TWO NOVEL TECHNOLOGIES FOR ENHANCING GROWTH OF RUMINANTS: DIETARY MELATONIN AND ANTIBODIES AGAINST INTERLEUKIN-10

by

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Dissertation submitted in partial fulfillment

of the requirements for the degree

Doctor of Philosophy

(Animal Science)

at the

University of Wisconsin-Madison

2015

Date of final oral examination: 8/25/2015

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ACKNOWLEDGEMENTS

I greatly appreciate working with all of the University of Wisconsin's Animal and Dairy Science faculty and staff. Gratitude goes to my committee members, Lou Armentano, Dave Combs, and Randy Shaver for their ability to teach me both the basic and applied nutrition concepts, and Mark Cook for instilling an entrepreneurial drive in me. I am indebted to Dan Schaefer, my advisor and mentor, for his great efforts towards making me a better student. Dan's experience and scientific knowledge encouraged my interest in learning, and his motivation pressed me to reach my goals as a graduate student.

Over my 5 years at UW, I have been able to collect a tremendous amount of data and thanks are extended to all of those who helped: the Arlington Agricultural Research Station staff of Dennis Anderson, Steve Arp, Ben Schwartz, Derald Stronach, and Todd Taylor; the Lancaster Agricultural Research Station staff of Arin Crooks and Ryan Schnieder; the Larry Smith Farm staff, especially Larry for his endless input in many aspects of beef production and animal health; and the Oregon F.A.N.G. members.

Lastly, special thanks are extended to all of my immediate and extended family members, as well as personal friends. Listing all of their names and attributes would be a novel in itself. I have received nothing but endless encouragement and playful banter over this amazing journey from everyone. I am thankful for the agricultural environment that I grew up in, and look forward to my future involvement in such a great area.

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ABSTRACT

Two novel technologies were evaluated for their effects on growth in young ruminants. Nine trials evaluated the effect of dietary melatonin on growth performance and carcass composition in growing cattle. Additionally, four trials evaluated dietary antibodies against interleukin-10 (aIL-10) on growth performance in both cattle and sheep.

Feeding melatonin to non-implanted beef heifers increased growth performance by 3.5% and hot carcass weight by 6 kg. However, when melatonin was fed to implanted beef steers, no difference in any growth parameter was observed. A meta-analysis of seven of the trials suggested feeding melatonin increased weight gain and gain efficiency each by 2%, however neither effect was less than $P < 0.15$. Further research is needed to re-assess the 2% difference in growth rate and gain efficiency, as the technology could aid in the production of livestock.

Maintaining health of food producing animals without the use of antibiotics is a concern for both farmers and consumers. Three trials that utilized grazing cattle or lambs were analyzed to assess if dietary aIL-10 fed for 14 d would alter animal growth rate. In two trials, when nematode infection was high, feeding aIL-10 increased animal growth rate compared to animals that had not received a de-worming agent. Furthermore, feeding aIL-10 for 14 d after steers arrived to the feedlot increased gain efficiency by 6% and antibiotic usage was numerically lower for the aIL-10 fed steers compared to the control treatment.

Data reported in this dissertation are foundational for both of the aforementioned technologies. Both technologies appear to aid in the production of livestock for human consumption by increasing animal growth parameters.

Chapter I

INTRODUCTION

Humans have consumed animal protein sources for over 1 million years and continue to do so currently. The domestication of animals such as the pig, chicken and cow, has allowed for many people to continue consuming such proteins, but today there is an economic cost and value to those protein sources. As the demand for animal protein continues to grow, it is economically advantageous for these protein sources to be grown with fewer feed calories. Any new technology that allows this to occur would hold value for the current production systems.

In 1954, a hormone called diethylstilbestrol (DES) was the first of its kind to be approved for use in cattle by the U.S. Food and Drug Administration (FDA). The oral administration of DES led to increased growth rate, less feed per unit weight gain, increased carcass leanness, and reduced carcass yield grade, all of which are of great value to producers thus the technology was rapidly adopted. Issues arose with oral DES, and in 1972 the FDA suspended its use. Although DES is currently unavailable to use, many other technologies have been approved for use which have similar beneficial effects, and these new technologies have been readily accepted by the beef industry. More than 90% of large (> 1,000 hd) feedlots use growth promoting implants, 71.2% feed tylosin, 90.1% use ionophores, and 32.6% use coccidiostats in their production systems (USDA, 2011), which illustrate the general acceptance of novel technologies in beef production systems.

Strategies to modify beef carcass composition and thereby improve efficiency of growth have focused on exogenous administration of steroid hormones (estrogen, progesterone, and trenbolone acetate) or B-agonist molecules (ractopamine and zilpaterol). One of the purposes of this dissertation is to explore an entirely different endocrine approach, which is focused on photoperiod effects that are manifested via melatonin.

Cattle are responsive to differing photoperiod lengths; longer photoperiods increase milk production in dairy cattle and supplemental lighting reduces age at puberty of heifers born between February and July, while a shorter photoperiod induces hair growth. The United States Department of Agriculture (USDA) Agriculture Marketing Service provides a database which includes data from more than 13 million harvested steers and heifers. This database records quantity (pounds) and quality (marbling scores) of harvested animals. A hierarchy system of marbling scores (Prime > Choice > Select, greatest marbling/ fat content) is used to assess the amount of intramuscular fat within the rib muscle of graded animals. It has been shown that a larger percentage of beef carcasses are graded as USDA Choice during the short photoperiod months compared to animals harvested in the summer, when a long photoperiod is present (Fig. I-1). In cattle, melatonin is a hormone that has diurnal variation, whereby levels are elevated during darkness and diminish during light exposure. This simple association suggests the hypothesis: administration of exogenous melatonin will increase beef carcass grade of feedlot animals and may alter growth performance. Additionally, melatonin has been suggested to affect reproduction, seasonal behavior, immunity, thermo-regulation, and possibly aging. It has also been postulated to be pathophysiologically associated with cancer, epilepsy, seasonal affective disorder, and ocular diseases (Yu and Reiter, 1993), but these pathologies will not be addressed in this document.

Few trials have used exogenous melatonin in cattle and typically small numbers of animals were used in the trials, probably due to the high cost of melatonin. Because little data is available, a primary objective was to determine if dietary melatonin alters animal growth or carcass characteristics. Secondary objectives were to assess if interactions existed between melatonin feeding and 1) animal gender, 2) season, or 3) anabolic implant status. The dataset collected is the largest known to date, in which dietary melatonin was studied in growing beef animals.

Maintaining adequate health in all livestock is a primary concern for farmers and there is growing interest in animal products that were raised without the use of antibiotics. Grazed pasture is an essential feed resource for the beef and sheep industries. However, this feed source also comes with the problem that grazed animals become infected with gastrointestinal parasites that consume blood-borne nutrients and/or cause diarrhea by the animal resulting in suppressed animal growth rates. Economic losses associated with parasitism in sheep are considered to be significant and 75% of sheep producers rate internal parasites as a main concern. Lawrence and Ibarburu (2007) estimated the value of anthelmintics in cattle to be between 166 and 201 ($/head) during the stocker phase and an additional 21- 24 ($/head) while in the feedlot. Furthermore, resistance to modern anthelmintics by nematode parasites is an increasing problem throughout the world, and has been largely documented in small ruminants and increasingly frequent observations have been reported in cattle (Gasbarre et al., 2009).

Upon entrance to the feedlot, morbidity and mortality from bovine respiratory disease (BRD) continues to be the most significant health problem facing the US beef cattle industry (Duff and Galyean, 2007), and is estimated to cost 13.90 ($/head), not including labor and associated handling costs (Snowder et al., 2006). In 2011, all feedlots (100%) within 12 states reported BRD treatments with an overall incidence of 16.2%, and an average cost of $23.60 per treatment (NAHMS, 2011). The use of dietary antibodies designed to bind gut interleukin-10 has not be researched, and its potential applications for controlling gastrointestinal parasites and BRD will be addressed. The main objective of the trials was to assess if dietary aIL-10 would increase growth rate and maintain adequate health of food producing ruminants.

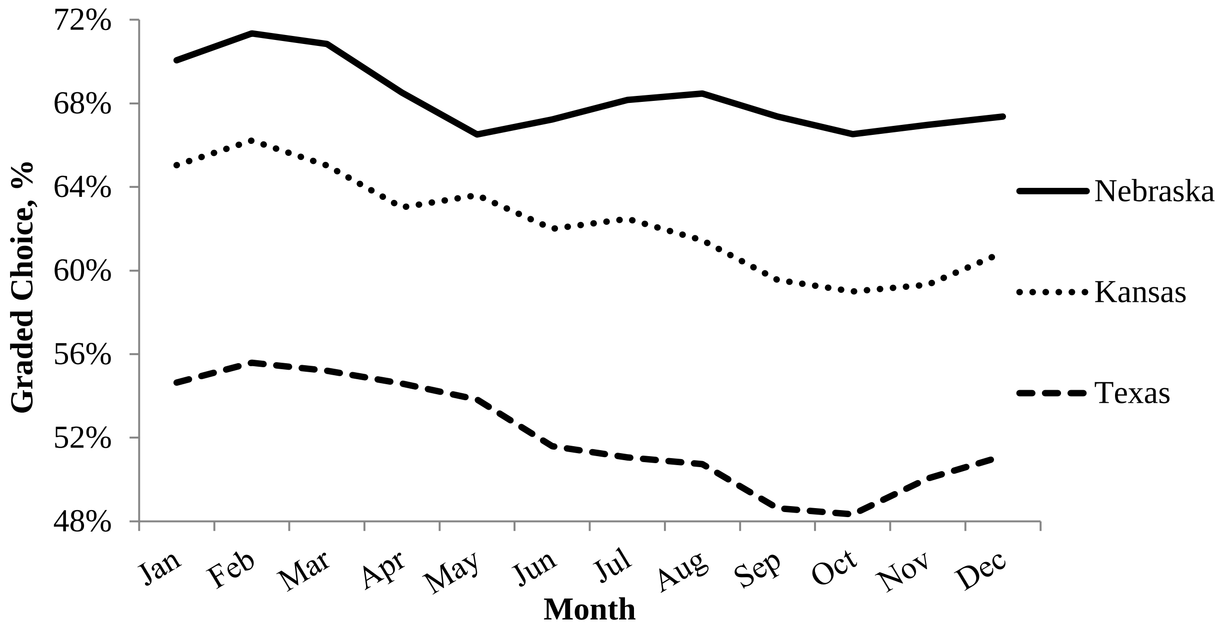

Figure I-1. Percentage of USDA inspected animals grading choice by state and month of year (adapted from USDA AMS website).

CHAPTER I REFERENCES

Duff, G.C., and M. L. Galyean. 2007. Board-invited review: Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Gasbarre, L.C., L.L. Smith, J.R. Lichtenfels, and P.A. Pilitt. 2009. The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. Vet. Parasitol. 166:281-285.

Lawrence, J.D., and M. Ibarburu. 2007. Economic analysis of pharmaceutical technologies in modern beef production in a bioeconomy era. Accessed 9 July 2015. http://www2.econ.iastate.edu/faculty/lawrence/pharma%202007%20update.pdf

NAHMS. 2011.Feedlot 2011 Part IV: Health and health management on U.S. feedlots with capacity of 1,000 or more head. USDA, APHIS, National Animal Health Monitoring System. Accessed 9 July 2015. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf

Snowder, G.D., L.D. Van Vleck, L.V. Cundiff, and G.L. Bennett. 2006. Bovine respiratory disease in feedlot cattle: Environmental, genetic, and economic factors. J. Anim. Sci. 84:1999-2008.

USDA. 2011. Feedlot 2011 part IV: health and health management on U.S. feedlots with a capacity of 1,000 or more head. Accessed 5 Jan. 2015. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf

USDA AMS. 2015. Choice Beef Percentage Graded (Figure). Accessed 18 Jun. 2015. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5077890

Yu, Hing-Sing, and R.J. Reiter. 1993. Melatonin: biosynthesis, physiological effects, and clinical applications. CRC, Boca Raton, FL.

Chapter II

LITERATURE REVIEW

MELATONIN HISTORICAL OVERVIEW

Melatonin (N-acetyl-5-methoxytryptamine) is a lipophilic molecule that was discovered in 1958 from bovine pineal extracts by Lerner et al. (1958). They used about 250,000 bovine pineal glands, weighing several kilograms for the attempted isolation. The pineal glands were defatted and lyophilized and the substance, which they named melatonin, was successfully extracted by ethyl acetate and subsequently purified. Bio assays were performed using isolated frog (*Rana pipiens)* skin darkened with caffeine and then the lightening effect was measured photometrically. They also showed that melatonin had neither adrenaline, nor noradrenaline-like activity on rat uterus and no serotonin-like effect on clam heart (Yu and Reiter, 1993). It was in 1959 that Lerner and colleagues determined the structure of melatonin (Lerner et al., 1959).

Given years of research it was deemed that melatonin was not restricted to only the pineal gland but could possibly be ubiquitous. Melatonin has been detected in multiple extrapineal tissues including the brain, retina, lens, cochlea, Harderian gland, airway epithelium, skin, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, immune system cells, carotid body, reproductive tract, and endothelial cells, and most of these tissues contain melatonin-synthesizing enzymes. It is also present in essentially all biological fluids including cerebrospinal fluid, saliva, bile, synovial fluid, amniotic fluid and breast milk (Acuna-Castroviejo et al., 2014). In 1976, Ozake and Lynch discovered melatonin in the plasma and urine of pinealectomized rats, but urinary excretion of melatonin decreased by 80% after pinealectomy and then did not exhibit a circadian variation. This suggests that in mammals, melatonin is mainly produced and secreted at night by the pineal gland, although other production sites have also been described such as the retina and the gut which do not contribute significantly to blood levels in mammals, but may be of local importance (Arendt, 1995).

The common absence of day and night variations in extrapineal melatonin synthesis suggests signaling pathways other than photoperiod regulation of this indoleamine (Venegas et al., 2012). Many extrapineal tissues have higher concentrations of melatonin than plasma throughout a 24 h period, and the intracellular melatonin from these tissues is not generally released into circulation (Venegas et al., 2012). These observations suggest that extrapineal melatonin acts locally, possibly protecting cells from oxidative and inflammatory damage. Because melatonin is known to be a highly effective antioxidant (Bonnefon-Rousselot et al, 2011), the lack of a circadian rhythm to melatonin's concentration in extrapineal tissues may be understandably irrelevant. Nevertheless, the nocturnal melatonin peak provides important photoperiodic information to every cell to ensure proper chronobiotic synchronization (Hardeland et al., 2012). However, the production of reactive oxygen or nitrogen species takes place mainly during metabolic, motor, and neural activity, when oxygen consumption is maximal; these activities do not necessarily occur at night in diurnal animals (Acuna-Castroviejo, 2014).

SYNTHESIS OF MELATONIN

For whatever species considered, melatonin is synthesized during the dark phase (Barrenetxe et al. 2004). In humans and other diurnal species, melatonin synthesis occurs during sleep, while in nocturnal species peak synthesis occurs during the active period. The duration and magnitude of the hormone secretion by the pineal gland is directly related to the length of darkness so it acts as a neuroendocrine mediator of the photoperiod (Reiter, R.J., 1991). Except for some tissues as the retina, extrapineal melatonin synthesis occurs through the same metabolic pathway as described in the pineal gland, although it is not regulated by photoperiod (Acuna-Castroviejo, 2014). Within the cell, tryptophan (TRP) is converted to melatonin via four enzymatic reactions:

TRP → 5-hydroxytryptophan → serotonin → N- acetylserotonin → melatonin.

1. TRP is actively transported across the cell membrane (Sugden, 1979) and tryptophan hydroxylase (TH) catalyzes the conversion of TRP to 5-hydroxytryptophan (5HTP). In the pineal, this step is believed to be rate limiting in serotonin biosynthesis (Yu and Reiter, 1993). TH activity in the rat exhibits a light-dark cycle with maximum activity towards the end of the dark phase of the cycle (Sitaram and Lees, 1978). TH activity is inhibited by ρ-chlorophenylalanine which reduces serotonin levels in the pineal gland (Bloom and Giarman, 1967).
2. The conversion of 5HTP to serotonin is accelerated by 5-hydroxytryptophan decarboxylase (5HTPD) which removes the terminal α-carboxyl group from 5HTP. The activity of 5HTPD in the pineal gland is higher than in most other tissues (Hakanson and Owman, 1966) and its activity remains unchanged throughout the light-dark cycle in the rat pineal gland (Snyder et al., 1965). A high level of serotonin is observed in the mammalian pineal gland (King et al., 1984) with a depression in the pineal level of this constituent associated with the dark phase of the light-dark cycle (Yu and Reiter, 1993).
3. *N*-acetyltransferase (NAT) catalyzes the transfer of an acetyl group from acetyl-COA to serotonin, which forms *N*-acetylserotonin (NAS). This step has been said to limit melatonin synthesis (Yu and Reiter, 1993). The activity of NAT in the rat pineal gland exhibits a 24 hour rhythm with peak night activity 20 to 100 fold greater than daytime levels, which results in increased pineal melatonin content during the night (Lewy, 1983).

Knockout mice that lack the NAT gene have a deficiency of melatonin in their pineal (Ebihara, et al., 1986).

4. The final step utilizes the enzyme hydroxyindole-*O*-methyltransferase (HIOMT), which constitutes 2-4% of the total soluble proteins in the pineal gland (Jackson, R.L. and Lovenberg, 1971). Transferring a methyl group from *S*-adenosyl methionine to NAS finalizes melatonin synthesis. In mammals, pineal HIOMT activity is high and does not appear to exhibit a diurnal variation (Sugden, 1989). As NAS level increases at night, melatonin synthesis is elevated by 2 to 12 fold where NAS is converted to melatonin by HIOMT (Yu and Reiter, 1993). Recently, HIOMT has been renamed *N*-acetylserotonin-*O*-methlytransferase.

This pathway is summarized in Figure II-1.

Neurological Stimuli

In the absence of light at night, an increase in melatonin biosynthesis in the pineal is stimulated by electrical signals originating from neurons in the suprachiasmatic nuclei (SCN) (Moore and Klein, 1974). The neurotransmitter at the postganglionic sympathetic nerve terminal is norepinephrine (NE), which is synthesized from tyrosine with the conversion being catalyzed by TH (Yu and Reiter, 1993). The combination of an increased impulse frequency (Taylor and Wilson, 1970) and TH activity at night (McGeer, E.G. and P.L. McGeer, 1966) results in greater NE released into the synaptic cleft. NE binds to α_1 and β-adrenergic receptors on the pinealocyte membrane and triggers a series of intracellular responses through specific transduction processes.

The receptors for NE on the pinealocyte are predominately the β type, and the density of these receptors on the membrane is greatest at night (Pangerl et al., 1990). Because NE also binds α receptors, both types are needed for a maximal response on the pinealocyte. Additional neurotransmitter receptors can be found on the pinealocyte membrane, such as the σ type, which is highly concentrated in the rat pineal (Jansen et al., 1990), but their function is not clear yet.

β-Adrenoceptor stimulation activates the enzyme adenylate cyclase via a stimulatory guanine nucleotide binding regulatory protein, Gs, resulting in the synthesis of cAMP (Sugden, 1989). It is believed that the increase in cAMP mediates the induction of NAT by activating a cAMP dependent protein kinase; however these steps are not well defined.

Using a pulse dose of melatonin (10 µg/ kg BW) and a direct radioimmunoassay, the production rate of melatonin synthesis was estimated to be approximately 400 ng/ kg BW, per 24 hours (Berthelot et al., 1990) for a mature dairy cow. Berthelot's 1990 data suggest a perfusion rate of 1,000 ng/kg BW per hour should achieve a steady state plasma concentration of 673 pg/ml after a delay of about 5 hours (five times the half-life). This is in agreement with the results obtained by others in sheep (Kennaway et al., 1982) and cattle (Stanisewski et al., 1988).

RECEPTORS AND BINDING SITES OF MELATONIN

Circulating melatonin can elicit an effect on tissues by two main pathways which include receptor- and non-receptor-mediated signaling. These receptors can be membrane or nuclear binding sites. There are two types of membrane receptors, MT1 and MT2, both of which are guanine nucleotide-binding regulatory protein (G-coupled) receptors and have been well characterized (Dubocovich and Markowska, 2005). Activation of MT1 receptors by melatonin leads to different responses, most of them depending on the inhibition of cAMP (Brydon et al., 1999). Binding of melatonin to MT2 receptors leads to cAMP and cGMP inhibition (Petit et al, 1999). Additionally, both MT1 and MT2 receptors can couple with phospholipase C-dependent pathways (Garcia-Lerganeda et al., 1997). MT1 receptors are related to reproductive and metabolic functions, and vasoconstriction, whereas MT2 receptors are involved in the control of circadian rhythms and dopamine release in the retina, and vasodilation, among other actions (Dubocovich and Markowska, 2005). Using a radio ligand suggests MT1 is a high affinity receptor (Ki=10-300 pM) and MT2 is a lower affinity receptor (Ki= 1-10 nM) (Dubocovich, M.L. 1985). A third melatonin binding site, formally known as MT3, has been identified as a quinone reductase 2, and it is related to the xenobiotic metabolism of the cell (Nosjean et al., 2000); this enzyme, however, does not fulfil the criteria for a melatonin receptor (Acuno-Castroviejo et al., 2014). Melatonin also interacts with cytosolic proteins including calmodulin and calreticulin, which are involved in the cytoskeleton regulation and control of nuclear receptors, respectively (Benitez-King et al., 1993). Binding sites in the nucleus have also been reported (Acuna-Castroviejo et al., 1994); they were later identified as retinoid-related orphan receptors (Becker-Andre et al., 1994).

