone or a ‘hiss’ or whistle; 3: Entry following physical provocation such as a slap on the rump; 4:

Entry following the use of an electric prod. Exit speed was assessed by recording the amount of time (s) for each individual animal to travel 1.63 m following release from the squeeze chute with an electronic timing system that utilized 2 laser trip lines, spaced 1.63 m apart, to start and stop a wireless timer (Polaris, FarmTek, Inc., Wylie, TX). The time was recorded in s and the distance was divided by the time recorded for each animal to determine the speed of each steer in m / s.

Blood collection and processing

Whole blood samples were collected from each of the 4 steers per pen that were monitored for physiological factors during restraint. A rope halter was used to restrain the head of each steer during blood sampling. The head of each animal was pulled upward and to the left to expose the right jugular vein for sampling. For each steer, 1 - 7mL blood collection tube with sodium fluoride potassium oxalate additive (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and 1 - 10 mL blood collection tube with no additive (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were collected with a single-use 18 ga. × 2.54 cm needle affixed to a reusable vacuumized blood collection tube hub. A new needle was used for each steer. Immediately after whole blood collection, both blood collection tubes were immediately placed on ice. After approximately 4 h, all blood collection tubes were centrifuged at 2,500 × *g* for 15 min. Immediately after centrifugation, serum and plasma were harvested from the blood collection tubes and transferred to polyethylene microcentrifuge tubes. The microcentrifuge tubes were immediately placed in a - 20° C freezer where they were held for up to 3 d before transport to Colorado State University, where they were stored at - 80° C for up to 8 weeks before analysis.

Plasma glucose and lactate determination

Plasma glucose and lactate are indicators of the metabolic status of an animal. Plasma glucose and lactate were determined using a benchtop biochemistry analyzer (YSI 2700 Select, YSI Inc., Life Sciences Division, Yellow Springs, OH) equipped with a D-glucose membrane (#2365, YSI Inc., Life Sciences Division, Yellow Springs, OH) and Lactate membrane (#2329, Inc., Life Sciences Division, Yellow Springs, OH). Assay results were reported in mmol / L.

Serum Urea N determination

Serum Urea N, an indicator of protein turnover in the mammalian body, was determined in duplicate using an enzymatic colorimetric kit (Stanbio Enzymatic Urea N Procedure No. 2050, Stanbio Laboratory, Boerne, TX) with assay procedures modified to facilitate the use of a 96-well microassay plate. This procedure followed a modification of the Berthelot reaction that ultimately results in the creation of a blue-green chromophore. Intensity of the color development was determined by recording the absorbance of standard and unknown samples at 600 nm wavelength and developing a standard curve from known concentrations to solve for unknown sample concentrations. Serum Urea N was reported in mg / dL.

Nonesterified fatty acid (NEFA) determination

Nonesterified fatty acid concentration, and indicator of lipid mobilization in the mammalian body, was determined in duplicate from serum samples using a commercially-available enzymatic colorimetric NEFA assay (Wako NEFA-HR(2), Wako Diagnostics, Richmond, VA). The assay was performed in 96-well microtiter plates and

optical absorbance was recorded at 550 nm. Maximum CV within duplicates was set at 15% for precision. NEFA concentrations were reported in mEq / L.

Serum cortisol determination

Serum cortisol concentration, which has been used to assess the response of animals to acute stressors, was determined under the guidance of Dr. Dennis Hallford at New Mexico State University. A radioimmunoassay was performed following the method outlined by Kiyma et al. (2004). Inter- and intra-assay CVs were 10.1% and 9.7%, respectively.

Plasma catecholamine determination

Plasma catecholamine (epinephrine and norepinephrine) concentrations, which are important for lipid metabolism and stress response in the bovine and may interact with the signaling pathways for βAA, were determined through the use of a commercially – available enzyme-linked immunosorbent assay (3-CAT ELISA #BA 10-1600, Labor Diagnostika Nord GmbH & Co., Nordhorn, Germany). The assay was performed in 96-well microtiter plates with antibody coatings specific to each specific catecholamine (epinephrine, norepinephrine). Results for norepinephrine and epinephrine are reported in ng / mL.

Rumen temperature monitoring

At the time of ear and electronic ID tagging, 4 steers per pen (N = 192) received a wireless rumen temperature bolus (Smartstock, LLC., Pawnee, OK) using a large-diameter cattle balling gun. Rumen temperature was recorded to assess the impact of βAA supplementation and IMP strategy on cattle core body temperatures. Each temperature bolus was programmed to transmit its environmental temperature hourly to 2

receivers located centrally within the block of pens where the steers resided. The receivers immediately transmitted the newest temperature reading from the bolus along with the previous 12 h of temperature readings. All temperature data were automatically entered into a Microsoft Excel® spreadsheet for collection.

Behavioral time-budgeting assessment

Behavioral time budgets were constructed for the cattle in the present study to identify any behavioral implications of βAA supplementation and IMP strategy. During the final 30 d of finishing, activity of cattle in all pens was continuously recorded with 2 high-resolution network video cameras (Model VSX-2MP-FE, 3xLOGIC, Westminster, CO) enclosed in climate-controlled casings that were affixed to the railing at the top of the SECRC grain elevator. Both cameras were equipped with 2.5 to 7.0 mm mega-pixel vari-focal lenses (Model BL2570M2CS, IQinVision, Inc., San Juan Capistrano, CA). All video data was recorded in 1280 X 1024 resolution with a hybrid digital / analog video recorder (Model PRO-8-LT-500, 3xLOGIC, Westminster, CO). Stored video was analyzed on a high-definition monitor (Model TC-P50G10, Panasonic North America, Seacaucus, NJ) that measured 127 cm diagonally to provide a large image for behavior detection.

Each pen of steers was scan sampled hourly between 0700 h and 1800 h on d 30, d 16, and d 4 prior to slaughter. Scan sampling entailed counting the number of animals expressing each of the following behaviors each hour: Head over bunk: head over the feed bunk; Head over water: head over the pen water source; Lying: the body of the animal in full contact with the ground; Standing: standing on all 4 legs but not moving; Walking: standing upright and moving; Agonistic behavior: head-to head contact

typically associated with fighting. The numerical count data was then converted to the percent of animals within a pen that expressed each behavior, which is analogous with total time budgeting in animals. The behaviors recorded were adapted from the behaviors listed by Mitlohner et al. (2001). Mitlohner et al. (2001) recommended scan intervals of less than 15 min when assessing the behavior of a single group of animals. Due to the structure of our study, with 8 pen replicates within each treatment combination, the hourly scan sampling performed in this study is analogous to 60 min/8 scans = 7.5 min scanning interval. All calculated percentages within the pen scan dataset were arcsine square root transformed to improve the distribution of variance before statistical analysis. Following analysis, all means were back transformed to a percentage basis for data reporting.

Respiration behavior scoring

During the final 28 d of the finishing period, respiratory, rumination and feeding behaviors of all steers in each pen were assessed daily at approximately 15:00 h. For each pen, the number of animals displaying the following respiratory behaviors was determined by a trained observer: Normal (Closed-mouth breathing without noticeable panting or 'head-bobbing' (< 60 breaths/min)); Elevated (Closed-mouth breathing with noticeable exaggerated respiration in the form of 'head-bobbing' (60 – 120 breaths/min)); Panting: (Open-mouth breathing with exaggerated respiration in the form of 'head-bobbing' (> 120 breaths/min)). Respiration score was based on exhibited behavior, not actual respiratory rate. Associated respiratory rates were developed from the collection of preliminary data and reference to Brown-Brandl et al. (2006), who determined that respiratory rate is an indicator of heat stress in cattle. Respiration scores were expressed

as the mean percentage of animals expressing each score within each pen for statistical analysis. All data were arcsine square root transformed prior to statistical analysis and all means were back transformed prior to reporting.

Water intake monitoring

Daily water intake was measured, as an indicator of thirst, by recording water meter readings from pen water sources. A single water source was provided for a pair of two pens and a total of 24 water meters were used in this study. Both pens that shared a common water source received the same βAAsupplementation and implant strategy combination. This configuration of water intake measurement resulted in the reduction of the number of experimental units during statistical analysis (N = 24 – 2 pen groups).

Slaughter and carcass data collection

Steers were slaughtered in 4 groups (repetitions 7 and 8, 5 and 6, 3 and 4, and 1 and 2). The 4 groups were slaughtered over a 2 wk period. On the first wk, 2 groups were slaughtered on 2 consecutive d. Two wks later, the remaining 2 groups were slaughtered on 2 consecutive d. All cattle were slaughtered using humane, conventional slaughter procedures under the supervision of USDA personnel. Hot carcass weights (HCW) were collected during the slaughter procedure and individual animal identification was maintained throughout the slaughter process to calculate individual animal dressing percentage.

Statistical analysis

All data were analyzed using a complete, randomized design with a 2 × 3 factorial treatment arrangement. Pen was the experimental unit for all data in this study (N = 48) with the exception of the daily water intake data, which was reported as the mean of 2

adjacent pens of cattle that shared the same water source and treatment assignment (N = 24). In all models for analysis of physiological, hormonal, and performance data, an implant strategy factor (CONT, IMP) and a β-adrenergic agonist supplementation factor (CONT, RAC, ZIL) were included. Sample collection day also was included in the model when appropriate, although no interaction effects were d. Since no interaction of implant strategy × βAA supplementation × week of study were suggested, body weight data was analyzed separately on each weight collection day. All behavior data were arcsine square root transformed to increase normality of data distribution before analysis. In all models, repetition (1 - 8) was included in the random statement of the model to control variation. Data were analyzed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with Kenward-Roger denominator degrees of freedom and AR1 covariate structure designated within the model. Rumen temperature data were analyzed using a different MIXED model that included the interaction effects of implant strategy (No IMP, IMP), βAA supplementation (No βAA, RAC, ZIL) and period of day (1-6). The random statements for the model included the intercepts for slaughter day, pen, and week × pen interaction. Satterthwaite denominator degrees of freedom and AR1 covariate structure were specified within the model. Means were separated using pairwise t-tests and differences were recognized at $\alpha < 0.05$.

In addition to the MIXED model, all data were analyzed for the main effects of βAA supplementation and implant strategy using contrasts in the MIXED procedure of SAS. Only pens that received one treatment factor were included in the analysis. A total of 24 pens were included in this randomized, complete, paired-comparison design. Means were separated using pairwise t-tests and differences were recognized at $\alpha < 0.05$.

RESULTS AND DISCUSSION

Mixed model results

Growth performance and feed efficiency

The impact of βAAsupplementation and implant strategy on the performance of cattle in this study can be observed in Table II-2. The study failed to detect differences ($P > 0.05$) due to βAAsupplementation on multiple indicators of cattle growth performance and ultimate carcass yield. These results were contrary to those reported by multiple authors (Bryant et al., 2010; Montgomery et al., 2009; Parr et al., 2011; Scramlin et al., 2010; Woerner et al., 2011). Vasconcelos et al. (2008) found no difference in final BW between feedlot cattle that had been treated with zilpaterol at the same dose and duration as the present study, but the investigators did recognize a drastic increase in HCW. Gruber et al. (2007) reported no difference in dressing percentage of pens of cattle that were either treated with no βAAor 200 $mg \times hd^{-1} \times d^{-1}$ of ractopamine for the final 28d of the finishing period. The lack of difference in growth performance between βAAsupplementation treatments was not typical and the exact cause of the lack of affect was not clearly understood. Further investigation regarding the interaction of ambient conditions and βAAsupplementation is warranted to explore the performance impact of feeding βAAduring summer months as opposed to cooler periods of the year. In addition, the weight sampling regimen in the current study maintained weekly weight collections during the final 28d of the finishing period, which may have introduced handling and human presence stress at a frequency that was difficult for the cattle to overcome. Both of these factors warrant further exploration.

