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Effects of central and peripheral administration of an acute phase protein, alpha-1-acid-glycoprotein, on feed intake and rectal temperature in sheep

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DEDICATION

To my wonderful Husband

Thank you for always being there to encourage me and always pushing me to do my best!

To my Mom

Thank you for molding me into the person I am today, I strive to be more like you!

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ABSTRACT

No mechanistic link has tied inflammation to suppression of feed intake. In rodents, an acute phase protein, α-1-acid glycoprotein (AGP), could provide this link by acting as a leptin receptor agonist. The objective of these studies was to determine the effects of AGP on food intake and rectal temperature in sheep. In the first experiment ewes (n=4) received 1 of 4 treatments [0 (control), 0.012 (low), 0.06 (medium), or 0.30 (high) mg / kg BW AGP] into the lateral ventricle. The study was repeated until all sheep received all treatments. In the second experiment ewes (n=10) received one of two treatments (0 and 3 mg / kg BW of AGP) intravenously. In the third experiment ewes (n = 19) received peripheral treatments (IV) of an antipyretic [0 (control) or 2.2 mg / kg BW flunixin meglumine] 30 minutes prior to receiving central treatments (ICV) [0 (control) or 0.3 mg / kg BW AGP]. In the first experiment there was no effect of treatment on feed intake rate ($P = 0.37$) or cumulative feed intake ($P = 0.31$). There was an effect of treatment on rectal temperature ($P = 0.002$) such that rectal temperatures were greater ($P < 0.05$) following the high dose of AGP. In the second experiment there was no effect of treatment on feed intake rate ($P = 0.98$), on cumulative feed intake ($P = 0.41$) or on rectal temperature ($P = 0.71$). In the third experiment there was an effect of central treatment ($P < 0.0001$) and an interaction of central treatment and time ($P < 0.0001$). There was no effect of peripheral treatments ($P = 0.93$) on rectal temperature, indicating that central AGP may increase rectal temperature in sheep by pathways that do not involve prostaglandins. Further research is needed to determine if AGP may be an important integrator of energy balance and inflammation.

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central treatment by time interaction (P < 0.0001) on rectal temperature such that ewes treated centrally with AGP had greater rectal temperatures at 2, 4, 6, 8, and 12 h relative to ewes treated centrally with control. There was no effect of intravenous treatment (P = 0.93), intravenous treatment by time interaction (P = 0.35), intravenous by central treatment (P = 0.54), or intravenous by central treatment by time interaction (P= 0.15) on rectal temperature. (*) indicates time points at which AGP treated ewes differed from CON treated ewes (P < 0.05). CON, control; FLU, flunixin meglumine; AGP, Alpha-1-acid glycoprotein 63

CHAPTER I

LITERATURE REVIEW

POSTPARTUM INFLAMMATION

Systemic inflammation is common in the first week of lactation. Inflammation is the host protective response to pathogenic (e.g., infection or non-pathogenic (e.g., stress)) damaging stimuli. Recently, Bradford and others (2015) highlighted the major differences between acute and sub-acute/metabolic inflammation in dairy cows. Briefly, inflammatory molecules promote increased blood flow to the infected tissue, immune cell infiltration and activation, and systemic responses, including increased body temperature, increased heart rate, and decreased feed intake. In contrast, "metabolic inflammation" is characterized by its sub-acute, chronic nature and subtle shifts in both immune cell and metabolic function. The presence of an inflammatory state in the postpartum period has been documented in a number of species, including cattle (Humblet et al., 2006, Mullins et al., 2012), mice (Gregor et al., 2013), pigs (Rosenbaum et al., 2012a, b), and humans (Disilvestro, 1986). Unlike inflammation associated with a local infection, the postpartum inflammatory state has no clear focal organ and does not necessarily induce the traditional signs of inflammation (fever, swelling, pain). Rather, inflammation during this period is systemic and sub-acute, consistent with the concept of metabolic inflammation. Although data are limited, mammary (Clarkson et al., 2004), liver (Loor et al., 2005), and adipose (Sadri et al., 2010, Gregor et al., 2013) tissue all show increased inflammatory signals in early lactation, and other metabolic organs are also likely affected.

One effect of cytokines is to activate production of acute phase proteins. Primarily produced by the liver, this class of proteins includes haptoglobin, serum amyloid A, C-

reactive protein, and alpha-1-acid glycoprotein (AGP). Proteins that participate in the acute phase response to infection are generally found in very low abundance in the bloodstream, but are greatly elevated during periods of systemic inflammation. The presence of an acute phase response in postpartum dairy cows is well established. Numerous studies in the past decade have demonstrated that inflammatory and acute phase mediators are elevated in the days after parturition, even in cows that are apparently healthy (Humblet et al., 2006, Bionaz et al., 2007, Huzzey et al., 2009, Graugnard et al., 2012, Mullins et al., 2012, Qu et al., 2014). This growing body of evidence suggests that either the processes of parturition and galactopoiesis induce inflammation directly or that infections or endotoxin affect far more postpartum cows than is currently recognized. Whatever the explanation, the prevalence of postpartum inflammation raises important questions about the implications of this inflammation on central regulation of feed intake.

Greater degrees of inflammation are associated with poor performance. Although most transition dairy cows apparently go through a period of inflammation, the magnitude of this inflammatory condition varies greatly between cows. Bertoni et al. (2008) assessed the importance of this variation by measuring a panel of inflammatory markers and separating transition cows into quartiles for degree of inflammation. Cows in the highest quartile had significantly lower milk yields, greater proportions of cows with health problems, and lower conception rates. One metric that has been used in this respect is paraoxanase, a plasma biomarker that is potently suppressed by a variety of inflammatory stimuli. Transition cows with high paraoxanase concentrations, in addition

to having lower concentrations of acute phase proteins and reactive oxygen metabolites, produced more milk, and had lower proportion of cows reporting at least one health problem (Bionaz et al., 2007). Because haptoglobin is the acute phase protein reaching the highest concentration in response to inflammation in ruminants, it has been widely used as an inflammatory marker. Recently, a greater magnitude of inflammatory response, measured in terms of circulating haptoglobin, during the transition period was associated with an impaired performance (Huzzey et al., 2015). Increments of 1 g/L of haptoglobin at -2, -1 and +1 weeks relative to parturition reduced whole-lactation milk yield by ~ 500 kg. Furthermore, increase of postpartum haptoglobin was negatively associated with hazard of conception (0.70 – 0.97, 95% coefficient interval). More recently (McCarthy et al., 2016), cows were categorized in 4 quartiles of inflammation based on plasma haptoglobin concentration during the first week postpartum. Interestingly, cows in Q2 and Q3 (moderate inflammation) had lower dry matter intake and BW postpartum, coupled with tendency for lower fat- and energy-corrected milk. These longitudinal studies strengthen the established links between postpartum inflammation and negative health and production outcomes. Additionally, incidence of diseases such as metritis, mastitis, and others have been evaluated using, the acute phase protein, AGP as a potential marker for disease incidence (Tamura et al., 1989, Eckersall et al., 2001, Sheldon et al., 2001, Cairoli et al., 2006), albeit using a more limited number of cows (n = 10 to 90) as compared with studies with haptoglobin cited above (n = 67 to 412). The limitation of associative data is that its interpretation is difficult because it does not demonstrate causation. While it is possible that these studies identified cows with

primary inflammatory conditions that impaired feed intake and overall performance, it is also possible that impaired intake exacerbates inflammation, impairing health and productivity. To carefully determine whether inflammation or intake is the primary cause of peripartum disorders, more controlled studies are required.

Feed Intake

In spite of the great body of research in dairy cattle and many other species to identify the role of inflammatory and acute phase mediators on metabolic function, little research has focused on studying their role in feed intake regulation. Feed intake regulation is a complex phenomenon integrating peripheral and central signals in the hypothalamus. Regulation of feed intake has been a focus of research for many years. In ruminants, attempts to develop models for intake prediction are mostly focused on physical and chemical factors (Ingvartsen and Andersen, 2000). Forbes extensively studied physical regulation of intake and attempted to study its interaction with metabolic regulators and neuronal signaling (Forbes, 1980, 1996). Studies across many decades have identified numerous metabolic compounds and hormones that influence hypothalamic signals of satiety or hunger (Ingvartsen and Andersen, 2000, Allen and Piantoni, 2013). The physiological mediators of metabolite responses are complex and diverse; among several proposed mechanisms of peripheral regulation of intake, the hepatic oxidation theory appears to be the best suited for ruminants accounting for differences in fuels absorbed and sites of absorption (Allen et al., 2009, Allen and Bradford, 2012).

Marked depression in feed intake is common during the peripartum period. After 17 years and despite of the great body of research we still are unable to answer a key question formulated by Drackley (1999): “what controls dry matter intake during the transition period?” Variability in feed intake during the first week postpartum is 30 to 40%, whereas DMI variation after peak lactation is only 6 to 10% (Drackley, 1999). Dairy cows at parturition face a drastic increase in nutrient requirements (2 to 4x for many nutrients) driven by the increased demands for milk production, which are compensated by increases in hepatic gluconeogenesis and mobilization of body stores of fat, protein, and calcium (Roche et al., 2013). Dry matter intake during the first week of lactation only increases by 30 to 50%, and if this increase is delayed, it forces the cow to mobilize excessive amount of body reserves, exacerbating the risk of metabolic disorders and disease development (Ingvartsen et al., 2003). Inflammation during the first week of lactation is associated with depressed feed intake in dairy cows (Bertoni et al., 2008, McCarthy et al., 2016).

Although no associations of intake and AGP have been reported for ruminants, a study involving dairy cows, Jafari et al. (2006), evaluated the infusion of three levels of glutamine on dry matter intake and plasma concentrations of AGP in transition cows. Using data from that study, a negative association was found between dry matter intake adaptation to lactation and the rise in plasma AGP after parturition. Using mean values for dry matter intake and AGP for days 7 to 21 ($n = 9$), it was found that AGP was negatively associated with dry matter intake ($r = 0.90$; $P = 0.001$).

Rectal Temperature

Fever, known by an elevated body temperature, is well known in response to inflammation or infections. Many exogenous pyrogens (ex: lipopolysaccharide; LPS) can cause a fever. The presence of these substances induce the production of proteins called cytokines. Cytokines such as, tumor necrosis factor alpha (TNF-α) and other interleukins, have been found in circulation (Kluger, 1991, Jansky et al., 1995), however changes in interleukin-6 concentrations in the rat best match the change in temperature produced by administration of LPS (Lemay et al., 1990). Cytokines have various routes to access the brain. Described in a review by Blomqvist and Engblom (2018) are four routes used to bypass the blood-brain-barrier and get the signals to the brain. Thermosensitive neurons in the hypothalamus are exposed to cytokines at the onset of a temperature. Researchers found that administering endogenous pyrogens released fever inducing prostaglandins (Feldberg and Saxena, 1971). Soon thereafter, Vane (1971) administered the antipyretic drug, aspirin, for prostaglandin inhibition and decreased the induced fever.

LEPTIN

Introduction

One of the most important endocrine factors is leptin, a cytokine-like hormone produced by adipose tissue. Leptin, known for its homeostatic regulation of adiposity, is one of the main contributors in energy homeostasis, proving that obesity can be a sign of resistance to its role (Ahima and Flier, 2000, Munzberg and Myers, 2005). Leptin exerts its suppression effects in intake regulation principally through its long-form receptor

(LepRb) in the hypothalamus. There are six isoforms of the leptin receptor, among those, LepRb is the only isoform with capacity to transmit leptin signaling and initiate the signaling response (Kwon et al., 2016). Neurons located in the hypothalamus monitor energy status by sensing concentrations of hormones (e.g., leptin; (Ingvartsen and Boisclair, 2001)) and nutrients (e.g., free fatty acids; (Obici et al., 2002)).

Leptin Background

The concept began with the discovery of two recessive mutations in mice, obese (ob) and diabetes (db), with the effects of hyperphagia, decreased energy expenditure, and early onset obesity (Ingalls et al., 1950). The joining of two mice, known as parabiosis, showed that when a normal wild-type mouse was joined with an ob/ob mouse, the obese model mouse began to lose weight (Hausberger, 1958). However, parabiosis of the db/db model found the wild-type mouse suffering from hypophagia (Coleman and Hummel, 1969, Coleman, 1973). This information led to the discovery of their role: the ob gene product was named leptin (from the Greek root, leptos, which translates meaning thin) because of its effects on leptin-deficient and normal mice (Halaas et al., 1995). The db locus is needed for response to the satiety factor.