Early observations using autoradiography, suggested 2-[^{125}I] iodomelatonin binding sites were localized in the SCN and hypothalamus of the rat (Vanecek et al., 1987). Further studies using a light microscope revealed that specific labelling was restricted to the pars tuberalis of the anterior pituitary in the rat (Williams, 1989). Despite the effects of melatonin on circadian rhythm, mediated by its action in the hypothalamus and pituitary, there is continuing interest in its effects on peripheral tissues.

DEGRADATION

The most important site for melatonin metabolism is within the liver. Here it undergoes a 6-hydroxylation followed by a sulfate or glucuronide conjugation. The amount of each of these compounds formed depends on the species (Barrenetxe et al., 2004). In humans, the major urinary metabolite is 6-sulfatoxymelatonin, and the measurement of its level provides a simple and reliable assessment of melatonin secretion (Kovacs et al., 2000). Melatonin may also be deacetylated to 5-methoxytryptamine, which has been demonstrated in rat liver slices in-vitro, though it is a minor pathway (Rogawski et al., 1979). Additionally, other metabolites such as NAS (melatonin precursor) (Leone and Silman, 1984), and in the brain 5-methoxykenurenamine (Hirata et al., 1974) may play a role in melatonin metabolism. Despite these alternative metabolic pathways, melatonin secreted into circulation by the pineal gland is mainly metabolized in the liver and kidney and excreted as hydroxymelatonin in urine (Yu and Reiter, 1993).

Berthelot et al. (1990) used a pulse IV dose of 10 µg / kg BW and determined the elimination half-life of melatonin was 67 minutes in mature dairy cows. In contrast, using the data published by Zinn et al. (1988) who fed a pulse dose of 4 mg/ 100 kg BW, the half-life is estimated to be approximately 6 hours. The calculated clearance value suggests for melatonin a first pass hepatic effect, and hepatic blood flow should be a major determinant in melatonin clearance (Berthelot et al., 1990). A possible consequence of a first pass hepatic effect should be the non-equivalency of oral versus other routes of melatonin administration, because melatonin can be altered to 6-sulfaoxymelatonin within the liver. Therefore, some of the observed effects could be attributed to melatonin metabolites rather than to the hormone itself.

MELATONIN AND IMMUNE FUNCTION

Photoperiod and tissue size

Early experiments to associate melatonin with the immune system were done by manipulations of photoperiod. As described above, varying light exposure has the ability to increase or decrease circulating levels of melatonin. Thus, increasing photoperiod can lower circulating melatonin and limiting light exposure can increase these levels. The immune system is comprised of a variety of lymphoid organs, the primary being the thymus and bone marrow, and secondary tissues such as spleen, tonsils, and lymph nodes. Located in front of the heart, but behind the sternum, the thymus allows immature T cells to mature and differentiate which is a vital part of the adaptive immune system. Additionally, in the spleen antigens can be processed and presented to appropriate B or T cells, and activated B cells can produce large amounts of antibodies which are released into the circulating blood. Laboratory rats maintained under constant light for 4 weeks revealed a decrease in thymic mass by more than 50% compared to their long day counterparts (Mahmoud et al. 1994). However, for rats that were maintained in constant dark, thymic mass increased by 300%, with the majority of the increase seen in the lymphatic tissue of the thymic medulla, as well as in the total number of thymocytes (Mahmoud et al., 1994). Laboratory rats are not traditionally thought of as photoperiodic animals since constant darkness does not alter steroid hormone concentrations in this species (Heidman and Sylvester, 1997). Therefore it is presumed the responses listed above are in relation to the effects of melatonin on immune function.

Other research has concurred with evidence of short day photoperiod stimulating immune function in a variety of rodent species (Nelson and Demas, 1996). Wurtman and Weisel (1969) found rats maintained in short days had a moderate increase in spleen weight compared to long day. This increase in spleen mass during short photoperiod has also been found in deer mice (*Peromyscus maniculatus*) (Vriend and Lauber, 1973), and Syrian hamsters (Brainard et al., 1985). Also, total splenic lymphocyte numbers and macrophage counts are significantly elevated in short day Syrian hamsters compared with long day animals (Brainard et al., 1985). Photoperiod did not alter humoral (serum antibody concentration) immunity in deer mice or Syrian hamsters in the above trial. However, when Maestroni and Pierpaoli (1981) exposed mice to continuous light for 3 generations, they displayed an impaired ability to mount an antibody response to T-cell dependent antigens. These mice also showed a depletion of T-cells in the cortex of the thymus gland, as well as atrophy of the white pulp of the spleen.

General Effects on Immune Function and Regulation

Removal of the source of endogenous melatonin can alter immune function in many animal species (Nelson et al., 2002). Functional pinealectomy via pharmacological inhibition of pineal gland function with the β-adrenergic antagonist propranolol (which inhibits pineal melatonin synthesis) or by ρ-chlorophenylalanine (which inhibits serotonin) results in atrophy of lymphatic tissue, as well as impairment in somatic growth and antibody production (Maestroni and Pierpoli, 1981). Exogenous melatonin administration restores normal immune functioning in these drug treated animals (Maestroni et al., 1987). Additional studies illustrate that pinealectomy reduces humoral immunity (Kuci et al., 1983), natural killer cell activity, interleukin (IL)-2 production (Del Gobbo et al., 1989), and collagen II-induced autoimmunity (Hansson et al., 1992).

There has been much research focused on the role of cytokines in mediating the photoperiodic effects on immune function. Withyachumnarnukul et al. (1990) observed that interferon (IFN)-γ can enhance melatonin production when added to cultured rat pinealocytes. Additionally, glycoprotein granulocyte colony-stimulating factor and granulocyte-macrophage colony stimulating factor can increase melatonin synthesis by the pineal gland both in vitro and in vivo (Zylinska et al., 1995). Other cytokines such as IL-1β can decrease circulating melatonin concentrations in rats in a dose dependent fashion (Mucha et al., 1994). These data, along with the relatively wide distribution of receptors for specific cytokines throughout the mammalian brain, suggest that cytokines play a critical role in a bidirectional communication between the pineal gland and the immune system (Cardinali et al., 2000).

Further trials have assessed the effect of exogenous melatonin to pineal intact animals and implications on various immune functions. It was observed that exogenous melatonin (0.01-10 mg/kg) can enhance both the primary and secondary responses to sheep red blood cells in mice but only when injections were given in the afternoon. Melatonin given at this time also increased the secondary, but not primary, response by cytotoxic T-cells in response to infection with vaccinia virus (Maestroni et al., 1988).

Antioxidant properties

The existence of the key enzymes of melatonin synthesis in many tissues, the presence of melatonin receptors in numerous tissues, and the discovery of the widespread antioxidant and anti-inflammatory properties of melatonin caused scientists to reclassify melatonin from being exclusively a hormone to that of a multi-tasking molecule (Acuna-Castroviejo et al., 2014).

In 1993, in vitro evidence was reported that melatonin was a voracious scavenger or inhibitor of the very toxic hydroxyl radical (Tan et al., 1993), therefore it was thought that melatonin might modulate the redox state of cells (Chen et al., 1993). Melatonin and other known radical scavengers such as glutathione and mannitol were added to determine the effectiveness of melatonin’s ability to neutralize the hydroxyl radical. Results showed melatonin to be the best of the three, where it was 5 times better at scavenging than glutathione and 15 times better than mannitol. This study also showed that naturally occurring analogs of melatonin were less effective, or totally ineffective as free radical scavengers (Poeggeler et al, 1994).

Further experiments tested the ability of melatonin to scavenge the peroxyl radical. Using an in vitro model, it was reported that melatonin was approximately twice as effective as Trolox (a water soluble analog of vitamin E) (Pieri et al, 1994). In all in vitro studies conducted thus far, melatonin has shown itself to be the most effective molecule against free radicals

(Watson, 1999). When melatonin neutralizes •OH it becomes an indolyl cation radical, which has been shown to have a low toxicity (Hardeland et al., 1993). This radical then has the capability to scavenge a superoxide anion and in the process is converted to N-acetyl-N-formyl-5-methoxykynuramine (Watson, 1999).

GASTROINESTINAL TRACT MELATONIN

In 1975, Raikhlin et al. immunocytochemically identified the presence of melatonin in enterochromaffin cells of the intestinal mucosa. They estimated melatonin content in the gastro-intestinal tract (GIT) to be roughly 400 times greater than in the pineal gland, and 10-100 times higher than in the plasma. Gastrointestinal melatonin amounts were initially found not to be controlled by the photoperiod, but by the rhythms in food intake (Raikhlin et al., 1975).

It was only 7 years after the discovery of melatonin that Quastel and Rahamimoff (1965) observed the distinct effect of this indole on decreasing peristalsis rate of the rat duodenum . This initial study was followed by a report of a strong antiserotonin action of melatonin when used in the rat stomach and ileum (Fioretti et al., 1972). This effect of melatonin as a natural inhibitor of serotonin action in the GIT and its influence on muscle tone were later confirmed by Bubenik (1986) in vitro. In their studies, melatonin was shown to relax smooth muscle and to counteract the spastic effect of serotonin (Watson, 1999). Later in vivo studies showed that melatonin increased food transit time which had been shortened in mice previously implanted with serotonin containing pellets (Bubenik and Dhanvantari, 1989). Using a histochemical technique, it was concluded that melatonin is most probably produced in the enterochromaffin cells of the GIT mucosa (Raikhlin et al., 1975, and Raikhlin and Kvetnoy, 1976). A follow up study revealed that melatonin could be found in the mucosa of the entire alimentary tract ranging from the esophagus to the rectum (Bubenik et al., 1976).

In early studies, the sensitivity of detection assays was so low that the removal of the pineal gland resulted in non-detectable levels of melatonin in blood (Watson, 1999). That supported the hypothesis that the pineal gland is the sole producer of melatonin (Ozaki and Lynch, 1976), however, as melatonin assays became more sensitive, a different picture emerged. In numerous studies which used radioimmunoassays and gas chromatography, pinealectomy abolished only the nighttime elevation of melatonin in plasma (Kennaway et al., 1977), but substantial daytime levels were still present (Watson, 1999). The discovery that HIOMT was present in GIT tissues (Quay and Ma, 1976) and that daytime administration of L-tryptophan increased rat portal vein melatonin levels by fourfold (Huether et al., 1992) suggest the GIT may be a primary source of daytime melatonin levels in plasma. Lastly, the GIT also has the ability to uptake exogenous melatonin which has been shown in studies (Kopin et al., 1961, and Bubenik, 1980). The mechanism of melatonin action in the GIT may include scavenging of free radicals, stimulation of the immune system, and the movement of fluids.

ENERGY BALANCE AND MELATONIN

Thermoregulation

Melatonin plays an important role in the regulation of seasonal thermoregulation including torpor (a bout of decreased physiological activity) and hibernation, being involved in the circadian thermoregulatory adjustments of body temperature in different animals (Saarela and Reiter, 1994). Because the pineal is the primary site for melatonin release into circulation, it may seem plausible that pineal size may correlate with the thermoregulation capability of a given species. Indeed, vertebrates that inhabit the high latitudes tend to have larger pineals while those living at lower latitudes have small pineals or, in a very few cases, an alleged absence of the gland (Ralph, 1975).

John and George (1991) researched the effects of melatonin and metabolic responses to varying ambient temperatures in pigeons. Melatonin implanted (MI) and control birds were exposed to temperatures gradually dropping from 34^{o} C to 2^{o} C. The MI pigeons maintained a lower body temperature than the controls throughout. This was in agreement with an earlier observation that MI nullifies or reverses the hyperthermic effect of a pinealectomy (John et al., 1978). In the 1991 study, it was shown that shivering activity increased at temperatures below 26^{o} C, but the rate of shivering in the MI treatment was relatively slow and MI birds had a lower heart beat rate. The role of melatonin was thus indicative of being a facilitator of vasodilation and increased blood flow so as to enhance peripheral heat dissipation. The peripheral vasodilatory action of melatonin has been shown in its capacity for lowering the vasoconstrictor effects induced by various constrictor agents in pinealectomized rats (Cunnane et al., 1980). These effects were also observed for dietary melatonin as Zeman et al. (2001) reported that female chicks fed 150 mg melatonin/kg feed reduced heat production by greater than 8%. This was in agreement with Apeldoorn et al. (1999) who fed 0 or 40 mg melatonin/ kg feed and showed feeding melatonin decreased the physical activity related heat production in broilers by greater than 8.5%.

Adipose Tissue

Studies performed in hibernating animals have shown that characteristic seasonal changes in fat mass observed in these animals depend on the photoperiod through the nocturnal release of melatonin (Barrenetxe et al., 2004). Melatonin may affect white and brown adipose tissues via its innervation through the sympathetic nervous system from brain to fat and thereby modulate adiposity. The SCN is one of the nuclei which project through the sympathetic nervous system to both white and brown adipose tissues (Bartness et al., 2001). The SCN express both MT1 and

MT2 receptor subtypes and MT1 receptors have been identified on the neurons of these nuclei which project to the white adipose tissue (Song and Bartness, 2001). Microinfusions of short day-like melatonin signals into the SCN in Siberian hamsters trigger short-day-like responses including decreased body fat (Bartness et al., 1993). In Siberian hamsters, short days increase the sympathetic drive on white adipose therefore an increase of norepinephrine turnover and consequently an increase of lipolysis which leads to the decrease of adiposity (Youngstrom and Bartness, 1995). In contrast, Syrian hamsters increase their fat mass when submitted to short days and the mechanism may be opposite of that described above (Bartness and Wade, 1985).

EXOGENOUS MELATONIN AND BODY WEIGHT ACCUMULATION

For nontropical species, the coming of winter represents a remarkable adaptational challenge in that extremely cold temperatures occur at a time when food supplies are in short supply (Osei et al., 1989). Such harsh environmental conditions have exerted evolutionary pressures for animals to develop mechanisms for conserving energy for survival (Thomson, 1950). There is a tremendous amount of evidence of a seasonal variation in appetite and body weight in humans with a tendency to increase in the fall and winter, and to decrease in the spring and summer (Attarzadeh, 1983).

In 1989 Osei et al. fed young chicks 0 or15 mg melatonin/kg diet and recorded differences in growth, feed intake, energy retention, triiodothyronine (T3) and thyroxine (T4) levels. When fed from day 7 to 14 post hatching, melatonin fed chicks increased growth rate by 19%, feed intake by 4%, and feed efficiency by 8%. Their determination of energy retention (percent of metabolizable energy retained in body) suggested melatonin-fed chicks increased energy retention by 23%; however they did not record body percentage of protein or fat. Hormone levels of both T3 and T4 were increased when melatonin was fed. Within this data set, they also showed pinealectomized chicks decreased the efficiency of energy utilization for growth, however treatment effects on maintenance energy requirements were not statistically different, but pinealectomized chicks were only 89% as efficient compared to the control counterparts.

The effect of feeding melatonin to growing bovine animals and the response on growth rate and carcass composition has only been published in one journal article to date. In 1988, Zinn et al. fed a pulse dose of melatonin (4 mg/ 100 kg BW) at 1330 hr to growing heifers in two trials for the purpose of determining effects on weight gain and carcass composition. Using a radioimmunoassay for melatonin, it was found that this administration method allowed for plasma melatonin concentrations to surpass 100 pg/ml for several hours after feeding and return to basal levels before 0800 hr the following day. During the dark period, control animals reached a peak of approximately 40 pg melatonin/ ml plasma, suggesting that the dose used was physiologically relevant. In both trials, no difference was detected in animal growth rate. Because of trial design, no analysis could be conducted on feed intake or comparison of BW gain/ feed intake. When melatonin was fed to pre-pubertal heifers it was determined that melatonin fed animals increased 9-10-11 rib fat percentage, but this was not detected when fed in the second trial that utilized fatter animals at the start of the trial. The authors speculated that feeding melatonin must begin before approaching maturity with respect to fatness in order to stimulate fat accretion.

Other researchers have used a subcutaneous implant designed to release 0.65 mg melatonin/d for a 5 week period to assess its effect on the attainment of puberty and growth rate of young, growing heifers. When Tortonese and Inskeep (1992) used this approach, no difference in growth rate during the 5 week implant period was observed, even though melatonin plasma concentration was fourfold greater in the implanted calves compared to the controls (69.5 vs 16.7 pg/ml, respectively).

MELATONIN AND OTHER CIRCULATING HORMONES

As hormones work in synchrony within the body it would be irrational to think melatonin does not mediate changes to other circulating hormones. Osie et al. (1989) observed when melatonin was fed to chicks there were elevated levels of T3 and T4, and an elevated T3:T4 ratio in circulation , however when melatonin was injected once subcutaneously in crossbred heifers T3 and T4 levels decreased but the T3:T4 ratio increased (Aggarwal et al., 2005). Others (Panda and Turner, 1968; Defronzo and Roth, 1972; and Thieblot 1965) have provided evidence that melatonin ascribes a stimulating effect on the thyroid via measures of increased thyroid stimulating hormone, thyroid DNA and RNA, as well as other histological changes when melatonin was administered to animals. Thyroid hormones are necessary for normal growth and development, and Chai (1970) has shown that selection for elevated thyroid activity resulted in a positively correlated response in body size of mice. It is therefore possible that growth responses associated with melatonin administration are due to a product of a hyper-active thyroid when compared to their control counterparts.

Similar to thyroidal effects, the adrenal appears to be affected by the pineal (Vaughan et al., 1982; Pavel et al., 1973). Under natural environmental conditions, it is most likely that an interaction exists between pineal and adrenomedullary hormones in cattle, similar to sheep (Arendt et al., 1981). When Aggarwal et al. (2005) injected melatonin into cattle, increases in plasma norepinephrine, dopamine, and epinephrine were detected but the increase in epinephrine was not as prolonged, and cortisol levels declined shortly after melatonin administration.

Evidence has shown the possibility that melatonin from the pineal gland is one factor controlling growth hormone secretion (Kasuya et al., 2006). These researchers injected 100 µg of melatonin into the third ventricle and reported that growth hormone concentration increased, however growth hormone releasing hormone was not altered. They suggest that melatonin stimulates growth hormone secretion possibly through the hypothalamus in cattle. In a follow up study, similar researchers (Kasuya et al., 2008) injected serotonin (0.1 mg) via the same route but did not see an increase in growth hormone levels, however, did observe an increase in prolactin.

INTERLEUKINS AND IMMUNITY

REVIEW OF CYTOKINES

Cytokine network

Cytokines are local messenger molecules that transmit information of importance between cells and have a major impact on growth regulation, cell division, differentiation, inflammation, and immunity. Immune and inflammatory responses can occur anywhere in the body in response to a wide variety of stimuli. They depend on the rapid and efficient recruitment of a variety of different blood cell types, with the exact type and relative proportion dependent upon the type of insult, and the chemotactic and adhesion response generated. In this rapidly changing environment, cytokines are produced that have both agonist and antagonist action. Therefore, the outcome of the immune/ inflammatory response depends on the net effect of these mediators (Balkwill, 2000).

Cross regulation of Th1 and Th2 cytokines

It has been suggested that helper T cells (Th) fall into two groups, Th1 and Th2, the former producing predominately interferon (INF)-γ and interleukin (IL)-2, and the latter producing IL-4 and IL-5 (Mire-Sluis and Thorpe, 1998). This subdivision proves functionally relevant, since Th1 cells mediate cellular immune responses and Th2 cells provide help for B cells in humoral immune responses (Mosmann, 1991). Some lymphokines produced by Th1 and Th2 cells can exert contradictory regulatory actions. In particular, IL-4 and INF-γ, often oppose one another's actions. The cytokine IL-10, which is mainly produced by Th2 cells, but also by macrophages and B cells, exerts a negative regulatory effect on some Th1 cell functions. By contrast, the Th1-derived cytokine IFN-γ, as well as IL-12, and IFN-α, which are not a product of Th1 cells but act as Th1-inducing cytokines, down-regulate the function of Th2 cells (Balkwill, 2000).

INTERLEUKIN-10

Cytokine properties

IL-10 was first identified by Fiorentio et al. (1988) as a product of Th2 cells that inhibited cytokine production from Th1 cells. The human IL-10 gene encodes a protein of 178 amino acids which includes 18 amino acids of hydrophobic signal sequence, and its predicted molecular mass is 18,647 daltons. An acidic treatment (below pH 5.5) will result in biologically inactive material (Windsor et al., 1998). This cytokine is produced by many types of cells including macrophages, dendritic cells, B cells, keratinocytes, mast cells, and several subsets to T cells including Tregs, Th2, and a population of Th17 cells (Galli et al., 2005; Grimbaldeston et al., 2007; and McGeachy et al., 2007), as well as certain non-hematopoietic cells like epithelial cells (Cella et al., 2009, and Jarry et al., 2008).

IL-10 enacts most of its immunosuppressive activity indirectly by effects on antigen presenting cells (APC) such as monocytes, dendritic cells, and macrophages. It limits proinflammatory cytokine and chemokine expression in APCs, but can also directly affect CD4+ T cells by limiting their activation, proliferation, and antigen-dependent production of cytokines such as IFNγ, IL-2, IL-4, IL-5, IL-13, and TNFα (Moore et al., 2001; Couper et al., 2008; Schandene et al., 1994; and Joss et al., 2000) Additionally, a study with *Mycobacterium* showed that autocrine IL-10 signaling in dendritic cells can prevent their trafficking to lymph nodes, which impedes the recruitment of naïve T cells to draining lymph nodes as well as polarization of these T cells to a Th1 phenotype (Demangel et al., 2002).