Administration of a terminal implant in the current study improved cattle performance (Table II-2) and consistently increased BW ($P < 0.01$). Dry matter intake (DMI) was greater for implanted steers than non-implanted steers ($P < 0.01$), which agreed with results reported by Parr et al. (2011), who used the same terminal implant (Revalor-XS) as that used in the present study. Greater HCW also was observed in implanted cattle than non-implanted cattle. Water intake was greater ($P < 0.01$) in implanted steers than non-implanted steers, which was likely a result of increased water turnover due to increased muscle mass and reduced body surface area relative to body mass. Results agreed with conclusions of Blackshaw and Blackshaw (1994) who determined that *Bos taurus* cattle increase water intake as heat stress increases. It is common knowledge that growth-promoting implants increase muscle mass, which may have increased the susceptibility of IMP steers to heat stress and increased water intake in our study. However, the severity of the heat stress incurred by the cattle in the IMP treatment group was difficult to quantify because increased water intake does not imply suffering on the part of the animal. In any case, increased water intake did indicate increased thirst.

Physiology, hormones, and metabolites

Table II-3 displays the main effects of βAAsupplementation and implant strategy on physiological, metabolic, and hormonal responses in feedlot steers. Cattle treated with ZIL were characterized as having reduced plasma glucose concentrations ($P < 0.01$) when compared to the None and RAC treatments. A stratified response in plasma lactate concentration was recognized with RAC cattle that were consistently characterized as having less plasma lactate than None cattle and ZIL cattle having less plasma lactate than

either None or RAC cattle ($P < 0.01$). Steers treated with ZIL displayed lower mean heart rates than None or RAC steers ($P < 0.01$). The observed difference in mean heart rates was indicative of a potential β-1 adrenoceptor antagonistic action caused by ZIL. In rats, β-1 adrenoceptor agonistic compounds increase cardiac output (heart hate and stroke volume) (Carlisle and Stock, 1992; Carlisle and Stock, 1993, Mersmann, 1998). Interestingly, Ekpe et al. (2000) found that β-1 adrenoceptor density in the myocardial membrane of sheep was reduced when feeder lambs were exposed to cold temperatures and restricted feed access. The authors did not identify any difference in β-1 adrenoceptor density between cold and warm environments when feeder lambs were granted *ad libitum* feed access. If the receptor expression on bovine and ovine mycardiocyte membrane is similar with respect to how it responds to changes in ambient and subsequent body temperature, the physiological data in the present study suggest that ZIL may have an antagonistic effect on cardiac β-1 adrenoceptor expression.

Implant strategy also influenced the physiological state of steers in this study (Table II-3). Implanted steers displayed less serum cortisol ($P < 0.01$) than their non-implanted counterparts. Other authors have noted a similar difference between steers and intact bulls as a result of androgenic cortisol suppression (Fritsche and Steinhart, 1998; Verkerk and Macmillan, 1997). Interestingly, heart rate was greater ($P < 0.01$) in implanted steers. Implanted steers also displayed reduced serum urea N ($P < 0.01$) relative to non-implanted steers.

Interaction effects for βAAsupplementation and implant strategy also were recognized relative to hormonal, metabolic, and physiological responses in the feedlot steers in this study (Table II-4). Steers that received RAC or ZIL displayed reduced

plasma epinephrine concentrations ($P = 0.04$) compared to negative controls, regardless of implant strategy. Bryant et al. (2010) reported no difference in plasma epinephrine between groups of steers that were treated with no β-AA, 100 $mg \times hd^{-1} \times d^{-1}$, or 200 $mg \times hd^{-1} \times d^{-1}$ of ractopamine for the final 28d of the finishing period. Bassett (1993) identified a reduction in plasma adrenaline concentrations in Suffolk rams that were infused with ritodrine, a β-2 adrenergic agonist, at a rate of 0.5 $\mu g \times kg^{-1} \times min^{-1}$ for 9 d. The author concluded that prolonged βAAtreatment had an attenuating affect on the response of heart function and metabolic rate to endogenous catecholamine secretion. Due to the ability of βAAto bind β adrenoceptors, which are typically bound by endogenous epinephrine and norepinephrine, it is very possible that βAAmay be capable of participating in negative feedback effects on the adrenal medulla, which would reduce endogenous epinephrine concentrations.

Steers receiving IMP / RAC and No IMP / ZIL displayed less serum NEFA ($P = 0.01$) than steers in all other treatment combinations (Table II-4). The reason for the observed interaction effect was not evident given current literature. Bryant et al. (2010) reported no effect of RAC supplementation with or without a TBA implant on NEFA concentrations in feedlot steers. Further investigation of the impact of the IMP/RAC and No IMP/ZIL treatment combinations on circulating NEFA concentrations is necessary to identify the metabolic modifications that ensue following their use.

Steers that received the No IMP / ZIL treatment combination had greater ($P < 0.01$) mean rectal temperatures than No IMP / No β-AA, No IMP / RAC, and IMP / ZIL steers (Table II-4). The remaining treatment combination effects on rectal temperature were intermediate and not different than any other treatment combinations ($P > 0.05$).

Figure II-1 shows the interaction of βAAsupplementation, implant strategy, and period of day on mean rumen temperature in feedlot steers. The time period from 16:01h to 20:00h contained a βAA × IMP × Period of day interaction effect as pens of steers in the IMP/RAC treatment combination displayed lower ($P < 0.05$) mean rumen temperatures than pens of steers in the No IMP/RAC and No IMP/ZIL treatments. No other interaction effects were recognized with regard to rumen temperature. These data suggested that heat mitigation is affected when ZIL is used without a TBA/E17β implant. The observed reduction in ability of ZIL-treated pens of steers to mitigate or counteract heat production, even when cardiac output decreased, was indicative of peripheral vasoconstriction. Other authors have acknowledged the ability of β adrenergic agonists to promote vasodilation (Carlisle and Stock, 1993; Kern et al., 2008; Guimaraes and Moura, 2001). Within the mammalian body, α-adrenoreceptors are largely responsible for promoting vasoconstriction. Endogenous adrenergic compounds, such as epinephrine, can functionally bind both α and β receptors (Guimaraes and Moura, 2001). Mills et al. (2003) identified disagreement among studies regarding the β adrenoceptor subtype specificity of ractopamine, which indicates incomplete scientific understanding of the functional specificity of compounds such as ractopamine and zilpaterol. It seems logical that adrenergic agonist compounds, such as ractopamine and zilpaterol, may also have some impact on α receptor function in vascular endothelium, but this response requires investigation. Our results suggest that such an interaction may exist.

Behavior

A significant βAAsupplementation × implant strategy effect was detected for chute entry score (Table II-4). Mean chute entry score for IMP/ZIL treated steers was

greater ($P < 0.05$) than steers in the No IMP/ZIL and IMP/No βAAtreatments. Although statistical significance was recognized, it is debatable if the observed differences in chute entry force score were practically important due to the small magnitude of differences in means. Baszczak et al. (2006) recognized a subtle difference in chute entry force scores in cattle treated with 200 $mg \times hd^{-1} \times d^{-1}$ for 28 d before slaughter. They also concluded that the difference they observed, although statistically significant, was not likely to be practically significant to livestock handlers in field conditions.

Additional βAAsupplementation × implant strategy interaction effects were detected for the occurrence of increased respiratory rates in the pens of feedlot steers in this study (Table II-5). Pens of steers in the No IMP/RAC treatment group displayed a greater prevalence of normal respiration rates than any other treatment group. A greater percentage ($P < 0.05$) of steers within the No IMP/ZIL treatment pens displayed panting respiration scores than all other treatment combinations with the exception of IMP/RAC, which was not different than No IMP/ZIL or IMP/ZIL ($P > 0.05$). Brown-Brandl et al. (2003, 2005) demonstrated that respiration rate was a reliable indicator of heat stress in feedlot cattle. Considering the observed prevalence of panting respiration scores that the treatment combinations of No IMP/ZIL and IMP/RAC displayed relative to all other treatment combinations, the combinations of No IMP/ZIL and IMP/RAC appear to increase susceptibility to heat stress in feedlot cattle. These data indicated that the cattle in the No IMP/ZIL treatments, which displayed greater rectal temperatures and rumen temperatures than other treatment combinations, were likely experiencing a heat mitigation challenge. The physiological explanation for increased respiratory rates, aside for the need to mitigate increased body heat production in these treatment combinations,

is not clear and further investigation at the tissue level is necessary to identify the interactive effects of these implant and βAAcombinations.

In-pen behavioral time budgets for the main effects of βAAsupplementation and implant strategy are shown in Table II-6. Pens of steers in the ZIL treatment group spent 1.1% more time with their head over the pen water supply than pens of steers that did not receive βAA($P < 0.05$). The mean amount of time with head over the water supply observed in this study was considerably greater than the amount of watering time described by Sowell et al. (1999). One of the major differences between Sowell et al. (1999) study and the current study was the time frame in which behavior sampling was conducted. In the current study, we conducted scan sampling from 0700h to 1800h on scan sampling days. Sowell et al. (1999) relied on Growsafe technology to monitor individual animal water intake over the entire 24-h day. Behavioral assessments were only possible in the present study during daylight hours (0700 to 1800) of each scan sampling day. Work by Sowell et al. (1999) suggested that cattle consume more water during the day than during the night, which may be part of the reason for such a discrepancy in watering time between the two studies. Sowell et al. (1999) did not cite the ambient conditions of the study, which are vitally important as they play a major role in heat stress conditions in cattle. Several authors have recognized the importance of considering ambient conditions when evaluating the amount of heat stress cattle experience (Brown-Brandl et al., 2006; Brown-Brandl et al., 2003; Hahn and Mader, 1997; Mitlohner et al., 2002; Ray and Roubicek, 1971). Pens of steers in the RAC treatment groups appeared to be more active than the No βAAand ZIL treatment groups as RAC treated pens spent less time lying and more time standing ($P < 0.05$) than the

other 2 treatment groups. Pens of steers in the IMP treatments also appeared to be more active as they spent approximately 3.3% less time lying ($P < 0.05$) and 0.82% more time walking ($P < 0.05$) than pens in the No IMP treatments. This observed increase in activity provided a partial explanation for the increased DMI observed for pens of IMP cattle, although it is likely that nutritional demand as a result of increased metabolic rate played a major role in DMI as well. In all cases, the percentage of time spent walking in our study was similar to the amount of time observed by Ishiwata et al. (2008) in Japanese black × Holstein steers in pen conditions. All cattle in the present study spent a much greater amount of time lying than the 32.2% reported by Ishiwata et al. (2008), even though the cattle were kept in pens with similar stocking densities. Many factors may have impacted this difference; i.e. steers in Ishiwata et al. (2008) were provided shade and temperatures that were considerably cooler than those in the present study. Ray and Roubicek (1971) observed that 60% to 80% of feedlot cattle spent their time lying down during the day, which was considerably greater than the amount of time that cattle in the present study spent lying. Blackshaw and Blackshaw (1994) reported that cattle without shade in heat-stressed conditions spent more time lying than cattle that were not heat-stressed. Results of the present study fall between those reported by Ray and Roubicek (1971) and Ishiwata et al. (2008). It was interesting to note that shade use approached 100% during the summer days in the study by Ray and Roubicek (1971). Mitlohner et al. (2002) observed greater incidence of respiration rates analogous to our "very elevated" classification in feedlot heifers that were not provided with shade in comparison to heifers provided with shade during the same time of year as the present study. An investigation of the interaction of shade, implant strategy, and

βAAsupplementation would be beneficial in future investigations of growth promotants on heat stress responses in feedlot steers.

Contrast results

During statistical analysis, paired-comparison contrasts were developed to assist in the identification of the true effects of βAAsupplementation and implant strategy. In this design, pens of cattle that were part of the IMP treatment group were excluded from the analysis of βAAsupplementation effects and pens that were assigned to the RAC and ZIL βAAsupplementation treatments were excluded from the implant strategy analysis. The results are shown in Table II-7.

Paired – comparison contrasts of behavioral responses of pens of steers to βAAsupplementation in the present study were similar to the results of the MIXED model analysis. Pens of steers in the ZIL treatment group spent more time ($P < 0.05$) with their head over the pen water supply than No βAAor RAC pens. Interestingly, significant differences in lying and standing behavior that were recognized in the MIXED model analysis were no longer significant in the paired-comparison contrasts. The significance of the βAAsupplementation effect on normal respiration also dropped below α in the paired-comparison contrasts. However, the effect of βAAsupplementation on panting was still detected. The paired-comparison contrasts revealed a much greater incidence of panting respiration scores in pens of steers treated with ZIL than RAC or No βAA($P < 0.05$).