Leptin Pathway in the Brain

For leptin, several signaling pathways can mediate the hypothalamic actions, with most effects thought to be via the JAK/STAT signaling pathway. After leptin binds to LepRb, downstream signaling through the sequential phosphorylation of the tyrosine kinase janus kinase (JAK) and the transcription factor signal transducer and activators of

transcription (STAT) is initiated. Phosphorylated STAT3 is translocated to the nucleus where it modulates the transcription of genes involved in feed intake, resulting in altered neuropeptide production and release (Vaisse et al., 1996). Leptin increases the transcription of proopiomelanocortin (POMC) which in turn stimulates the synthesis of α-melanocyte stimulating hormone (α-MSH), which exerts its anorexigenic (intake inhibition) effect by binding to the melanocortin-4 receptor (MC4R) in the hypothalamus (Ellacott and Cone, 2004). Additionally, leptin inhibits the neuronal activity of agouti-related protein (AgRP) and reduces the expression of neuropeptide Y (NPY) and AgRP, both orexigenic (intake-promoting) neuropeptides. The net effect of leptin signaling is therefore a decrease in feed intake (Myers et al., 2008).

Leptin in Rodents

With the center of leptin research established in the rodent model, the mechanisms and purpose of this hormone are fairly clear. However, further research continuously discovers more about it. There are both central and peripheral mechanisms that control leptin's effect on food intake and energy expenditure. Leptin receptors are expressed in most tissues of the rodent. In rodent models, mutations in the leptin and leptin receptor genes can result in morbid obesity, infertility, and insulin resistance (Chua et al., 1996, White et al., 1997, Farooqi, 2002, Perry et al., 2014). It has been determined that administration of central leptin in rats increases body temperature (Luheshi et al., 1999, Turek et al., 2004, Skibicka and Grill, 2009). As with inflammatory studies in the past, Luheshi et al. (1999) administered a cyclo-oxygenase inhibitor, flurbiprofen, and

was able to block the leptin-induced temperature via the prostaglandin pathway. More recently, researchers are using leptin, in a diabetic model, to find a mechanism that will help diminish type I diabetes (Kadota et al., 2018).

Leptin in Ruminants

Leptin properties in the ruminant model has shown similar results as human and rodent research (Chilliard et al., 2001). For example, ovine leptin given centrally to goats and sheep was found to reduce food intake (Clarke et al., 2001). Plasma leptin is higher in sheep and cows with higher body fatness (Blache et al., 2000, Delavaud et al., 2000). These few examples in ruminant species are parallel with those in mice and humans. However, the factor that sets them apart is the discovery of leptin levels, unlike in non-ruminant studies, does not increase in response to inflammatory indicators. Rodent studies have shown that administration of LPS peripherally causes an increase of plasma leptin levels, suggesting a role of leptin in anorexia induced by endotoxin (Francis et al., 2000). In sheep, however, peripheral administration of LPS had no effect on plasma leptin concentrations but sheep still showed signs of anorexia and fever (Soliman et al., 2001)). Soliman and others (2002) then continued by replicating this experiment in cattle. The plasma leptin concentrations from these cows were not changed for 8 hours, showing almost the same concentration as those in saline-injected controls. These discoveries suggest that leptin may not be involved in anorexia and fever in ruminants with inflammatory status.

ALPHA-1-ACID-GLYCOPROTEIN

Introduction

Alpha-1-acid-glycoprotein, (AGP) also known as orosomucoid, is a versatile acute phase protein with diverse roles. From rodent studies, it is now known that AGP exerts some anti-inflammatory and immunomodulatory properties (Hochepied et al., 2003). More recently, AGP has been identified as a protein capable of integrating inflammatory and metabolic signals to modulate immune responses, protecting adipose tissue from excessive inflammation by improving glucose and insulin tolerance in mice (Lee et al., 2010). It has also been reported that AGP was consistently found in high concentrations around parturition, and has been associated with greater incidence of several diseases in cattle (Tamura et al., 1989, Eckersall et al., 2001, Sheldon et al., 2001, Cairoli et al., 2006). In the early 1990's a new role for AGP was discovered by serendipity when Bellinger et al. (1990) were investigating the purity of extracted satietin, a putative hypophagic endocrine factor in plasma. Bellinger and coworkers found that the extracted satietin was contaminated with other plasma proteins (e.g., albumin and AGP). After further purification and isolation, they determined that centrally administered purified AGP was also able to induce hypophagia in rodents.

Alpha-1-acid-glycoprotein in the Leptin Pathway

Since the early 1990's, the putative role of AGP was not evaluated further until a recently published paper in *Diabetes* (Sun et al., 2016) thoroughly documented the role of AGP as a hypophagic compound, signaling via the long form leptin receptor to reduce

feed intake. Sun and Coworkers (2016) fed C57BL/6 or db/db mice with a normal diet. Feed intake was reduced in C57BL/6 mice but not in db/db in response to intraperitoneal infusions of AGP for four consecutive days. The lack of hypophagic effect of AGP in db/db mice led authors to hypothesize that AGP signals via LepR. Subsequently, they silenced LepR in the hypothalamus of C57BL/6 mice and demonstrated that the hypophagic effects of AGP were blocked.

This discovery immediately found ruminant researchers discussing the possibility of AGP playing a role in hypophagia of cattle under inflammatory conditions. The Bradford group has been investigating the systemic effects of inflammation during the immediate postpartum period in cattle (Bradford et al., 2015). To mimic systemic inflammation, the Bradford group subcutaneously administered recombinant TNFα to 33 transition cows assigned to control (carrier), low dose (1.5 μg/kg BW), or high dose (3.0 μg/kg) daily from day 1 to 7 of lactation (Yuan et al., 2013). This treatment protocol performed did not induce pyrexia or other acute responses because the subcutaneous route of administration slowed the appearance of the protein in circulation. The mild elevation in plasma TNFα nevertheless induced an increase in plasma haptoglobin and had a dramatic impact on dry matter intake and milk production by significantly decreasing dry matter intake and milk yield throughout the first week of lactation. Bradford et al. (2015) results were inconsistent with the hypothesis that inflammation in early lactation primarily influenced liver function; rather, inflammation during this period altered mammary and/or neurological function greatly.

CONCLUSIONS

In summary, if AGP acts as a LepRb agonist in ruminants, as reported in mice (Sun et al., 2016), it's increase during peripartum inflammation may be the link between inflammation and hypophagia that is a common precursor to common metabolic and infectious disorders in early lactation dairy cows. Research regarding the possibilities of alpha-1-acid-glycoprotein in inflammatory models is ongoing. This should give a greater insight to the many diverse roles that leptin and its receptor play in the ruminant model, just as recently discovered by serendipity in the rodent model.

CHAPTER II

EFFECTS OF CENTRAL AND PERIPHERAL ADMINISTRATION OF AN ACUTE PHASE PROTEIN, ALPHA-1-ACID-GLYCOPROTEIN, ON FEED INTAKE AND RECTAL TEMPERATURE IN SHEEP

ABSTRACT

In rodents, an acute phase protein, α-1-acid glycoprotein (AGP), could provide this link by acting as a leptin receptor agonist. Serum concentrations of AGP rise markedly during inflammation. The objective of this study was to determine the effects of AGP on feed intake and rectal temperature in sheep. Ewes were ovariectomized, implanted with a cannula into a lateral ventricle of the brain, and kept indoors in individual pens. Feed intake and rectal temperature was determined for sheep in all experiments. In the first experiment ewes (n = 4) received one of four treatments [0 (control), 0.012 (low), 0.06 (medium), or 0.30 (high) mg / kg BW AGP] into the lateral ventricle. The study was repeated until all sheep received all treatments. In the second experiment ewes (n = 10) received one of two treatments (0 and 3 mg / kg BW of AGP) intravenously. In the third experiment ewes (n = 19) received peripheral treatments (IV) of an antipyretic [0 (control) or 2.2 mg / kg BW flunixin meglumine (FLU)] 30 minutes prior to receiving central AGP [0 (control) or 0.3 mg / kg BW of AGP]. All data were analyzed using a mixed model analysis of variance, SAS software v9.4 (SAS Institute Inc., Cary, NC), and tested for effects of treatment, time, and the interaction of treatment and time. Cumulative feed intake following administration of treatments was tested for effects of treatment. In the first experiment there was no effect of treatment ($P = 0.37$) on feed intake rate and no effect of treatment ($P = 0.31$) on cumulative feed intake. There was an effect of treatment ($P = 0.002$) on rectal temperature such that rectal temperatures were greater ($P < 0.05$) following the high dose of centrally administered AGP. In the second experiment there was no effect of treatment on feed intake rate ($P = 0.98$), on

cumulative feed intake ($P = 0.41$) or on rectal temperature ($P = 0.71$). In the third experiment there was an effect of central treatment ($P < 0.0001$) and an interaction of central treatment and time ($P < 0.0001$) on rectal temperature such that ewes treated centrally with AGP had greater rectal temperatures. There was no effect of peripheral treatments ($P = 0.93$) on rectal temperature. These results indicate that central AGP increases rectal temperature in sheep by pathways that do not involve prostaglandins. Further research is needed to determine if AGP may be an important integrator of energy balance and inflammation.

Key words: acute phase protein; orosomucoid; rectal temperature; feed intake; sheep

INTRODUCTION

Both inflammation and sub-optimal feed intake are common in livestock during the transition from late gestation to lactation, and both conditions are associated with greater risk for removal from the herd and less productivity (Bradford et al., 2015). Along with decreased feed intake, fever is also associated with inflammation (Ingvartsen and Andersen, 2000, Sartin et al., 2008).

Leptin, an adipokine synthesized and released into circulation in proportion to the amount of body fat, plays a crucial role in the regulation of food intake and energy expenditure (Elmquist et al., 1998, Friedman and Halaas, 1998, Ahima and Flier, 2000, Blache et al., 2000, Delavaud et al., 2000, Ehrhardt et al., 2000). Administration of leptin reduces food intake, increases energy expenditure and body temperature (Halaas et al., 1995, Barb et al., 1998, Henry et al., 1999, Morrison et al., 2001, Henry et al., 2008, Skibicka and Grill, 2009, Henry et al., 2011). In rodents and primates leptin gene expression in adipocytes and/or circulating concentrations of leptin are increased by bacterial endotoxin and pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β (Grunfeld et al., 1996, Finck et al., 1998, Francis et al., 1999, Landman et al., 2003). Therefore, leptin is an important hormone regulating food intake, metabolism, and body temperature in sick animals (Harden et al., 2006).

Although leptin regulates food intake (Chilliard et al., 2005) and endocrine events in ruminants (Morrison et al., 2001, Daniel et al., 2002, Zieba et al., 2005), plasma leptin in ruminants is unchanged by endotoxin/inflammation (lipopolysaccharide, LPS) (Soliman et al., 2001, Daniel et al., 2002, Soliman et al., 2002), leaving questions about

the mechanisms by which inflammation and/or endotoxin (lipopolysaccharide; LPS) induces changes in food intake, metabolism, and body temperature in domestic species, like sheep and cattle. One response of animals to inflammation and models of inflammation (endotoxin administration) is the synthesis and secretion of acute phase proteins into circulation. These proteins are generally produced by the liver and normally are found in low abundance in the bloodstream, but are greatly elevated during periods of systemic inflammation (Murata et al., 2004, Petersen et al., 2004). Alpha-1-acid glycoprotein (AGP) is an acute phase protein with diverse roles. In rodents, AGP has anti-inflammatory and immunomodulatory properties (Hochepied et al., 2003). Bellinger and co-workers determined that central administration of AGP induced hypophagia in rodents (Bellinger and Mendel, 1990). The putative role of AGP was not evaluated further until a recent publication (Sun et al., 2016) thoroughly demonstrated the role of AGP as a hypophagic compound, signaling via the hypothalamic leptin receptor (LepR) to reduce feed intake in rodents. While these findings are interesting, it is exciting to consider the potential role of AGP as a link between inflammation, appetite, and temperature regulation in ruminants, as they lack the typical inflammation-induced leptin response observed in rodents and humans. Therefore, AGP may play an even more important role in the effects of inflammation on appetite and temperature regulation in ruminants than other species. The objectives of this study were to determine the effects of central and peripheral AGP administration on feed intake and rectal temperature in sheep and to begin to elucidate the mechanism(s) by which AGP increased rectal temperature in sheep.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the University of Tennessee approved, in protocol number 2514-0217, all procedures and protocols used throughout this experiment.