IL-10 and intestinal inflammation

Several studies in the last 20 years have confirmed the importance of IL-10 in mediating intestinal homeostasis (Kuhn et al., 1993; Davidson et al., 1996; Asseman et al., 1999; Zhou et al., 2004; and Jarry et al., 2008). In 1993, Kuhn et al. observed that IL-10 knock-out mice ($IL10^{-/-}$) developed a spontaneous, progressive enterocolitis starting in the cecum, ascending colon and transverse colon, that subsequently extended to the descending colon and rectum and eventually also affected the small intestine. Treatment of exogenous IL-10 prevented disease development when given to weanlings (Berg et al., 1996), although IL-10 treatment of adult $IL10^{-/-}$ with established disease attenuated, but could not fully reverse, clinical pathology. Intestinal inflammation in $IL10^{-/-}$ mice was mediated by Th1 cells, as adoptive transfer of Th1 cells isolated from the inflamed colons of $IL10^{-/-}$ into IL-10-sufficient mice recapitulated the disease seen in $IL10^{-/-}$ mice (Davidson et al., 1996). Furthermore, blockage of IFN-γ, the signature cytokine to Th1 cells, ameliorated disease in $IL10^{-/-}$ mice (Berg et al., 1996). This intestinal inflammation in $IL10^{-/-}$ mice is dependent on the presence of intestinal bacteria (Sellon et al., 1998).

MUCOSAL IMMUNITY

Interconnection of the respiratory tract and intestinal immunity

Most infectious agents that infect animals do so via mucosal sites, principally the digestive, respiratory and genitourinary tracts. Immune defenses at mucosal surfaces therefore constitute a very vital part of the overall protective responses against these invading pathogens (Ravindran et al., 2011). The respiratory and gastrointestinal tracts have many structural similarities (Mestecky, 1987). Both have an extensive, highly vascularized, luminal surface area (Kuebler, 2005) which is protected by a selective epithelial barrier (Keely et al., 2010) and an overlying mucus-gel layer (Keely et al., 2008) from commensal bacteria, pathogens and foreign antigens. These epithelial surfaces cover a sub-mucosal layer of loose connective tissue and mucosa-associated lymphoid tissue, consisting of resident lymphocytes. This lymphoid tissue regulates antigen sampling, lymphocyte trafficking and mucosal host defense (Holt, 1993). Respiratory and gastrointestinal epithelia share a common embryonic origin in the primitive foregut (Shu et al., 2007) which may account for their similarities. However, it is most likely that it is the similar inflammatory and immune components of these organs that are the cause of the overlap in pathological changes during respiratory and intestinal mucosal diseases (Keely et al., 2012).

In 1975, Rudzik et al. transferred either Peyer's patch cells or cells from bronchus-associated lymphoid tissue to homologous, lethally irradiated recipients. This resulted in repopulation of both the intestinal and the bronchial lamina propria. These findings led to the hypothesis of a common mucosal immune system (McDermott and Bienenstock, 1979), with a proposal that cells primed in one mucosal inductive site may home to many different, if not all, mucosal effector sites. It has been elaborated in animal models (Ruedel et al., 1996 and Allen et al., 2000) that intestinal priming can lead to protection of the lower respiratory tract. Zuercher et al. (2002) suggest that cross-protection in the common mucosal immune system is mediated by trafficking of B cells and effector memory cytotoxic T cells.

THESIS OBJECTIVES

The main objective of this research was to evaluate dietary melatonin or dietary antibodies against interleukin-10 in growing ruminants, and to determine the effect of melatonin on growth performance and carcass characteristics, and the effect of the antibodies on growth performance in the face of challenges by gastrointestinal nematodes or bovine respiratory pathogens.

CHAPTER II REFERENCES

Acuna-Castroviejo, D., G. Escames, and C. Venegas. 2014. Extrapineal melatonin: sources, regulation and potential functions. Cell Mol. Life Sci. 71:2997-3025.

Acuna-Castroviejo, D., R.J. Reiter, A. Menendez Pelaez, M.I. Pablos. and A. Burgos. 1994. Characterization of high-affinity melatonin binding sites in purified cell nuclei of rat liver. J. Pineal Fes 16:100-112.

Aggarwal, A., R.C. Upadhyay, S.V. Singh, and P. Kumar. 2005. Adrenal-thyroid pineal interaction and effect of exogenous melatonin during summer in crossbred cattle. Indian J. Anim. Sci. 75:915-921.

Allen, J.S., G. Dougan, and R.A. Strugnell. 2000. Kinetics of the mucosal antibody secreting cell response and evidence of specific lymphocyte migration to the lung after oral immunization with attenuated S. enterica var. typhimurium. FEMS Immunol. Med. Microbiol. 27:275-281.

Apeldoorn, E.J., J.W. Schrama, M.M. Mashaly, and H.K. Parmentier. 1999. Effect of melatonin and lighting schedule on energy metabolism in broiler chicks. Poult. Sci. 78:223-229.

Arendt, J. 1995. Melatonin and the mammalian pineal gland. Chapmann and Hall. London. 201-285.

Arendt, J., A.M. Symons and C. Land. 1981. Pineal function in the sheep: for a possible mechanism mediating seasonal reproductive activity. Experientia. 37:584-586.

Asseman, C., S. Mauze, M.W. Leach, R.L. Coffman and F. Powrie. 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J. Exp. Med. 190:995-1004.

Attarzadeh, F., 1983. Season variation in stature and body weight. Int. J. Orthodontics. 21:3-12.

Balkwill, F., 2000. The Cytokine Network. Oxford University Press. New York, New York.

Barrenetxe, J., P. Delagrange, and J.L. Martinez. 2004. Physiological and metabolic functions of melatonin. J. Phys. Biochem. 60:61-72.

Bartness, T.J. and G.N. Wade. 1985. Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci. Biobehav. Rev. 9:599-612.

Bartness. T.J. J.B. Powers, M.H. Hastings, E.L. Bittman and B.D. Goldman. 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses. 15:161-190.

Bartness, T. J., C.K. Song and G.E. Demas. 2001. SCN efferents to peripheral tissues: implications for biological rhythms. J Biol. Rhythms. 16:196-204.

Becker-Andre, M., I. Wiesenber, N. Schaeren-Weimers, E. Andre, M. Missbach, J.H. Saurat, and C. Carlberg. 1994. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J. Bio Chem. 269:28531-28534.

Benitez-King, G., L. Huerto-Delgadillo, and F. Anton-Tay. 1993. Binding of 3H-melatonin to calmodulin. Life Sci. 53:201-207.

Berg, J.J., N. Davidson, R. Kuhn, W. Muller, S. Menon, G. Holland, L. Thompson-Snipes, M.W. Leach, and D. Rennick. 1996. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ TH1-like responses. J. Clin. Investig. 98:1010-1020.

Berthelot, X., M. Laurentie, J.P. Ravault, J. Ferney, and P.L. Toutain. 1990. Circadian profile and production rate of melatonin in the cow. Dom. Anim. Endocrinol. 7:315-322

Bloom, F.E. and N.J. Giarman. 1967. Fine structure of granular vesicles in pineal autonomic nerve endings after depletion. Anat. Rec. 157:351

Bonnefont-Rousselot D., F. Collin, D. Jore, and M. Gardes-Albert. 2011. Reaction mechanism of melatonin oxidation by reactive oxygen species in vitro. J Pineal Res. 50:328-335.

Brydon, L., F. Roka, L. Petit, M. Tissot, P. Barrett, P.J. Morgan, C. Nanoff, A.D. Strossberg, and R. Jockers. 1999. Dual signaling of human mel1a melatonin receptors via G(i2), G(i3), and G(q/11) proteins. Mol. Endocrinol. 13:2025-2038.

Bubenik, G.A., G.M. Brown, and L.J.Grota. 1977. Immunohistological localization of melatonin in the rat digestive tract. Experientia. 33:662-663.

Bubenik, G.A. 1980. Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Hormone Res. 12:313-323.

Bubenik, G.A. and S. Dhanvantari. 1989. The influence of serotonin and melatonin on some parameters of gastrointestinal activity. J. Pineal Res. 7:333-344.

Cardinali, D.P., R.A. Cutera, and A.I. Esquifino. 2000. Psychoimmune neuroendocrine integrative mechanisms revisited. Biol. Signals. Recep. 9:215-230.

Cella, M., A. Fuchs, W. Vermi, F. Facchetti, K. Otero, J.K. Lennerz, J.M. Doherty, J.C. Mills, and M. Colonna. 2009. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature. 457:722-725.

Chai, C.K., 1970. Selective breeding for thyroid ^{131}I uptake in mice. Genetics. 64:29-40.

Chen, L.D. D.X. Tan, R.J. Reiter, K. Yaga, B. Poeggeler, P. Kumar, and J.P. Chambers. 1993. In vivo and in vitro effects of the pineal gland and melatonin on [Ca, Mg] dependent ATPase in cardiac sarcolemma. J. Pineal Res. 14:178-183.

Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180:5771-5777.

Cunnane, S.C., M. Manku, M. Oka, and D.F. Horralein. 1980. Enhanced vascular reactivity to various constrictor agents following pinealectomy in the rat: role of melatonin. Can. J. Physiol. Pharmacol. 58:287.

Davidson, N.J., M.W. Leach, M.M. Fort, L. Thompson-Snipes, R. Kuhn, W. Muller, D.J. Berg, and D.M. Rennick. 1996. T helper cell 1-type CD4+ T Cells, but not B cells mediate colitis in interleukin-10 deficient mice. J. Exp. Med. 184:241-251.

Defronzo, R.A., and W.D. Roth. 1972. Evidence for the existence of a pineal-adrenal and pineal-thyroid axis. Acta. Endocrinol. 70:31-42.

Del Gobbo, V., V. Bibri, N. Villani, R. Calio, and B. Fistico. 1989. Pinealectomy inhibits interleukin-2 production and natural killer activity in mice. Int. J. Immunopharmacol. 11:567-577.

Demangel, C., P. Bertolino, and W.J. Britton. 2002. Autocrine IL-10 impairs dendritic cells (DC) derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. Eur. J. Immunol. 32:994-1002.

Dubocovich, M.L. 1985. Trends Pharmacol Sci. 16:50-56.

Dubocovich M.L., and M. Markowska. 2005. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine. 27:101-110.

Ebihara, S., T. Marks, D.J. Hudson, and M. Menaker. 1986. Genetic control of melatonin synthesis in the pineal gland of the mouse. Science. 231:491-493.

Fiorentino, D.F., M.W. Bond, and T.R. Mosmann. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J. Exp. Med. 17:2081-2095.

Fioretti, M.C., E. Menconi, and L. Martini. 1972. Affinity of melatonin for serotonin receptors in the isolated rat stomach. Riv. Farmacol. Terap. 3:383-194.

Galli, S.J., J. Kaleskikoff, J.W. Brimbaldeston, A.M. Piliponsky, C.M. Williams, and M. Tsai. 2005. Mast cells as “tunable” effector and immunoregulatory cells: recent advances. Annu. Rev. Immunol. 23:749-786.

Garcia-Perganeda, A., D. Pozo, J.M. Duerrero, J.R. Calvo. 1997. Signal transduction for melatonin in human lymphocytes: involvement of a pertussis toxin-sensitive G protein. J. Immunol. 159:3774-3781.

Grimbaldeston, M.A., S. Nakae, J. Kaleskikoff, M. Tsai, and S.J. Galli. 2007. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. Nat. Immunol. 8:1095-1104.

Hakanson, R. and C. Owman. 1966. Pineal DOPA decarboxylase and monoamine oxidase activities as related to the monoamines stores. J. Neurochem. 13:597-605

Hansson, I., R. Holmdahl, and R.Mattsson. 1992. The pineal hormone melatonin exaggerates development of collagen induced arthritis in mice. J. Neuroimmunol. 39:23-31.

Hardeland, R., R.J. Reiter, B. Poeggeler and T.X. Tan. 1993. The significance of the metabolism of the neurohormone melatonin: protection and formation of bioactive substances. Neurosci. Biobehav. Rev. 17:347-357.

Hardeland R., J.A. Madrid, D.X. Tan, and R.J. Reiter. 2012. Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling. J. Pineal Res. 52:139-166.

Heideman, P.D., and C.J. Sylvester. 1997. Reproductive photoresponsiveness in unmanipulated male Fischer 344 laboratory rats. Biol. Repro. 57:134-138.

Hirata, F. O. Hayaishi, T. Tokuyama, and S. Senoh. 1974. In vitro and in vivo formation of two new metabolites of melatonin. J. Biol. Chem. 249:1311-1313.

Holt, P.G. 1993. Development of bronchus associated lymphoid tissue (BALT) in human lung disease: a normal host defense mechanism awaiting therapeutic exploitation? Thorax. 48:1097-1098.

Huether, G., B. Poegeller, R. Reimer, and A. George. 1992. Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci. 51:945-

Jansen, K.L.R., M. Dragunow, and R.L.M Faull. 1990. Sigma receptors are highly concentrated in the rat pineal. Brain Res. 507:158-160.

Jarry, A., C. Bossard, C. Bou-Hanna, D. Masson, E. Espaze, M.G. Denis, and C.L. Laboisse. 2008. Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. J. Clin. Invest. 118:1132-1142.

John, T.M., S. Itoh, and J.C. George. 1978. On the role of the pineal in thermoregulation in the pigeon. Hormone Res. 9:41.

John, T.M. and J.C. George. 1991. Physiological responses of melatonin-implanted pigeons to changes in ambient temperature. Photobiology. Plenum Press, NY. pg 597.

Joss, A., M. Akdis, A. Faith, K. Blaser, and C.A. Akdis. 2000. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. Eur. J. Immunol. 30:1683-1690.

Kasuya, E. S. Kushibiki, M. Sutoh, T. Saito, S. Ito, K. Yayou, R. Sakumoto and K. Hodate. 2006. Effect of melatonin injected into the third ventricle on growth hormone secretion in Holstein steers. J. Vet. Med. Sci. 68:1075-1080.

Kasuya, E., S. Kushibiki, K. Yayou, S. Ito, T. Saito, K. Hodate, and M. Sutoh. 2008. Effects of serotonin injected into the third ventricle on prolactin and growth hormone secretion in Holstein steers. Anim. Sci. J. 79:362-367.

Keely, S., L.E. Glover, T. Weissmueller, C.F. MacManus, S. Fillon, and B. Fennimore. 2010. Hypoxia inducible factor-dependent regulation of platelet-activating factor receptor as a route for gram-positive bacterial translocation across epithelia. Mol. Biol. Cell. 21:538-546.

Keely, S., L.A. Rawlinson, D.M. Haddleton, and D.J. Brayden. 2008. A tertiary amino-containing polymethacrylate polymer protects mucus-covered intestinal epithelial monolayers against pathogenic challenge. Pharm. Res. 25:1193-1201.

Keely, S., N.J. Talley, and P.M. Hansbro. 2012. Pulmonary-intestinal cross talk in mucosal inflammatory disease. Mucosal Immunol. 5:7-18.

Kennaway, D.J., K.G. Frith, G. Phillipou, C.E. Matthews, and R.F. Seamark. 1977. A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography mass spectrometry. Endocrinology. 110:119-127.

Kennaway, D.J., T.A. Gilmore, and R.F. Seamark. 1982. Effect of melatonin feeding on serum prolactin and gonadotropin levels and the onset of seasonal estrous cyclicity in sheep. Endocrinology. 110:1766-1772.

Konturek, K.J. P.C. Konturek, I. Brzozowska, M. Pawlik, Z. Sliwowski, G.A. Bubenik and W.W. Pawlik. 2007. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract. J. Physiol. Pharmacol. 58:381-405.

Kopin, I.J., C.M. Pare, J. Axelrod. and H. Weissbach. 1961.The fate of melatonin. J. Biol. Chem. 236:3072-3075.

Kovacs, J., W. Brodner, V. Kirchlechner, T. Arif, and F. Waldhauser. 2000. Measurement of urinary melatonin: a useful tool for monitoring serum melatonin after its oral administration. J. Clin. Endocrinol. Metab. 85:666-670.

King, T.S., R.W. Steger, R. Steinlechner, and R.J. Reiter. 1984. Day-night differences in the estimated rates of 5-hydrotryptamine turnover in the rat pineal gland. Exp. Brain Res. 54:432-436.

Kuci, S., J. Becker, and G. Verr. 1983. Circadian variations of the immunomodulatory role of the pineal gland. Neuroendocrinol. Lett. 10:65-79.

Kuebler, W.M. 2005. Inflammatory pathways and microvascular response in the lung. Pharmacol. Rep. 57:196-205.

Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Mueller. 1993. Interleukin-10 deficient mice develop chronic enterocolitis. Cell. 75:263-274.

Lerner, A.B., J.D. Case, Y. Takahashi, T.H. Lee, and W. Mori. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. J. Am. Chem. Soc. 80: 2587-2588.

Lerner, A.B., J.D. Case and R.V. Heinzelman. 1959. The structure of melatonin. J. Am. Chem. Soc. 80: 6084-6085

Leone, R.M. and R.E. Silman. 1984. Melatonin can be differentially metabolized in the rat to produce N-acetylserotonin in addition to 6-hydroxymelatonin. Endocrinology. 114:1825-1832.

Lewy, A.J. 1983. Biochemistry and regulation of mammalian melatonin production, in the *Pineal Gland*. Elsevier Biomedical, New York. 77-79.

Jackson, R.L and W. Lovenberg. 1971. Isolation and characterization of multiple forms of hydroxyindole-*O*-methyltransferase. J. Biol. Chem. 246:4280-4285.

Maestroni,G.J., A. Conti, and W. Pierpaoli. 1987. Role of the pineal gland in immunity. II. Melatonin enhances the antibody response via an opiatergic mechanism. Clin. Exp. Immunol. 68:384-391.

Maestroni, G.J., A. Conti, and W. Pierpaoli. 1988. Role of the pineal gland in immunity. III. Melatonin antagonizes the immunosuppressive effect of acute stress via an opiatergic mechanism. Immunology. 63:465-469.

Maestroni, G.J., and W. Pierpaoli. 1981. Pharmacological control of the hormonally mediated immune-response. In: Psychoneuroimmunology. 405-425.

Mahmoud, I., Salman, S.S. and A. Alkhateeb. 1994. Continuous darkness and continuous light induce structural-changes in the rat thymus. J. Anat. 185:143-149.

McDermott, M.R., and J. Bienenstock. 1979. Evidence for a common mucosal immune system. I. Migration of immunoblasts into intestinal, respiratory and genital tissue. J. Immunol. 122:1892-1899.

McGeachy, M.J., K.S. Bak-Jensen, Y. Chen, C.M. Tato, W. Blumenschein, T. McClanahan, and D.J. Cua. 2007. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain Th-17 cell mediated pathology. Nat. Immunol. 8:1390-1397.

McGeer, E.G. and P.L. McGeer. 1966. Circadian rhythm in pineal tyrosine hydroxylase. Science. 153:73-74

Mestecky. J. 1987. The common mucosal immune system and current strategies for induction of immune response in external secretions. J. Clin. Immunol. 7:265-276.

Moore, K.W., R. de Waal Malefyt, R.L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immuno. 19:683-765.

Moore, R.Y., and D.C. Klein. 1974. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin *N*-acetyltransferase activity. Brain Res. 71: 17-33.

Mosmann, T.R., 1991. Cytokine secretion patterns and cross regulation of T cell subsets Immunol. Res. 10:183-188.

Mucha, S., K. Zylinska, G. Zerek-Meten, J. Swietoslawski, and H. Stepien. 1994. Effect of interleukin-1 on in vivo melatonin secretion by the pineal gland in rats. Adv. Pineal Res. 7:177-181.

Nelson, R.J., G.E. Demas, S.L. Klein, and L.J. Kriegsfeld. 2002. Seasonal patterns of stress, immune function, and disease. Cambridge University Press. New York, NY.

Nosjean, O., M. Ferro, F. Coge, P. Beauverger, J.M. Henlin, F. Lefoulon, J.L. Fauchere, P. Delagrange, E. Canet, and J.A. Boutin. 2000. Identification of the melatonin-binding site MT3 as the quinone reductase 2. J. Biol Chem. 275:31311-31317.

Osei, P., K.R. Robbins, and H.V. Shirley. 1989. Effects of exogenous melatonin on growth and energy metabolism of chickens. Nutr. Res. 9:69-81.

Ozaki, Y. and H.J. Lynch. 1976. Presence of melatonin in plasma and urine of pinealectomized rats. Endocrinology. 99:641-644.

Panda, J.N. and C.W. Turner. 1968. The role of melatonin in the regulation of thyrotrophin secretion. Acta. Endocrino. 57:363-373.

Pangerl, B., A. Pangerl, and R.J. Reiter. 1990. Circadian variations of adrenergic receptors in the mammalian pineal gland: a review. J. Neural Transmissions. 81:17-29

Pavel, S., L. Matrescu, and M. Petrescu. 1973. Central corticotropin inhibition by arginine vasotocin in the mouse. Neuroend.. 12:371-375.

Petit L., I. Lacroix, A.D. Strossberg, and R. Jockers. 1999. Differential signaling of human Mel1a and Mel1b melatonin receptors through the cyclic guanosine 3'-5'-monophosphate pathway. Biochem Pharmacol. 58:633-639.

Pieri, C., M. Marra, F. Moroni, R. Decchioni and F. Marcheselli. 1994. Melatonin: a peroxyl radical scavenger more potent than vitamin E. Life Sci. 55:271-276.