The paired-comparison contrasts also detected a difference in dressing percentage that was not revealed in the MIXED model analysis. Dressing percentage was not

different between RAC and ZIL ($P > 0.05$), but both treatments produced greater mean dressing percentages than the No βAAtreatment ($P < 0.05$).

Physiological differences also were detected between βAAtreatments in the paired-comparison contrasts. Plasma epinephrine and glucose concentrations were greater ($P < 0.05$) in the No βAAtreatment group than either the RAC or ZIL treatments. In the MIXED analysis, a significant βAAsupplementation × implant strategy interaction was detected for plasma glucose concentration, but the means for implant strategy treatment within each βAAsupplementation treatment appeared to follow a similar pattern to the βAAsupplementation effects revealed in the paired-comparison contrasts. Pens of cattle that received the ZIL treatment also displayed depressed mean heart rate and plasma lactate concentration ($P < 0.05$). Mean rumen temperature was greater ($P < 0.05$) in pens of cattle in the ZIL treatment than the No βAAtreatment, but no difference ($P < 0.05$) was detected between RAC and the other 2 βAAtreatments. The observed differences of βAAsupplementation treatments appeared similar to those observed in the No IMP treatments shown in Figure II-1, although the differences were not significant in Figure II-1.

Multiple similarities in implant strategy effects were recognized between the MIXED and paired-comparison contrast analyses (Table II-7). Pens of cattle in the IMP treatment displayed increased dry matter intake (DMI), dressing percentage, hot carcass weight, and water intake ($P < 0.05$). Decreased serum cortisol and plasma glucose concentrations also were detected among pens of cattle treated with IMP versus No IMP ($P < 0.05$). Most of these differences also were detected using the MIXED analysis procedures, with the exception of dressing percentage and plasma glucose concentration.

IMPLICATIONS

Use of specific combinations of implant strategy and βAA supplement appeared to affect physiological and behavioral responses of feedlot steers in varying manners. Results suggested that the combinations of IMP/RAC and No IMP/ ZIL presented the greatest risk for heat mitigation difficulty. A negative effect of ZIL on heart rate also was detected, which revealed a potential antagonistic influence of ZIL on myocardial function. Perhaps the most surprising results of the entire study were the relative lack of performance response in cattle that were treated with βAA. The observed lack of performance response was not easy to explain because such a lack of performance in cattle that have been treated with βAA has not been well documented in literature. However, the observed discrepancy between the growth performance recorded in the present study and the results of others presents an important set of issues that require investigation. Effects of weekly weight collections on endogenous catecholamine concentrations perhaps resulted from human presence and handling stress that may have had an inhibitory effect on the function of β adrenergic receptors. Additionally, the impact of β-adrenergic agonist supplementation and implant strategy on the ability of cattle to cope with increased ambient temperature conditions must be identified as these are two factors that can easily be managed by feedlot operators if the proper courses of action regarding heat mitigation and response to handling and human presence are controlled. In summary, it does not appear to be cost effective to feed a product that does not positively influence growth and performance but displays negative influence on heat tolerance. Therefore, further investigation to identify factors that reduce the efficacy of

βAA supplements or implicate animal welfare must be conducted to identify periods of time or conditions during which βAA should be limited or carefully adjusted.

Table II-1. Composition of finishing diets for pens of feedlot steers on trial during the final 28 d before slaughter.

	Diet[1]		
	CONT (Diet 100316)	RAC (Diet 100326)	ZIL (Diet 100336)
Number of pens	16	16	16
Ingredient, %			
Corn Grain, Flaked	76.13	76.10	75.10
Corn Silage, 50% Grain	9.87	9.87	9.87
Steep Liquor	3.00	3.00	3.00
Distillers Grain with solubles	4.47	4.47	3.95
Tallow	2.93	2.93	2.99
Supplement[2,3]	3.60	3.61	3.60
βAA Supplement	0.00	0.02	1.49
Calculated Chemical Composition[4]			
DM, %	67.74	67.74	67.89
CP, %	13.50	13.50	13.50
Non-protein N, %	3.50	3.50	3.50
Neutral Detergent Fiber, %	13.40	13.40	13.59
Forage NDF, %	4.00	4.00	4.00
Crude Fiber, %	4.94	4.94	5.00
ME, Mcal·kg^{-1}	3.25	3.25	3.20
NEm, Mcal·kg^{-1}	2.17	2.17	2.17
NEg, Mcal·kg^{-1}	1.48	1.48	1.48
Ca, %	0.70	0.70	0.70
P, %	0.36	0.36	0.36
K, %	0.70	0.70	0.70
Mg, %	0.25	0.25	0.25
Zn, ppm	150	150	150
Vitamin A, IU/kg	2200	2200	2200
Vitamin E, IU/kg	33	33	33

[1]Diet: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.
[2]All supplements were formulated to provide monensin at a rate of 33 mg/kg DM.
[3]All supplements were formulated to provide tylosin at a rate of 11 mg/kg DM.
[4]Diets were formulated to meet or exceed the nutrient requirements of feedlot steers (NRC, 1996).

Table II-2. Least squares means for the main effects of β-adrenergic agonist supplementation and implant strategy on growth performance, feed intake and efficiency, and carcass yield of pens of feedlot steers.

	β-adrenergic agonist[1]			Implant strategy[2]			*P*-value		
Dependent variable	None	RAC	ZIL	None	IMP	SEM	βAA	IMP	βAA × IMP
Number of pens	16	16	16	24	24	-	-	-	-
ADG[3], kg	1.73	1.87	1.83	1.65	1.96	0.14	0.79	0.07	0.56
BW[4], kg									
d 0	520.0	515.0	519.0	506.6[b]	529.4[a]	9.3	0.35	<0.01	0.13
d 7	539.7	536.8	537.1	527.4[b]	548.4[a]	10.9	0.65	<0.01	0.69
d 14	545.1	546.5	546.7	533.2[b]	559.0[a]	11.5	0.93	<0.01	0.41
d 21	559.0	558.2	560.9	545.9[b]	572.9[a]	11.4	0.80	<0.01	0.90
d 28	564.8	562.9	567.0	549.1[b]	580.8[a]	10.5	0.59	<0.01	0.76
DMI, kg / d	8.79	8.76	8.66	8.44[b]	9.03[a]	0.17	0.11	<0.01	0.89
Dressing Percent	63.5	63.9	64.2	63.7	64.0	0.2	0.10	0.24	0.16
G:F	0.20	0.22	0.21	0.20	0.22	0.02	0.77	0.18	0.56
HCW, kg	359.0	359.7	364.1	349.9[b]	371.9[a]	6.9	0.21	<0.01	0.88
Water intake, $\ell \cdot hd^{-1} \cdot d^{-1}$	33.33	34.07	32.56	31.61[b]	35.03[a]	1.68	0.63	<0.01	0.68

[a,b] Means in the same row within a main effect that do not have a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] ADG, Average Daily Gain reported as the average over the entire 28d period prior to slaughter.

[4] Weights were collected weekly during the final 28d of the finishing period. The beginning of the 28d period is denoted as d0. Weights were decreased by 4% to represent a standard industry shrink.

Table II-3. Least squares means for the main effects of implant strategy and β-adrenergic agonist supplementation on physiological, metabolic, and hormonal responses during the last 28 d on feed by finishing steers.

	β-adrenergic agonist[1]			Implant strategy[2]			*P*-value		
Dependent variable	None	RAC	ZIL	None	IMP	SEM	βAA	IMP	βAA × IMP
Number of pens	16	16	16	24	24	-	-	-	-
Hormones and metabolites									
Serum Cortisol, ng/mL	40.60	38.74	39.64	48.59[a]	30.73[b]	1.47	0.65	<0.01	0.72
Plasma Glucose, mmol / L	3.31[a]	3.03[a]	2.32[b]	3.04	2.74	2.89	<0.01	0.06	0.11
Plasma Lactate, mmol / L	4.79[a]	4.60[b]	4.33[c]	4.56	4.58	0.12	<0.01	0.79	0.14
Plasma Norepinephrine, ng / mL	0.23	0.22	0.20	0.20	0.24	0.02	0.46	0.07	0.19
Serum Urea N, mg / dL	10.40	9.99	9.81	10.70[a]	9.44[b]	0.38	0.35	<0.01	0.48
Physiological parameters									
Heart rate, beats/min	135.7[a]	133.6[a]	120.1[b]	126.8[b]	132.8[a]	2.2	<0.01	0.01	0.29
Respiration rate, breaths/min	49.4	49.5	49.4	49.3	49.6	0.9	>0.99	0.71	0.74

[a,b,c] Means in the same row within a main effect that do not have a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

Table II-4. Least squares means for interaction effects of implant strategy and β-adrenergic agonist supplementation on behavioral, physiological, metabolic, and hormonal parameters during the last 28 d on feed by finishing steers.

	No BAA[1]		RAC[1]		ZIL[1]			*P*-value
Item	No IMP[2]	IMP[2]	No IMP[2]	IMP[2]	No IMP[2]	IMP[2]	Pooled SEM	BAA × IMP
Number of pens	8	8	8	8	8	8	-	-
Behavior								
Chute entry score	2.08^{ab}	1.93^{b}	2.03^{ab}	2.09^{ab}	1.97^{b}	2.18^{a}	0.07	<0.01
Hormones and metabolites								
Plasma Epinephrine, ng/mL	2.77^{a}	2.10^{abc}	1.47^{bc}	1.96^{bc}	1.38^{c}	1.63^{c}	0.27	0.04
Serum NEFA[3], mEq/L	0.24^{a}	0.24^{a}	0.23^{a}	0.18^{b}	0.20^{b}	0.24^{a}	0.02	0.01
Physiological parameter								
Rectal temperature, °C	39.51^{b}	39.63^{ab}	39.51^{b}	39.68^{ab}	39.73^{a}	39.54^{b}	0.08	<0.01