Animals and Maintenance

Non-lactating, non-pregnant mature (≥1 yr of age) Suffolk or Suffolk-cross female sheep were ovariectomized to avoid cyclic variations in plasma concentrations of steroids that may affect feed intake (Laker et al., 2011).Animals were allowed at least 2 weeks for recovery before subsequent surgeries and at least 1 month before used in any experiments. Each sheep was then fitted with an intracerebroventricular (ICV) catheter into a lateral ventricle of the brain as previously described (Whitlock et al., 2010). Twice each week during the entire duration of the ICV catheter presence, antibiotics were administered in order to prevent potential infections and help maintain catheter patency. Catheter patency was assessed each time antibiotics were administered centrally by attempting to withdraw approximately 1 mL of cerebrospinal fluid (CSF) from the subcutaneous port and ICV catheter unit. The ICV antibiotic "cocktail" included Gentamycin[a] (40 mg / mL) and Vancomycin[a] HCl (equivalent to 500 mg of Vancomycin). Approximately 100 µL of the antibiotic cocktail [Gentamycin (5 mg / mL) and Vancomycin (10 mg / mL)] was administered through the port and ICV catheter and subsequently flushed with approximately 250 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride[a]. Based upon previous experience, at least 80% of the ICV catheters and subcutaneous ports were expected to remain patent for subsequent experimental use,

therefore additional animals were prepared in anticipation of a failure rate of no more than 20%. For all ICV injections, the skin above the port site was aseptically prepared with 70% isopropyl alcohol prior to all central treatment and treatments were administered through the skin and into the port via a 25-gauge Huber Point needle[b] followed with 250 µl of sterile 0.9% saline[a] to flush the port and catheter. Ewes were kept indoors in individual pens (approximately 3 m^2) with an environment consisting of a 12-hour light/dark photoperiod and approximately 22-24°C. Ewes were fed a diet (Table 1) calculated to meet 100% of daily maintenance requirements (NRC, 1989) and had *ad libitum* water.

Experiment 1- Effects of Central Administration of α-1-acid-glycoprotein on Feed Intake and Rectal Temperature in Sheep

Ovariectomized sheep (n = 4) weighing 79.0 ± 5.0 (SD) kg, with patent ICV catheters and ports, received 1 of 4 treatments [0 (control), 0.012 (low), 0.060 (medium), or 0.30 (high) mg / kg BW AGP (AGP from bovine plasma)[c]] administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride[a] into the lateral ventricle. The day before each experimental period body weights were determined. A 10-d interval was allowed between experimental periods and each period lasted 5 days, with 3 days for basal measure of feed intake and rectal temperature and 2 days for measurement of feed intake and rectal temperature after experimental treatment administration. Experimental treatments were administered as a single bolus reconstituted in 500 µL of pyrogen-free saline[a] (Daniel et al., 2016). Feed intake and rectal temperature were determined at -72, -48, -24, 0, 2, 4, 6, 8, 12, 24, 36, and 48 h relative to treatment (time 0: administration of

the respective treatments). The study was repeated until all sheep received all treatments with a 10-d washout period between treatments.

Experiment 2- Effects of Peripheral Administration of α-1-acid-glycoprotein on Feed Intake and Rectal Temperature in Sheep

Ewes (n = 10) weighing 78.5 ± 23.7 (SD) kg were randomly assigned to two treatments (0 and 3.0 mg / kg BW of AGP[c]). Approximately 24 hours prior to treatment administration, sheep body weights were determined and the area over each animal's jugular vein was desensitized by SC administration of 2% lidocaine hydrochloride solution[d] prior to inserting a catheter[e] into the vein. The catheter was attached to an extension set[f] and then sutured to the ewe's neck. The catheters and extension sets were flushed with heparinized saline (0.9% sodium chloride) solution (20 U of heparin / mL). Each ewe was infused via IV catheters with one of 2 doses (0 or 3.0 mg / kg BW) of a commercially available AGP extracted from bovine plasma[c]. The dose of AGP was selected based on the greatest dose administered centrally in experiment one (0.30 mg / kg BW). The dose administered IV in this experiment was approximately 10-fold greater than the greatest dose administered centrally in experiment one. Using a 10-fold greater dose has been utilized in previous experiments when going from central to peripheral treatment administration (Whitlock et al., 2010). AGP was administered as a single bolus reconstituted in approximately 1 mL of pyrogen-free saline[a] (Daniel et al., 2016). The sheep were offered a known amount of fresh feed ad libitum. Feed was weighed and replaced with fresh feed and rectal temperatures were determined at -72, -48, -24, 0, 2, 4, 6, 8, 12, 24, 36 and 48 h (time 0: administration of the respective treatments).

Following a washout period ≥10 days, sheep were included in subsequent experimentations (Experiment 3).

Experiment 3- Effects of Peripheral Administration of a Non-Steroidal Anti-Inflammatory, Flunixin Meglumine, and Central Administration of α-1-acid-glycoprotein on Feed Intake and Rectal Temperature in Sheep

Ewes (n = 19; weighing 85.4 ± 19.4 kg) previously ovariectomized and implanted with an ICV catheter and port were used in this experiment. Approximately 24 hours prior to treatment administration, body weights were determined and jugular catheters[e] were inserted and maintained (as described previously in experiment 2). Sheep were assigned to receive peripheral treatments [administered IV; 0 (SAL[a]; equal volume of SAL to match the other IV treatment) or 2.2 mg / kg BW flunixin meglumine (FLU[g]; a cyclooxygenase inhibitory non-steroidal anti-inflammatory drug)] 30 minutes prior to receiving centrally [administered ICV through a port and catheter into the lateral ventricle of the brain; 500 uL of SAL[a] or 25 mg AGP[c] (approximately 0.3 µg / kg BW) administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride[a]]. Feed intake and rectal temperature was determined at -48, -24, -2, 0, 2, 4, 6, 8, 12, and 24 h relative to AGP treatment (time 0: administration of the AGP treatments).

Statistical Analysis

To determine the effects on food intake rate and rectal temperature throughout the experiments, data were analyzed using the GLIMMIX procedure for mixed model analysis of variance[h] and tested for fixed effects of treatment, time, and their interactions. Cumulative feed intake following administration of treatments was tested for fixed effect

of treatment using the GLIMMIX procedure for mixed model analysis of variance[h]. Values of $P < 0.05$ were considered significant.

RESULTS

Experiment 1

Central administration of AGP did not affect rate of feed intake ($P = 0.37$; Figure 1) or 48-hour cumulative feed intake ($P = 0.31$; Figure 2). There was an effect of time ($P < 0.0001$) but no interaction of central treatment and time ($P = 0.97$) on feed intake rate. Central administration of AGP increased rectal temperature ($P = 0.002$) and maximum rectal temperatures in the high dose AGP group were approximately one degree greater compared to controls at eight hours, the time at which rectal temperatures reached their peak, and were greater ($P < 0.05$) following administration of the high dose compared to all other doses administered (Figure 3). There was an effect of time ($P < 0.001$) on rectal temperature and there was a tendency for an interaction of treatment and time ($P = 0.07$).

Experiment 2

Peripheral administration of AGP did not affect rate of feed intake ($P = 0.98$; Figure 4) or 48-hour cumulative feed intake ($P = 0.41$; Figure 5). There was an effect of time ($P = 0.004$) but no interaction of peripheral treatment and time ($P = 0.77$) on feed intake rate. Unlike central AGP, peripheral administration of AGP did not increase rectal temperature ($P = 0.71$; Figure 6). There was no effect of time ($P = 0.10$) or an interaction of treatment and time ($P = 0.91$) on rectal temperature.

Experiment 3

Central administration of AGP did not affect rate of feed intake (P = 0.18; Figure 7) or 24-hour cumulative feed intake (P = 0.79; Figure 8). There was no effect of time (P = 0.28) or an interaction of central treatment and time (P = 0.64). Moreover, peripheral administration of AGP did not affect rate of feed intake (P = 0.88; Figure 7) or 24-hour cumulative feed intake (P = 0.92; Figure 8). The interaction of peripheral treatment and time (P = 0.21), peripheral by central treatment (P = 0.42), or peripheral by central treatment by time interaction (P= 0.50) did not affect feed intake rate. The interaction of peripheral and central treatments did not affect 24-hour cumulative feed intake (P = 0.32).

Although feed intake was not affected, central administration of AGP increased rectal temperature (P < 0.0001). There was an effect of time (P < 0.001) and an interaction of central AGP administration and time (P < 0.0001) on rectal temperature, such that rectal temperatures in the AGP group were greater than controls (P < 0.05) at 2, 4, 6, 8, and 12 hours following central treatment administration (Figure 9). Peripheral flunixin meglumine administration had no effect (P = 0.93) on rectal temperatures. Moreover, there was no interaction of peripheral treatment and time (P = 0.35), peripheral and central treatment (P = 0.54), or peripheral, central treatment and time (P= 0.15) on rectal temperature.

DISCUSSION

Identifying the direct causes of intake depression is critical to preventing a cascade of disorders in animals undergoing exaggerated systemic inflammation. Sun and

coworkers clearly demonstrated in rodents that AGP could potentially provide a link between inflammation and reduced feed intake via the leptin receptor (2016). Our results show that AGP can induce an increase in rectal temperature in the ruminant model, sheep. Our discovery of an effect of AGP on rectal temperature was found to not be dependent on the prostaglandin pathway. While feed intake rate was not affected by AGP administration, 24-hour cumulative feed intake was numerically greater, although not significant, following the highest dose of centrally administered AGP.

Feed Intake

It is well-documented that during the transition period dairy cows undergo a marked systemic inflammation accompanied by an array of responses, some of which may be adaptive and others pathological. One of the most dramatic responses putatively tied to exaggerated peripartum inflammation is suppressed feed intake, but the mechanisms that underlie this relationship are poorly understood.

The acute phase protein AGP is elevated in early lactation in cattle (Cairoli et al., 2006). A recent study in mice demonstrated that AGP has potent hypophagic effects (Sun et al., 2016). Therefore, AGP is a candidate for inflammation-induced suppression of food intake in ruminants. Interestingly, while AGP suppressed feed intake in ob/ob, mice it had no effect on feed intake in db/db mice (Sun et al., 2016) leading the authors to speculate that the effects of AGP on feed intake were mediated through the LepR. Sun et al. (2016) confirmed their speculation when administration of AGP failed to affect feed intake in mice with temporarily silenced LepR. Further, they ultimately demonstrated with rat hypothalamic tissue and GT1-7 cells that AGP bound to and activated the LepR.

In our first study, while numerically the greatest dose of AGP centrally administered to sheep had the least cumulative food intake 48 hours later, the effect of AGP on feed intake was not significant. Potentially the central route of AGP administration has no effect or less effect on feed intake. However, it was observed previously that central administration of AGP decreased feed intake in rats (Bellinger and Mendel, 1990). While studying the effects of satietin on feed intake in rats, Bellinger and Mendel inadvertently administered AGP. Ultimately they determined that the satietin they were administering contained albumin and AGP, and when AGP alone was administered centrally to rats, food intake decreased (Bellinger and Mendel, 1990). Therefore, it is unlikely that the route by which we administered AGP to our sheep in this first experiment was the reason feed intake was not significantly decreased.

Possibly the lack of an effect of centrally administered AGP on feed intake in sheep was because the maximum dose given was not sufficient to elicit this response. When compared to the dose of AGP administered centrally to rats (50 μg; or approximately 0.20 mg / kg BW; (Bellinger and Mendel, 1990)), the maximum dose and dose used in experiment one and three of this study, respectively, were greater (approximately 25 mg; or approximately 0.30 mg / kg BW). Therefore, the difference in response as it relates to feed intake between rats and sheep following central administration of AGP cannot simply be explained by differences in doses used. That being stated, it is still possible that a greater central dose of AGP is needed to suppress/reduce feed intake in ruminants. If AGP truly has a role in inflammation induced suppression of food intake in sheep, maybe the concentrations (in plasma and

possibly in CSF) achieved during systemic inflammation in this species, and necessary to affect feed intake, exceed that administered in our model.

Our original intentions were to administer a greater dose of AGP centrally if there was no observed/significant effect of AGP on feed intake in the initial experiment. However, subsequent studies of the effects of greater doses of centrally administered AGP were not completed because of the unexpected increase in rectal temperature following treatment administration. Once we understand better the mechanism(s) by which AGP increases rectal temperature in sheep we will likely administer greater doses centrally.