Poeggeler, B., S. Saarela, R.J. Reiter, D.X. Tan, L.K. Chen, and L.R. Barlow-Walden. 1994. Melatonin: a highly potent endogenous radical scavenger and electron donor. New aspects of the oxidation chemistry of this indole assessed in vivo. Ann. N.Y. Acad. Sci. 738:419-420.

Quastel, M.R. and R. Rahamimoff. 1965. Effect of melatonin on spontaneous contraction and response to 5-hydroxytryptamine of rat isolated duodenum. Br. J. Pharmacol. 24:455-461.

Quay, W.B. and Y.H. Ma. 1976. Demonstration of gastrointestinal hydroxyindole-O-methyltransferase. IRCS Med. Sci. 4:563-571

Raikhlin, N.T., I.M. Kvetnoy, and V.N. Tolkachev. 1975. Melatonin may be synthesized in enterochromaffin cells. Nature. 255:344-345.

Raikhlin, N.T. and I.M. Kvetnoy. 1976. Melatonin and enterochromaffin cells. Acta Histochem. Cytochem. 55:19-24.

Ralph, C.L. 1975. The pineal gland and geographical distribution of animals. Int. J. Biometeor. 19:289-303.

Ravindran, R., R. Rappuoli, and F. Bagnoli. 2011. Mucosal vaccines. In Vaccine Design Innovative Approaches and novel strategies. Caister Academic Press: Norfolk, UK. 171-209.

Reiter, R.J. 1991. Pineal Melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr. Rev. 12:151-180.

Rogawski, M.A., R.H. Roth, and G.K. Aghajanian. 1979. Melatonin: deacetylation to 5-methoxytryptamine by liver by not brain aryl acylamidase. J. Neurochem. 32:1219-1226.

Rudzik, O., R.L. Clancy, D.Y.E. Perey, R.P. Day, and J. Bienenstock. 1975. Repopulation with IgA containing cells of bronchial and intestinal lamina propria after transfer of homologous Peyer’s patch and bronchial lymphocytes. J. Immunol. 114:1599-1604.

Ruedel, C., C. Rieser, N. Koffler, G. Wick, and H. Wolf. 1996. Humoral and cellular immune responses in the murine respiratory tract following oral immunization with cholera toxin or Escherichia coli heat-labile enterotoxin. Vaccine. 14:792-798.

Saarela S. and R.J. Reiter. 1994. Function of melatonin in thermoregulatory processes. Life Sci. 54:295-311.

Schandene, L., C. Alonso-Venga, F. Willems, C. Derard, A. Delvaux, T. Velu, R. Devos, and M. Goldman. 1994. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. J. Immunol. 152:4368-4374.

Sellon, R.K., S. Tonkonogy, M. Schultz, L.A. Dieleman, W. Genther, E. Balish, D.M. Rennick, and R.B. Sarton. 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infec. Immun. 66:5224-5231.

Shu, W., M.M. Lu, Y. Zhang, PW. Tucker, D. Zhou, and E.E. Morrisey. Foxp2 and Foxp1 cooperatively regulate lung and esophagus development. Development. 127:2763-2722.

Sitaram, B.R. and G.L. Lees. 1978. Diurnal rhythm and turnover of tryptophan hydroxylase in pineal gland of the rat. J. Neurochem. 31:1021-1026.

Mire-Sluis, A.R. and R. Thorpe. 1998. Cytokines. Academic Press. San Diego, CA.

Snyder, S.H, J. Axelrod, R.J.Wurtman, and J.E. Fischer. 1965. Control of 5-hydroxytryptophan decarboxylase activity in the rat pineal by sympathetic nerves. J. Pharmacol. Exp. Therap. 147:371-375.

Song, C.K. and T.J. Bartness. 2001. CNS sympathetic outflow neurons to white fat that express MEL receptors may mediate seasonal adiposity. Am. J. Physiol. Regulatory Comp. Physiol. 281:666-672.

Stanisiewski, E.P., L.T. Chapin, N.K. Ames, S.A. Zinn, and H.A. Tucker. 1988. Melatonin and prolactin concentrations in blood of cattle exposed to 8, 16, or 24 hours of daily light. J. Anim. Sci. 66:727-734.

Sugden, R. 1979. Circadian changes in rat pineal tryptophan content: lack of correlation with serum tryptophan. J. Neurochem. 33:811-813.

Sugden, R. 1989. Melatonin biosynthesis in the mammalian pineal gland. Experientia. 45:922-932.

Tan, D.X., L.D. Chen, B. Poeggeler, L.C. Manchester, and R.J. Reiter. 1993. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrine J. 1:57-60.

Taylor, A.N., and R.W. Wilson. 1970. Electrophysiological evidence for the action of light on the pineal gland of the rat. Experientia. 26:267-269.

Thieblot, L., Physiology of the pineal body. 1965. Prog. Brain Res. 10:479-488.

Thomson., A.L., 1950. Factors determining the breeding seasons of birds: an introductory review. Ibis. 92:173-184.

Tortonese, D.J., and E.K. Inskeep. 1992. Effects of melatonin treatment on the attainment of puberty in heifers. J. Anim. Sci. 70:2822-2827.

Vanecek, J., A. Pavlik, and H. Illnerova. 1987. Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res. 435:359-363.

Vaughan, M.K., M.C. Powanda, B. Richardson, T.S. King, L.Y. Johnson, and R.J. Reiter. 1982. Chronic exposure to short photoperiod inhibits free thyroxine index and plasma levels of TSH, T4, T3, and cholesterol in female Syrian hamsters. Comp. Biochem. Phys. 71:615-618.

Venegas, C., J.A. Garcia, G. Escames, F. Ortiz, A. Lopez, C. Doerrier, L. Garcia Corzo, L.C. Lopez, R.J. Reiter, and D. Acuna-Castroviejo. 2012. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J. Pineal Res. 52:217-227.

Watson, R., 1999. Melatonin in the promotion of health. CRC, Boca Raton, FL.

Williams, L.M. 1989. Melatonin binding sites in the rat brain and pituitary mapped by in-vitro autoradiography. J. Mol. Endocr. 3:71-75.

Windsor, T.R., N.J. Murgolo, and R. Syto. 1998. Structural and biological stability of the human Interleukin-10 homodimer. Biochemistry. 37:16943-16951.

Withyachumnarnkul, B., K.O. Nonaka, C. Santana, A.M. Attia, and R.J. Reiter. 1990. Interferon-gamma modulates melatonin production in the rat pineal gland in organ culture. J. Interfer. Res. 10:403-411.

Youngstrom, T.G. and T.J. Bartness. 1995. Catecholaminergic innervation of white adipose-tissue in Siberian hamsters. Am. J. Physiol. 268:744-751.

Yu, Hing-Sing, and R.J. Reiter. 1993. Melatonin: biosynthesis, physiological effects, and clinical applications. CRC, Boca Raton, FL.

Zeman, M. J. Buyse, I. Hefichova, and E. Decuypere. 2001. Melatonin decreases heat production in female broiler chickens. Acta Vet. (Brno.) 70:15-18.

Zhou, P., C. Streutker, R. Borojevic, Y. Wang, and K. Croitoru. 2004. IL-10 modulates intestinal damage and epithelial cell apoptosis in T cell mediated enteropathy. Am. J. Physiol. Gastrointest. Liver Physiol. 287:G599-604.

Zinn, S.A., L.T. Chapin, W.J. Enright, A.L. Schroeder, E.P. Stanisiewski and H.A. Tucker. 1988. Growth, carcass composition and plasma melatonin in postpubertal beef heifers fed melatonin. J. Anim. Sci. 66:21-27.

Zuercher, A.W., H. Jiang, M.C. Thurnheer, C.F. Cuff, and J.J. Cebra. 2002. Distinct mechanisms for cross-protection of the upper versus lower respiratory tract through intestinal priming. J. Immunol. 169:3920-3925.

Zylinska, K., J. Komorowski, T. Robk, S. Mucha, and H. Stepien. 1995. Effect of granulocyte-macrophage colony stimulating factor and granulocyte colony stimulating factor on melatonin secretion in rats. In vivo and in vitro studies. J. Neuroimmunol. 56:187-190.

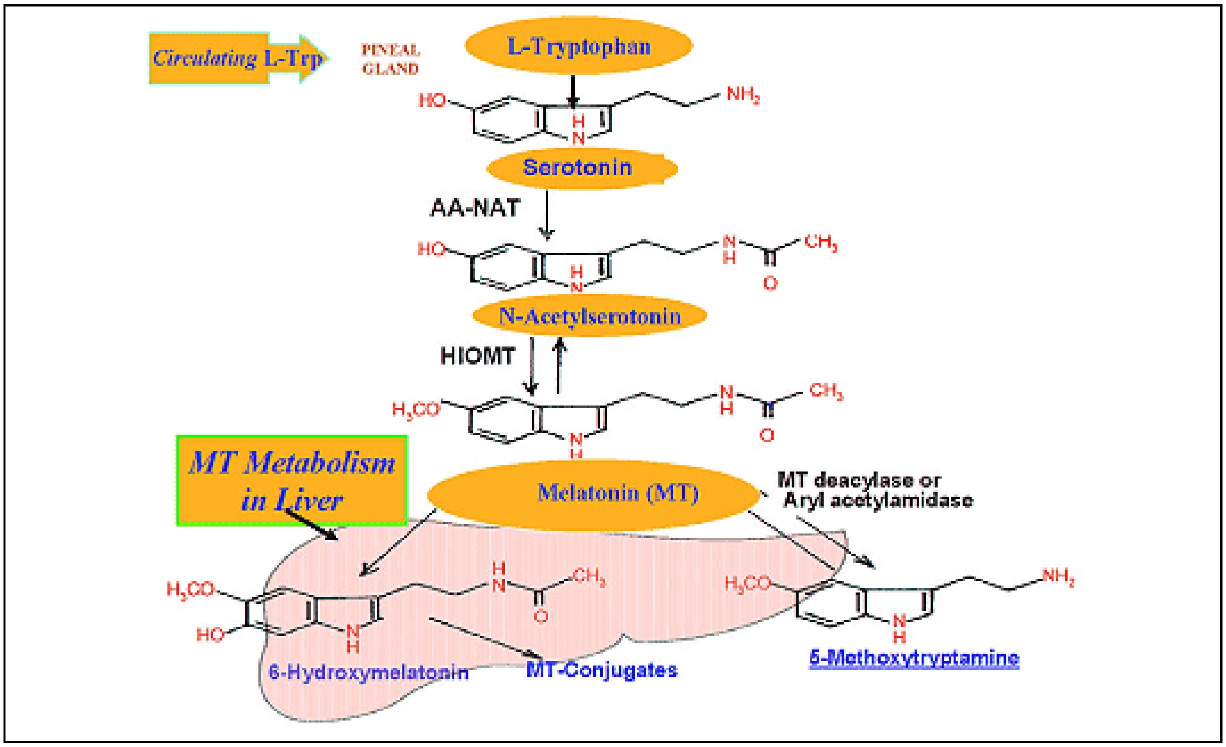

Figure II-1. Diagram of melatonin synthesis and degradation. Originally published in Konturek et al., 2007.

CHAPTER III. Dietary melatonin and implications on growth characteristics in young cattle

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ABSTRACT

The effects of dietary melatonin (MEL) supplementation on growth parameters and carcass composition were studied in 9 trials using growing steers and heifers. Trials were designed to compare treatments of control vs dietary MEL (0 vs 20 to 200 mg MEL · $hd^{-1} \cdot d^{-1}$). All trials except for one, utilized beef breed influenced cattle which were primarily black hided. A total of 229 animals were fed the control treatment, while 373 animals were fed a diet with MEL added at various levels, and the experimental unit was either animal or pen (6 hd) depending on experimental design. When non-implanted heifers were fed MEL for slick bunk management in the feedlot, both trials yielded a positive linear tendency ($P \leq 0.12$) between MEL level and ADG (≥ 3.5%) or HCW (6 kg), and no negative effects on carcass composition ($P \geq 0.25$) were noted. However, stocker heifers fed MEL in a daily feed supplement had similar ($P = 0.32$) ADG compared to control animals (1.09 vs 1.05 kg/d, respectively). Interestingly, when heifers were program fed and given a fixed amount of feed daily, the addition of dietary MEL did not alter growth or feed efficiency ($P \geq 0.69$) and no effects were found on carcass characteristics ($P \geq 0.38$). Analysis of 4 beef steer trials revealed no differences ($P \geq 0.36$) in growth rate when fed for 92 to 161 d during the finishing phase. In one trial which used heavy Holstein steers, a tendency ($P = 0.09$) for MEL fed animals to have a lower final BW than the control treatment was observed. Three trials were designed to determine if an interaction existed between dietary MEL and implant status, and no interaction was evident ($P \geq 0.43$) for growth performance. Within the finishing phase, a single terminal implant increased ($P \leq 0.01$) steer ADG and G:F (17 and 14%, respectively). Anabolic implant use in steers increased performance, while dietary MEL did not alter steer growth or carcass composition. However, MEL may increase ADG in non-implanted heifers.

INTRODUCTION

At least two climatic variables affect rate of body growth and reproduction in mammals. For example, ambient temperatures beyond the thermoneutral zone of animals reduce growth rates and reproductive efficiency. In addition, daily photoperiod has long been known to affect reproductive efficiency of many species (Tucker et al., 1984). The majority of cattle in the United States raised for beef are housed prior to slaughter in an environment partially or completely exposed to natural photoperiods and temperatures. The use of fully enclosed and climate controlled structures to minimize the negative effects of seasonal variation is not readily accepted in the beef industry, but has been implemented in poultry and pork production systems. It seems credible that the use of endocrine acting molecules which mimic photoperiod may modulate bovine biological functions.

Fluctuations of daily photoperiod have been shown to alter plasma melatonin (MEL) levels in cattle. Plasma MEL levels are lowest during periods of light and increase during darkness (Critser et al., 1988 and Stanisiewski et al., 1988). Zinn et al. (1988) concluded that feeding MEL at 4 mg/100 kg BW in the middle of the light period can increase carcass fatness if MEL feeding begins before heifers approach maturity with respect to fatness. Heifers have also been reported to have a higher marbling score if placed in the feedlot in December compared to June, despite having similar breed composition and starting weight (Kreikemeier and Mader, 2004). This result agrees with the seasonality of the percentage of finished cattle that grade Choice reported by the USDA (2015). The USDA observed that the highest percentage of animals grading Choice was in February, which coincides with animals fed during the shortest photoperiod. Feeding growing chicks MEL at 15 mg/kg diet increased weight gain and feed efficiency, but feeding MEL to gilts or heifers has shown no effect on growth parameters

(Diekman et al., 1991 and Zinn et al., 1988). The main objective of the present study is to determine if dietary MEL alters animal growth performance or carcass composition in growing cattle.

MATERIALS AND METHODS

All animal procedures and protocols were approved by the University of Wisconsin College of Agricultural and Life Sciences Animal Care and Use Committee.

Melatonin Procurement and Purity

Ten kilograms of MEL were procured from Luotian Xinpusheng Pharmaceutical Co. Ltd., Hubei, China and analyzed for impurities prior to the start of the trials. Two samples were taken to the University of Wisconsin Biotechnology Center for analysis via liquid chromatography mass spectrometry time of flight. Melatonin was diluted with 75% acetonitrile-0.1% formic acid to 10 μg/ml, and injected at 20 μl. Figure III-1 displays the mass abundance from samples A and B, and suggests a purity of > 98% with minor impurities present.

Experiments 1 and 2

Animals and Experimental Design. For each experiment, 100 beef heifers were acquired from a Wisconsin auction barn and transported 160 km to the Beef Nutrition research farm located on the Arlington Agricultural Research Station. Heifers were handled 3 days after arrival when processing procedures included testing for cattle that were persistently infected with bovine viral diarrhea virus (PI-BVD), and administration of Bovi-Shield Gold 5 (Zoetis, Exton, PA), Vision 7 (Bayer Corporation, Pittsburg, PA), and an ivermectin (Ivomec, Merial, Duluth, GA) pour-on. Animals were weighed on d -1 and 0 to determine initial body weight (BW). The heifers used for the experiments were confirmed not pregnant or a free-martin (via rectal palpation by a licensed veterinarian), free of PI-BVD, and black hided, and possessed a temperament score of 4 or lower (Grandin, 1993). Treatments were designed to provide 0, 20 and 100 mg MEL · hd^{-1} · d^{-1} (Exp. 1) or 0, 100, and 200 mg MEL · hd^{-1} · d^{-1} (Exp. 2). For each experiment, a total of 90 heifers were stratified into 5 weight blocks based upon initial BW. Groups of six heifers from each weight block were assigned to a bedded pen inside a confinement barn. All pens were completely under a roof and only the south side of the barn was open to natural photoperiod. Pen size and design were similar for all treatments and allowed a minimum of 7.1 m^2 and 0.6 m of linear bunk space per animal. Additional artificial lighting was similar across all pens and was given from 0600 h to sunrise to aid in daily management practices. Trials were initiated on 22 October and 15 April for experiments 1 and 2, respectively. Experiments 1 and 2 occurred during short and long photoperiod months, respectively.

Within 21 days, heifers were transitioned onto a high concentrate diet which was fed for the duration of the trial and can be seen in Table 1. Feeding of a total mixed ration (TMR) was done with a Rissler TMR cart (New Enterprise, PA) to allow ad libitum intake, and was delivered twice daily (0700 and 1100 h). The initial TMR feed delivery was a fixed amount of 3.30 kg DM/hd and the remainder of the daily feed allotment was fed at the second delivery when MEL carrier was added to the TMR. Finishing diets were sampled (approximately 1 kg) every week during the experiment. Weekly diets were composited by 4 week periods for analysis. Every 28 d, MEL was diluted in ground corn with multiple steps at no less than 7.5% dilution. Final mixtures were bagged and stored at room temperature until fed. Each carrier replaced cracked corn when mixing occurred for their respective treatments to supply 0.45 kg (as-is) carrier per hd daily.

Sample Collection. Pen feed delivery and feed refusals were recorded daily, and dry matter intake (DMI) was calculated. Average daily gain (ADG) and gain to feed ratio (G:F) were determined using pen DMI and BW gain over the duration of the trial. Animals were weighed every 28 d and final shrunk BW was the average of 2 consecutive pre-feeding weights. Melatonin was removed from the feed 2 d prior to animal slaughter. Animals were harvested with USDA-FSIS approval at a commercial abattoir when 12^{th} rib back-fat was estimated to be 1.3 cm for the entire slaughter block. The 2 heaviest initial BW blocks were harvested on d 93, and the remaining 3 blocks were harvested on d 113 for Exp. 1, and the 3 heaviest weight blocks were harvested on d 149 while the remaining 2 blocks were harvested on d 163 for Exp. 2. Hot carcass weight (HCW) was recorded at slaughter and used to determine actual dressing percentage. After carcasses were chilled for 48 h, the following measurements were obtained by two trained university personnel: 1) longissimus muscle (LM) area, taken by direct grid reading of the LM at the 12^{th} rib; 2) subcutaneous fat over the LM at the 12^{th} rib taken at a location ¾ the lateral length from the chine bone end; 3) estimated kidney, pelvic, and heart fat (KPH) as a percentage of carcass weight; and 4) marbling score (USDA, 1965). Yield grade was calculated based on USDA standards (USDA, 1997). Animal performance was used to predict dietary NEm and NEg, which were estimated using the procedure of Zinn et al. (1998), and equations published by Guiroy et al. (2002) were used to estimate carcass empty body fat percentage and shrunk body weight when an animal is estimated to be 28% empty body fat (adjusted final body weight).

Tissue Collection and Analysis. For both trials, on d 0 the area between the *tuber coxae* (hook bone) and *tuber ischiadicum* (pin bone) was clipped to remove hair to a residual height of 5 mm. During experiment 1, hair was clipped on d 56 from a 200 cm^2 area in this region, to a height of 5 mm and collected from 3 heifers per pen, and the remaining 3 heifers were clipped on d 85 to determine accumulated hair weight. The designated area was covered with an appropriate sized container, the perimeter clipped, and the remaining hair was collected (Fig. III-2). Once hair was collected it was dried at 55°C for 48 h, and weighed. During experiment 2, no appreciable hair growth was observed for any of the treatments and collection was not conducted due to the low abundance of clippable hair.

After carcass measurements had been collected, one steak slice sample, approximately 1 cm thick, was cut from the carcass's right side at the exposed surface of the 12th rib. The samples were chilled on ice and transported to the Meat Science and Muscle Biology Laboratory of the University of Wisconsin-Madison. Steak slices were trimmed of all external fat, and ground twice through an 8 mm grinding plate, mixed by hand and frozen until further analysis was conducted. Duplicate sub-samples, each weighing approximately 8 grams, were dried at 55^{o}C for 72 h to determine DM, and then washed with petroleum ether in a Soxhlet extraction apparatus for 7 days, followed by a 48 h, 55^{o}C drying event. The amount of fat lost during the process was computed by weight difference.