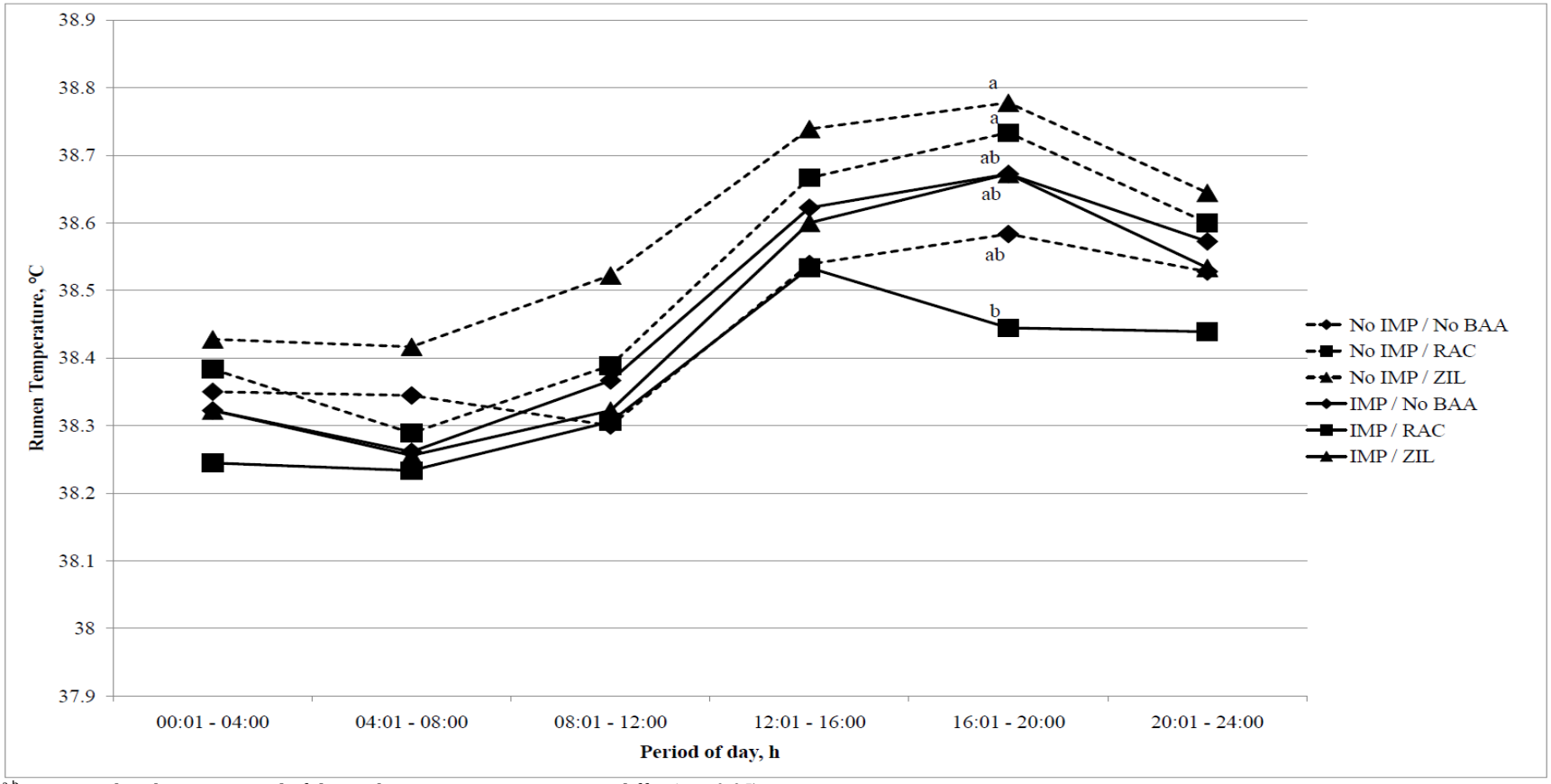
[a,b,c] Means in the same row within a main effect that do not have a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] Serum Nonesterified Fatty Acids

Figure II-1. Least squares means for interaction effects of β-adrenergic agonist supplementation[1], implant strategy[2], and period of day[3] on the mean rumen temperatures of pens of feedlot steers (N=48).



[a,b] Means within the same period of day without a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] Data collected during each day were averaged within the 6 – 4 h periods of time shown on the x-axis of the figure.

Table II-5. Least squares means for interaction effects of β-adrenergic agonist supplementation[1] and implant strategy[2] on behavioral respiration rate scores[3] at approximately 1500h during the last 28 d on feed by finishing steers.

	No BAA		RAC		ZIL			*P*-value
Item	No IMP	IMP	No IMP	IMP	No IMP	IMP	Pooled SEM	BAA × IMP
Number of pens	8	8	8	8	8	8	-	-
Feeding, %	3.17	3.55	3.34	3.83	4.86	4.60	0.05	0.89
Normal, %	22.37^{b}	23.19^{b}	28.22^{a}	21.47^{b}	20.58^{b}	21.83^{b}	0.05	0.02
Elevated, %	70.92	69.25	65.71	70.41	71.13	69.48	0.04	0.11
Panting, %	0.01^{c}	0.06^{c}	0.04^{c}	0.39^{ab}	0.46^{a}	0.12^{bc}	0.02	<0.01
Ruminating, %	1.46	1.43	0.53	1.23	0.60	1.06	0.04	0.57

[a,b,c] Means in the same row without a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] Respiration scores were collected for all animals in all pens at approximately 1500 h each day during the β-adrenergic agonist supplementation period. The number of animals exhibiting each of the following mutually exclusive respiratory patterns was recorded: Normal (Closed-mouth breathing without noticeable panting (< 60 breaths/min)); Elevated (Closed-mouth breathing with noticeable exaggerated respiration and head-bobbing (60 – 120 breaths/min)) ; Panting: (Open-mouth breathing with exaggerated respiration and head bobbing (> 120 breaths/min)). Respiration score was based on exhibited behavior, not actual respiratory rate. Associated respiratory rates were developed from the collection of preliminary data and reference to Brown-Brandl et al. (2006). Respiration scores are expressed as the mean percentage of animals expressing each score. Occurrence of rumination (visible chewing not at the feed bunk) and feeding (eating diet from feed bunk) were also collected for all animals in all pens at approximately 1500 h each day during the β-adrenergic agonist supplementation period. Data are expressed as the mean percentage of animals expressing each activity.

Table II-6. Least squares means for the main effects of β-adrenergic agonist supplementation and implant strategy on chute exit speed and behavior time budgeting by pens of feedlot steers during the final 28d of the finishing period.

	β-adrenergic agonist[1]			Implant strategy[2]			*P*-value		
Dependent variable	None	RAC	ZIL	None	IMP	SEM	βAA	IMP	βAA × IMP
Number of pens	16	16	16	24	24	-	-	-	-
Chute exit speed, $m \cdot s^{-1}$	2.10	2.13	2.04	2.11	2.07	0.04	0.37	0.45	0.09
Behavior scans[3]									
Agonistic behavior, %	0.09	0.14	0.03	0.07	0.09	0.01	0.42	0.83	0.15
Head over bunk, %	14.14	14.99	14.80	14.69	14.59	0.02	0.60	0.89	0.17
Head over water, %	3.69[b]	4.24[ab]	4.78[a]	4.49	3.97	0.004	0.02	0.10	0.38
Lying, %	49.90[a]	42.88[b]	48.39[a]	48.70[a]	45.41[b]	0.04	<0.01	0.05	0.88
Standing, %	29.00[b]	33.56[a]	28.97[b]	29.10	31.90	0.06	0.03	0.08	0.95
Walking, %	1.11	1.67	1.40	1.00[b]	1.82[a]	0.02	0.42	0.02	0.49

[a,b] Means in the same row without a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] Pen scan sampling was performed hourly each week on all 48 pens of cattle during the 28-d β-adrenergic agonist supplementation period. The number of animals in each pen displaying each of the following activities as recorded: Agonistic behavior: head-to head contact typically associated with fighting; Head over bunk: head over the feed bunk; Head over water: head over the water source; Lying: the body of the animal in full contact with the ground; Standing: standing on all 4 legs but not moving; Walking: standing upright and moving. Behaviors are expressed as the mean percentage of animals expressing each activity, which is indicative of overall time budgeting.

Table II-7. Least squares means for the paired-comparison contrast effects of β-adrenergic agonist supplementation and implant strategy on behavioral, performance, and physiological responses in feedlot cattle.

	β-adrenergic agonist[1]				Implant strategy[2]			*P*-value	
Dependent variable	None	RAC	ZIL	SEM	None	IMP	SEM	βAA	IMP
Number of pens	8	8	8	-	8	8	-	-	-
Behavior									
Chute behavior									
Chute entry score	2.08	2.03	1.97	0.07	2.08	1.93	0.07	0.39	0.15
Chute exit speed, m/s	2.19	2.08	2.06	0.07	2.19	2.01	0.07	0.15	0.06
Pen behavior scans[3]									
Agonistic behavior, %	0.05	0.59	0.02	0.04	0.05	0.34	0.04	0.35	0.18
Head over bunk, %	14.18	14.74	17.73	0.03	14.18	14.75	0.03	0.21	0.74
Head over water, %	3.79[b]	4.50[b]	5.89[a]	0.01	3.79	3.86	0.01	<0.01	0.89
Lying, %	50.93	44.74	46.70	0.06	50.93	47.93	0.06	0.08	0.37
Standing, %	28.83	32.52	28.22	0.08	28.83	30.33	0.08	0.64	0.68
Walking, %	0.94	1.88	0.54	0.03	0.94	2.04	0.03	0.70	0.08
Respiratory behavior[4]									
Feeding, %	3.17	3.34	4.86	0.05	3.17	3.55	0.05	0.39	0.75
Normal, %	22.37	28.22	19.36	0.05	22.37	23.19	0.05	0.58	0.76
Elevated, %	70.92	65.71	71.13	0.04	70.92	69.25	0.04	0.29	0.53
Panting, %	0.01[b]	0.04[b]	0.46[a]	0.02	0.01	0.06	0.02	0.03	0.40
Ruminating, %	1.29	0.43	0.49	0.04	1.29	1.25	0.04	0.06	0.95
Performance									
ADG[5], kg	1.51	1.84	1.60	0.27	1.51	1.94	0.27	0.53	0.26
BW[6], kg									
d 0	511.0	499.1	509.6	9.7	511.0	529.0	9.7	0.58	0.20
d 7	530.8	525.1	526.3	11.2	530.8	548.6	11.2	0.71	0.27
d 14	533.2	530.2	526.2	11.9	533.2	556.9	11.9	0.99	0.17
d 21	546.2	543.6	547.9	11.9	546.2	571.8	11.9	0.98	0.13
d 28	550.2	545.4	551.6	10.9	550.2	579.4	10.9	0.90	0.07
DMI, kg / d	8.49	8.47	8.35	0.07	8.49[b]	9.07[a]	0.07	0.30	<0.01
Dressing Percent	63.1[b]	64.1[a]	64.0[a]	0.03	63.1[b]	64.0[a]	0.03	0.02	0.05
G:F	0.18	0.22	0.19	0.03	0.18	0.22	0.03	0.53	0.39
HCW, kg	347.2	349.5	353.1	7.3	347.2[b]	370.7[a]	7.3	0.65	0.03
Water intake, $\ell \cdot hd^{-1} \cdot d^{-1}$	30.98	33.16	30.80	1.60	30.98[b]	35.73[a]	1.60	0.61	0.04

Physiology and biochemistry									
Serum Cortisol, ng / mL	49.13	47.15	49.49	2.07	49.13[a]	32.08[b]	2.07	0.75	<0.01
Plasma Epinephrine, ng / mL	2.78[a]	1.43[b]	1.33[b]	0.26	2.78	2.17	0.26	<0.01	0.11
Plasma Glucose, mmol / L	3.64[a]	2.96[b]	2.54[b]	0.22	3.64[a]	3.00[b]	0.22	<0.01	0.04
Heart rate, beats/min	132.3[a]	133.2[a]	115.4[b]	3.3	132.3	139.5	3.3	0.05	0.13
Plasma Lactate, mmol / L	4.87[a]	4.59[a]	4.23[b]	0.11	4.87	4.71	0.11	<0.01	0.29
Plasma Norepinephrine, ng / mL	0.20	0.23	0.17	0.03	0.20	0.26	0.03	0.94	0.09
Plasma NEFA[7], mEq / L	0.24	0.23	0.20	0.02	0.24	0.24	0.02	0.18	0.85
Rectal temperature, °C	39.51	39.51	39.73	0.07	39.51	39.63	0.07	0.22	0.25
Respiration rate, breaths/min	49.8	49.8	49.3	1.3	48.8	50.0	1.3	0.65	0.49
Rumen temperature, °C	38.44[b]	38.51[ab]	38.57[a]	0.03	38.44	38.47	0.03	<0.01	0.43
Serum Urea N, mg / dL	10.83	10.91	10.34	0.46	10.83	9.96	0.46	0.72	0.19

[a,b] Means in the same row without a common superscript differ ($P \leq 0.05$).

[1] β-adrenergic agonist supplementation: CONT: no β-adrenergic agonist supplementation; RAC: supplemented daily with ractopamine hydrochloride (200 mg/animal/d) for the final 28 d of finishing; ZIL: supplemented daily with zilpaterol hydrochloride (75 mg/animal/d) for 20 d during the final 23 d of finishing with a 3-d withdrawal.

[2] Implant strategy: CONT: No growth promoting implant was used; IMP: Animals were treated with a terminal implant containing 200 mg trenbolone acetate and 40 mg estradiol-17β at initial processing (Revalor XS, Intervet/Schering-Plough Animal Health, DeSoto, KS).