The effects of AGP on feed intake in mice by Sun et al., (2016) were observed following peripheral administration (intraperitoneal and IV). Thus, we administered AGP peripherally in our second experiment. The dose of AGP used in the second experiment (~3.0 mg / kg IV; ~ 250 mg) was 10-fold greater than the greatest dose used in our first experiment (~0.30 mg / kg ICV; ~25 mg) to determine if the lack of an effect was possibly a result of the route by which the treatments were administered. However, similar to the first experiment, AGP had no effect on feed intake in sheep (cumulative or feed intake rate). Sun et al. (2016) administered 100 mg / kg IV or ~ 2 mg total to reduce feed intake in mice. Based upon their reported body weights, assumed blood volumes, and previously reported circulating concentrations of AGP in mice (Kopf et al., 1994), it is likely that the 2 mg total dose of AGP administered to the mice increased their plasma AGP by approximately 670%. In cattle, serum concentrations of AGP vary. Serum concentrations of AGP range from approximately 200 to 450 mg / L in healthy cattle and

increase to ≥ 1000 mg / L during acute inflammation (Ceciliani et al., 2012). Recently we observed a negative association between feed intake and serum AGP concentrations in periparturient dairy cattle (unpublished). In that study a reduction in periparturient intake of feed in dairy cattle was associated with an increase of approximately 40% in plasma AGP. While this association does not necessarily confirm causation, it is interesting to consider in light of what was previously observed and reported regarding AGP and feed intake by Sun and others (2016).

While there are very few recorded reference ranges for serum concentrations of AGP in sheep, a 30 day experiment was conducted with the concentrations of the control sheep being approximately 100 mg / L (Eckersall et al., 2007). Concentrations in other species (mice, rats, and swine) are similar to those observed/reported in cattle (Pous et al., 1990, Caperna et al., 2017). Therefore, it is likely that serum concentrations of AGP in healthy sheep are not that divergent from other species. Comparatively, if our assumption is true, that the normal circulating concentration of AGP in sheep is approximately 300 mg / L, the IV dose administered to the sheep in this study likely increased their (concentration and total) serum AGP by 10 to 13%. If AGP does suppress feed intake in ruminants during inflammation, it is possible that the IV dose of AGP administered to our sheep was not comparable to increases in serum or plasma normally achieved during systemic inflammation associated with suppressed feed intake. Therefore, it is possible that a greater IV dose could have an effect on feed intake in sheep. Subsequent analyses and validation of AGP assays for serum and plasma of sheep associated with our experiments are underway and will likely provide greater insight into the circulating

concentrations of AGP in healthy and diseased (inflammation models) animals in this species.

Additionally, bovine AGP includes at least five glycosylations and potentially eight phosphorylation sites (Ceciliani et al., 2012). Being similar in humans and rats who have six glycosylations (Fournier et al., 2000). Moreover, it is clear that systemic inflammation alters AGP glycosylation (Kreisman and Cobb, 2012). Therefore, it is possible that there are unique AGP glycosylations associated with inflammation that make the AGP molecule respond differently at the site of the LepR. Because of the complexity of this protein and changes associated with inflammation, we cannot rule out that the observations made during our studies resulted from variation/diversity in glycosylation of the AGP administered to our animals. However, we did use the same lot of AGP for all studies reported herein.

Rectal Temperature

An unexpected and previously unreported effect of central administration of AGP was increased rectal temperature in sheep. While this was a surprising and interesting observation, the mechanism(s) by which AGP increased rectal temperature in sheep was not immediately clear/obvious. Fever, an elevation in body temperature, may be the result of many infectious or inflammatory diseases. To elicit a fever, the infection/inflammatory cause triggers cytokine production which then use specific routes crossing the blood-brain-barrier to initiate a febrile response. A rise in body temperature is necessary in many cases for the body to recover from or fight back against the initial foreign cause (for more in-depth review see (Evans et al., 2015)).

During the first experiment, we observed that central administration of AGP increased rectal temperature in sheep. Previous research regarding the effects of AGP on feed intake did not determine body temperature; therefore, this is a novel and unexpected finding. One potential concern regarding the increased rectal temperature in response to central AGP administration was that the administered bovine AGP might have been contaminated with endotoxin. To help ensure that the increase temperature in response to central AGP was not because of contamination with endotoxin, we determined that our AGP did not contain endotoxin using a limulus amebocyte lysate test. Moreover, the timing of the increased rectal temperature following central administration of AGP was not consistent with previous endotoxin models, in that the increase in temperature was later than what would have been observed following endotoxin administration (Kabaroff et al., 2006, Feng et al., 2010, Ranjan et al., 2011). Once we confirmed that the AGP did not contain endotoxin we turned our attention to other possible mechanisms.

It has been determined that central leptin administration in rats not only reduced feed intake but also increased body temperature (Luheshi et al., 1999, Turek et al., 2004, Skibicka and Grill, 2009). If the effect of AGP to suppress food intake in rodents is mediated through the LepR as supported by Sun and others (2016), then it is reasonable to speculate that AGP, like leptin, could increase body temperature. It could be that the effects of AGP or leptin on body temperature in rodents and/or sheep are more sensitive than the effects of either of them on feed intake. However, Luheshi et al. (1999) administered various quantities of leptin centrally to rats and found that even their lowest dose of 0.4 ug / rat caused a significant decrease in food intake, but did not induce a

temperature. Even given this information, we cannot rule out the possible differences between species. We concluded that we should administer AGP by a different route based on the previous work in mice.

Unlike central, peripheral administration of AGP had no effect on rectal temperatures. The peripheral dose of AGP needed to increase rectal temperature may not have been achieved. When administering leptin peripherally in rodents, concentrations that caused an increase in temperature were 1 mg / rat (~3.5 mg / kg) albeit at a lower increase then the central route (Luheshi et al., 1999). Potentially more than a 10-fold greater dose, such as a 100-fold greater dose than the central dose would get the same results as the central doses. Given that central administration of AGP increased rectal temperature in sheep in experiment one, we speculate that the dose given IV in this study may not have been sufficient to elicit a response to affect feed intake or rectal temperature.

While studying the effects of leptin on feed intake and body temperature in rats, Luheshi et al. (1999) administered a cyclo-oxygenase inhibitor, flurbiprofen, and found it had no effect on feed intake but was able to block the leptin induced increased temperature. Therefore, to help elucidate the mechanism(s) by which central administration of AGP increases rectal temperature in sheep we designed an experiment to eliminate pyrogenic/fever causing pathways that involve prostaglandins. We administered flunixin meglumine, a non-steroidal anti-inflammatory drug and potent cyclo-oxygenase inhibitor that has antipyrogenic effects involving inhibition of endogenous pyrogens (IL-1 and TNF) and prostaglandin PGE2 (McKellar et al., 1989,

Lees and Taylor, 1991). Pretreatment with flunixin meglumine had no effect on increased rectal temperature following central administration of AGP. To keep our experimental models the same we continued to monitor feed intake and found there was no effect on feed intake. Because we observed a tendency to reduce cumulative feed intake 48 hour post treatment, we regret not determining/measuring feed intake and rectal temperatures out to 48 hours in this subsequent study. We concluded that central AGP administration increases rectal temperature in sheep by a mechanism(s) independent of prostaglandin synthesis.

Therefore, it is plausible that central administration of AGP is having an effect on body temperature/rectal temperature through actions at the LepR. Moreover, if this effect is mediated through the LepR, then it is likely that increased rectal temperature following central AGP is affecting the melanocortin system through actions at the LepR to ultimately increase body temperature (Turek et al., 2004, Skibicka and Grill, 2009). Subsequent studies designed to determine the mechanism(s) by which AGP increases rectal temperature in sheep might focus on the effects of blocking hypothalamic melanocortin receptors prior to central administration of AGP.

CONCLUSIONS

In summary, although there are many open questions regarding alpha-1-acid glycoproteins' effects on feed intake, we did discover a novel effect on rectal temperature. While AGP did not decrease feed intake in our sheep model, central administration of AGP did increase rectal temperature in sheep. It has been stated that leptin induced increase in body temperature in rats will plateau even when given up to 10

times greater than the normal plasma concentrations of leptin (Luheshi et al., 1999). Therefore, it is plausible to assume, if the temperature response was maximal in this study, that higher amounts of AGP may decrease feed intake in sheep while not causing any greater increase in rectal temperature. Furthermore, administration of flunixin meglumine did not abolish the increase rectal temperature brought on by central administration of AGP, removing the possibility of prostaglandin pathways involved in AGP-stimulated rectal temperature and it would be reasonable to move forward by researching AGP actions on the melanocortin pathway in this area. Alpha-1-acid glycoprotein may be an important integrator of inflammation, food intake and body temperature in ruminants, however, further research is needed to completely elucidate and confirm its role in these areas.

CHAPTER III

CONCLUSIONS

Inflammation and reduced feed intake are common in dairy cows in the transition to lactation, and both conditions are associated with risk for removal from the herd and less productivity. Poor feed intake is associated with other negative outcomes detrimental to lactation efficiency and the well-being of dairy cows (Grummer, 1995, Huzzey et al., 2007, Goldhawk et al., 2009). Alpha-1-acid glycoprotein (AGP) has been shown to signal via the leptin receptor in rodents to reduce feed intake (Sun et al., 2016). It has been discovered that AGP (Cairoli et al., 2006), but not leptin, increases as a result of an inflammatory response in ruminants, therefore we hypothesized that AGP may be the factor reducing feed intake during the transition period in ruminants. The objectives were to test the effects of AGP (1) centrally and (2) peripherally on feed intake and rectal temperature then (3) determine the mechanism(s) in which central administration of AGP increases rectal temperature.

In the first experiment, it was hypothesized that central AGP would affect feed intake in the ruminant model, sheep. Feed intake was not reduced. This could be because of the route of administration although in rodents both central and peripheral routes of AGP reduced feed intake so this is unlikely. The greatest dose of AGP had the least cumulative food intake 48 hours post treatment, but the effect was not significant. Potentially a higher dose would elicit a response, however a increase in rectal temperature was discovered at this dose. Another possible explanation is the many glycosylations of AGP may cause the protein to react differently. The increase in rectal temperature is a novel finding. Other research monitoring AGP effect on feed intake did

not record body temperature. High amounts of leptin can cause an increase in body temperature, therefore, although not expected, it can be understood.

In the second experiment, we hypothesized peripheral AGP would affect feed intake and body temperature in sheep. Using 10 times the highest dose given in experiment one, there was no effect on feed intake or rectal temperature. It may be that the AGP concentrations, in plasma, during an inflammatory response are much higher then what was given. Potentially a greater dose is needed peripherally since the dose administered by this route did not induce a fever.

In the third experiment, flunixin meglumine, a cyclo-oxygenase inhibitor was administered peripherally 30 minutes prior to central AGP. The administration of peripheral flunixin meglumine had no effect on the temperature rise via central AGP. There was once again a rise in temperature such that ewes treated centrally with AGP had greater rectal temperatures at 2, 4, 6, 8, and 12 hours relative to ewes treated centrally with saline. These results suggest AGP using a mechanism(s) independent of the prostaglandin pathway to increase temperature.

Alpha-1-acid glycoprotein could be a potential marker for inflammatory or infectious disease in ruminants. Although AGP did not reduce feed intake in these models, the induction of a rectal temperature was discovered. The temperature is not induced through the prostaglandin pathway and future research could focus on the melanocortin pathway. If the fever can be abolished further research can be achieved with higher doses.

REFERENCES

Ahima, R. S. and J. S. Flier. 2000. Leptin. Annu. Rev. Physiol. 62:413-437.

Allen, M. S. and B. J. Bradford. 2012. Control of food intake by metabolism of fuels: a comparison across species. Proc. Nutr. Soc. 71(3):401-409.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. The hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of Animal Science 87(10):3317-3334.

Allen, M. S. and P. Piantoni. 2013. Metabolic Control of Feed Intake Implications for Metabolic Disease of Fresh Cows. Vet. Clin. N. Am.-Food Anim. Pract. 29(2):279-+.

Barb, C. R., X. Yan, M. J. Azain, R. R. Kraeling, G. B. Rampacek, and T. G. Ramsay. 1998. Recombinant porcine leptin reduces feed intake and stimulates growth hormone secretion in swine. Domest. Anim. Endocrinol. 15(1):77-86.