Experiments 3 and 4

Animals and Experimental Design. For each experiment, 100 beef steers were acquired from a Wisconsin auction barn and transported 160 km to the Beef Nutrition research farm located on the Arlington Agricultural Research Station. Steers were handled 3 days after arrival when processing methods included testing for PI-BVD, palpation of scrotum to ensure removal of testes, administration of Bovi-Shield Gold 5 (Zoetis Animal Health, New York, NY), Vision 7 (Bayer Corporation, Pittsburg, PA), and injectable Dectomax parasiticide (Zoetis Animal Health). Animals were weighed on d -1 and 0 pre-feeding to determine initial BW. The steers used for the trial were free of PI-BVD and black hided, and had a temperament score of 4 or lower (Grandin, 1993). All steers received a single implant of 120 mg trenbolone acetate (TBA) and 24 mg estradiol (Revalor-S, Intervet, Millsboro, DE) on d 0 (experiment 3) or d 28 (experiment 4). Treatments were designed to provide 0, 100 and 200 mg MEL · hd^{-1} · d^{-1}. For each experiment a total of 90 steers were stratified into 5 weight blocks based upon initial BW. Groups of six steers from each weight block were assigned to a bedded pen inside a confinement barn. All pens were completely under a roof and only the south side of the barn was open to natural photoperiod. Pen size and design were similar for all treatments and allowed a minimum of 7.1 m^2 and 0.6 m of linear bunk space per animal. Additional artificial lighting was similar across all pens and was used from 0600 h to sunrise to aid in daily management practices. Trials were initiated on 19 March and 23 October for experiments 3 and 4, respectively. Experiments 3 and 4 occurred during long and short photoperiod months, respectively.

Within 21 days, steers were transitioned onto a high concentrate diet which was fed for the duration of the trial and can be seen in Table 1, but no MGA was fed to the steers. Feeding was done to allow ad libitum intake, and was delivered once daily (0800 h). Finishing diets were sampled (approximately 1 kg) every week during the experiment. Weekly diets were composited by 4 week periods for analysis. Every 28 d, MEL was diluted into ground corn with multiple steps at no less than 7.5% dilution. Final mixtures were bagged and stored at room temperature until fed. Each carrier replaced cracked corn when mixing occurred for their respective treatments to supply 0.45 kg (as-is) carrier per hd daily. One steer was removed from trial 3 prior to harvest due to a foot injury (100 mg treatment). Additionally during trial 4, one steer died due to bloat (200 mg treatment), and one steer (control treatment) was removed due to excessive weight loss and being non-responsive to antibiotic treatment.

Sample Collection. Pen feed delivery and feed refusal were recorded daily, and DMI was calculated. Average daily gain (ADG) and G:F were determined using pen DMI and BW gain over the duration of the trial. Animals were weighed every 28 d and final BW was the average of 2 consecutive pre-feeding weights. Melatonin was removed from the feed 2 d prior to animal slaughter. Animals were harvested with USDA-FSIS approval at a commercial abattoir when estimated 12th rib back-fat was estimated to be 1.3 cm for the entire slaughter group. The 3 heaviest initial BW blocks were harvested on d 92, and the remaining 2 blocks were harvested on d 127 for Exp. 3, and the 3 heaviest weight blocks were harvested on d 139 while the remaining 2 blocks were harvested on d 161 for Exp. 4. Hot carcass weight was recorded at slaughter and used to determine actual dressing percentage. After carcasses were chilled for 48 h, the following measurements were obtained by two trained university personnel: 1) longissimus muscle area, taken by direct grid reading of the LM at the 12th rib; 2) subcutaneous fat over the LM at the 12th rib taken at a location ¾ the lateral length from the chine bone end; 3) KPH as a percentage of carcass weight; and 4) marbling score (USDA, 1965). Yield grade was calculated based on USDA standards (USDA, 1997). Pen data was used to predict dietary NEm and NEg values, and these were estimated using the procedure of Zinn et al. (1998), and equations published by Guiroy et al. (2002) were used to estimate carcass empty body fat percentage and shrunk body weight when an animal is estimated to be 28% empty body fat (adjusted final body weight).

Urine Collection and Analysis. On d 154 (26 March) during experiment 4, a urine sample (5-20 mls) was collected from 3 animals in each pen of the lightest 2 blocks. Samples were collected from 1400 h to1600 h, stored on ice, transported 35 km to the Animal Science Building, and were frozen (0^{o} C) until analyzed. Urine was analyzed for 6-hydroxymelatonin-sulfate using a melatonin-sulfate urine ELISA kit (IBL International Corp., Toronto, ON), and the recommended procedures were followed. At the same time when steers were being handled, a blood sample was collected from the jugular vein. The blood was collected in 10-ml vacuum tubes containing Na-heparin (Vacutainer, No. 6480, green stopper, Becton Dickinson, Franklin Lakes, NJ) and transported to the laboratory while stored on ice for 3 h. Tubes were then centrifuged at 2,500 × *g* for 20 min. A 1 mL aliquot of the supernatant was stored frozen until further analysis took place. Blood plasma serotonin was measured using the Serotonin Enzyme Immunoassay kit (IM1749, Immunotech, Beckman Coulter, Marseille Cedex 9, France). The assay was conducted per the manufacturer's instructions except the blood plasma was diluted 1:100 for sample values to fall within the standard curve of the assay

Experiments 5 and 6

Animals and Experimental Design. For each experiment, 40 beef steers were acquired from a Wisconsin auction barn and transported 160 km to the Beef Nutrition research farm located on the Arlington Agricultural Research Station. Animals were handled 3 days after arrival when processing methods included testing for PI-BVD, palpation of scrotum to ensure removal of testes, and administration of Bovi-Shield Gold 5 (Zoetis Animal Health, New York, NY), Vision 7 (Bayer Corporation, Pittsburg, PA), and injectable Dectomax parasiticide (Zoetis Animal Health). Animals were weighed on d -1 and 0 pre-feeding to determine initial BW. Steers used for the trial were free of PI-BVD, black hided, and had a temperament score of 4 or lower (Grandin, 1993). Trial design was a 2 x 2 factorial design with 10 replicates per treatment, and main factors consisted of dietary MEL inclusion and growth promoting implant status. Treatments were designed to provide 0 or 100 mg MEL $\cdot$ hd^{-1} $\cdot$ d^{-1} (Exp. 5) and 0 or 200 mg $\cdot$ hd^{-1} $\cdot$ d^{-1} (Exp. 6) and compared non-implanted vs implanted steers (Revalor-S, 120 mg TBA and 24 mg estradiol; Intervet, Millsboro, DE). Steers assigned to be implanted, received a single implant on d 0 (experiment 5) and d 28 (experiment 6). For each experiment, the 40 steers were stratified into 5 weight blocks based upon initial BW. Steers were randomly assigned to an individual bedded pen inside a confinement barn. All pens were completely under roof and had minimal exposure to natural sunlight. Pen size and design were similar for all treatments and allowed a minimum of 7.1 m^2 and 0.6 m of linear bunk space per animal. Additional artificial lighting was similar across all pens and was used from 0600 h to sunrise to aid in daily management practices. Trials were initiated on 19 March and 23 October for experiments 5 and 6, respectively. Experiments 5 and 6 occurred during long and short photoperiod months, respectively.

Within 21 days of experiment initiation, steers were transitioned onto a high concentrate diet which was fed for the duration of the trial and can be seen in Table 1, but no MGA was fed to the steers. Feeding was done to allow ad libitum intake, and was delivered once daily (0800 h). Finishing diets were sampled (approximately 1 kg) every week during the experiment. Weekly diets were composited by 4 week periods for analysis. Every 28 d, MEL was diluted into ground corn with multiple steps at no less than 7.5% dilution. Final mixtures were bagged and stored at room temperature until fed. Each carrier replaced cracked corn when mixing occurred for their respective treatments to supply 0.45 kg (as-is) carrier per hd daily. In trial 6, one steer was removed from the trial prior to harvest due to a physical leg injury (200 treatment), and 1 steer due to extremely low feed intake (control treatment). Additionally, during experiment 6 on d 28, when palpating ears to ensure proper implant technique, 1 steer was found containing an implant that was not assigned, and the animal was then considered to be part of the implanted treatment.

Sample Collection. Pen feed delivery and feed refusal were recorded daily, and DMI was calculated. Average daily gain (ADG) and G:F were determined using pen DMI and BW gain over the duration of the trial. Animals were weighed every 28 d and final BW was the average of 2 consecutive pre-feeding weights. Melatonin was removed from the feed 2 d prior to animal slaughter. Animals were harvested with USDA-FSIS approval at a commercial abattoir when 12^{th} rib back-fat was estimated to be 1.3 cm. The heaviest initial BW block was harvested on d 92, and the remaining 4 blocks were harvested on d 127 for Exp. 5. The 4 heaviest weight blocks were harvested on d 125 while the remaining block was harvested on d 139 for Exp. 6. Hot carcass weight was recorded at slaughter and used to determine actual dressing percentage. After carcasses were chilled for 48 h, the following measurements were obtained by two trained university personnel: 1) longissimus muscle area, taken by direct grid reading of the LM at the 12^{th} rib; 2) subcutaneous fat over the LM at the 12^{th} rib taken at a location ¾ the lateral length from the chine bone end; 3) KPH as a percentage of carcass weight; and 4) marbling score (USDA, 1965). Yield grade was calculated based on USDA standards (USDA, 1997). Pen data was used to predict dietary NEm and NEg values, which were estimated using the procedure of Zinn et al. (1998), and equations published by Guiroy et al. (2002) were used to estimate carcass empty body fat percentage and shrunk body weight when an animal is estimated to be 28% empty body fat (adjusted final body weight).

Experiment 7

Animals and Experimental Design. Seventy two beef heifers were acquired from a Wisconsin auction barn and transported 160 km to the Beef Nutrition research farm located on the Arlington Agricultural Research Station. Heifers were handled 3 days after arrival when processing methods included testing for PI-BVD, and administration of Bovi-Shield Gold 5 (Zoetis, Exton, PA), Vision 7 (Bayer Corporation, Pittsburg, PA), and injectable Dectomax parasiticide (Zoetis Animal Health). Animals were weighed on d -1 and 0 to determine initial BW. The heifers used for the trial were confirmed not pregnant or a free-martin (via rectal palpation by a licensed veterinarian), free of PI-BVD and black hided, and had a temperament score of 4 or lower (Grandin, 1993). Treatments were designed to provide 0, 100 and 200 mg MEL $\cdot$ $hd^{-1} \cdot d^{-1}$. Heifers were stratified into 4 weight blocks based upon initial BW. Groups of six heifers from each weight block were assigned to a bedded pen inside a confinement barn. All pens were completely under a roof and only the south side of the barn was open to natural photoperiod. Pen size and design were similar for all treatments and allowed a minimum of 7.1 m^2 and 0.6 m of linear bunk space per animal. Additional artificial lighting was similar across all pens and was used from 0600 h to sunrise to aid in daily management practices. The trial was initiated on 5 November 2015.

Within 21 days of experiment initiation, heifers were transitioned onto a high concentrate diet which was fed for the duration of the trial and can be seen in Table III-1. In contrast to previous experiments, a fixed amount of feed was fed daily across all treatments. For the first 42 d, within each block feed deliveries were designed to allow heifers to gain 1.48 kg BW/d (actual gains = 1.46 kg BW/d) based upon equations by the NRC (2000). Following this period, feed deliveries for each block were increased by no more than 0.25 kg DM $\cdot$ $hd^{-1} \cdot wk^{-1}$ to maintain similar intakes for each treatment within each block. Feeding of a TMR was done with a Rissler TMR cart (New Enterprise, PA) and was delivered twice daily (0700 and 1100 h). Initial TMR feed delivery was a fixed amount of 3.30 kg DM/hd and the residual was fed at the second delivery when MEL carrier was added to the TMR. Finishing diets were sampled (approximately 1 kg) every week during the experiment. Weekly diets were composited by 4 week periods for analysis. Every 28 d, MEL was diluted in ground corn with multiple steps at no less than 7.5% dilution. Final mixtures were bagged and stored at room temperature until fed. Each carrier replaced cracked corn when mixing occurred for their respective treatments to supply 0.45 kg (as-is) carrier per hd daily.

Sample Collection. Pen feed delivery and feed refusal were recorded daily, and DMI was calculated. Average daily gain (ADG) and G:F were determined using pen DMI and BW gain over the duration of the trial. Animals were weighed every 28 d and final shrunk BW was the average of 2 consecutive pre-feeding weights. Melatonin was removed from the feed 2 d prior to animal slaughter. Animals were harvested with USDA-FSIS approval at a commercial abattoir when 12^{th} rib back-fat was estimated to be 1.3 cm. The 2 heavy initial BW blocks were harvested on d 112, and the remaining 2 blocks were harvested on d 142. Hot carcass weight was recorded at slaughter and used to determine actual dressing percentage. After carcasses were chilled for 48 h, the following measurements were obtained by two trained university personnel: 1) longissimus muscle area, taken by direct grid reading of the LM at the 12^{th} rib; 2) subcutaneous fat over the LM at the 12^{th} rib taken at a location ¾ the lateral length from the chine bone end; 3) KPH as a percentage of carcass weight; and 4) marbling score (USDA, 1965). Yield grade was calculated based on USDA standards (USDA, 1997). Animal performance was used to predict dietary NEm and NEg which were estimated using the procedure of Zinn et al. (1998).

Experiment 8

Animals and Experimental Design. Fifty four heifers were procured from a nearby (<20 km) Wisconsin auction barn and transported to the Lancaster Agricultural Research Station. Animals were acclimated to a cemented and sheltered feedlot for 21 d and during this period were trained to consume feed from Calan gated bunks. Five days after arrival, heifers were administered Bovi-Shield Gold 5 (Zoetis Animal Health, New York, NY) and Vision 7 (Bayer

Corporation, Pittsburg, PA), and tested for PI-BVD. Animals were weighed on d -1 and 0 pre-feeding to determine initial BW, and on d 0 were administered a pinkeye vaccine (Piliguard, Merck Animal Health, Summit, NJ) an ivermectin (Ivomec, Merial, Duluth, GA) pour-on, and a 40 mg TBA and 8 mg estradiol-17β implant (Revalor-G, Merck Animal Health) if assigned. Trial design was a 2 x 2 x 2 factorial design with unbalanced replicates, (numbers in parentheses equal heifers assigned to treatment) with factors of 1) grazing only (18) vs. supplemented (36), 2) control (27) vs implanted with Revalor-G (27), and 3) control supplement (18) vs supplement containing 100 mg MEL $\cdot$ hd^{-1} $\cdot$ d^{-1} (18).

After initial BW was determined, heifers were balanced by BW across 3 blocks, and all treatments were replicated similarly across blocks. Each block was a grazing unit that had access to free choice water and mineral, was rotated to new paddocks twice a week, and contained Calan gated bunks with 12 gates where supplements were offered daily. Paddock size was variable to maintain a similar forage allowance across blocks and season. Paddock size was calculated as ha = [(hd × average BW, kg × 0.03 × grazing d)/(pasture height, cm × 36 × 0.5)]. The values of 0.03, 36, and 0.5 represent; estimated forage intake of 3% BW, 36 kg biomass DM $\cdot$ cm^{-1} $\cdot$ ha^{-1}, and 50% grazing efficiency. The supplement was offered at 2.3 kg as-is $\cdot$ hd^{-1} $\cdot$ d^{-1}, and composition can be seen in Table III-2. The MEL containing supplement was prepared like the control supplement, but MEL diluted in ground corn was added during the mixing process. Feed orts were measured daily and discarded. Minimal feed was discarded as all supplemented animals consumed over 98.5% of the overall offered supplement. Animals were allowed to graze for a total of 138 d, weighed approximately every 28 d, and final BW was calculated as the average of 2 non-fasted BW collected on consecutive days. Feed efficiency of the supplemented treatment group was calculated as: [individual animal weight change-average weight change of 6 grazed animals within block)/amount of feed consumed by the individual animal].

Sample Collection and Laboratory Analysis. Blood samples were obtained from all heifers on d 68 of the trial. As per a typical day, animals were fed their respective supplements at approximately 0830 h. The provided supplements were readily ingested (within 30 min) by all animals except one, which had a residual ort amount of 1 kg, and its blood sample was not used for analysis. Blood was collected from animals approximately 3 h post feeding. The blood was collected in 10-ml vacuum tubes containing Na-heparin (Vacutainer, No. 6480, green stopper, Becton Dickinson, Franklin Lakes, NJ) and transported to the laboratory while stored on ice for 3 h. Tubes were then centrifuged at 2,500 × *g* for 20 min. A 1 mL aliquot of the supernatant was stored frozen until further analysis took place. Blood plasma serotonin was measured using the Serotonin Enzyme Immunoassay kit (IM1749, Immunotech, Beckman Coulter, Marseille Cedex 9, France). The assay was conducted per the manufacturer's instructions except the blood plasma was diluted 1:100 for sample values to fall within the standard curve of the assay. Six plasma samples were randomly selected from heifers fed the control supplement, and 5 samples from heifers fed the MEL containing supplement for serotonin analysis.

Experiment 9

Animals and Experimental Design. Sixty Holstein steers were procured from a single source at 225 kg in April 2014 and allowed to graze, or fed hay when pasture forage was limited, from procurement to late August. Animals were moved to a feedlot and administered Bovi-Shield Gold 5 (Zoetis Animal Health, New York, NY), Vision 7 (Bayer Corporation, Pittsburg, PA), and injectable Dectomax parasiticide (Zoetis Animal Health). Steers were weighed on d -1 and 0 pre-feeding to determine initial BW, and blocked by weight. Groups of 10 steers from each weight block were assigned to a bedded pen inside a partially confined barn. Pen size and design were similar for all treatments and allowed a minimum of 17.8 m^2 and 0.7 m of linear bunk space per animal. The trial was initiated on 5 September, and MEL treatments were started on d 26 and provided 0 or 200 mg MEL $\cdot$ $hd^{-1} \cdot d^{-1}$. Over a 21 d period, steers were transitioned to a high concentrate diet (Table III-3) and were fed for 244 days. Within each pen, similar steroidal implant treatments were administered which were as follows: 1) non-implanted, 2) implanted on d 0 with Encore (43.9 mg estradiol; Elanco Animal Health, Greenfield, IN) and 3) implanted with Encore, Revalor-S (120 mg TBA and 24 mg estradiol; Intervet, Millsboro, DE), and Revalor-S on d 0, 109, and 169, respectively).

Sample Collection. Pen feed delivery and feed refusal were recorded daily, and DMI was calculated. Average daily gain (ADG) and G:F were determined using pen DMI and BW gain over the duration of the trial. Animals were weighed every 28 d and final BW was the average of 2 consecutive pre-feeding weights. Melatonin was removed from the feed 2 d prior to animal slaughter. Animals were harvested with USDA-FSIS approval at a commercial abattoir when average BW reached 705 kg. Hot carcass weight was recorded at slaughter and used to determine actual dressing percentage. After carcasses were chilled for 48 h, the following measurements were obtained by two trained university personnel: 1) longissimus muscle area, taken by direct grid reading of the LM at the 12^{th} rib; 2) subcutaneous fat over the LM at the 12^{th} rib taken at a location ¾ the lateral length from the chine bone end; 3) KPH as a percentage of carcass weight; and 4) marbling score (USDA, 1965). Yield grade was calculated based on USDA standards (USDA, 1997). Pen data was used to predict dietary NEm and NEg values, and were estimated using the procedure of Zinn et al. (1998), and equations published by Guiroy et al. (2002) were used to estimate carcass empty body fat percentage and shrunk body weight when an animal is estimated to be 28% empty body fat (adjusted final body weight).

Experiment 10.

Data collection. This experiment uses data collected from experiments 1 to 6, and 8. These trials were selected as they all utilized beef breed type genetics and allowed animals to consume feed to a slick bunk, which is ad libitum feed consumption within a 24-hr period. In each trial, a treatment mean was calculated using the LSMEANS statement in SAS for all measured variables. Within each trial, means for the control and highest level of MEL treatment were used for analysis. In order to conduct a percentage change analysis, all control values were set to equal 100 and MEL values were calculated as MEL/control within each trial.

Statistical Analysis

Experiments 1 and 2. Each experiment was analyzed individually since MEL levels were different. Data were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Pen was the experimental unit for all analyses. Class variables considered were treatment and block, and block was recognized as a random effect. The LSMEANS option was used to generate treatment means. An orthogonal contrast was used to determine if a linear effect of MEL inclusion level existed and used values of -0.5345, -0.2673, and 0.8018 for experiment 1, and values of 1, 0, and -1 for experiment 2. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiments 3 and 4. Data were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Pen was the experimental unit for all analyses. Class variables considered were experiment, treatment and block, while experiment and block were recognized as random effects. The LSMEANS option was used to generate treatment means. An orthogonal contrast was used to determine if a linear effect of MEL inclusion level existed. Significance was declared at $P <$ 0.05, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq$ 0.05), treatment means were separated using the PDIFF statement in SAS.

Experiments 5 and 6. Steer performance and carcass characteristics were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.). Animal was the experimental unit for all analyses. Main effects were tested if no interaction between the two factors existed at $P > 0.10$. Class variables considered were trial, implant status, dietary MEL and block, while trial and block were recognized as random effects. Although dietary MEL level differed between trials, data were analyzed comparing fed vs not fed MEL. The LSMEANS option was used to generate treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 7. Heifer performance and carcass characteristics were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Pen was the experimental unit for all analyses. Class variables considered were treatment and block, and block was recognized as a random effect. The LSMEANS option was used to generate treatment means. An orthogonal contrast was used to determine if a linear effect of MEL inclusion level existed. Significance was declared at $P <$ 0.05, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq$ 0.05), treatment means were separated using the PDIFF statement in SAS.