[3] Pen scan sampling was performed hourly each week on all 48 pens of cattle during the 28-d β-adrenergic agonist supplementation period. The number of animals in each pen displaying each of the following activities as recorded: Agonistic behavior: head-to head contact typically associated with fighting; Head over bunk: head over the feed bunk; Head over water: head over the water source; Lying: the body of the animal in full contact with the ground; Standing: standing on all 4 legs but not moving; Walking: standing upright and moving. Behaviors are expressed as the mean percentage of animals expressing each activity, which is indicative of overall time budgeting.

[4]Respiration scores were collected for all animals in all pens at approximately 1500 h each day during the β-adrenergic agonist supplementation period. The number of animals exhibiting each of the following mutually exclusive respiratory patterns was recorded: Normal (Closed-mouth breathing without noticeable panting (< 60 breaths/min)); Elevated (Closed-mouth breathing with noticeable exaggerated respiration and head-bobbing (60 – 120 breaths/min)) ; Panting: (Open-mouth breathing with exaggerated respiration and head bobbing (> 120 breaths/min)). Respiration score was based on exhibited behavior, not actual respiratory rate. Associated respiratory rates were developed from the collection of preliminary data and reference to Brown-Brandl et al. (2006). Respiration scores are expressed as the mean percentage of animals expressing each score. Occurrence of rumination (visible chewing not at the feed bunk) and feeding (eating diet from feed bunk) were also collected for all animals in all pens at approximately 1500 h each day during the β-adrenergic agonist supplementation period. Data are expressed as the mean percentage of animals expressing each activity.

[5] ADG, Average Daily Gain reported as the average over the entire 28d period prior to slaughter.

[6]Weights were collected weekly during the final 28d of the finishing period. The beginning of the 28d period is denoted as d0. Weights were decreased by 4% to represent a standard industry shrink.

[7] Serum Nonesterified Fatty Acids

Chapter III : Head-only followed by cardiac arrest electric stunning is an effective alternative to head-only electrical stunning in pigs.

INTRODUCTION

Head-only electrical stunning is a reversible stunning method used in many small commercial slaughter plants. When head-only electrical stunning is used, exsanguination should occur before 15 s have elapsed following the end of the stun (Anil, 1991; Wotton & Gregory, 1986). Effective and complete bleeding is very important to ensure rapid death and meat quality in slaughtered livestock (Anil et al., 1997; Anil et al., 2000; Grandin, 2001). The 15 s interval can be met with rapid shackling and hoisting or bleeding on a platform or the floor prior to shackling and hoisting. In small North American slaughter plants, the authors have observed hoist speeds that may be too slow to achieve a short enough stun to bleed interval. In addition, bleeding on the floor can be awkward and difficult and may result in poor bleeding or employee injury. Most North American slaughter inspection personnel do not condone bleeding on the floor because it presents an additional vector for microbial carcass contamination.

An extension of the insensible period between stunning and bleeding would be useful in ensuring the welfare of electrically stunned slaughter pigs. Some small slaughter plants have begun to use a two-stage stun where head-only stunning is immediately followed by application of the stunning wand to the chest of the pig near the heart (head/heart method). The head/heart method may improve pig welfare by killing the stunned animal before it can recover consciousness.

The hypothesis of this study was that pigs stunned using the head/heart method would display fewer signs of sensibility, earlier loss of detectable heartbeat, and meat quality that was not different than head-only stunned pigs. The objective of this study was to evaluate signs of return to sensibility, stun to bleed time, blood lactate

concentration, muscle pH, drip loss, and fresh meat color to validate the head/heart electrical stunning method for small slaughter establishments.

MATERIALS AND METHODS

Animal use protocol

All procedures involving live animals in this study were approved by the Animal Care and Use Committees at both collaborating universities because one purpose of this study was to validate modifications of commonly used industry procedures that would improve pig welfare under commercial conditions. The Colorado State University Animal Care and Use Committee approval number was 08-341A-01. The University of Wisconsin protocol number was A1377.

Description of animals and stunning method

Eighty-nine pigs (market barrows and gilts, various breeds, mean HCW = 88.5 ± 10.4 kg) from 18 farms in Southwestern WI were slaughtered on three days over three weeks in a WI state-inspected meat processing facility. All pigs arrived at the plant on the morning of each slaughter day. All farms of origin were located within 172 km of the slaughter facility. The farms of origin varied with regard to management practices, feeding regimens, and outdoor access. At the time of slaughter, groups of two to eight pigs, with an average group size of five pigs, were moved 8 m from lairage to a stunning pen on the slaughter floor of the processing facility. Electric prods were used to move approximately 16 – 20% of the pigs. Prior to stunning, each pig was dampened with a light mist of water, such that the pig was not dripping. All pigs were stunned with a commonly-used fixed-voltage electric hog stunner (Model "ES", Best and Donovan Corp., Cincinnati, OH, USA) equipped with Model 9600100 stunning wand assembly.

Stunner output during application to a pig was 313 volts and mean current was 2.3 amperes at 60 cycles/s. Two stunning treatments (HEAD, the "head-only" method, 6s application of stunning wand in the hollow behind the ears; HEAD/HEART, the "head/heart" method, 3s application of stunning wand behind the ears followed by 3s application of stunning wand to the ventral region of the ribcage directly caudal to the junction of the humerus and scapula while the stunned pig was in lateral recumbancy) were applied in an alternating manner in the slaughter order such that every other pig received the same treatment. Each pig was stunned while standing in the holding pen. No restraint device was used. Upon conclusion, all stuns immediately resulted in tonic and clonic convulsive activity indicative of epileptiform brain activity and insensibility (Blackmore and Newhook, 1982; Anil, 1991; Anil et al., 1997).

Stun to bleed time, loss of detectable heartbeat, and blood lactate determination

Immediately after stun completion, a digital stopwatch was started to measure stun to bleed time and time to loss of detectable heartbeat. Loss of detectable heartbeat by stethoscope was performed, immediately after commencement of exsanguination, by a veterinarian that was blinded to the applied stunning treatment. Immediately after commencement of exsanguination, an exsanguination blood sample was captured in a 7mL blood collection tube with sodium fluoride potassium oxalate additive (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for blood lactate measurement at the end of the slaughter shift. Blood lactate was measured with a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany) as described by Edwards (2009).

Assessment of insensibility

Signs of return to sensibility were monitored beginning immediately after shackling, hoisting, and the commencement of exsanguination, which was approximately 30 s after the conclusion of the stun and within 2 s of the beginning of bleeding. Monitoring continued until slaughter procedures began approximately 4 min after the commencement of bleeding. Clonic spasms were still present at the commencement of exsanguination, which is indicative of insensibility (Anil, 1991). Monitoring continued until pigs were moved to a cradle for skinning. The following signs of return to sensibility were monitored: corneal reflex (eye blink from touch), dazzle response (eye blink from application of a bright light to the eye), nose twitching without stimulus, rhythmic breathing, natural blinking, eye tracking to a moving object, and righting reflex. Signs of sensibility were monitored by a trained observer. All pigs in the study were monitored by the same observer to eliminate interobserver variation.

Corneal reflex was evaluated by physical touch of the cornea of the eye with the blunt end of an LED pen light (Streamlight 65018, Eagleville, PA). Any blink or twitch of the eye lid associated with this test was considered a corneal reflex. Dazzle response was measured by shining an LED pen light (light output: 10 lumens, Streamlight 65018, Eagleville, PA, USA) directly into the eye of the stunned pig at a maximum distance of 15.2 cm. In order to be considered a reaction, the eyelid of the pig needed to close or partially close when the light was applied, then open or partially open when the light was removed. Nose twitching without stimulus was defined as movement of the snout without a touch stimulus. Rhythmic breathing was recorded when two or more sequences of expansion and contraction of the rib cage were observed. Rhythmic breathing was detected visually and confirmed by stethoscope during heartbeat monitoring. Natural

blinking was defined as the full closure and reopening of the eyelids as observed in sensible pigs in lairage with no stimulus applied to their eyes by an observer. Eye tracking to a moving object was recorded if the eye moved to focus on an object passed in front of the eye. The pen light that was used for dazzle response and corneal reflex was moved side to side perpendicular to the line of sight of the pig at a distance of 15.24 cm. The pen light was turned off for this test. Eye tracking was only recorded if the eye followed the object (i.e. rolling of the eyes was not a response). The righting reflex response is arching of the back in an attempt to regain a lateral position with the floor. Nose twitch, rhythmic breathing, natural blinking, eye tracking, and righting reflex were considered indicators of sensibility and animals showing these signs were immediately re-stunned with a hand-held captive bolt stunner.

Fresh meat color and pH assessment

Forty-eight hours following slaughter, carcasses were butchered for further processing by the plant. Meat color and pH was assessed at this time. Color was determined using a colorimeter (Model CR300, 8 mm aperture, Minolta Camera Co., Ltd., Osaka, Japan) standardized against a white (No. 20933026 CIE L* 97.91, a* -0.70, b* +2.44) calibration plate. The colorimeter was used to determine Commission Internationale d'Eclairage color space (**CIE L* a* b*)** values on the cut surface of the *Gluteus medius* in triplicate and *Gluteus accessorius* in duplicate on the ham side of the ham/loin junction of the left side of each carcass. The cut surface was allowed to bloom for 15 min, which was the maximum amount of time the plant could delay the processing of the hams. Color space values of the *Longissimus thoracis* were also collected in triplicate from a sample pork chop collected from the 10^{th} rib area of the left side of 23

carcasses in the final week of the study. Sample pork chops were allowed to bloom for 30 min before color was measured. For each carcass, the replicate color space values for each muscle were averaged to yield a single set of values for each muscle for statistical analysis. *Longissimus thoracis* pH was determined in duplicate 48 h postmortem with a pH meter (Accumet AR 50, Fischer Scientific, Pittsburgh, PA) equipped with a glass-encased pH probe (Orion 910600, Thermo Electron Corp., Beverly, MA) using a 10 g sample of the pork chop used for color assessment homogenized in 90 mL of distilled, deionized water.

Drip loss determination

Drip loss was determined using methods similar to those outlined by Honikel and Hamm (1995). Following color analysis, a 5 cm x 5 cm x 2.5 cm cube was cut from the center of each sample pork chop. Each cube was weighed and suspended in an air-tight container by a fishing hook and fishing line attached to the lid of the container such that no surface within the container made contact with the suspended sample. All containers were placed in a walk-in cooler and maintained at 2 to 4°C until reweighing 48h later.

Statistical analysis

Continuous data were analyzed in two paired comparison designs. The first analysis compared the two stunning treatments (HEAD, HEAD/HEART). The second analysis compared the group means of pigs from the 18 farms in the study. Individual animal was the experimental unit for the analysis of treatment and farm effects. In both designs, all main effects and interactions were included in the models using the GLIMMIX procedure of SAS for generalized linear mixed models with Kenward-Roger denominator degrees of freedom designated within the model (SAS, 2008). Interaction

effects that were declared not significant at $\alpha > 0.05$ were removed from the model in a stepwise manner until only significant interactions and all main effects remained in the model. Significant differences in treatment effects and farm effects on continuous data were realized at $\alpha < 0.05$.

Categorical data for treatment effects on signs of return to sensibility were analyzed using two-sided Fisher's exact tests in the frequency procedure of SAS (SAS, 2008). Categorical data in this study were binomially distributed (yes, no). Significant differences in frequency of incidence of signs of return to sensibility were realized at $\alpha < 0.05$.

RESULTS AND DISCUSSION

The head/heart method (HEAD/HEART) eliminated rhythmic breathing, natural blinking, eye tracking to a moving object, and righting reflex in the pigs observed in this study (Table 1). HEAD pigs showed increased ($P < 0.05$) incidence of nose twitch in contrast to HEAD/HEART pigs. No difference ($P > 0.05$) was observed in the incidence of corneal reflex or dazzle response between stunning treatments. However, the occurrence of the corneal reflex and dazzle response were common regardless of stun treatment. Anil (2004) found that corneal reflexes returned in head-only stunned pigs after 37 ± 12 s had elapsed if exsanguination was not performed. Other authors have reported similar times to the return of corneal reflexes in electrically-stunned calves, sheep, and pigs (Anil, 1991; Anil and McKinstry, 1992; McKinstry and Anil, 2004). The time between stunning and the beginning of bleeding in our study was 32 – 33 s, which may allow adequate time for the corneal reflex to redevelop before the commencement of exsanguination.