Bellinger, L. L. and V. E. Mendel. 1990. THE EFFECTS OF SEMI-PURIFIED AND HPLC-PURIFIED HUMAN SATIETIN AND ALPHA-1-GLYCOPROTEIN ON INGESTION AND BODY-WEIGHT. Brain Res. Bull. 25(6):941-947.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91(9):3300-3310.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90(4):1740-1750.

Blache, D., R. L. Tellam, L. M. Chagas, M. A. Blackberry, P. E. Vercoe, and G. B. Martin. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. J. Endocrinol. 165(3):625-637.

Blomqvist, A. and D. Engblom. 2018. Neural Mechanisms of Inflammation-Induced Fever. Neuroscientist 24(4):381-399.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J Dairy Sci 98(10):6631-6650.

Cairoli, F., M. Battocchio, M. C. Veronesi, D. Brambilla, F. Conserva, I. Eberini, R. Wait, and E. Gianazza. 2006. Serum protein pattern during cow pregnancy: Acute-phase proteins increase in the peripartum period. Electrophoresis 27(8):1617-1625.

Caperna, T. J., A. E. Shannon, M. Stoll, S. Kahl, L. A. Blomberg, J. L. Vallet, and T. G. Ramsay. 2017. A sandwich ELISA for porcine alpha-1 acid glycoprotein (pAGP, ORM-1) and further demonstration of its use to evaluate growth potential in newborn pigs. Domest. Anim. Endocrinol. 60:75-82.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J. Proteomics 75(14):4207-4231.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Leroux, J. Djiane, and F. Bocquier. 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest. Anim. Endocrinol. 21(4):271-295.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest. Anim. Endocrinol. 29(1):3-22.

Chua, S. C., W. K. Chung, X. S. WuPeng, Y. Y. Zhang, S. M. Liu, L. Tartaglia, and R. L. Leibel. 1996. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271(5251):994-996.

Clarke, I. J., B. Henry, J. Iqbal, and J. W. Goding. 2001. Leptin and the regulation of food intake and the neuroendocrine axis in sheep. Clin. Exp. Pharmacol. Physiol. 28(1-2):106-107.

Clarkson, R. W. E., M. T. Wayland, J. Lee, T. Freeman, and C. J. Watson. 2004. Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. Breast Cancer Res. 6(2):R92-R109.

Coleman, D. L. 1973. EFFECTS OF PARABIOSIS OF OBESE WITH DIABETES AND NORMAL MICE. Diabetologia 9(4):294-298.

Coleman, D. L. and K. P. Hummel. 1969. EFFECTS OF PARABIOSIS OF NORMAL WITH GENETICALLY DIABETIC MICE. Am. J. Physiol. 217(5):1298-&.

Daniel, J. A., B. K. Whitlock, J. A. Baker, B. Steele, C. D. Morrison, D. H. Keisler, and J. L. Sartin. 2002. Effect of body fat mass and nutritional status on 24-hour leptin profiles in ewes. Journal of Animal Science 80(4):1083-1089.

Daniel, J. A., B. K. Whitlock, D. L. Marks, J. A. Gard, and J. L. Sartin. 2016. Leukemia inhibitory factor as a mediator of lipopolysaccharide effects on appetite and selected hormones and metabolites. Journal of Animal Science 94(7):2789-2797.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165(2):519-526.

Disilvestro, R. A. 1986. PLASMA-LEVELS OF IMMUNOREACTIVE CERULOPLASMIN AND OTHER ACUTE PHASE PROTEINS DURING LACTATION. Proc. Soc. Exp. Biol. Med. 183(2):257-261.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82(11):2259-2273.

Eckersall, P. D., F. P. Lawson, L. Bence, M. M. Waterston, T. L. Lang, W. Donachie, and M. C. Fontaine. 2007. Acute phase protein response in an experimental model of ovine caseous lymphadenitis. BMC Veterinary Research 3(1):35.

Eckersall, P. D., F. J. Young, C. McComb, C. J. Hogarth, S. Safi, A. Weber, T. McDonald, A. M. Nolan, and J. L. Fitzpatrick. 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec. 148(2):35-+.

Ehrhardt, R. A., R. M. Slepetis, J. Siegal-Willott, M. E. Van Amburgh, A. W. Bell, and Y. R. Boisclair. 2000. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. J. Endocrinol. 166(3):519-528.

Ellacott, K. L. J. and R. D. Cone. 2004. The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. Recent Progress in Hormone Research, Vol 59 59:395-408.

Elmquist, J. K., E. Maratos-Flier, C. B. Saper, and J. S. Flier. 1998. Unraveling the central nervous system pathways underlying responses to leptin. Nat. Neurosci. 1(6):445-450.

Evans, S. S., E. A. Repasky, and D. T. Fisher. 2015. Fever and the thermal regulation of immunity: the immune system feels the heat. Nat. Rev. Immunol. 15(6):335-349.

Farooqi, I. S. 2002. Leptin and the onset of puberty: Insights from rodent and human genetics. Semin. Reprod. Med. 20(2):139-144.

Feldberg, W. and P. N. Saxena. 1971. FEVER PRODUCED BY PROSTAGLANDIN-E1. J. Physiol.-London 217(3):547-&.

Feng, S. Y. S., T. Samarasinghe, D. J. Phillips, T. Alexiou, J. H. Hollis, V. Y. H. Yu, and A. M. Walker. 2010. Acute and chronic effects of endotoxin on cerebral circulation in lambs. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 298(3):R760-R766.

Finck, B. N., K. W. Kelley, R. Dantzer, and R. W. Johnson. 1998. In vivo and in vitro evidence for the involvement of tumor necrosis factor-alpha in the induction of leptin by lipopolysaccharide. Endocrinology 139(5):2278-2283.

Forbes, J. M. 1980. A MODEL OF THE SHORT-TERM CONTROL OF FEEDING IN THE RUMINANT - EFFECTS OF CHANGING ANIMAL OR FEED CHARACTERISTICS. Appetite 1(1):21-41.

Forbes, J. M. 1996. Integration of regulatory signals controlling forage intake in ruminants. Journal of Animal Science 74(12):3029-3035.

Fournier, T., N. Medjoubi-N, and D. Porquet. 2000. Alpha-1-acid glycoprotein. Biochim. Biophys. Acta-Protein Struct. Molec. Enzym. 1482(1-2):157-171.

Francis, J., P. S. MohanKumar, S. M. J. MohanKumar, and S. K. Quadri. 1999. Systemic administration of lipopolysaccharide increases plasma leptin levels - Blockade by soluble interleukin-1 receptor. Endocrine 10(3):291-295.

Francis, J., S. M. J. MohanKumar, and P. S. MohanKumar. 2000. Correlations of norepinephrine release in the paraventricular nucleus with plasma corticosterone and leptin after systemic lipopolysaccharide: blockade by soluble IL-1 receptor. Brain Res. 867(1-2):180-187.

Friedman, J. M. and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature 395(6704):763-770.

Goldhawk, C., N. Chapinal, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Prepartum feeding behavior is an early indicator of subclinical ketosis. J. Dairy Sci. 92(10):4971-4977.

Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Loor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. J. Dairy Sci. 95(4):1749-1758.

Gregor, M. F., E. S. Misch, L. Yang, S. Hummasti, K. E. Inouye, A. H. Lee, B. Bierie, and G. S. Hotamisligil. 2013. The Role of Adipocyte XBP1 in Metabolic Regulation during Lactation. Cell Reports 3(5):1430-1439.

Grummer, R. R. 1995. IMPACT OF CHANGES IN ORGANIC NUTRIENT METABOLISM ON FEEDING THE TRANSITION DAIRY-COW. Journal of Animal Science 73(9):2820-2833.

Grunfeld, C., C. Zhao, J. Fuller, A. Pollock, A. Moser, J. Friedman, and K. R. Feingold. 1996. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters - A role for leptin in the anorexia of infection. J. Clin. Invest. 97(9):2152-2157.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. 1995. WEIGHT-REDUCING EFFECTS OF THE PLASMA-PROTEIN ENCODED BY THE OBESE GENE. Science 269(5223):543-546.

Harden, L. M., I. du Plessis, S. Poole, and H. P. Laburn. 2006. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. Physiol. Behav. 89(2):146-155.

Hausberger, F. X. 1958. PARABIOSIS AND TRANSPLANTATION EXPERIMENTS IN HEREDITARILY OBESE MICE. Anat. Rec. 130(2):313-313.

Henry, B. A., Z. B. Andrews, A. Rao, and I. J. Clarke. 2011. Central Leptin Activates Mitochondrial Function and Increases Heat Production in Skeletal Muscle. Endocrinology 152(7):2609-2618.

Henry, B. A., F. R. Dunshea, M. Gould, and I. J. Clarke. 2008. Profiling postprandial thermogenesis in muscle and fat of sheep and the central effect of leptin administration. Endocrinology 149(4):2019-2026.

Henry, B. A., J. W. Goding, W. S. Alexander, A. J. Tilbrook, B. J. Canny, F. Dunshea, A. Rao, A. Mansell, and I. J. Clarke. 1999. Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: Evidence for a dissociation of effects on appetite and neuroendocrine function. Endocrinology 140(3):1175-1182.

Hochepied, T., F. G. Berger, H. Baumann, and C. Libert. 2003. alpha(1)-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. Cytokine Growth Factor Rev. 14(1):25-34.

Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. 35(2):188-193.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92(2):621-625.

Huzzey, J. M., S. Mann, D. V. Nydam, R. J. Grant, and T. R. Overton. 2015. Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. Prev. Vet. Med. 120(3-4):291-297.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J. Dairy Sci. 90(7):3220-3233.

Ingalls, A. M., M. M. Dickie, and G. D. Snell. 1950. OBESE, A NEW MUTATION IN THE HOUSE MOUSE. J. Hered. 41(12):317-318.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83(7):1573-1597.

Ingvartsen, K. L. and Y. R. Boisclair. 2001. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. Domest. Anim. Endocrinol. 21(4):215-250.

Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest. Prod. Sci. 83(2-3):277-308.

Jafari, A., D. G. V. Emmanuel, R. J. Christopherson, J. R. Thompson, G. K. Murdoch, J. Woodward, C. J. Field, and B. N. Ametaj. 2006. Parenteral administration of glutamine modulates acute phase response in postparturient dairy cows. J. Dairy Sci. 89(12):4660-4668.

Jansky, L., S. Vybiral, D. Pospisilova, J. Roth, J. Dornand, E. Zeisberger, and J. Kaminkova. 1995. PRODUCTION OF SYSTEMIC AND HYPOTHALAMIC CYTOKINES DURING THE EARLY PHASE OF ENDOTOXIN FEVER. Neuroendocrinology 62(1):55-61.

Kabaroff, L. C., A. Rodriguez, M. Quinton, H. Boermans, and N. A. Karrow. 2006. Assessment of the ovine acute phase response and hepatic gene expression in response to Escherichia coli endotoxin. Vet. Immunol. Immunopathol. 113(1-2):113-124.

Kadota, R., K. Sugita, K. Uchida, H. Yamada, M. Yamashita, and H. Kimura. 2018. A mathematical model of type 1 diabetes involving leptin effects on glucose metabolism. J. Theor. Biol. 456:213-223.

Kluger, M. J. 1991. FEVER - ROLE OF PYROGENS AND CRYOGENS. Physiol Rev 71(1):93-127.

Kopf, M., H. Baumann, G. Freer, M. Freudenberg, M. Lamers, T. Kishimoto, R. Zinkernagel, H. Bluethmann, and G. Kohler. 1994. IMPAIRED IMMUNE AND ACUTE-PHASE RESPONSES IN INTERLEUKIN-6-DEFICIENT MICE. Nature 368(6469):339-342.

Kreisman, L. S. C. and B. A. Cobb. 2012. Infection, inflammation and host carbohydrates: A Glyco-Evasion Hypothesis. Glycobiology 22(8):1019-1030.

Kwon, O., K. W. Kim, and M. S. Kim. 2016. Leptin signalling pathways in hypothalamic neurons. Cell. Mol. Life Sci. 73(7):1457-1477.

Laker, R. C., B. A. Henry, G. D. Wadley, I. J. Clarke, B. J. Canny, and G. K. McConell. 2011. Central infusion of leptin does not increase AMPK signaling in skeletal muscle of sheep. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 300(2):R511-R518.

Landman, R. E., J. J. Puder, E. Xiao, P. U. Freda, M. Ferin, and S. L. Wardlaw. 2003. Endotoxin stimulates leptin in the human and nonhuman primate. J. Clin. Endocrinol. Metab. 88(3):1285-1291.