Experiment 8. Heifer performance was analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.). Animal was the experimental unit for all analyses. Class variables considered were treatment and block while block was recognized as a random effect. Orthogonal contrasts were used to test treatment effects: 1) supplemented vs grazed, 2) control vs MEL supplemented, and 3) non-implanted vs implanted. The LSMEANS option was used to generate treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 9. Steer performance was analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.). Pen was experimental unit for all analyses. Class variables considered were treatment and block while block was recognized as a random effect. The LSMEANS option was used to generate treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Additional analysis to compare implant regimen was conducted. Steer performance was analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.). Steer was the experimental unit for all analyses. Class variables considered were implant status, MEL level, and block, while block was recognized as a random effect. The interaction between implant status and MEL was not significant ($P \geq 0.39$) therefore the interaction was removed from the model and both variables were considered random. The LSMEANS option was used to generate individual treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 10. Variables measured were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.). Trial was the experimental unit for all analyses. Class variables considered were trial, sex, and treatment while trial and sex were recognized as random effects. The LSMEANS option was used to generate treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

RESULTS

Experiment 1. The heifers in experiment 1 were slaughtered in two drafts, after 93 and 113 d, and average days on feed (DOF) for the entire population was 103 d. During the trial, growth rate tended to increase in a linear dose dependent fashion ($P = 0.06$, Table III-4), and no difference was observed for DMI ($P = 0.20$) when MEL was fed to finishing heifers. Feed efficiency, and predicted dietary energy value also increased as MEL level increased ($P < 0.02$). Carcass characteristics of MEL fed heifers can be seen in Table III-5. Hot carcass weight tended to increase ($P = 0.09$) for MEL fed heifers, which is representative of accelerated ADG. Despite increasing growth rate, feeding MEL tended to lower KPH percentage ($P = 0.06$), without affecting 12th rib fat depth or LM area ($P > 0.17$). Neither marbling score nor longissimus lumborum ether extractable lipid content ($P > 0.31$) were affected by feeding MEL, however their association was positive and seemingly linked (Fig. III-3). Using the observed carcass measurements, predictions of whole animal composition were made. Empty body fat percentage tended to decrease ($P = 0.07$) and adjusted final body weight was increased ($P = 0.02$) with increasing level of MEL. Feeding MEL did not alter 56 d accumulated hip hair weight ($P > 0.05$), but 85 d accumulated hair weight was greater for control fed vs MEL fed animals ($P = 0.05$, Fig. III-4).

Experiment 2. After an average of 156 DOF, MEL fed heifers were heavier (570 vs 562 kg, Table III-6) than control heifers ($P = 0.11$), but no differences in feed intake or G:F were observed ($P > 0.29$). Dietary energy calculations were not different for any of the treatments in this trial ($P > 0.88$). Carcass characteristics of heifers can be seen in Table III-7, and suggests HCW increased when MEL was fed to heifers ($P = 0.05$). However, no other differences in carcass composition were observed for the various treatments. The association between marbling score and ether extractable lipid was positive (Fig III-3), however the R^2 was lower than observed in experiment 1, or reported by Dow et al. (2011, $R^2 = 0.82$).

Experiments 3 and 4. No interaction between trial and treatment was observed for any of the response variables of interest ($P > 0.26$) and the interaction was removed from the final model and both trials were analyzed together. Days on feed averaged 130 between the 2 trials, and no differences in performance were detected ($P \geq 0.19$, Table III-8). Steer growth rate was numerically 2% greater (2.16 vs 2.11 kg/d) when fed 200 mg $\cdot$ hd^{-1} $\cdot$ d^{-1} compared to the control treatment, but these values were not considered different ($P = 0.26$). The 200 mg level also had numerically greater ($P = 0.19$) feed intake (11.5 vs 11.3 kg/d) than the control treatment, but all treatments had similar G:F efficiency ($P = 0.92$), and no difference was found for calculated diet energy density ($P \geq 0.63$). Carcass composition of the MEL treatments can be found in Table III-9. As suggested by the numerically greater ADG for the 200 mg treatment, HCW tended ($P = 0.10$) to increase when a higher level of MEL was fed. However, with increasing HCW, an increase of 12th rib fat depth was observed for MEL fed steers (*P* = 0.01). Yield grade and empty body fat percentage both increased for MEL fed animals (*P* = 0.01), a result of increased 12th rib fat depth, as it is a major contributing factor to the calculations. Despite increasing fat depth, feeding MEL did not alter marbling score (*P* = 0.74). Urine 6-hydroxymelatonin-sulfate increased (*P* = 0.01) as feeding level of MEL increased, but no difference in plasma serotonin levels was observed (*P* = 0.16) as can be seen in Fig. III-5.

Experiments 5 and 6. No interactions between either implant status or MEL level within experiment were observed ($P > 0.10$), therefore interactions were not used in the final model. Data from both experiments were analyzed as one data set. Feeding MEL did not alter steer performance, but did tend to lower feed intake (11.4 vs 11.1 kg/d, *P* = 0.07) compared to the control treatment (Table III-10). Using an anabolic implant increased final BW (599 vs 634 kg), ADG (1.74 vs 2.03 kg/d), and G:F (0.157 vs 0.179) compared to the control treatment ($P \leq 0.01$). Feed intake was similar between implanted and non-implanted animals (*P* = 0.17).

Hot carcass weight was increased (359 vs 382 kg, Table III-11) when an implant was administered compared to control steers (*P* = 0.001), but feeding MEL did not affect HCW (*P* = 0.19). Loin muscle area and AFBW were increased when steers where implanted ($P < 0.01$), while KPH and yield grade decreased ($P < 0.03$), and marbling score tended to decrease (*P* = 0.08) compared to their control counterparts. Feeding MEL lowered marbling score (606 vs 644) and empty body fat percentage (30.4 vs 31.2%) compared to the non-MEL fed steers ($P \leq 0.05$), respectively. An interaction between implant status and MEL feeding level tended to exist for marbling score (*P* = 0.09).

Experiment 7. Heifer performance when program fed for 130 d can be found in Table III-12. Growth rate was adequate (1.66 kg/d) for being program fed, however, adding MEL to the diet did not alter growth rate (*P* = 0.97). Feed efficiency was not different (P = 0.91), nor was calculated energy density of the diet (*P* > 0.76). As indicated by similar final BW and no differences in dressing percentage (*P* = 0.56), HCW was similar for all treatments (*P* ≥ 0.70, Table III-13). All other carcass measurements were similar (*P* ≥ 0.25), but LM area tended (*P* = 0.12) to increase as MEL feeding level increased.

Experiment 8. Stocker heifer performance when allowed to graze cool season pastures for 138 d can be found in Table III-14. Supplemental feeding of 2.3 kg as-fed · $hd^{-1} \cdot d^{-1}$ increased (*P* ≤ 0.01) final BW and overall ADG (1.06 vs 0.89 kg/d) compared to non-supplemented control heifers. Implanting heifers with 40 mg TBA and 8 mg estradiol-17β numerically increased ADG by 5% (1.03 vs 0.98) but was not considered statistically different (*P* = 0.24). Feeding MEL did not alter final BW or overall ADG (*P* ≥ 0.32) vs control fed animals. Plasma serotonin levels were not affected by feeding MEL (*P* = 0.31) to grazing heifers. Chemical composition of the grazed sward structure changed during the trial (Fig. III-6), and is similar to other values of Midwestern pastures (Schaefer et al., 2014). Macro nutrients were all above the requirement (NRC, 2000) except for energy which is believed to have limited growth since the corn-containing supplement stimulated ADG. Heifer growth rate was greatest early in the grazing season (Fig. III-7) and moderated during the remainder of the trial, except for the third period which had surprisingly high growth rates. Feed efficiency was most favorable early in the growing season, decreased during the summer, and rebounded for the last grazing period.

Experiment 9. Holstein steers were fed for 244 d and 2 MEL feeding levels were started on d 27. Feeding MEL tended to lower final BW (*P* = 0.09) and numerically lowered ADG 4.7% (*P* = 0.18) compared to non-MEL fed steers. Feed intake tended (*P* = 0.08) to decrease in MEL fed steers vs the control treatment (10.6 vs 11.1 kg/d); however, no difference was found in

G:F or energy density of the diet ($P \geq 0.63$). Hot carcass weight was similar ($P = 0.18$) for both MEL feeding levels (413.7 vs 406.4 kg) for 0 and 200 mg · hd^{-1} · d^{-1}, respectively. No other differences were observed between treatments for either MEL feeding level ($P \geq 0.20$).

Effects of implant strategy on Holstein steer growth rate can be found in Table III-15. No data was collected for feed intake and the associated calculations. Implanting steers, regardless of the regimen, increased final BW and ADG ($P = 0.001$) compared to the non-implanted control treatment. When a more aggressive implant regimen was used, consisting of multiple TBA containing implants, final BW and ADG tended to increase ($P \leq 0.09$). Implanting steers increased HCW and LM area ($P = 0.001$) compared to the controls, and increased further with a more aggressive regimen ($P \leq 0.04$, Table III-16). Marbling score was numerically lower for implanted steers but was not considered significantly different ($P = 0.34$). Adjusted final body weight increased ($P = 0.01$) as implant regimen intensified. Figure III-8 shows steer performance variables as animal BW increased over time. Feed intake continued to climb during the first 180 d but plateaued once steers reached approximately 575 kg. Growth rate was greatest during the second period which is the first 28-d period on the final diet, and then declined into the range of 1.25 to 1.5 kg d^{-1}. Interestingly, ADG increased in the second to last period and final period, which is commonly thought to be the time when animal growth rate is lowest. Feed efficiency responded in a similar fashion as steer ADG. An interaction between implant treatment and feed period was significant ($P = 0.02$) and can be seen in Fig. III-9. During d 0 to 108, the Encore and E/S/S cattle were both under the influence of an Encore implant, and the ADG of these 2 treatments did not differ, but were greater than the control. Following d 109 and d 196, Revalor-S was administered to the E/S/S treatment, and this resulted in an ADG that exceeded the ADG of Encore steers. The interaction confirms the benefit of re-implanting with

Revalor-S. Conversely, it indicates that Encore with its proposed 400 d payout period does not support a growth rate as fast as that supported by Revalor-S implants.

Experiment 10. Analysis of 7 experiments in which MEL was fed to finish-phase steers and heifers revealed no differences ($P \geq 0.16$, Table III-17) in any animal growth response when animals were fed 100 or 200 mg MEL · $hd^{-1} \cdot d^{-1}$. However, feeding MEL to growing animals had a numerical increase in ADG and G:F by 2% over the controls. Feeding MEL had no effect on any of the observed carcass measurements ($P \geq 0.18$, Table III-18).

DISCUSSION

Experiment 1. Accelerated growth rate of heifers fed MEL has not been observed in previously conducted trials (Zinn et al., 1988). The earlier experiment fed 4 mg MEL/100 kg BW, or a calculated dosage of approximately 14 mg MEL · $hd^{-1} \cdot d^{-1}$, while the current trial fed 20 or 100 mg daily. Additionally, Zinn et al. (1988), only fed heifers MEL for 63 d, and harvest occurred at 375 kg, whereas the current trial was initiated when heifers weighed 381 kg and MEL was fed for 103 d. The positive effect of feeding MEL on feed efficiency has not been reported for any cattle study, but has been reported in chick studies (Osei et al., 1989) when MEL was added to the diet at 15 mg/kg diet. Calculated energy density based upon actual performance data was greater for MEL fed heifers. Enright et al. (2000) fed MEL to Friesian steers at 3 mg/100 kg BW daily, and observed no effect on in vivo dry matter or organic matter digestibility. This type of trial should be repeated with cattle fed 100 or 200 mg MEL hd^{-1} d^{-1}. If similar results are observed, it would be indicative that MEL increases energy retention via a post-absorptive mechanism. Trials using rats (Mustonen et al., 2002) and mink (Nieminen et al., 2001) have reported increased liver glucose-6-phosphatase activity for MEL implanted female animals, but no data is available for cattle. The current trial observed a decrease in empty body fat percentage with increasing MEL level and no effect on marbling score or LM fat percentage, which contrasts with the findings of Zinn et al. (1988), who suggested MEL increased fat in the 9-10-11 rib section and carcass. Heifers fed MEL increased AFBW by 27 kg, a response which is similar to the effect TBA implants have on heifers (Guiroy et al., 2002). Wettemann et al. (1990) fed 4 mg MEL/100 kg BW to steers that were exposed to a natural photoperiod in summer, and observed after 8 or 12 wk shoulder hair weight was greater for MEL fed steers. Our data, collected in the winter, does not support the inference that dietary MEL stimulates hair accretion, which could imply hair growth is at a maximum during the winter and cannot be accelerated.

Experiment 2. Growth rate of heifers fed MEL was numerically 3.5% greater than control fed heifers, but was not considered significantly different. When MEL was fed to heifers during the short photoperiod (Exp. 1) heifer growth rate was 6% greater than the controls. Osei et al. (1989) reported a greater weight gain response to dietary MEL when fed to chicks in 16L:8D rather than a constant lighting schedule. It appears that animal growth response to dietary MEL may be modified by lighting schedule or season. The effect of photoperiod on heifer growth rate has been variable. Zinn et al. (1986) reported accelerated growth rate of postpubertal heifers when exposed to a shorter photoperiod, most of which was carcass fat. However, others (Peters et al., 1978; Petitclerc et al., 1983, 1984) observed long-day photoperiods stimulate BW gain and carcass protein accretion. Despite the higher levels of MEL fed in this trial than experiment 1, no difference was observed for feed efficiency or diet energy density. Hot carcass weight was 6 kg greater for MEL fed animals which is in agreement with results reported for experiment 1. However, no differences were found across treatments for

AFBW. Wettemann et al. (1990) fed 4 mg MEL/100 kg BW to steers that were exposed to a natural photoperiod in summer, and observed after 8 or 12 wk shoulder hair weight was greater for MEL fed steers. The current trial also took place during the summer, but no appreciable hair growth was detected for any of the treatments.

Experiments 3 and 4. When both trials were analyzed together, no differences were found for any growth performance variable. However, when experiments were analyzed individually, ADG was similar (P = 0.90; 2.28, 2.26, and 2.28 kg/d for 0, 100 and 200 mg MEL, respectively) for all treatments in experiment 3 (long photoperiod), but tended (P = 0.11) to increase (1.93, 1.94, and 2.04 kg/d for 0, 100, and 200 mg MEL, respectively) for MEL fed steers in experiment 4 (short photoperiod). As observed in experiments 1 and 2, and discussed in the prior section, there may be an interaction between dietary MEL and season/photoperiod to alter animal growth rate. In the present study, we report an increase in 12th rib fat depth, calculated yield grade, and empty body fat percentage when MEL was fed to finishing steers. Zinn et al. (1989) observed no differences in steer carcass composition when animals were housed under differing lighting regimens (long vs short), which contrasts their earlier data (1986) using postpubertal heifers. Perhaps there is a sexual dimorphism in cattle regarding their responsiveness to MEL, as has been observed in mink (Niemien et al., 2001). Urine 6-hydroxymelatonin-sufate increased with increasing level of dietary MEL, which indicates that MEL tolerated rumen and gastrointestinal conditions and was absorbed. Both 6-hydroxymelatonin-sulfate and *N*-acetylserotonin-sulfate account for over 90% of total MEL metabolites in human (Tian et al., 2015), however the primary excretory metabolites of MEL in cattle are unknown. Tian et al. (2015) also observed species differences for total intrinsic clearance rate of MEL sulfation (monkey > rat > dog > human > pig > mouse), suggesting more in depth research needs to be conducted in cattle.

Experiments 5 and 6. Because photoperiod altered body growth in peripubertal bulls but not in prepubertal bulls or steers, (Roche and Boland, 1980; Tucker et al., 1984), Zinn et al. (1989) postulated photoperiod induced changes in growth may be dependent on gonadal steroids. Our trial design, which utilized a synthetic androgen (trenbolone acetate), found no interactions between dietary MEL and implant status for any growth parameter. Feed intake tended to be lower for MEL fed steers, and marbling score and empty body fat percentage were also lower for MEL fed steers, all observations which had not previously been identified (trials 1 to 4). Implanting steers once increased ADG by 0.29 kg/d and G:F by 0.02 which is remarkably close to 0.25 kg/d and 0.02 values reported by Wileman et al. (2009) in a meta-analysis. However, feed intakes were similar for treatments in these trials, compared to the proposed increase of 0.53 kg/d (Wileman et al., 2009) or 8% (Fox et al., 1988) when animals were implanted. Steer AFBW increased 43 kg when implanted, which is greater than the 30 kg increase reported by Guiory et al., (2002), but is within in the 25 to 45 kg range recommended by the NRC (2000).

Experiment 7. Programming intake has been shown to increase feed efficiency via limiting intake without altering final BW compared to ad libitum fed counterparts (Loerch and Fluharty, 1998). Loerch and Fluharty (1998) suggested daily gains of 1.13, 1.36 and maximum kg/d for BW intervals of 300 to 397, 397 to 488, and 488 to 540 kg. The present trial fed to achieve growth rates of 1.48 kg/d for the initial 42 d, followed by 1.70 kg/d for 28 d, and then increased feed allowance not to exceed 2.15% BW until slaughter. Actual ADG was 1.48, 1.95, and 1.62 kg/d for the three discussed periods. Feeding MEL to heifers when intake was fixed did not alter any growth performance variable which contrasts with the numerical increases in ADG by 6 and 3.5%, respectively, in experiments 1 and 2. Additionally, no differences were detected for any carcass measurements when fed differing levels of MEL in the present study. It is possible that the slight feed restriction caused an increase in cortisol levels which has been previously observed in cows withheld from feed for 30 h (Bourguet et al., 2011). Infusing MEL was determined to have a calming effect on isolated ewes, and also lowered plasma cortisol levels (Guesdon et al, 2013); however, no data is available for the association between MEL and cortisol in cattle.

Experiment 8. Providing supplemental energy feedstuffs to stockers grazing cool season swards increased final BW and ADG, which has been reported previously (Anderson et al., 1988). The apparent G:F was not affected by an androgenic implant or feeding MEL, and overall seemed low and possibly not economically viable. However, no measurements of forage intake were made, therefore true G:F may have been quite favorable if forage intake was lower for the supplemented heifers. Variation from year to year in apparent G:F has been observed (Frieser et al., 2007) and this variable relies heavily on performance of non-supplemented control animals. When non-supplemented animal growth is low, apparent G:F is typically favorable. Conversely, when non-supplemented animal growth rate is high, apparent G:F may be low, but stocking rate could possibly be increased. Providing cattle grazing small grain pastures with starch or readily digestible fiber supplements can increase stocking rate (33%) and ADG (0.15 kg/d), compared with non-supplemented animals (Horn et al., 1995). Adding MEL to the supplement numerically increased ADG by 4.3% (0.04 kg/d), which is comparable to trials 1 and 2. Implanting heifers with 40 mg TBA and 8 mg estradiol numerically increased growth rate by 4.8% (0.05 kg/d). As a percentage this is lower, but the ADG benefit is numerically similar to others (20.5%, 0.05 kg/d; Paisley et al., 1999) and is similar to what we have observed when stocker steer growth rate was approximately 1.1 kg/d (4%, 0.10 kg/d; Schaefer, data not published). A power analysis using the reported data suggests 56 experimental units are needed to detect a 0.05 kg/d difference in gain; assuming a similar SEM, alpha = 1.96, and beta = 1.28.

Experiment 9. Feeding MEL to Holstein steers tended to lower final BW by 17 kg, a result which has not been observed previously (experiments 1 to 8). Despite being slightly lighter at slaughter, MEL fed Holstein steers did not have any different carcass measurement compared to the controls, which is representative of trials 1 to 8 being variable, and non-definitive. A single implant of 43.9 mg estradiol (Encore) increased steer growth rate by 20% or 0.25 kg/d in the current 244 d trial. In addition to the Encore implant, re-implanting twice with 120 mg TBA and 4 mg estradiol (Revalor-S) tended to further increase steer final BW. Beckett (2002) observed an increase in ADG by 8%, or 0.11 kg/d, when light Holstein steers were fed for 276 d and administered a single Encore implant. Similar to Beckett's findings, HCW and yield grade increased, while LM area remained unchanged when implanted with Encore. Although Beckett only used a single terminal implant containing 120 TBA and 24 mg estradiol in addition to the Encore implant, and the current trial utilized 2 Revalor-S implants. Both datasets report a further increase in final BW, ADG, LM area, and numerically lower marbling scores than a single Encore implant. The single dose implant Encore is labeled to last 400 d, but the interaction between implant regimen and days on feed questions the payout period of the implant. The Encore treatment had greater ADG in both the initial (18%) and middle (22%) weigh periods, but was not statistically different from the control treatment during the last period (11%) which started on d 196.

Experiment 10. Analysis using 7 MEL trials increased statistical sensitivity, but no MEL effects were found to be significant for any response variable. However, economic advantages may exist for feeding MEL to growing animals as ADG and G:F were 2% numerically greater than the control treatments. For each 1% increase in ADG or G:F an increase in profit of $8 or $12 per animal space can be realized within the feedlot annually (personal calculations), but this does not account for the added cost of MEL. A power analysis using a common SEM of experiments 1 to 4 (0.05 and 0.003 for ADG and G:F, respectively) suggests 107 experimental units per treatment (6 hd pens) are needed to detect a 2% difference in growth, and 42 experimental units per treatment (6 hd pens) are needed to detect a 2% difference in G:F when using alpha = 1.96, and beta = 1.28.

CONCLUSIONS

The results of these studies suggest that implanting feedlot steers continues to be a sustainable technology by increasing weight gain without negatively affecting carcass composition. Feeding up to 200 mg MEL $\cdot$ hd^{-1} $\cdot$ d^{-1} to growing cattle is safe and has no detrimental effects on growth or carcass composition. The 2% numerical increase observed in ADG and G:F when feeding MEL is potentially reliable; however, the sensitivity of our trials could not detect such small differences with confidence. Minimal research observing the effects of exogenous MEL in cattle has been conducted, and our data suggests a better understanding is needed.