The presence of a corneal reflex in livestock that have been electrically stunned in a manner that induces epileptiform activity and cardiac failure may not be an indicator of sensibility on the part of the stunned animal. Blackmore and Newhook (1982) recorded an immediate decline in blood pressure following head to back electrical stunning to undetectable levels within 35 s in sheep. The authors have observed that corneal reflexes can be evoked from animals that show no other signs of insensibility. In our study, pigs that were stunned with the HEAD/HEART method commonly displayed corneal reflexes following the commencement of exsanguination. Corneal reflexes have been observed beginning 10 – 20s after head to back stunning and subsequent cardiac failure in sheep and calves (Blackmore and Newhook, 1982). Blackmore and Petersen (1981) monitored corneal reflexes in 35 calves stunned using a head to back stunning wand and 2 amperes of current for 5s. In the study, 60% of calves displayed corneal reflexes for a mean duration of 59s, although none of the calves displayed a detectable heartbeat by stethoscope. A maximum of 225 s elapsed before loss of corneal reflexes occurred in one calf in the study. The elimination of rhythmic breathing and detectable heart beat in HEAD/HEART pigs in our study does not support the likelihood of a return to sensibility. In sheep and calves that were electrically stunned using a head to back method, insensibility was permanent in all animals that did not have a detectable heartbeat (Blackmore and Newhook, 1982). There is a need to do further research on these residual brain stem reflexes.

In a previous study of six large commercial pork slaughter facilities, the fourth author did not observe corneal reflexes in electrically stunned pigs (Grandin, 2001; Grandin, unpublished data). However, the author evaluated corneal reflexes

approximately 60 seconds after stunning, which was 15 – 30 s following the commencement of exsanguination. The blood loss sustained before the observer tested corneal reflexes may have been sufficient to abolish brain stem activity, which is responsible for corneal reflexes. We evaluated the corneal reflex beginning approximately 2 s after the commencement of exsanguination. It is possible that our observations fell within a period of recurrence for the corneal reflex before it was abolished by brainstem hypoxia as a result of cardiac arrest or blood loss. Further research is necessary to identify the cause and duration of corneal reflex recurrence following electrical stunning when cardiac arrest has occurred. Other authors have identified the application of a painful stimulus to the nose of stunned pigs as a reliable test of sensibility (Anil, 1991; Anil et al., 1997; Grandin, 2009). Additional research regarding this method is also warranted.

No difference ($P > 0.05$) was observed in stun to bleed time between HEAD and HEAD/HEART (Table 2). HEAD pigs maintained a detectable heartbeat for more time ($P < 0.01$) than HEAD/HEART pigs. No HEAD/HEART pigs maintained a detectable heartbeat beyond the time when heartbeat monitoring began. Blackmore and Newhook (1982) also observed immediate loss of detectable heartbeat by electrocardiogram in head to back electrically stunned calves and sheep. The authors also reported failure to induce cardiac arrest in three calves stunned with the head to back device. It was recommended that a stethoscope be used to detect cardiac arrest in animals stunned using this method (Blackmore and Newhook, 1982).

No difference ($P > 0.05$) was observed in blood lactate concentration between stunning treatments. A farm effect ($P < 0.05$) was observed with regard to blood lactate

concentration. The variation in muscle fiber type and diameter between breeds appears to have a major influence on lactate production (Reiner et al., 2002). Handling stress and short transport time have also been linked to increased blood lactate concentration (Allison et al., 2006; Hambrecht et al., 2004; Apple et al., 2005). In summary, multiple factors are responsible for preslaughter blood lactate concentration differences, but it is impossible to identify the factors responsible for the effects observed in this study.

Petechial hemorrhages were not observed in the *L. thoracis* samples collected in this study. The fourth author has been able to reduce petechial hemorrhages in pigs by stopping double stunning. In our study, the stunner operator was highly skilled and double stunning was not observed. In addition, the head to back stunning method studied by Wotton et al. (1992) includes the *Longissimus thoracis* in the electrical circuit, which causes violent muscle contraction in the muscle, resulting in broken vertebrae and hemorrhaging. The head/heart method begins with a head-only stun, which the first, fourth, and seventh authors have observed to not typically cause petechial hemorrhages at 313 volts constant output. The second stage is applied separate to the head only stun and the primary muscles in the circuit are the intercostals. Since these muscles are much smaller than the *Longissimus,* the contraction and resultant carcass damage is far less. This is part of the reason why petechial hemorrhages were not detected. In addition, our study was performed during a period of consistent ambient temperatures, which the fourth author has observed as a period of less petechial hemorrhage occurrence. Multiple factors influence petechial hemorrhages. Kirton and Frazerhurst (1983) found that season, time of day, and double stunning all affected petechial hemorrhages in lambs

No difference ($P > 0.05$) was observed in CIE L*a*b* color space values for *L. thoracis*, *G. medius*, or *G. accessorius*, 48 h postmortem *L. thoracis* pH, or 48 h drip loss percentage between stunning treatments (Table 3). A farm effect ($P < 0.05$) was observed in *G. medius* L* value. Farm effects ($P < 0.05$) were also observed for CIE a* and b* values in *L. thoracis*, *G. medius*, and *G. accessorius*. Breed has been shown to impact pork drip loss and color stability (Van Heugten et al., 2003; Edwards et al., 2003). Hambrecht et al. (2004) cited preslaughter stress as a significant contributor to variation in fresh pork color and drip loss.

The HEAD/HEART method induced cardiac arrest and abolished righting reflex, eye tracking to moving objects, natural blinking, and rhythmic breathing. This method also significantly reduced the occurrence of involuntary nose twitch. Corneal reflex was observed in the majority of pigs in this study, regardless of stunning treatment. The recurrence of the corneal reflex in the present study merits further investigation, but the corneal reflex should not be used as an indicator of sensibility in pigs that have sustained cardiac arrest. Blood lactate concentrations were not different between stunning treatments. Objective measurement of meat color, drip loss, and pH did not identify meat quality differences between stunning treatments. Differences in blood lactate concentration, meat color, and drip loss were identified between farms of origin. These differences warrant further study of between-farm factors that influence these variables. This study has identified the HEAD/HEART method as a valid stunning procedure for small slaughter facilities to ensure maintenance of insensibility in electrically-stunned pigs.

IMPLICATIONS

The maintenance of insensibility during pig slaughter is essential for compliance with humane slaughter regulations. In small slaughter facilities, where stun to bleed time is increased due to slow hoist speed, the head-only stunning method is not capable of rendering animals unconscious for an adequate period of time. A two stage stunning method, where a standard head-only stun is immediately followed by stunning wand application to the cardiac region of the pig's chest, ensured the maintenance of insensibility during ensuing slaughter procedures. This stunning procedure had a very distinct advantage to render pigs unconscious and insensible to pain because heartbeat, rhythmic breathing, natural blinking, eye tracking to a moving object, and righting reflex were all abolished by this method. However, a corneal reflex was present in the majority of pigs, regardless of stunning treatment. It is unlikely that the presence of a corneal reflex without additional signs of sensibility is indicative of consciousness on the part of the stunned animal (Blackmore and Newhook, 1982). However, additional research is necessary regarding the occurrence brainstem reflexes following epileptiform activity and concurrent cardiac arrest in electrically-stunned swine. Overall, no differences in meat quality or plant efficiency were recognized between stunning treatments. This study identifies the head/heart method as a more effective stunning method than the head only method with regard to animal welfare.

Table III-1. Stun treatment effects on incidence of signs of return to sensibility in electrically stunned pigs (n=89).

	Stun treatment[1]		
Dependent variable (%)[2]	HEAD	HEAD/ HEART	p - value
Corneal reflex	93.8	85.0	0.29
Dazzle response	38.8	42.5	0.83
Nose twitch	26.5[a]	5.0[b]	0.01
Rhythmic breathing	12.2[a]	0.0[b]	0.03
Natural blinking	40.8[a]	0.0[b]	< 0.01
Eye tracking to moving object	32.7[a]	0.0[b]	< 0.01
Righting reflex	14.3[a]	0.0[b]	0.02

[a,b] Percentages under Stun treatment within a row with unlike superscripts are different ($P < 0.05$).

[1] Stun treatment: HEAD: 6 second electrical stun applied behind ears; HEAD/HEART: 3 second electrical stun applied behind ears followed by 3 second electrical stun applied directly caudal to the forelimb and dorsal to the sternum.

[2] Signs of return to sensibility were monitored continuously by a trained observer beginning approximately 2 s after the commencement of exsanguination and ending approximately 4 min later. Corneal reflex: eye blink in response to physical contact of the cornea with the end of a penlight; Dazzle response: eye lid movement in response to a penlight beam applied to the eye at 15.2 cm distance; Nose twitch: twitching of the nose without provocation; Rhythmic breathing: more than one successive expansion/contraction of the rib cage; Natural blinking: closure and reopening of the eyelid without provocation; Eye tracking to moving object: eye movement following the movement of a penlight held perpendicular to the line of sight at 15.24 cm distance and moved from side to side; Righting reflex: arching of the back in an attempt to regain a lateral position with the floor.

Table III-2. Least Squares means for stun treatment and farm effects on stun to bleed time, time to loss of heartbeat, and blood lactate concentration in electrically stunned pigs.

	Stun treatment[1]				Farm effects[2]				
Dependent variable	HEAD	HEAD/ HEART	S.E.	p - value	Farm mean	Farm minimum	Farm maximum	S.E.	p - value
Stun to bleed (s) (n=89)	32	33	1	0.44	34	27	46	6	0.21
Time to loss of heartbeat (s) (n=79)	121[a]	0[b]	5	< 0.01	62	46	87	18	0.07
Blood lactate (mmol/l) (n=88)	8.8	7.8	0.7	0.15	6.8	2.7[b]	18.1[a]	3.2	< 0.01

[a,b] Means under Stun treatment or Farm effects within a row with unlike superscripts are different ($P < 0.05$).

[1] Stun treatment: HEAD: 6 second electrical stun applied behind ears; HEAD/HEART: 3 second electrical stun applied behind ears followed by 3 second electrical stun applied directly caudal to the forelimb and dorsal to the sternum.

[2] Farm effects: Pigs from 16 farms were included. Maximum and minimum individual farm means are reported.

Table III-3. Least Squares means for stun treatment and farm effects on CIE L*a*b* color space values, 48h postmortem pH, and 48h drip loss of selected pork muscles.

	Stun treatment[1]				Farm effects[2]				
Dependent variable	HEAD	HEAD/ HEART	S.E.	p - value	Farm mean	Farm minimum	Farm maximum	S.E.	p - value
Longissimus thoracis (n=23)									
L*	54.71	54.49	0.98	0.85	54.83	51.98	56.48	1.82	0.71
a*	18.39	18.93	0.31	0.16	18.32	15.85^{b}	20.59^{a}	0.70	<0.01
b*	7.57	6.92	0.54	0.32	6.93	4.95^{b}	10.75^{a}	1.22	0.03
48 h postmortem pH	5.58	5.53	0.04	0.34	5.54	5.46	5.67	0.11	0.66
48 h drip loss (%)	5.7	6.8	0.80	0.3	4.7	2.7^{b}	13.0^{a}	2.1	0.01
Gluteus medius (n=82)									
L*	50.41	50.65	0.63	0.72	51.93	46.64^{b}	56.73^{a}	2.85	0.04
a*	-	-	-	-	19.62	18.72^{b}	21.68^{a}	0.87	<0.01
b*	-	-	-	-	4.78	3.43^{b}	7.50^{a}	0.70	<0.01
Gluteus Accessorius (n=82)									
L*	43.38	43.54	0.58	0.80	43.53	40.67	46.37	2.23	0.79
a*	-	-	-	-	22.08	18.39^{b}	24.75^{a}	1.13	<0.01
b*	5.72	5.64	0.21	0.70	5.24	2.91^{b}	7.61^{a}	0.96	<0.01
Two-way interactions									
Gluteus Medius									
a*									
Week 1	19.63	19.95	0.32		-	-	-	-	
Week 2	20.35	19.67	0.26		-	-	-	-	
Week 3	20.35	20.73	0.29		-	-	-	-	
b*									
Week 1	4.52^{ay}	4.17^{ay}	0.25		-	-	-	-	
Week 2	4.75^{axy}	5.14^{ax}	0.20		-	-	-	-	
Week 3	5.52^{ax}	5.34^{ax}	0.22		-	-	-	-	
Gluteus Accessorius (n=82)									
a*									
Week 1	20.99^{ax}	21.87^{ax}	0.41		-	-	-	-	
Week 2	22.67^{ax}	22.02^{ax}	0.33		-	-	-	-	
Week 3	22.34^{axy}	22.52^{ax}	0.38		-	-	-	-	

^{a,b} Means under Stun treatment or Farm minimum and Farm maximum within a row with unlike superscripts are different ($P < 0.05$).