Lee, Y. S., J. W. Choi, I. Hwang, J. W. Lee, J. H. Lee, A. Y. Kim, J. Y. Huh, Y. J. Koh, G. Y. Koh, H. J. Son, H. Masuzaki, K. Hotta, A. A. Alfadda, and J. B. Kim. 2010. Adipocytokine Orosomucoid Integrates Inflammatory and Metabolic Signals to Preserve Energy Homeostasis by Resolving Immoderate Inflammation. J. Biol. Chem. 285(29):22174-22185.

Lees, P. and P. M. Taylor. 1991. PHARMACODYNAMICS AND PHARMACOKINETICS OF FLUNIXIN IN THE CAT. Br. Vet. J. 147(4):298-305.

Lemay, L. G., A. J. Vander, and M. J. Kluger. 1990. ROLE OF INTERLEUKIN-6 IN FEVER IN RATS. Am. J. Physiol. 258(3):R798-R803.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. J. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23(2):217-226.

Luheshi, G. N., J. D. Gardner, D. A. Rushforth, A. S. Loudon, and N. J. Rothwell. 1999. Leptin actions on food intake and body temperature are mediated by IL-1. Proc. Natl. Acad. Sci. U. S. A. 96(12):7047-7052.

McCarthy, M. M., T. Yasui, M. J. B. Felippe, and T. R. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci. 99(1):680-700.

McKellar, Q. A., E. A. Galbraith, J. A. Bogan, C. S. Russell, R. E. Hooke, and P. Lees. 1989. FLUNIXIN PHARMACOKINETICS AND SERUM THROMBOXANE INHIBITION IN THE DOG. Vet. Rec. 124(25):651-654.

Morrison, C. D., J. A. Daniel, B. J. Holmberg, J. Djiane, N. Raver, A. Gertler, and D. H. Keisler. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168(2):317-324.

Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95(3):1323-1336.

Munzberg, H. and M. G. Myers. 2005. Molecular and anatomical determinants of central leptin resistance. Nat. Neurosci. 8(5):566-570.

Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J. 168(1):28-40.

Myers, M. G., M. A. Cowley, and H. Munzberg. 2008. Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. 70:537-556.

NRC. 1989. Recommended dietary allowances. 10th ed. National Academies Press, Washington, DC.

Obici, S., Z. H. Feng, Y. Morgan, D. Stein, G. Karkanias, and L. Rossetti. 2002. Central administration of oleic acid inhibits glucose production and food intake. Diabetes 51(2):271-275.

Perry, R. J., X. M. Zhang, D. Y. Zhang, N. Kumashiro, J. P. G. Camporez, G. W. Cline, D. L. Rothman, and G. I. Shulman. 2014. Leptin reverses diabetes by suppression of the hypothalamic-pituitary-adrenal axis. Nat. Med. 20(7):759-763.

Petersen, H. H., J. P. Nielsen, and P. M. H. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35(2):163-187.

Pous, C., J. P. Giroud, C. Damais, D. Raichvarg, and L. Chauvelotmoachon. 1990. EFFECT OF RECOMBINANT HUMAN INTERLEUKIN-1-BETA AND TUMOR NECROSIS FACTOR-ALPHA ON LIVER CYTOCHROME-P-450 AND SERUM ALPHA-1-ACID GLYCOPROTEIN CONCENTRATIONS IN THE RAT. Drug Metab. Dispos. 18(4):467-470.

Qu, Y., A. N. Fadden, M. G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 97(7):4151-4165.

Ranjan, R., B. K. Roy, A. Ranjan, and S. K. Singh. 2011. Effect of E. coli endotoxin induced fever on the pharmacokinetic profile and dosage regimen of ceftriaxone in sheep (Ovis aries). Vet. Arh. 81(4):423-432.

Roche, J. R., A. W. Bell, T. R. Overton, and J. J. Loor. 2013. Nutritional management of the transition cow in the 21st century - a paradigm shift in thinking. Anim. Prod. Sci. 53(9):1000-1023.

Rosenbaum, S., R. Ringseis, S. Hillen, S. Becker, G. Erhardt, G. Reiner, and K. Eder. 2012a. Genome-wide transcript profiling indicates induction of energy-generating pathways and an adaptive immune response in the liver of sows during lactation. Comp. Biochem. Physiol. D-Genomics Proteomics 7(4):370-381.

Rosenbaum, S., R. Ringseis, S. Hillen, S. Becker, G. Erhardt, G. Reiner, and K. Eder. 2012b. The stress signalling pathway nuclear factor E2-related factor 2 is activated in the liver of sows during lactation. Acta Vet. Scand. 54:5.

Sadri, H., R. M. Bruckmaier, H. R. Rahmani, G. R. Ghorbani, I. Morel, and H. A. van Dorland. 2010. Gene expression of tumour necrosis factor and insulin signalling-related factors in subcutaneous adipose tissue during the dry period and in early lactation in dairy cows. J. Anim. Physiol. Anim. Nutr. 94(5):e194-e202.

Sartin, J. L., D. L. Marks, C. D. McMahon, J. A. Daniel, P. Levasseur, C. G. Wagner, B. K. Whitlock, and B. P. Steele. 2008. Central role of the melanocortin-4 receptors in appetite regulation after endotoxin. J Anim Sci 86(10):2557-2567.

Sheldon, I. M., D. E. Noakes, A. Rycroft, and H. Dobson. 2001. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet. Rec. 148(6):172-175.

Skibicka, K. P. and H. J. Grill. 2009. Hindbrain Leptin Stimulation Induces Anorexia and Hyperthermia Mediated by Hindbrain Melanocortin Receptors. Endocrinology 150(4):1705-1711.

Soliman, M., S. Abdelhady, L. Fattouh, K. Ishioka, H. Kitamura, K. Kimura, and M. Saito. 2001. No alteration in serum leptin levels during acute endotoxemia in sheep. J. Vet. Med. Sci. 63(10):1143-1145.

Soliman, M., K. Ishioka, K. Kimura, S. Kushibiki, and M. Saito. 2002. Plasma leptin responses to lipopolysaccharide and tumor necrosis factor alpha in cows. Jpn. J. Vet. Res. 50(2-3):107-114.

Sun, Y., Y. L. Yang, Z. Qin, J. Y. Cai, X. M. Guo, Y. Tang, J. J. Wan, D. F. Su, and X. Liu. 2016. The Acute-Phase Protein Orosomucoid Regulates Food Intake and Energy Homeostasis via Leptin Receptor Signaling Pathway. Diabetes 65(6):1630-1641.

Tamura, K., T. Yatsu, H. Itoh, and Y. Motoi. 1989. ISOLATION, CHARACTERIZATION, AND QUANTITATIVE MEASUREMENT OF SERUM ALPH-1-ACID GLYCOPROTEIN IN CATTLE. Japanese Journal of Veterinary Science 51(5):987-994.

Turek, V. F., D. H. Olster, K. R. Gililland, M. Sheehy, A. Ettenberg, and H. J. Carlisle. 2004. The effects of melanocortin agonists leptin-induced fever in and antagonists on rats. J. Therm. Biol. 29(7-8):423-430.

Vaisse, C., J. L. Halaas, C. M. Horvath, J. E. Darnell, M. Stoffel, and J. M. Friedman. 1996. Leptin activation of Stat3 in the hypothalamus of wildtype and ob/ob mice but not db/db mice. Nature Genet. 14(1):95-97.

Vane, J. R. 1971. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. Nature New Biology 231:232.

White, D. W., Y. P. Wang, S. C. Chua, J. P. Morgenstern, R. L. Leibel, H. Baumann, and L. A. Tartaglia. 1997. Constitutive and impaired signaling of leptin receptors containing the Gln->Pro extracellular domain fatty mutation. Proc. Natl. Acad. Sci. U. S. A. 94(20):10657-10662.

Whitlock, B. K., J. A. Daniel, B. P. Steele, and J. L. Sartin. 2010. Changes in plasma concentrations of growth hormone and luteinizing hormone in ewes following central and peripheral treatment with kisspeptin. J. Dairy Sci. 93:112-112.

Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNF alpha Altered Inflammatory Responses, Impaired Health and Productivity, but Did Not Affect Glucose or Lipid Metabolism in Early-Lactation Dairy Cows. PLoS One 8(11):11.

Zieba, D. A., M. Amstalden, and G. L. Williams. 2005. Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest. Anim. Endocrinol. 29(1):166-185.

APPENDIX

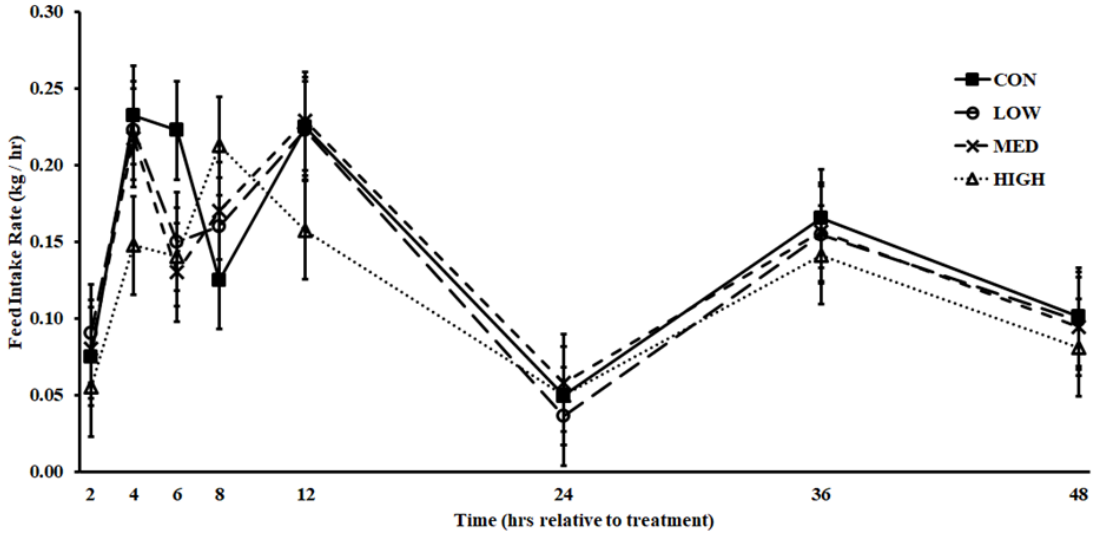

Figure 1. Effects of central α-1-acid-glycoprotein (AGP) administration on feed intake rate (kg / hour) in sheep (+/- SEM). Ewes were treated with 1 of 4 treatments [0 (control; n = 4), 0.012 (low; n = 4), 0.060 (medium; n = 4), or 0.30 (high; n = 4) mg / kg BW AGP] administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride into the lateral ventricle at time 0 hrs to yield four possible treatment groups. The study was repeated until all sheep received all treatments with a 10-day washout period between treatments. There was an effect of time ($P < 0.0001$) and no effect of treatment ($P = 0.37$), or treatment x time interaction ($P = 0.97$) on feed intake rate. CON, control; LOW, low; MED, medium; HIGH, high.