CHAPTER III REFERENCES

Anderson, S.J., J.K. Merrill, and T.J. Klopfenstein. 1988. Soybean hulls as an energy supplement for the grazing ruminant. J. Anim. Sci. 66:2959-2964.

Beckett, J. 2002. Comparison of implant programs in long-fed Holstein calves: Evaluation of Encore, a long acting estradiol implant. Vet-life Technical Bulletin. Data accessed 29 July 2015. http://www.extension.umn.edu/agriculture/dairy/beef/implant-strategies.pdf

Bourguet, C., V. Deiss, A. Boissy, S. Andanson, and E.M.C. Terlouw. 2011. Effects of feed deprivation on behavioral reactivity and physiological status in Holstein cattle. J. Anim. Sci. 89:3272-3285.

Critser, J. K., T. M. Block, B. W. Kirkpatrick, M. J. Lindstrom, and E. R. Hauser. 1988. The effect of photoperiod on diurnal rhythms of serum gonadotropins, prolactin and melatonin in ovariectomized heifers. Domest. Anim. Endocrinol. 5:23−34.

Diekman, M.A., J.A. Clapper, M.L. Green, and D.K. Stouffer. 1991. Reduction in age of puberty in gilts consuming melatonin during decreasing or increasing daylength. J. Anim. Sci. 69:2524-2531.

Dow, D.L., B.R. Wiegand, M.R. Ellersieck, and C.L. Lorenzen. 2011. Prediction of fat percentage within marbling score on beef longissimus muscle using 3 different fat determination methods. J. Anim. Sci. 89:1173-1179.

Enright, W.J., A.P. Moloney, and S.A. Zinn. 2000. A note on feed digestibility, nitrogen retention and serum concentrations of melatonin in Friesian steers fed melatonin. Ir. J. Agric. Food Res. 39: 451–455.

Fieser, B.G., G.W. Horn, and J.T. Edwards. 2007. Effects of energy, mineral supplementation, or both, in combination with monensin on performance of steers grazing winter wheat pasture. J. Anim. Sci. 85:3470-3480.

Fox, D.G., C.J. Sniffen, and J.D. O'Connor. 1988. Adjusting nutrient requirements of beef cattle for animal and environmental variations. J. Anim. Sci. 66:1475-1495.

Grandin, T. 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36:1–9.

Gueson, V., B. Malpaux, P. Delagrange, M. Spedding, F. Cornilleau, D. Chesneau, J. Haller, and E. Chaillou. 2013. Rapid effects of melatonin on hormonal and behavioral stressful responses in ewes. Psychoneuroendocrinology. 38:1426–1434.

Guiroy, P.J., L.O. Tedeschi, D.G. Fox, and J.P. Hutcheson. 2002. The effects of implant strategy on finished body weight of beef cattle. J. Anim. Sci. 80:1791-1800.

Horn, G. W., M. D. Cravey, F. T. McCollum, C. A. Strasia, E. G. Krenzer Jr., and P. L. Claypool. 1995. Influence of high-starch vs high-fiber energy supplements on performance of stocker cattle grazing wheat pasture and subsequent feedlot performance. J. Anim. Sci. 73:45-54.

Kreikemeier, W.M. and T.L. Mader. 2004. Effects of growth-promoting agents and season on yearling feedlot heifer performance. J. Anim. Sci. 82:2481-2488.

Loerch, S.C. and F.L. Fluharty. 1998. Effects of programming intake on performance and carcass characteristics on feedlot cattle. J. Anim. Sci. 76:371-377.

Mustonen, A.M., P. Nieminen, and H. Hyvarinen, 2002. Effects of continuous light and MEL treatment on energy metabolism of the rat. J. Endocrinol. Invest. 25:716–723.

National Research Council. 2000. Nutrient requirements of beef cattle. 7th revised ed. Natl. Academy Press, Washington, DC.

Nieminen P, R. Kakela, A-M. Mustonen, H. Hyvarinen, and J. Asikainen. 2001. Exogenous melatonin affects lipids and enzyme activities in mink (*Mustela vison*) liver. Comp. Biochem. Physiol. 128C:203–211.

Osei, P., K.R. Robbins, and H.V. Shirley. 1989. Effects of exogenous melatonin on growth and energy metabolism of chickens. Nutr. Res. 9:69-81.

Peters, R.R., L.T. Chapin, K.B. Leining, and H.A. Tucker. 1978. Supplemental lighting simulates growth and lactation in cattle. Science. 199:911-912.

Petitclerc, D., L.T. Chapin, R.S. Emery, and H.A. Tucker. 1983. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 57:892-898.

Petitclerc, D., L.T. Chapin, and H.A. Tucker. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 58:913-919.

Roche, J.F. and M.P. Boland. 1980. Effect of extended photoperiod in winter on growth rate of Friesian male cattle. Ir. J. Agric. Res. 19:85-91.

SAS Institute. 2008. SAS for Windows. Release Version 9.3. SAS inst., Cary, NC.

Schaefer, M.R., K.A. Albrecht, and D.M. Schaefer. 2014. Stocker steer performance on tall fescue or meadow fescue alone or in binary mixture with white clover. Agron. J. 106:1902-1910.

Stanisiewski, E.P., L.T. Chapin, N.K. Ames, S.A. Zinn, and H.A. Tucker. 1988. Melatonin and prolactin concentrations in blood of cattle exposed to 8, 16, or 24 hours of daily light. J. Anim. Sci. 66:727-734.

Tian, X., X. Huo, P. Dong, B. Wu, X. Wang, C. Wang, K. Liu, and X. Ma. 2015. Sulfation of melatonin: Enzymatic characterization, differences of organs, species and genders, and bioactivity variation. Biochem. Pharmacol. 94:282-296.

Tucker, H.A. D. Petitclerc, and S.A. Zinn. 1984. The influence of photoperiod on body weight gain, body composition, nutrient intake, and hormone secretion. J. Anim. Sci. 59:1610-1620.

USDA, 1965. Official United States Standards for Grades of Carcass Beef. C&MS, SRA 99, USDA, Washington, DC.

USDA, 1997. United States Standards for Grades of Carcass Beef. AMS-USDA, Washington, DC.

USDA AMS. 2015. Choice Beef Percentage Graded (Figure). Accessed 18 Jun. 2015. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5077890

Wettemann, R.P., S.C. Smith, K.S. Lusby, and H.A. Tucker. 1990. Hair growth of cattle is stimulated by melatonin. Animal science research report, Agricultural Experiment Station, Oklahoma. 346-348. Accessed 30 July, 2015. http://beefextension.com/research_reports/1990rr/90_57.pdf

Wileman, B.W., D.U. Thomson, C.D. Reinhardt, and D.G. Renter. 2009. Analysis of modern technologies commonly used in beef cattle production: Conventional beef production versus nonconventional production using meta-analysis. 87:3418-3426.

Zinn, S.A., R.W. Purchas, L.T. Chapin, D. Petitclerc, R.A. Merkel, W.G. Bergen, and H.W. Tucker. 1986. Effects of photoperiod on growth, carcass composition, prolactin, growth hormone, and cortisol in prepubertal and postpubertal Holstein heifers. J. Anim. Sci. 63:1804-1815.

Zinn, R. A., E. G. Alvarez, M. F. Montano, A. Plascencia, and J.E. Ramirez. 1998. Influence of tempering on the feeding value of rolled corn in finishing diets for feedlot cattle. J. Anim. Sci.76:2239–2246.

Zinn, S.A., L.T. Chapin, W.J. Enright, A.L. Schroeder, E.P. Stanisiewski, and H.A. Tucker. 1988. Growth, carcass composition and plasma melatonin in postpubertal beef heifers fed melatonin. J. Anim. Sci. 66:21-27.

Zinn, S.A., L.T. Chapin, W.J. Enright, and H.A. Tucker. 1989. Failure of photoperiod to alter body growth and carcass composition in beef steers. J. Anim. Sci. 67:1249-1257.

Table III-1. Ingredient and chemical composition of the finishing diet used when feeding supplemental melatonin to feedlot animals in experiments. 1-7.

	Percentage, DM basis
Cracked corn[a]	70.90
Corn silage	15.00
DDGS[b]	5.84
Wheat midds[b]	5.00
Urea[b]	1.00
Limestone[b]	1.43
Salt[b]	0.20
Calcium sulfate[b]	0.09
Micro ingredients[bc]	0.63
MGA 200[d]	0.01
Chemical Composition	
CP	12.9
NDF	17.7
Fat	3.87
Calcium	0.65
Phosphorus	0.37
Potassium	0.63
Ash	4.49
NEm, Mcal/ kg	2.13
NEg, Mcal/kg	1.46

[a]Melatonin carrier was substituted for cracked corn when daily TMR was mixed
[b]Ingredients mixed together as one pelleted supplement
[c]Designed to provide NRC requirements for micro minerals and provide 2790, 390, and 25 IU/kg diet DM vitamins A, D, and E, respectively, and 22 g monensin and 11 g tylosin /1,000 kg diet DM.
[d]Only fed to heifers in 0.45 kg cracked corn as-fed to deliver 0.50 mg melengestrol acetate/d

Table III-2. Ingredient composition of pelleted supplement fed to heifers grazing cool season pastures in experiment 8.

	Percentage, As-Is Basis
Ground corn	48.2
DDGS	47.2
Limestone	2.0
Liquid molasses	2.6

Supplement offered at 2.3 kg as-is · $hd^{-1} \cdot d^{-1}$

Table III-3. Ingredient and chemical composition of the finishing diet when feeding Holstein steers for 244 days in experiment 9.

	Percentage, DM basis
Cracked corn	56.00
Corn Silage	20.00
DDGS	20.00
Pelleted supplement[1]	4.00
Chemical Composition	
CP	12.6
NDF	19.9
Fat	4.3
Calcium	0.63
Phosphorus	0.44
Potassium	0.79
Ash	4.92
NEm, Mcal/ kg	2.10
NEg, Mcal/kg	1.43

[1]Designed to meet NRC requirements for micro minerals and provide 2150, 211, and 2 IU/kg diet DM vitamins A, D, and E, respectively, and 28 g monensin /1,000 kg diet DM.

Table III-4. Effect of dietary melatonin inclusion on crossbred beef heifer feedlot performance when fed for 93 or 113 days (Exp. 1).

	Treatment[1]				*P*-Value	
Item	0	20	100	SEM	Treatment	Linear contrast
No. of pens (heifers)	5 (30)	5 (30)	5 (30)			
Growth and intake						
Initial BW, kg	381	381	381	13	0.78	0.51
Final BW, kg	574	578	586	10	0.27	0.12
ADG, kg	1.84	1.88	1.95	0.04	0.17	0.06
DMI, kg	11.8	11.8	11.6	0.4	0.41	0.20
G:F	0.156[b]	0.160[ab]	0.169[a]	0.05	0.05	0.02
Energy of diet[2]						
NEm, Mcal/kg	2.05[b]	2.07[b]	2.13[a]	0.03	0.02	0.01
NEg, Mcal/kg	1.36[b]	1.41[ab]	1.45[a]	0.03	0.03	0.01

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.

[1] 0, 20 and 100 represent 0, 20 or 100 mg melatonin · $hd^{-1} \cdot d^{-1}$.

[2] Calculated from actual performance data using equation by Zinn et al. (1998).

Table III-5. Effect of dietary melatonin inclusion on crossbred beef heifer carcass characteristics when fed for 93 or 113 days (Exp. 1).

	Treatment[1]				*P*-Value	
Item	0	20	100	SEM	Treatment	Linear contrast
No. of pens (heifers)	5 (30)	5 (30)	5 (30)			
HCW, kg	354	355	361	6	0.21	0.09
DP[2], %	61.8	61.5	61.6	0.2	0.71	0.75
Fat depth, cm	1.54	1.46	1.34	0.09	0.35	0.17
LM, cm^2	82.8	83.2	85.0	1.4	0.44	0.21
KPH, %	2.0	2.0	1.9	0.1	0.15	0.06
Yield grade[3]	3.2	3.2	3.0	0.1	0.27	0.11
Marbling[4], units	636	659	623	15	0.28	0.30
Empty Body Fat, %[5]	31.4	31.3	30.3	0.4	0.18	0.07
AFBW[6], kg	503^b	506^b	530^a	7.8	0.04	0.02
LLDM[7], %	35.0	36.0	35.8	0.4	0.27	0.40
LLEEAS[8],%	7.2	8.4	7.0	0.4	0.11	0.30

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.
[1] 0, 20 and 100 represent 0, 20 or 100 mg melatonin · hd^{-1} · d^{-1}.
[2] DP = dressing percentage, calculated as final BW collected on the farm, not shrunk/ HCW.
[3] Calculated from carcass measurements.
[4] Marbling score units, 500 = USDA small^{00}, 600 = USDA modest^{00}, 700 = USDA moderate^{00}.
[5 and 6] Calculated from carcass measurements using equations published by Guiroy et al., 2002, AFBW = adjusted final body weight.
[7] LLDM = longissimus lumborum dry matter.
[8] LLEEAS = longissimus lumborum ether extract expressed as-is (water included).

Table III-6. Effect of dietary melatonin inclusion on crossbred beef heifer feedlot performance (Exp. 2).

Item	Treatment[1]			SEM	*P*-Value	
	0	100	200		Treatment	Linear contrast
No. of pens (heifers)	5 (30)	5 (30)	5 (30)			
Growth and intake						
Initial BW, kg	300	300	300	10	0.29	0.71
Final BW, kg	561	563	570	9	0.22	0.11
ADG, kg	1.69	1.70	1.75	0.03	0.24	0.12
DMI, kg	9.9	9.9	10.0	0.3	0.45	0.29
G:F	0.172	0.173	0.174	0.003	0.71	0.42
Energy of diet[2]						
NEm, Mcal/kg	2.07	2.08	2.07	0.03	0.97	0.87
NEg, Mcal/kg	1.41	1.41	1.41	0.02	0.97	0.88

[1] 0, 100 and 200 represent 0, 100 or 100 mg melatonin · $hd^{-1} \cdot d^{-1}$

[2] Calculated from actual performance data using equation by Zinn et al. (1998).

Table III-7. Effect of dietary melatonin inclusion on crossbred beef heifer carcass characteristics when fed for 149 or 163 days during the long photoperiod months (Exp. 2).

	Treatment[1]				*P*-Value	
Item	0	100	200	SEM	Treatment	Linear contrast
No. of pens (heifers)	5 (30)	5 (30)	5 (30)			
HCW, kg	342	343	348	6	0.10	0.05
DP[2], %	60.9	60.9	61.1	0.3	0.77	0.51
Fat depth, cm	1.65	1.60	1.71	0.04	0.13	0.25
LM, cm^2	72.9	74.0	74.5	1.4	0.70	0.42
KPH, %	2.0	1.9	2.0	0.1	0.31	0.68
Yield grade[3]	3.8	3.7	3.8	0.1	0.34	0.68
Marbling[4], units	663	656	665	12	0.86	0.91
Empty Body Fat, %[5]	32.6	32.3	33.0	0.2	0.13	0.31
AFBW[6], kg	464.8	472.4	469.2	9.2	0.47	0.48
LLDM[7], %	42.6	43.2	43.0	0.4	0.59	0.46
LLEEAS[8],%	9.6	10.4	10.3	0.4	0.41	0.31

[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin · hd^{-1} · d^{-1}.
[2] DP = dressing percentage, calculated as final BW collected on the farm, not shrunk/ HCW.
[3] Calculated from carcass measurements.
[4] Marbling score units, 500 = USDA small^{00}, 600 = USDA modest^{00}, 700 = USDA moderate^{00}.
[5 and 6] Calculated from carcass measurements using equations published by Guiroy et al., 2002, AFBW = adjusted final body weight.
[7] LLDM = longissimus lumborum dry matter.
[8] LLEEAS = longissimus lumborum ether extract expressed as-is (water included).

Table III-8. Effect of dietary melatonin inclusion on crossbred beef steer feedlot performance (Exp. 3 and 4).

Item	Treatment[1] 0	100	200	SEM	*P*-Value Treatment	Linear contrast
No. of pens (steers)	10 (59)	10 (59)	10 (59)			
Growth and intake						
Initial BW, kg	367	367	367	42	0.44	0.36
Final BW, kg	630	630	639	17	0.33	0.22
ADG, kg	2.11	2.10	2.16	0.2	0.36	0.26
DMI, kg	11.3	11.3	11.5	0.9	0.33	0.19
G:F	0.187	0.186	0.187	0.002	0.93	0.92
Energy of diet[2]						
NEm, Mcal/kg	2.18	2.17	2.17	0.02	0.89	0.63
NEg, Mcal/kg	1.50	1.50	1.49	0.02	0.89	0.64

[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin · hd^{-1} · d^{-1}.

[2] Calculated from actual performance data using equation by Zinn et al. (1998).

Table III-9. Effect of dietary melatonin inclusion on crossbred beef steer carcass characteristics (Exp. 3 and 4).

	Treatment[1]				*P*-Value	
Item	0	100	200	SEM	Treatment	Linear contrast
No. of pens (steers)	10 (59)	10 (59)	10 (59)			
HCW, kg	379	380	386	9	0.21	0.10
DP[2], %	61.5	61.6	61.8	1.5	0.56	0.29
Fat depth, cm	1.24[b]	1.34[ab]	1.41[a]	0.12	0.04	0.01
LM, cm^2	87.1	85.6	84.9	1.4	0.53	0.28
KPH, %	1.9	1.9	1.9	0.1	0.72	0.46
Yield grade[3]	2.9[b]	3.1[ab]	3.3[a]	0.2	0.01	0.01
Marbling[4], units	563	551	566	10	0.20	0.74
Empty Body Fat, %[5]	29.7[b]	30.1[ab]	30.7[a]	0.6	0.01	0.01
AFBW[6], kg	567	561	560	8	0.57	0.33

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.
[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin $\cdot$ $hd^{-1} \cdot d^{-1}$.
[2] DP= dressing percentage, calculated as final BW collected on the farm, not shrunk/ HCW.
[3] Calculated from carcass measurements.
[4] Marbling score units, 500 = USDA small[00], 600 = USDA modest[00], 700 = USDA moderate[00].
[5 and 6] Calculated from carcass measurements using equations published by Guiroy et al., 2002, AFBW= adjusted final body weight.

Table III-10. Least square means showing the effect of dietary melatonin or implant status on animal performance of individually fed crossbred beef steers in experiments 5 and 6.

	Treatment[1]					*P* - Value		
Item	NI-0	I-0	NI-100	I-100	SEM	Implant	MEL	Implant* MEL
No. of steers	18	21	19	20				
Growth and intake								
Initial BW, kg	389	389	387	386	15	0.80	0.85	0.94
Final BW, kg	601[b]	637[a]	597[b]	631[a]	10	0.001	0.44	0.82
ADG, kg	1.75[b]	2.05[a]	1.73[b]	2.01[a]	0.11	0.001	0.59	0.78
DMI, kg	11.2	11.5	11.0	11.2	0.6	0.17	0.07	0.97
G:F	0.155[b]	0.178[a]	0.158[b]	0.179[a]	0.004	0.001	0.51	0.76
Energy of diet								
NEm,[2] Mcal/kg	1.99[b]	2.13[a]	2.02[b]	2.16[a]	0.05	0.001	0.23	0.99
NEg,[2] Mcal/kg	1.33[b]	1.46[a]	1.36[b]	1.49[a]	0.04	0.001	0.25	0.94

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.

[1] NI = non-implanted, I = implanted with Revalor-S on day 0 or 28 of experiment, 0 = 0 mg of MEL fed $\cdot$ $hd^{-1} \cdot d^{-1}$, and 100 represents animals fed 100 or 200 mg melatonin $\cdot$ $hd^{-1} \cdot d^{-1}$.

[2] Calculated from actual performance data using equation by Zinn et al. (1998)

Table III-11. Least square means showing the effect of implant status or dietary melatonin on carcass composition of individually fed crossbred beef steers in experiments 5 and 6.

	Treatment[1]					*P* - Value		
Item	NI-0	I-0	NI-100	I-100	SEM	Implant	MEL	Implant* MEL
No. of steers	18	21	19	20				
HCW, kg	362^{b}	385^{a}	356^{b}	380^{a}	6	0.001	0.19	0.87
DP[2], %	61.6	61.7	60.9	61.5	1.5	0.34	0.16	0.39
Fat depth, cm	1.40	1.36	1.36	1.29	0.2	0.41	0.44	0.86
LM, cm^2	77.4^{b}	85.4^{a}	79.1^{b}	87.8^{a}	1.9	0.001	0.23	0.83
KPH, %	2.17	1.98	2.10	1.98	0.1	0.03	0.57	0.60
Yield grade[3]	3.5^{a}	3.2^{ab}	3.3^{ab}	3.1^{b}	0.3	0.03	0.13	0.84
Marbling[4], units	669^{a}	619^{b}	607^{b}	606^{b}	24	0.08	0.01	0.09
Empty Body Fat, %[5]	31.6^{a}	30.9^{ab}	30.7^{ab}	30.2^{b}	1.3	0.14	0.05	0.76
AFBW[6], kg	509^{b}	553^{a}	515^{b}	558^{a}	17	0.001	0.51	0.89

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.