^{x,y}Means for two-way interactions within a column with unlike superscripts are different ($P > 0.05$).

[1] Stun treatment: HEAD: 6 second electrical stun applied behind ears; HEAD/HEART: 3 second electrical stun applied behind ears followed by 3 second electrical stun applied directly caudal to the forelimb and dorsal to the sternum.

[2] Farm effects: Pigs from 18 farms were included in G. medius and G. accessorius assessment. Seven farms were included in L. thoracis assessment. Maximum and minimum individual farm means

[3]Interactions: Two-way interactions (Week of study * Stun treatment) observed for Gluteus medius a* and b* values and Gluteus accessorius a* values.

Chapter IV : The relationship of production level, age, and lactation status with indicators of animal welfare, metabolic status, and carcass characteristics of dairy cows: A single-farm case study.

INTRODUCTION

During the decade between National Animal Health Monitoring System (NAHMS) dairy industry surveys, increases in the incidence of mastitis, reproductive problems, lameness, and cow mortality were detected (NAHMS, 1996; NAHMS, 2007). The most common producer-identified cow health problems in the 1996 NAHMS survey were clinical mastitis (13.4 ± 0.3%), reproductive problems (11.6 ± 0.3%), and lameness (10.5 ± 0.3%) (NAHMS, 1996). In the 2006 NAHMS survey, the three most common producer-identified dairy health problems were clinical mastitis (16.5 ± 0.5%), lameness (14.0 ± 0.4%), and infertility problems (12.9 ± 0.3%) (NAHMS, 2007). Due to the common occurrence of these health conditions, udder or mastitis problems, lameness or injury, and reproductive problems accounted for 23.0 ± 0.6, 16.0 ± 0.4, and 26.3 ± 0.4 percent of culled dairy cattle during 2006, respectively (NAHMS, 2007). Within the population of cows that were sold for slaughter in 1995, the most common reasons for culling were reproductive problems (26.7 ± 0.8%) and udder or mastitis problems (26.5 ± 0.5%), followed by poor production not related to disease, mastitis, or lameness and injury (22.4 ± 0.8%), and lameness or injury (15.0 ± 0.6%) (NAHMS, 1996). The underlying factors in cases of lameness, mastitis, infertility and mortality vary widely, but a growing consensus has developed regarding the importance of management, environment, and genetic predisposition in the occurrence of such conditions (Cook and Nordlund, 2009; Dechow and Goodling, 2008; Dobson et al., 2008; Evans et al., 2006, Green et al., 2008; Hadley et al., 2006; Hansen, 2000). To promote dairy animal welfare on farms, potential markers for dairy welfare issues must be identified.

The Cooperatives Working Together (CWT) Dairy Herd Retirement program presents a valuable opportunity for dairy industry stakeholders and dairy scientists to

comprehensively assess the current state of the U.S. dairy herd on a large scale that could help to increase the depth of the NAHMS evaluations. The present study was conducted as the first of a potential series of comprehensive analyses of production records, on-farm metabolic status through blood sampling, body condition and locomotion scores, and carcass characteristics of dairy cows at various stages of lactation. The objective of the present study was to assess a single dairy enrolled in the Cooperatives Working Together (CWT) Dairy Herd Retirement program to identify important variables in performing large-scale dairy welfare assessments.

MATERIALS AND METHODS

Animal use protocol

All procedures involving live animals in this study were approved by the Colorado State University Animal Care and Use Committee. The approved protocol number was 09-175A-01.

Description of animals

A complete Northern Colorado dairy herd selected for liquidation in the Cooperatives Working Together (CWT) Dairy Herd Retirement Program was assessed over a 3 d period in June, 2009. The dairy herd in this study consisted of approximately 1300 cows. On the first two days of the farm assessment, blood samples were collected from approximately 200 cows of various ages and stages of lactation for analysis of nonesterified fatty acid (NEFA), glucose, and lactate concentrations. On the third day of the on-farm assessment, body condition score (BCS) and locomotion score was recorded for 572 cows. BCS was assessed according to the BCS scoring system characterized by Edmonson et al. (1989). Locomotion score was assessed according to the system

described by Thomsen et al. (2008). All BCS and locomotion score data was collected by two trained observers and scores were checked for consistency on approximately 20 cows before data collection began.

Blood collection and processing

Whole blood samples were collected from approximately 200 cows via tail venipuncture on the farm while the cows were restrained in headlocks during feeding. For each cow, 1 - 7mL blood collection tube with sodium fluoride potassium oxalate additive (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and 1 - 10 mL blood collection tube with no additive (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were collected with a single-use 20 ga. × 2.54 cm needle affixed to a reusable vacuumized blood collection tube hub. A new needle was used for each cow. Within 1 hr of whole blood collection, both blood collection tubes were placed on ice. After approximately 6 h, all blood collection tubes were centrifuged at 2,500 × *g* for 15 min. Immediately after centrifugation, serum and plasma were harvested from the blood collection tubes and transferred to polyethylene microcentrifuge tubes. The microcentrifuge tubes were immediately placed in a - 80° C freezer where they were held at - 80° C until analysis.

Following the commencement of slaughter procedures, exsanguination blood samples were collected in the same type of blood collection tubes used for on-farm blood sample collection. Following collection, exsanguination blood samples were processed following the same procedures outlined previously. Approximately 350 exsanguination blood samples were collected. Thirty-six matching samples between the farm and plant were generated from this sampling method. The matched samples were analyzed to

assess differences in glucose, lactate, and NEFA concentrations between the farm and plant.

Plasma glucose and lactate determination

Plasma glucose and lactate were determined using a benchtop biochemistry analyzer (YSI 2700 Select, YSI Inc., Life Sciences Division, Yellow Springs, OH) equipped with a D-glucose membrane (#2365, YSI Inc., Life Sciences Division, Yellow Springs, OH) and Lactate membrane (#2329, Inc., Life Sciences Division, Yellow Springs, OH). Assay results were reported in mmol / L.

Nonesterified fatty acid (NEFA) determination

Nonesterified fatty acid concentration was determined in duplicate from serum samples using a commercially-available enzymatic colorimetric NEFA assay (Wako NEFA-HR(2), Wako Diagnostics, Richmond, VA). The assay was performed in 96-well microtiter plates and optical absorbance was recorded at 550 nm. NEFA results were reported in mmol / L.

Liver sample collection and crude fat determination

Fresh liver samples were collected approximately 45 min following stunning at the slaughter establishment. A 10 g liver sample was placed in a 50 mL screw-cap conical tube labeled with the slaughter plant ID, then immediately placed in liquid N. Liver samples remained frozen until crude fat analysis was performed using the Folch method. One hundred fifteen liver samples were assayed.

Carcass characteristic assessment

Following humane slaughter, hot carcass weight was collected from plant records. Approximately 300 carcasses also were assessed for musculature and fat cover using a

modified version of a European scoring system for mature cow carcasses. For muscling score, the following criteria were used to assign muscling score: 1: Concave profile of the *Longissimus* from the ribs to the top of the spinous processes and very concave profile of the back of the round; 2: Slightly concave profile of the *Longissimus* from the ribs to the top of the spinous processes and slightly concave profile of the back of the round; 3: Angular profile of the *Longissimus* from the ribs to the top of the spinous processes and angular profile of the back of the round; 4: Slightly convex profile of the *Longissimus* from the ribs to the top of the spinous processes and slightly convex profile of the back of the round; 5: Convex profile of the *Longissimus* from the ribs to the top of the spinous processes and convex profile of the back of the round. Finish score was based on the amount of fat covering the exterior surfaces of the carcass. The following definitions characterize the Finish Score: 1: No fat cover on the carcass; 2: Fat covering 80% of the topline of the carcass but the muscles of the round are clearly discernable; 3: Fat covering more than 80% of the topline of the carcass and some of the plate and the muscles of the round are still discernable; 4: The entire carcass is covered with some fat, the muscles of the round are not easily discerned and the neck region of the carcass has some lean exposed; 5: The entire carcass is covered with fat and muscle definition is very difficult to detect.

Collection of dairy farm records

On the final week of herd occupancy at the dairy, all Dairycomp 305 cowfiles were collected and the following data were extracted for analysis: Previous lactation 305d milk, daily milk production, lactation number, days in milk (DIM), days open, services

per conception, times treated for mastitis in previous lactation, and times treated for lameness in previous lactation.

Statistical analysis

All continuous data were analyzed using paired comparison designs. Individual animal was the experimental unit for all data in this study. In all models, DIM, previous 305 d milk, daily production level, or lactation number were the fixed effects included in the analyses. Each fixed effect was analyzed in its own model. All data were analyzed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with Kenward-Roger denominator degrees of freedom and AR1 covariate structure designated within the model. Means were separated using pairwise t-tests and differences were recognized at $\alpha < 0.05$. The occurrence of broken tails was assessed with Fisher's Exact Tests for the fixed effects of lactation number and locomotion score. Differences within Fisher's Exact Tests were recognized at $\alpha < 0.05$.

RESULTS AND DISCUSSION

The impact of lactation number on several indicators of metabolic status, animal welfare, and carcass quality are shown in Table IV-1. Interestingly, BCS was significantly reduced ($P = 0.013$) in cows that were on their 3rd and 4th lactations. Banos and Coffey (2010) have indicated that heifers in their first 2 lactations may be susceptible to low energy balance due to the energetic demand of growth that still exists during the first two lactations in most Holstein dairy cows. At this time, it is unclear why a greater energy demand during the 3rd and 4th lactations would occur if growth typically ends near the end of the 2nd lactation, however, the increase in mastitis treatments ($P = 0.016$) recognized in the 5th lactation may indicate the presence of an underlying low-grade

chronic mastitis in many of the cows in this herd over lactations 3 and 4. The presence of a disease state can be detrimental to the maintenance of body condition. Brotherstone et al. (2007) recognized a positive correlation between growth rate at weaning and the occurrence of mastitis during production in Holstein dairy cows. Overall, the occurrence of mastitis in this particular dairy herd does not appear to be outside of the normal prevalence cited by other authors (Banos and Coffey, 2010; Brotherstone et al, 2007, Dechow et al., 2004). However, it appears that the actual prevalence of mastitis may be difficult to detect as Frigo et al. (2009) recognized mastitis prevalence rates of 16.3% and 30.5% in two university research dairies that were considered well-managed.

Locomotion score increased ($P < 0.0001$) with progressing years of lactation (Table IV-1). The discussion by Dechow et al. (2004) concurs with the results in the present study in the fact that decreasing BCS is associated with increasing occurrence of foot and leg health issues. Overall, it appears that the greatest impact of lactation number can be recognized at the 3rd and following lactations. However, the underlying causes for the issues that are observed at that time may have developed over the preceding two lactations.