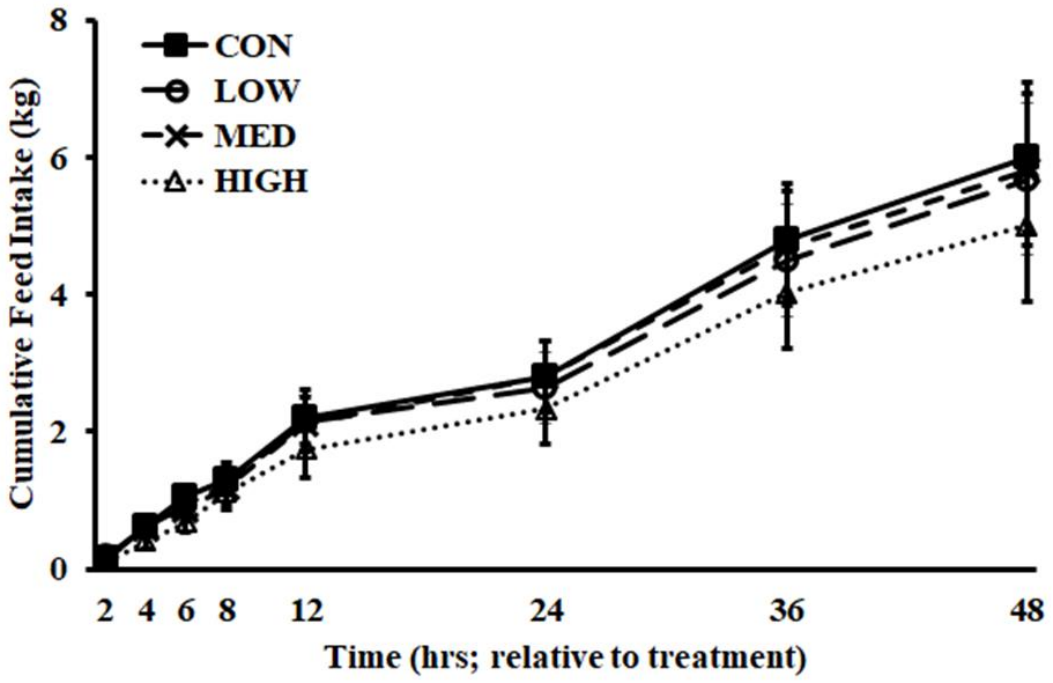

Figure 2. Effects of central α-1-acid-glycoprotein (AGP) administration on cumulative feed intake (kg) in sheep (+/- SEM). Ewes were treated with 1 of 4 treatments [0 (control; n = 4), 0.012 (low; n = 4), 0.060 (medium; n = 4), or 0.30 (high; n = 4) mg / kg BW AGP] administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride into the lateral ventricle at time 0 hrs to yield four possible treatment groups. The study was repeated until all sheep received all treatments with a 10-day washout period between treatments. There was no effect of treatment (P = 0.31) on 48 hour cumulative feed intake. CON, control; LOW, low; MED, medium; HIGH, high.

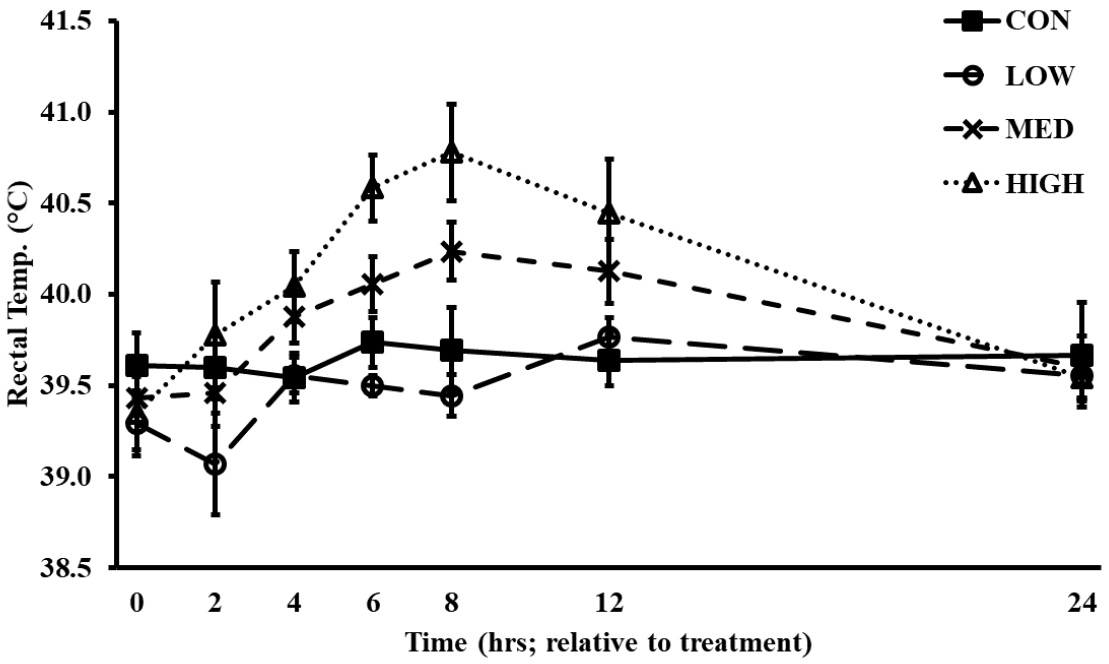

Figure 3. Effects of central α-1-acid-glycoprotein (AGP) administration on rectal temperatures (°C) in sheep (+/- SEM). Ewes were treated with 1 of 4 treatments [0 (control; n = 4), 0.012 (low; n = 4), 0.060 (medium; n = 4), or 0.30 (high; n = 4) mg / kg BW AGP] administered in 500 µL of sterile, nonpyrogenic, isotonic, 0.9% sodium chloride into the lateral ventricle at time 0 hrs to yield four possible treatment groups. The study was repeated until all sheep received all treatments with a 10-day washout period between treatments. There was a tendency for an interaction of treatment and time ($P = 0.07$), and there was an effect of time ($P < 0.0001$) and treatment ($P = 0.002$) on rectal temperature. Rectal temperatures were greater ($P < 0.05$) with high dose compared to other doses given. CON, control; LOW, low; MED, medium; HIGH, high.

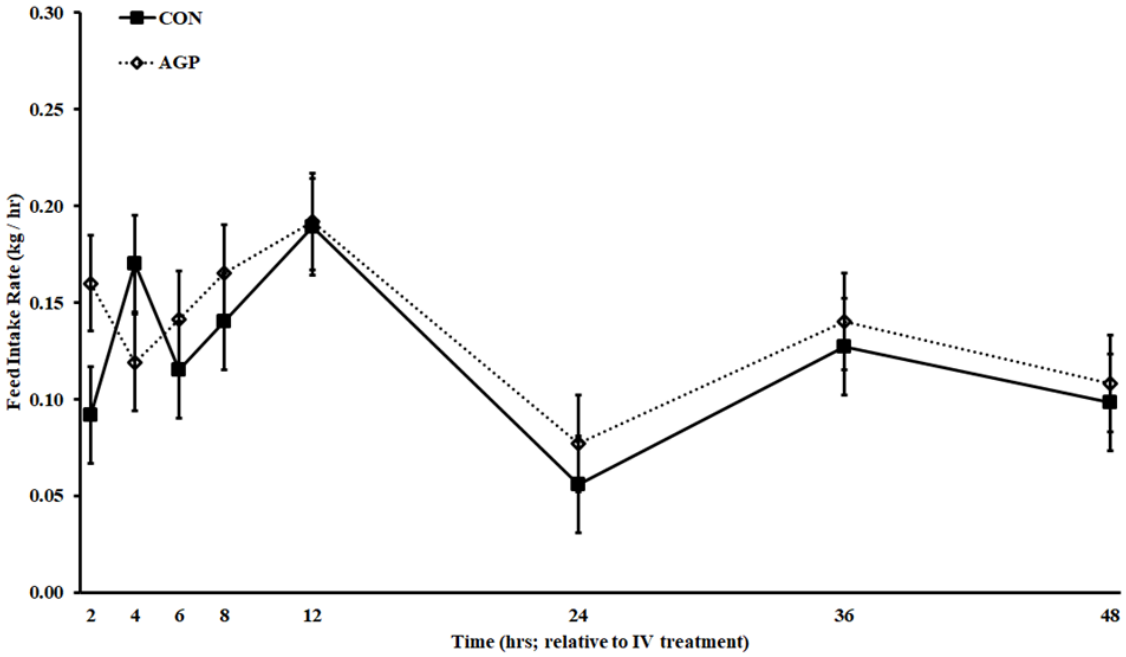

Figure 4. Effects of peripheral α-1-acid-glycoprotein (AGP) administration on feed intake rate (kg / hour) in sheep (+/- SEM). Ewes were treated with one of two treatments [0 (control; n = 5) and 3.0 (AGP; n = 5) mg / kg BW of AGP] infused via IV catheters at time 0 hrs to yield two possible treatment groups. The dose of AGP was selected based on the greatest dose administered centrally in experiment one (0.30 mg / kg BW). There was no effect of IV treatment (P = 0.98) or treatment x time interaction (P = 0.77), but there was an effect of time (P = 0.004) on feed intake rate. CON, control.

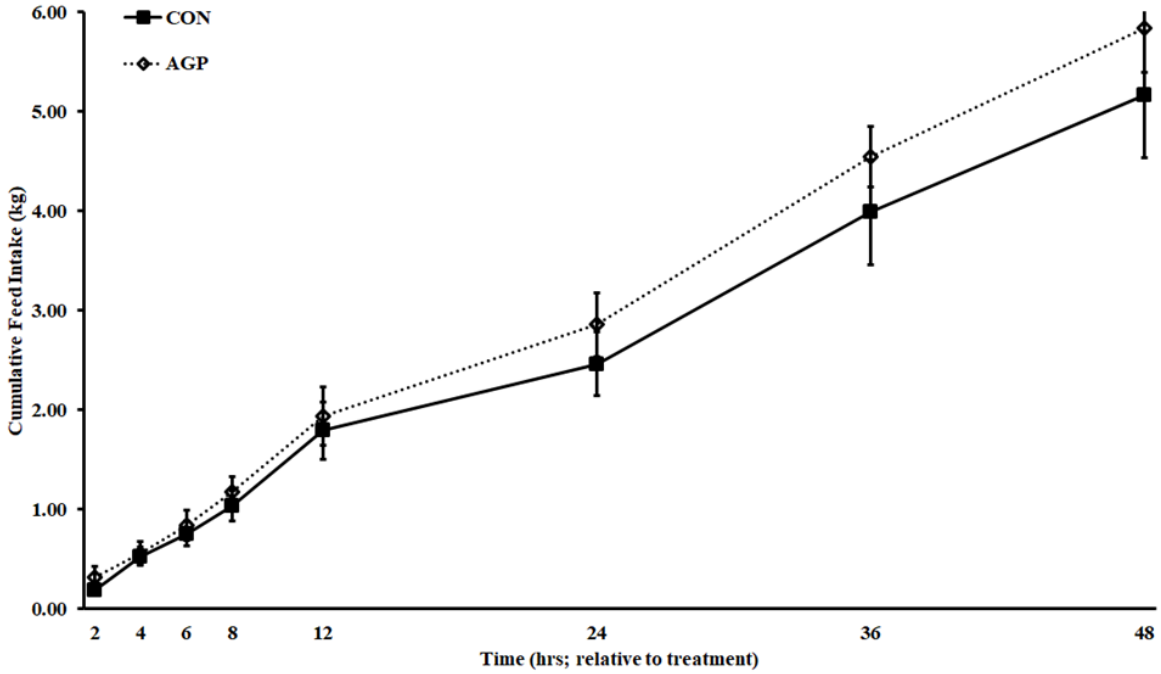

Figure 5. Effects of peripheral α-1-acid-glycoprotein (AGP) administration on cumulative feed intake (kg) in sheep (+/- SEM). Ewes were treated with one of two treatments [0 (control; n = 5) and 3.0 (AGP; n = 5) mg / kg BW of AGP] infused via IV catheters at time 0 hrs to yield two possible treatment groups. The dose of AGP was selected based on the greatest dose administered centrally in experiment one (0.30 mg / kg BW). There was no effect of IV treatment (P = 0.41) on 48 hour cumulative feed intake. CON, control.