Table IV-2 shows the effects of days in milk on the measured parameters of dairy cow health and carcass characteristics. Mean BCS followed a typical decrease followed by a gradual increase that is recognized as normal for Holstein dairy cows ($P < 0.0001$) (Heins et al., 2008). Hot carcass weight and Finish score followed a similar pattern to BCS ($P < 0.01$), suggesting their potential as measures of cow body condition status on a *post-hoc* basis. The validation of these measures was not within the scope of the current study, but they may have potential as indicators of cull cow body condition status during

postmortem evaluation at the slaughter establishment. The number of times cows were treated for lameness appeared to increase ($P < 0.0001$) over the course of lactation, which could be a multi-faceted issue. Other authors have cited metabolic, environmental, and genetic factors that underlie the occurrence of lameness (Cook and Nordlund, 2009; Dechow and Goodling, 2008).

Cows that produced less daily milk displayed greater BCS than medium and high producing cows ($P < 0.0001$) (Table IV-3). These results make logical sense as greater energy mobilization must occur to support greater milk excretion levels (Banos and Coffey, 2010; Heins et al., 2008). Low producing cows received more treatments for mastitis ($P = 0.019$) and remained open longer ($P < 0.0001$) than medium and high producing cows. Low and high producing cows received the greatest number of services per conception ($P = 0.014$). These results are indicative of the balance between minimal and maximal milk production that must be maintained to ensure cow health and reproductive receptivity (Banos and Coffey, 2010). Hot carcass weight and finish score followed the same trend as BCS, which is more surprising since the factors are directly related. Cows with previous lactation 305 d milk production greater than 11,589.5 kg displayed greater locomotion scores ($P < 0.0001$), which indicate an increased prevalence of lameness within these cattle. The metabolic load placed on such high-producing animals may be impacting foot and leg health through nutritional implications caused by the increased feed intake necessary to support production (Krause and Oetzel, 2006).

Within this particular dairy herd, an astounding incidence of broken tails were recognized. In total, the mean percentage of broken tails was 44.1%. The relationship of locomotion score and lactation number with broken tail prevalence can be observed in

Table IV-4 and Table IV-5, respectively. Overall, the percentage of cows with broken tails increased ($P < 0.0001$) with increasing lactation number and increasing locomotion score ($P < 0.0001$). These data suggest that tail twisting was a common method of coercing cows to move during handling and that tail twisting to the point of breakage was an increasingly common occurrence as locomotion score increased. There is likely an interaction effect between locomotion score and lactation number as we could expect tail breaks to accumulate over the total herd life. To our knowledge, this is the first quantification of this type of abuse in dairy industry – related research.

IMPLICATIONS

Several factors impact the health and well-being of dairy cows. It has been demonstrated in past research that production measures can be useful in assessing the welfare of dairy cattle. Our results agree with this concept, however, it is obvious that one cannot rely on production parameters alone to identify on-farm welfare issues. The occurrence of broken tails in the present study is a clear indication of poor management regard for animal welfare and a lack of employee training in humane handling practices. Multiple factors identified in the current case study warrant further investigation in future research endeavors. The interaction between animal lameness and risk of abusive handling is of particular interest because both lameness and abusive handling are animal welfare issues that must be addressed by farm managers, but the identification of an association between the two factors punctuates the need for management attention. In addition, the relationship of production level, BCS, and health status of the animal warrants investigation. Clearly, a cow with a greater BCS is not always the healthiest animal, which has been demonstrated in the current case study by the increased number

of mastitis treatments and days open in low producing cows with greater BCS scores. Overall, this study has clearly demonstrated the importance of management in maintaining dairy animal welfare and identified factors of interest in evaluating dairy animal welfare.

Table IV-1. Least squares means for the impact of lactation number on multiple indicators of dairy animal welfare, metabolic status, and carcass characteristics.

	Lactation									
Dependent variable	1	2	3	4	5	6	7	8	S. E.	*P* - value
Visual assessment										
BCS	2.71[a]	2.81[a]	2.58[bc]	2.48[b]	2.72[a]	3.00[a]	3.25[a]	1.75[c]	0.21	0.013
Locomotion score	1.61[c]	1.79[bc]	2.24[b]	2.67[b]	2.50[b]	3.00[abc]	1.00[c]	5.00[a]	0.43	< 0.0001
DairyComp 305 current lactation data										
Times treated for lameness	1.0	0.6	0.7	0.6	0.4	0.0	0.5	2.0	0.5	0.167
Times treated for mastitis	0.2[c]	0.3[bc]	0.5[b]	0.6[abc]	1.1[a]	1.0[abc]	0.0[abc]	1.0[abc]	0.4	0.016
Times bred	1.7	2.2	1.9	1.6	2.0	2.0	1.0	2.0	0.5	0.216
Days open	123.0	120.6	103.8	159.1	118.3	98.0	102.0	82.0	30.2	0.324
Metabolic indicators										
On – farm antemortem										
Plasma glucose (mmol / L)	3.34	3.30	3.06	3.46	3.19	-	-	-	0.28	0.928
Plasma lactate (mmol / L)	1.50	1.30	0.74	1.00	0.62	-	-	-	0.67	0.797
Plasma NEFA (mEq / L)	0.18	0.17	0.49	0.03	0.03	-	-	-	0.16	0.521
In – plant postmortem										
Plasma glucose (mmol / L)	10.85	12.30	12.95	17.85	19.75	-	-	-	3.80	0.415
Plasma lactate (mmol / L)	7.08	8.48	9.22	9.55	7.58	-	-	-	1.16	0.371
Plasma NEFA (mEq / L)	0.18	0.13	0.05	0.11	0.25	-	-	-	0.08	0.616
Liver Crude Fat (%)	5.85	6.47	10.30	5.36	8.69	-	4.34	-	2.47	0.472
Carcass characteristics										
Hot carcass weight (kg)	272.6	280.8	291.5	294.7	289.6	316.2	303.5	293.0	44.7	0.251
Finish score (1 - 5)	2.02	2.15	1.94	1.93	1.75	3.00	2.25	2.00	0.24	0.115
Muscle score (1 – 5)	2.67	2.58	2.59	2.43	2.56	3.50	3.00	3.00	0.24	0.319

[a,b,c] Means within a row with unlike superscripts are different ($P < 0.05$).

Table IV-2. Least squares means for the impact of days in milk (DIM) on multiple indicators of dairy animal welfare, metabolic status, and carcass characteristics.

	Days in milk									
Dependent variable	Dry	1 – 60	61 – 120	121 – 180	181 – 240	241 – 300	301 – 360	360 +	S. E.	*P* - value
Visual assessment										
BCS	3.57[a]	2.57[bc]	2.72[b]	2.58[c]	2.64[bc]	2.68[bc]	2.79[b]	2.79[b]	0.10	< 0.0001
Locomotion score	1.53[bcd]	1.00[b]	1.57[bd]	1.97[ac]	2.12[a]	2.16[a]	1.94[ad]	2.00[ab]	0.23	0.006
DairyComp 305 current lactation data										
Times treated for lameness	1.4[ab]	0.3[cde]	0.4[e]	0.6[e]	1.0[bcd]	1.3[ad]	1.6[a]	1.3[ace]	0.3	< 0.0001
Times treated for mastitis	0.4	0.3	0.1	0.5	0.3	0.3	0.2	0.3	0.19	0.238
Metabolic indicators										
On – farm antemortem										
Plasma glucose (mmol / L)	-	-	3.25	3.27	3.38	3.39	-	3.19	0.26	0.978
Plasma lactate (mmol / L)	-	-	1.09	1.37	1.30	1.72	-	0.99	0.65	0.972
Plasma NEFA (mEq / L)	-	-	0.06	0.12	0.25	0.41	-	0.34	0.14	0.468
In – plant postmortem										
Plasma glucose (mmol / L)	-	-	12.55	11.29	14.27	11.8	-	12.50	4.13	0.950
Plasma lactate (mmol / L)	-	-	7.34	7.65	7.40	10.75	-	7.56	1.08	0.412
Plasma NEFA (mEq / L)	-	-	0.14	0.19	0.15	0.11	-	0.08	0.08	0.849
Liver Crude Fat (%)	11.75	5.65	5.78	8.93	-	-	-	-	1.55	0.054
Carcass characteristics										
Hot carcass weight (kg)	345.8	263.3[bc]	574.4[c]	260.5[c]	288.3[b]	291.7[b]	295.0[b]	331.5[a]	9.1	< 0.0001
Finish score (1 - 5)	2.33[ab]	1.93[bcd]	1.95[cd]	1.93[d]	2.10[bcd]	2.21[ac]	2.15[ad]	2.57[a]	0.12	0.004
Muscle score (1 – 5)	3.00	2.64	2.60	2.55	2.61	2.61	2.59	2.93	0.12	0.108

[a,b] Means under Days in milk period within a row with unlike superscripts are different ($P < 0.05$).

Table IV-3. Least squares means for the impact of daily milk production and previous lactation 305 d milk on multiple indicators of dairy animal welfare, metabolic status, and carcass characteristics.

	Daily milk (kg)					Previous 305 d milk (kg)				
Dependent variable	< 22.8	22.8 – 45.4	> 45.4	S. E.	*P* - value	< 6917.5	6,917.5 – 11,589.5	> 11,589.5	S. E.	*P* - value
Visual assessment										
BCS	3.08[a]	2.70[b]	2.51[c]	0.07	< 0.0001	2.76	2.71	2.56	0.06	0.127
Locomotion score	1.58	1.86	2.03	0.15	0.281	1.58[b]	1.79[b]	2.59[a]	0.13	< 0.0001
DairyComp 305 current lactation data										
Times treated for lameness	0.9	0.8	0.4	0.2	0.055	1.0	0.8	0.6	0.1	0.313
Times treated for mastitis	0.7[a]	0.3[b]	0.1[b]	0.1	0.019	0.6	0.2	0.3	0.1	0.037
Times bred	2.4[a]	1.8[b]	2.2[a]	0.2	0.014	1.7	1.9	2.1	0.2	0.275
Days open	192[a]	119[b]	102[b]	10	< 0.0001	116	123	125	9	0.793
Metabolic indicators										
On – farm antemortem										
Plasma glucose (mmol / L)	-	3.33	3.14	0.16	0.436	3.67[a]	3.20[b]	3.22[ab]	0.16	0.049
Plasma lactate (mmol / L)	-	1.35	1.03	0.40	0.605	1.67	1.20	1.32	0.51	0.689
Plasma NEFA (mEq / L)	-	0.17	0.28	0.10	0.488	0.16	0.20	0.06	0.13	0.795
In – plant postmortem										
Plasma glucose (mmol / L)	-	12.77	10.53	2.52	0.573	13.10	12.68	8.10	3.27	0.688
Plasma lactate (mmol / L)	-	7.54	8.96	0.74	0.238	8.53	7.42	8.70	0.98	0.473
Plasma NEFA (mEq / L)	-	0.17	0.07	0.04	0.140	0.12	0.18	0.08	0.06	0.490
Liver Crude Fat (%)	14.38	6.54	5.35	1.76	0.057	8.51	6.28	3.98	2.18	0.498
Carcass characteristics										
Hot carcass weight (kg)	330.4[a]	276.3[ab]	264.6[b]	9.5	< 0.0001	281.8	276.3	286.5	5.8	0.393
Finish score (1 - 5)	2.34[a]	2.05[b]	1.79[c]	0.08	0.0005	2.16	2.01	1.97	0.07	0.179
Muscle score (1 – 5)	2.87[a]	2.62[b]	2.42[c]	0.08	0.006	2.76[a]	2.61[ab]	2.43[b]	0.07	0.020

[a,b] Means under Daily milk and Previous 305 d milk within a row with unlike superscripts are different ($P < 0.05$).

Table IV-4. Fisher's exact means for the impact of lactation number on the prevalence of broken tails.

	Lactation							
Dependent variable	1	2	3	4	5	6	7	*P* - value
Normal	72.9	50.9	50.0	38.1	38.1	12.5	40.0	< 0.0001
Broken	27.1	49.1	50.0	61.9	61.9	87.5	60.0	-

Table IV-5. Fisher's exact means for the impact of locomotion score on the prevalence of broken tails.

	Locomotion score					
Dependent variable	1	2	3	4	5	*P* - value
Normal	66.7	53.1	40.0	54.8	30.8	< 0.0001
Broken	33.3	46.9	60.0	45.2	69.2	-

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