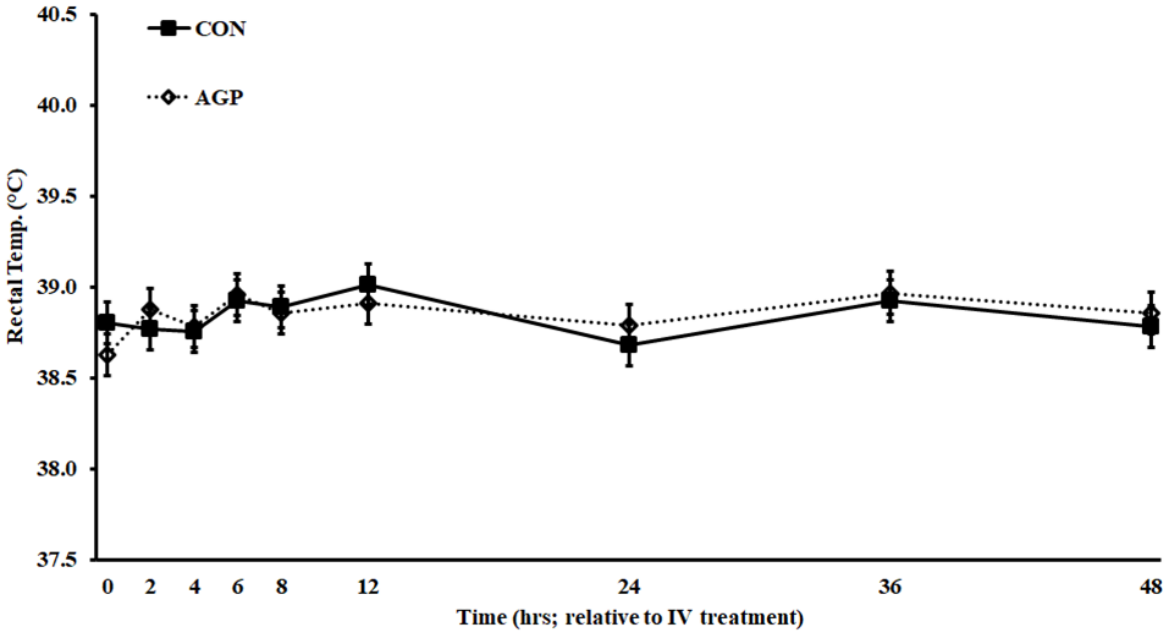

Figure 6. Effects of peripheral α-1-acid-glycoprotein (AGP) administration on rectal temperatures (°C) in sheep (+/- SEM). Ewes were treated with one of two treatments [0 (control; n = 5) and 3.0 (AGP; n = 5) mg / kg BW of AGP] infused via IV catheters at time 0 hrs to yield two possible treatment groups. The dose of AGP was selected based on the greatest dose administered centrally in experiment one (0.30 mg / kg BW). There was no effect of treatment (P = 0.71), time (P = 0.10), or treatment x time interaction (P = 0.91) on rectal temperature. CON, control.

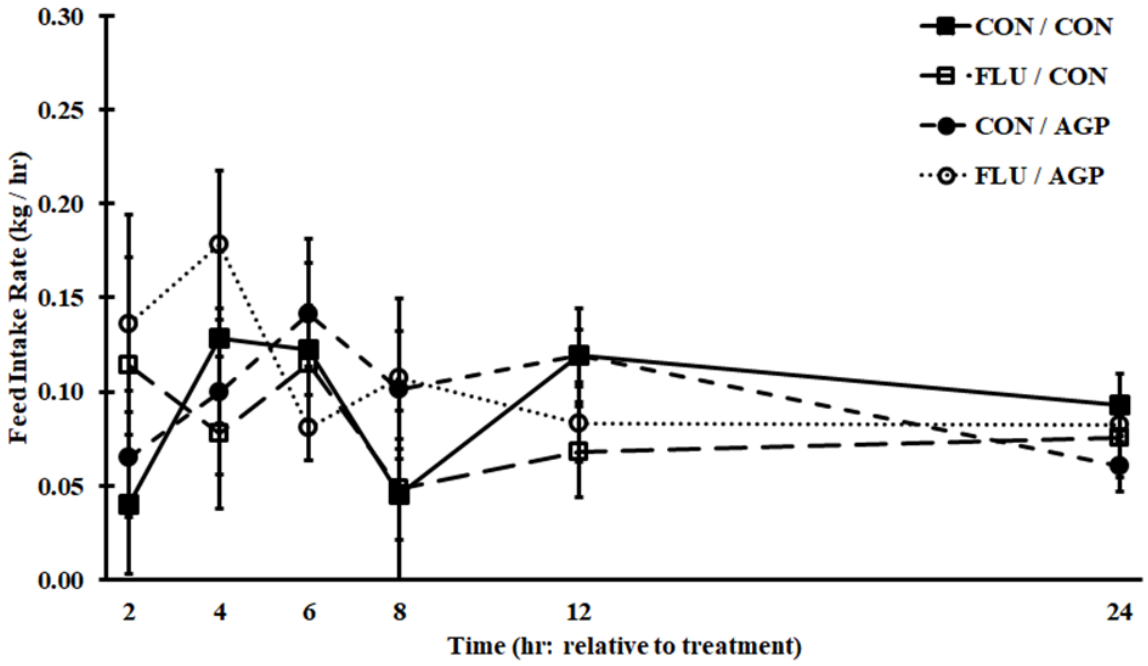

Figure 7. Effects of central α-1-acid-glycoprotein (AGP) and peripheral flunixin meglumine (FLU) administration on feed intake rate (kg / hour) in sheep (+/- SEM). Ewes were treated with peripheral treatments [administered IV; 0 (control) or 2.2 mg / kg BWFLU] 30 minutes prior to receiving centrally [administered ICV through a port and catheter into the lateral ventricle of the brain; 0 (control) or 25 mg AGP (approximately 0.30 mg / kg BW) to yield four possible treatment combinations (CON / CON, n=4; FLU / CON, n=5; CON / AGP, n =5; FLU / AGP, n=5). There was no effect of central treatment administration (P = 0.18), time (P = 0.28) or central treatment by time interaction (P = 0.64) on feed intake rate. Moreover, there was no effect of intravenous treatment (P = 0.88), intravenous treatment by time interaction (P = 0.21), intravenous by central treatment (P = 0.42), or intravenous by central treatment by time interaction (P= 0.50) on feed intake rate. CON, control.

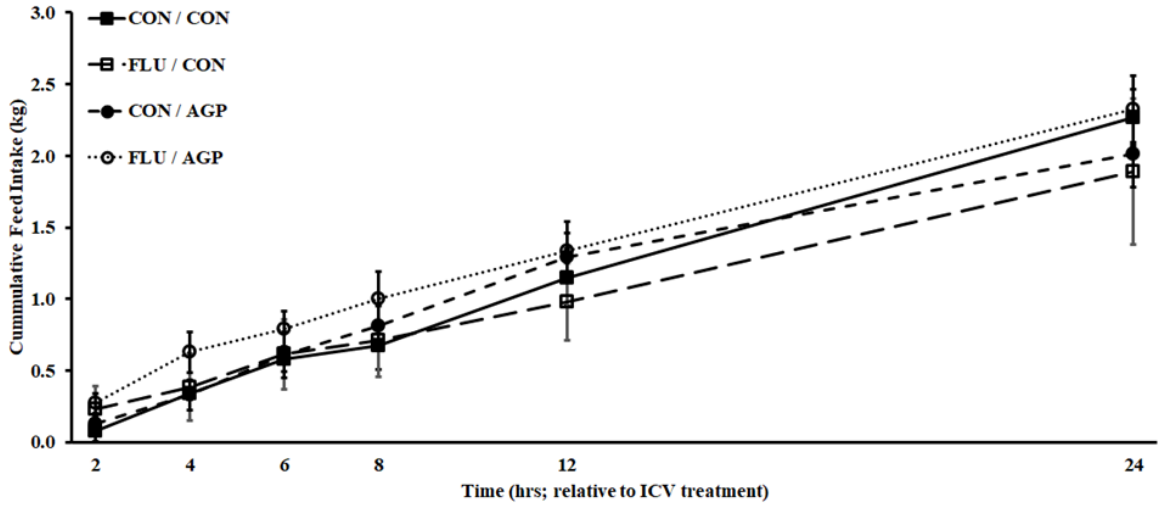

Figure 8. Effects of central α-1-acid-glycoprotein (AGP) and peripheral flunixin meglumine (FLU) administration on cumulative feed intake (kg) in sheep (+/- SEM). Ewes were treated with peripheral treatments [administered IV; 0 (control) or 2.2 mg / kg BWFLU] 30 minutes prior to receiving centrally [administered ICV through a port and catheter into the lateral ventricle of the brain; 0 (control) or 25 mg AGP (approximately 0.30 mg / kg BW) to yield four possible treatment combinations (CON / CON, n=4; FLU / CON, n=5; CON / AGP, n =5; FLU / AGP, n=5). There was no effect of central treatment (P = 0.79), intravenous treatment (P = 0.92) or their interaction (P = 0.32) on 24 hour cumulative feed intake. CON, control.

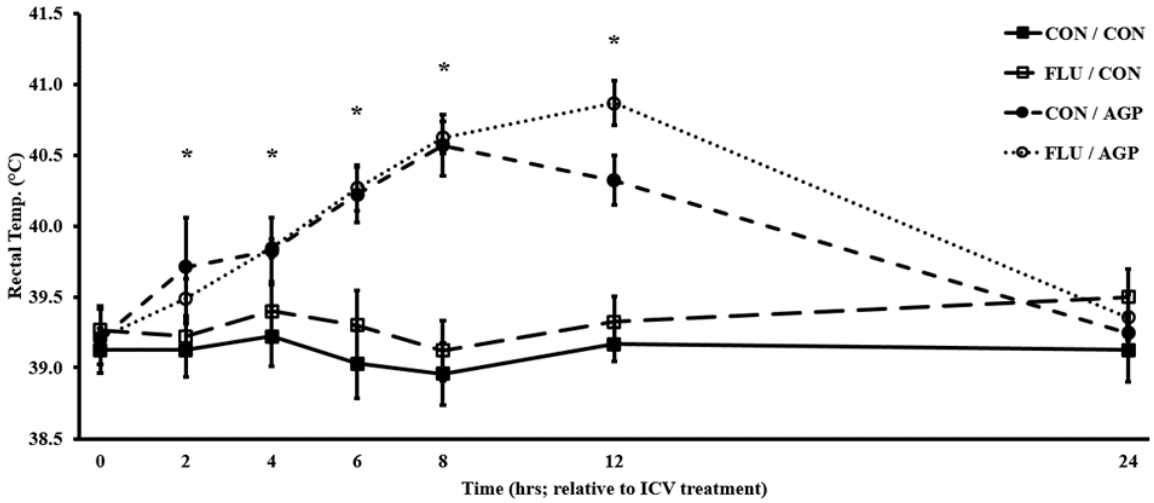

Figure 9. Effects of central α-1-acid-glycoprotein (AGP) and peripheral flunixin meglumine (FLU) administration on rectal temperatures (°C) in sheep (+/- SEM). Ewes were treated with peripheral treatments [administered IV; 0 (control) or 2.2 mg / kg BWFLU] 30 minutes prior to receiving centrally [administered ICV through a port and catheter into the lateral ventricle of the brain; 0 (control) or 25 mg AGP (approximately 0.30 mg / kg BW) to yield four possible treatment combinations (CON / CON, n=4; FLU / CON, n=5; CON / AGP, n =5; FLU / AGP, n=5). There was an effect of central treatment administration ($P < 0.0001$), time ($P < 0.0001$) and central treatment by time interaction ($P < 0.0001$) on rectal temperature such that ewes treated centrally with AGP had greater rectal temperatures at 2, 4, 6, 8, and 12 h relative to ewes treated centrally with control. There was no effect of intravenous treatment ($P = 0.93$), intravenous treatment by time interaction ($P = 0.35$), intravenous by central treatment ($P = 0.54$), or intravenous by central treatment by time interaction ($P= 0.15$) on rectal temperature. (*)

indicates time points at which AGP treated ewes differed from CON treated ewes ($P < 0.05$). CON, control.

Table 1. Feed ration composition (DM) of sheep

Ingredient	%
Cotton seed hulls	25.00
Molasses	7.00
Cracked corn	56.00
Soybean meal	10.00
Calcium phosphate	0.20
Limestone calcium	1.00
Ade premix	0.10
TM salt	0.50
Dynamate	0.20
Nutrient composition	
Dry matter, %	89.7
CP, %	11.7
ADF, %	24.30
aNDF, %	35.8

Table # 1. Continued

Ingredient	%
TDN, %	75.00
Calcium, %	0.74
Phosphorus, %	0.37
Potassium, %	1.08
Magnesium, %	0.23
Sodium, %	0.26
Sulfur, %	0.25
Aluminum, mg/kg	132.00
Cobalt, mg/kg	<0.20
Copper, mg/kg	5.06
Iron, mg/kg	157.00
Magnesium, mg/kg	18.70
Molybdenum, mg/kg	1.22
Zinc, mg/kg	30.70

*DM reported on an as fed basis

VITA

Brittany Antone (Gregg) Tipton was born on April 12, 1990 in Greeneville, TN. Brittany graduated from South Greene High School in 2008 and continued her education at Hiwassee College in Madisonville, TN. There, she pursued a Bachelor's of Art degree in Equine Management and Training. She then went on to University of Tennessee for a Bachelor's of Science degree in Animal Science and graduated in 2017. In Fall 2017, Brittany began studying for her Master of Science under the direction of Dr. Brian Whitlock and focusing her research on feed intake and inflammation of transition dairy cattle as it relates to an acute phase protein, alpha-1-acid glycoprotein.