[1] NI = non-implanted, I=implanted with Revalor-S on day 0 or 28 of experiment, 0 = 0 mg of MEL fed $\cdot$ $hd^{-1} \cdot d^{-1}$, and 100 represents animals fed 100 or 200 mg melatonin $\cdot$ $hd^{-1} \cdot d^{-1}$.

[2] DP = dressing percentage, calculated as final BW collected on the farm / HCW.

[3] Calculated from carcass measurements.

[4] Marbling score units, 500 = USDA $small^{00}$, 600 = USDA $modest^{00}$, 700 = USDA $moderate^{00}$.

[5 and 6] Calculated from carcass measurements using equations published by Guiroy et al., 2002, AFBW= adjusted final body weight.

Table III-12. Least square means showing the effect of dietary melatonin on feedlot performance in crossbred beef heifers when program fed in experiment 7.

	Treatment[1]				*P*-Value	
Item	0	100	200	SEM	Treatment	Linear contrast
No. of pens (heifers)	4 (24)	4 (24)	4 (24)			
Growth and intake						
Initial BW, kg	352	352	352	20	0.37	0.54
Final BW, kg	568	567	566	13	0.99	0.89
ADG, kg	1.66	1.67	1.65	0.04	0.97	0.97
DMI, kg	9.8	9.8	9.9	0.4	0.53	0.29
G:F	0.170	0.170	0.168	0.005	0.91	0.69
Energy of diet						
NEm,[2] Mcal/kg	2.14	2.13	2.12	0.03	0.76	0.48
NEg,[2] Mcal/kg	1.47	1.46	1.45	0.03	0.76	0.48

[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin · hd^{-1} · d^{-1}.
[2] Calculated from actual performance data using equation by Zinn et al. (1998)

Table III-13. Least square means showing the effect of dietary melatonin on carcass characteristics in crossbred beef heifers in experiment 7.

	Treatment[1]				*P*-Value	
Item	0	100	200	SEM	Treatment	Linear contrast
No. of pens (heifers)	4 (24)	4 (24)	4 (24)			
HCW, kg	343	343	341	7	0.70	0.47
DP[2], %	60.5	60.5	60.1	0.4	0.56	0.38
Fat depth, cm	1.50	1.36	1.43	0.08	0.54	0.61
LM, cm^2	77.4	79.5	79.6	1.5	0.19	0.12
KPH, %	2.2	2.2	2.2	0.1	0.52	0.56
Yield grade[3]	3.4	3.2	3.2	0.1	0.29	0.25
Marbling[4], units	627	626	630	15	0.98	0.89
Empty Body Fat, %[5]	31.3	30.5	30.8	0.4	0.43	0.45
AFBW[6], kg	487	500	491	15	0.62	0.78

[a,b] Within a row, means without a common superscript differ at $P < 0.05$.
[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin · $hd^{-1} \cdot d^{-1}$.
[2] DP = dressing percentage, calculated as final BW collected on the farm, not shrunk/ HCW.
[3] Calculated from carcass measurements.
[4] Marbling score units, 500 = USDA small^{00}, 600 = USDA modest^{00}, 700 = USDA moderate^{00}.
[5 and 6] Calculated from carcass measurements using equations published by Guiroy et al., 2002, AFBW = adjusted final body weight.

Table III-14. Stocker heifer growth performance and plasma serotonin level when allowed to graze cool season pastures for 138 days (experiment 8).

Treatment[1]	No. of heifers	Animal performance variables, kg					Plasma, ng/ml[3]
		Initial BW	Final BW	Overall ADG[2]	Total DMI	G:F	Serotonin
SUPP-0-0	9	287	430^{ab}	1.04^{ab}	281.9^{a}	0.086	76
SUPP-100-0	9	286	431^{ab}	1.05^{ab}	281.7^{a}	0.086	123
SUPP-0-1	9	286	429^{ab}	1.04^{ab}	282.1^{a}	0.074	-
SUPP-100-1	9	287	442^{a}	1.12^{a}	281.9^{a}	0.116	-
GRAZ-0-0	9	286	403^{b}	0.85^{c}	0^{b}	-	-
GRAZ-0-1	9	286	414^{ab}	0.92^{bc}	0^{b}	-	-
SEM		9	10	0.05	0.2	0.032	31.9
P-Values							
Treatment		1.0	0.13	0.01	0.001	0.47	0.31
SUPP vs GRAZ		0.98	0.01	0.001	0.001	-	-
Melatonin 0 vs 100		0.97	0.49	0.32	0.34	0.28	0.31
Implant 0 vs 1		0.95	0.41	0.24	0.44	0.63	-

[a-c]Within a column, means without a common superscript differ at $P < 0.05$.
[1]SUPP = supplemented at 2.3 kg as-fed · $hd^{-1} \cdot d^{-1}$, GRAZ = heifers only allowed to graze pastures as feed source, middle "0" or "100" = mg melatonin · $hd^{-1} \cdot d^{-1}$, and last "0" = no implant given, while last "1" = implanted with Revalor-G on d 0.
[2]No interaction between implant status and melatonin level was observed ($P = 0.43$).
[3]Blood samples obtained on d 68, two hours post feeding. n = 6 for SUPP-0-0, and n = 5 for SUPP-100-0.

Table III-15. Least square means showing the main effects of dietary melatonin and implant regimen on feedlot performance in Holstein steers when fed for 244 days in experiment 9.

	Melatonin [1]			Implant Regimen[2]				*P*-Value[3]		
Item	0	200	SEM	Control	Encore	E/S/S	SEM	Melatonin	Implant	Encore vs E/S/S
Replicates[4]	3	3		19	20	20				
Growth and intake										
Initial BW, kg	352	352	12.3	351	353	351	9	0.85	0.80	0.66
Final BW, kg	714	697	5.5	656^{b}	719^{a}	740^{a}	9	0.09	0.001	0.09
ADG, kg	1.49	1.42	0.061	1.25^{b}	1.50^{b}	1.60^{b}	0.05	0.18	0.001	0.06
DMI, kg	11.1	10.6	0.14	-	-	-	-	0.08	-	
G:F	0.134	0.133	0.006	-	-	-	-	0.76	-	
Energy of diet										
NEm,[5] Mcal/kg	1.88	1.90	0.027	-	-	-	-	0.67	-	
NEg,[5] Mcal/kg	1.24	1.25	0.025	-	-	-	-	0.63	-	

[1] 0 and 200 represent 0 or 200 mg fed · hd^{-1} · d^{-1}, and melatonin feeding started on d 27 of the trial.
[2] Control = non-implanted, Encore = Encore given on d 0, and E/S/S = implanted with Encore, Revalor-S, and Revalor-S on d 0, 109, and 169, respectively.
[3] No interaction was observed between dietary melatonin level and implant regimen ($P > 0.42$) and was removed from the final model.
[4] Experimental unit was pen for 0 and 200, while animal was used for implant regimen.
[5] Calculated from actual performance data using equation by Zinn et al. (1998)

Table III-16. Least square means showing the main effect of dietary melatonin or implant regimen on carcass characteristics in Holstein steers fed for 244 days in experiment 9.

	Melatonin[1]			Implant Regimen[2]				*P*-Value[3]		
Item	0	200	SEM	Control	Encore	E/S/S	SEM	Melatonin	Implant	Encore vs E/S/S
Replicates	3	3		19	20	20				
HCW, kg	414	406	3.16	379[c]	416[b]	434[a]	6	0.18	0.001	0.04
DP[3], %	57.9	58.3	0.37	57.7	58.0	58.6	0.4	0.53	0.13	0.15
Fat depth, cm	0.49	0.51	0.034	0.45	0.54	0.51	0.03	0.67	0.13	0.58
LM, cm^2	77.9	78.4	1.45	74.6[b]	75.4[b]	84.2[a]	1.7	0.77	0.001	0.001
KPH, %	2.5	2.3	0.08	2.5	2.3	2.35	0.1	0.20	0.53	0.79
Yield grade[5]	3.1	3.0	0.06	2.9[b]	3.2[a]	2.9[b]	0.1	0.26	0.01	0.01
Marbling[6], units	682	671	8.0	696	680	655	20	0.39	0.34	0.39
Empty Body Fat[7], %	28.4	28.2	0.15	27.8	28.8	28.3	0.3	0.27	0.06	0.16
AFBW[8], kg	637.9	630.4	3.78	595.7[c]	635.2[b]	670.1[a]	7.6	0.23	0.01	0.01

[1] 0 and 200 represent 0 or 200 mg melatonin · hd^{-1} · d^{-1}, and melatonin feeding started on d 27 of the trial.
[2] Control = non-implanted, Encore = Encore given on d 0, and E/S/S = implanted with Encore, Revalor-S, and Revalor-S on d 0, 109, and 169, respectively.
[3] No interaction was observed between dietary melatonin level and implant regimen (*P* > 0.42) and was removed from the final model
[4] DP = dressing percentage, calculated as final BW collected on the farm, not shrunk/ HCW.
[5] Calculated from carcass measurements.
[6] Marbling score units, 500 = USDA small^{00}, 600 = USDA modest^{00}, 700 = USDA moderate^{00}.
[7 and 8] Calculated from carcass measurements using equations published by Guiroy et al. (2002), AFBW = adjusted final body weight.

III-17. Percentage change of animal growth variables when fed melatonin compared to controls in experiments 1 to 6 and 8.

	Treatment[1]			*P*-Value
Item	Control	Melatonin	SEM	Treatment
No. of trials (total animals)	7 (176)	7 (176)		
Growth and intake				
Initial BW	100.0	100.0	0.1	0.36
Final BW	100.0	100.2	0.8	0.87
ADG	100.0	102.3	1.3	0.16
DMI	100.0	99.8	0.7	0.84
G:F	100.0	102.2	1.1	0.18
Energy of diet[2]				
NEm	100.0	100.9	0.5	0.26
NEg	100.0	101.5	0.8	0.24

[1] Control represents 0 and melatonin represents 100 or 200 mg melatonin · $hd^{-1} \cdot d^{-1}$.
[2] Calculated from actual performance data using equation by Zinn et al. (1998)

Table III-18. Percentage change of animal carcass variables when fed MEL compared to controls in experiments 1 to 6 and 8.

	Treatment[1]			P-Value
Item	Control	Melatonin	SEM	Treatment
No. of trials (total animals)	7 (176)	7 (176)		
HCW	100.0	100.7	0.6	0.40
DP[2]	100.0	99.9	0.2	0.84
Fat depth	100.0	101.7	3.0	0.68
LM	100.0	100.9	0.7	0.41
KPH	100.0	99.1	0.9	0.51
Yield grade	100.0	100.5	2.4	0.88
Marbling	100.0	98.0	0.9	0.18
Empty Body Fat	100.0	99.9	0.8	0.97
AFBW[2]	100.0	101.0	0.9	0.39

[1] 0, 100 and 200 represent 0, 100 or 200 mg melatonin · $hd^{-1} \cdot d^{-1}$.
[2] AFBW = adjusted final body weight.

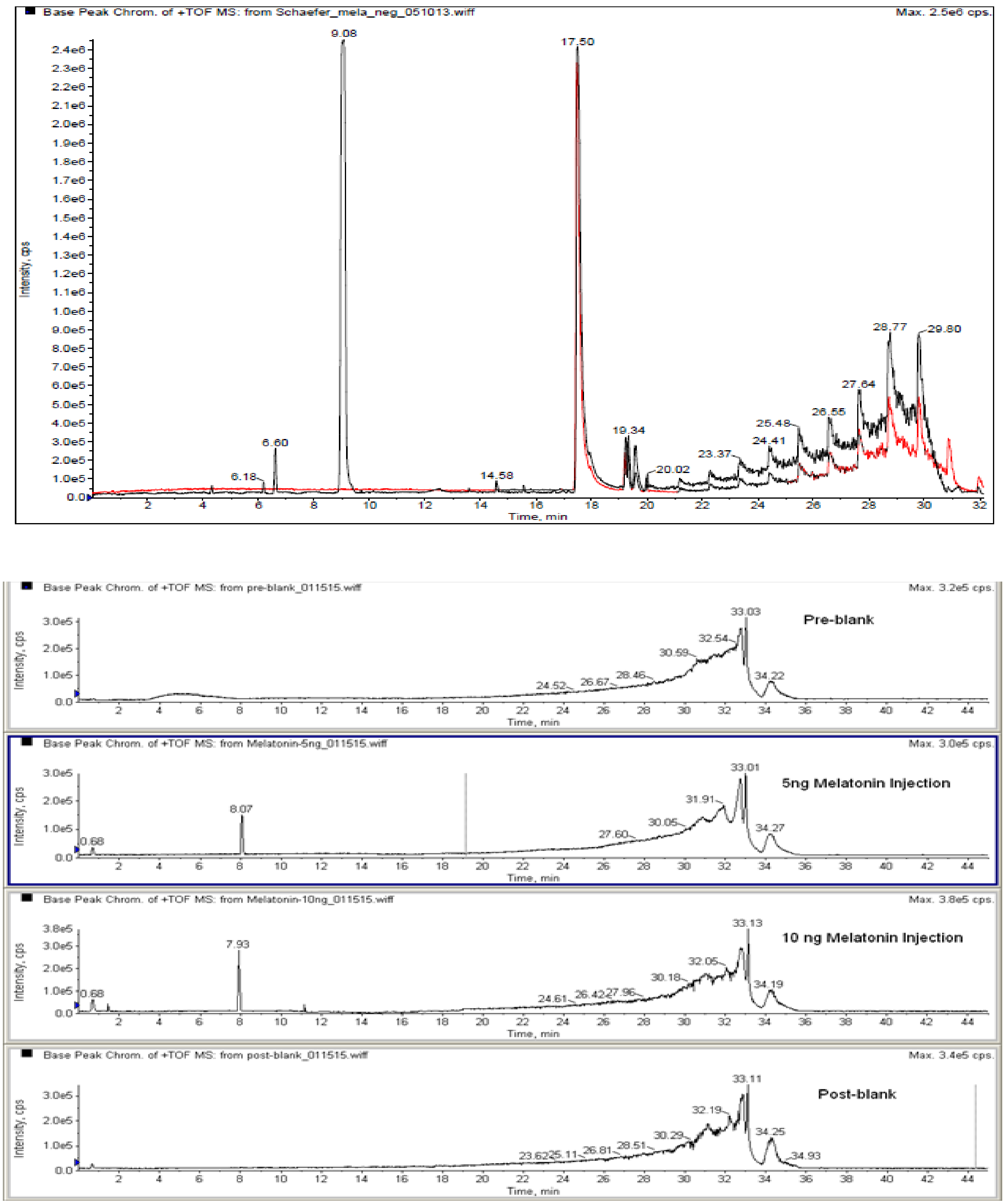

Figure III-1. Analysis of two melatonin samples (A and B, top and bottom respectively) and potential impurities using LC/MS.

Figure III-2. Images representing the technique used to collect hair samples from the rump of growing heifers in experiment 1.

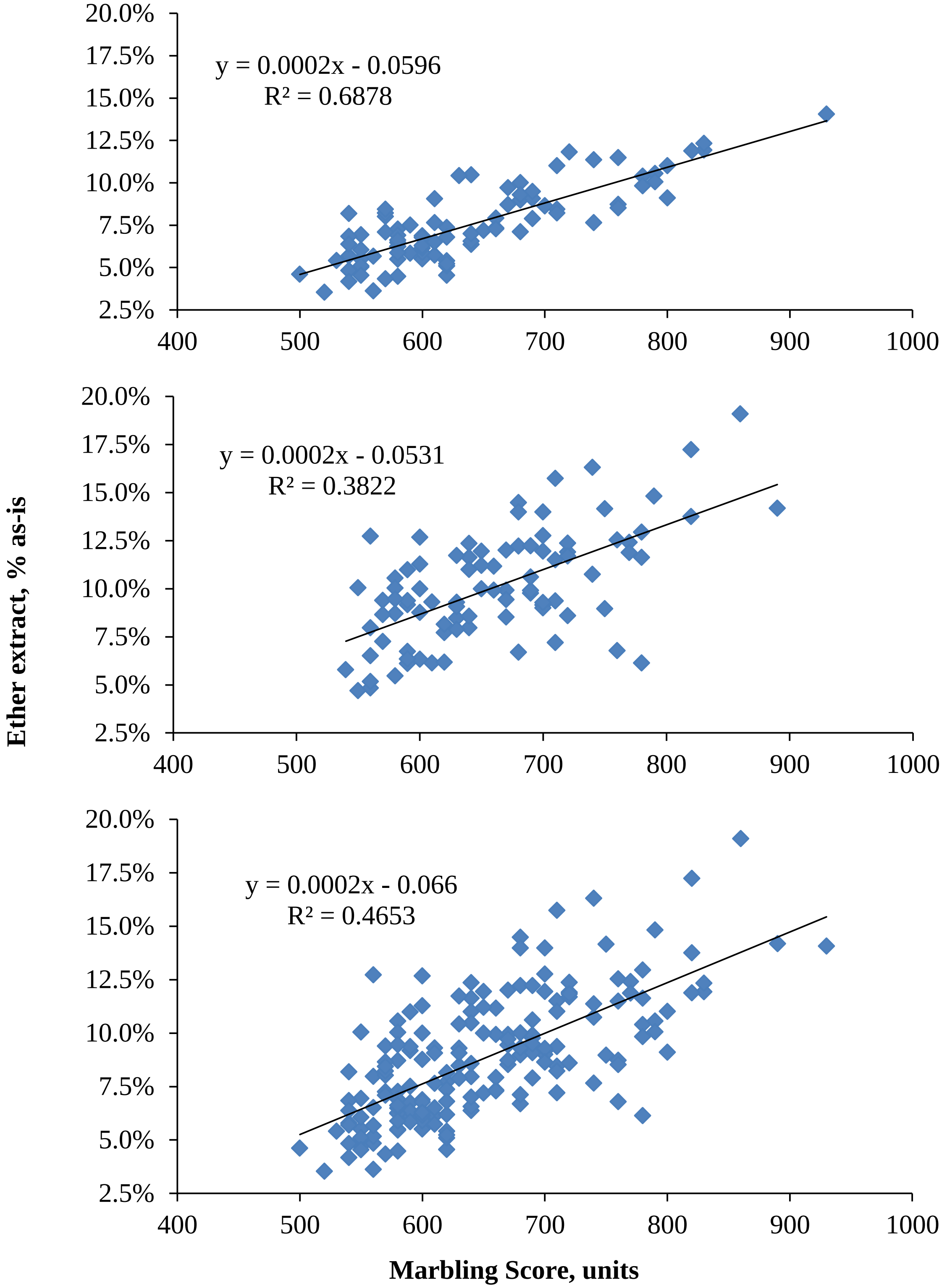

Figure III-3. Correlation between marbling score and as-is ether extract percentage in Exp. 1 (top), Exp. 2 (middle) and combining the two data sets (bottom).

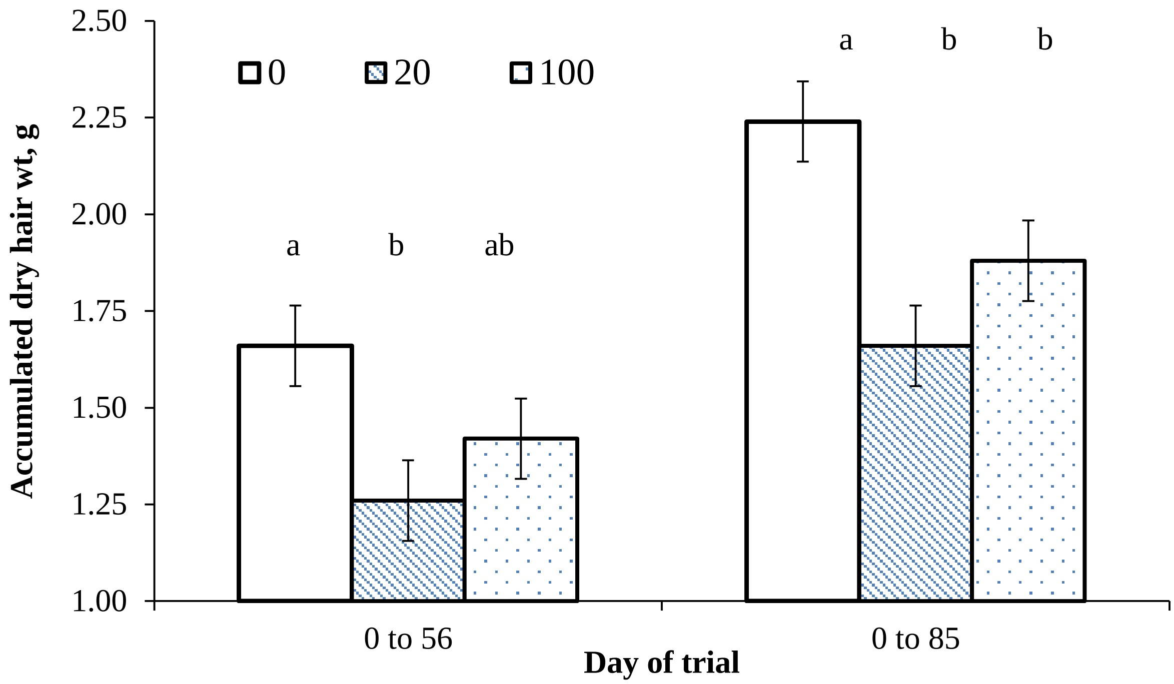

Figure III-4. Hip hair growth per 200 cm^2 when fed 0, 20, or 100 mg melatonin · hd^{-1} · d^{-1} in feedlot heifers during a short photoperiod (Exp. 1). No linear effect was observed for either day, SEM = 0.10.

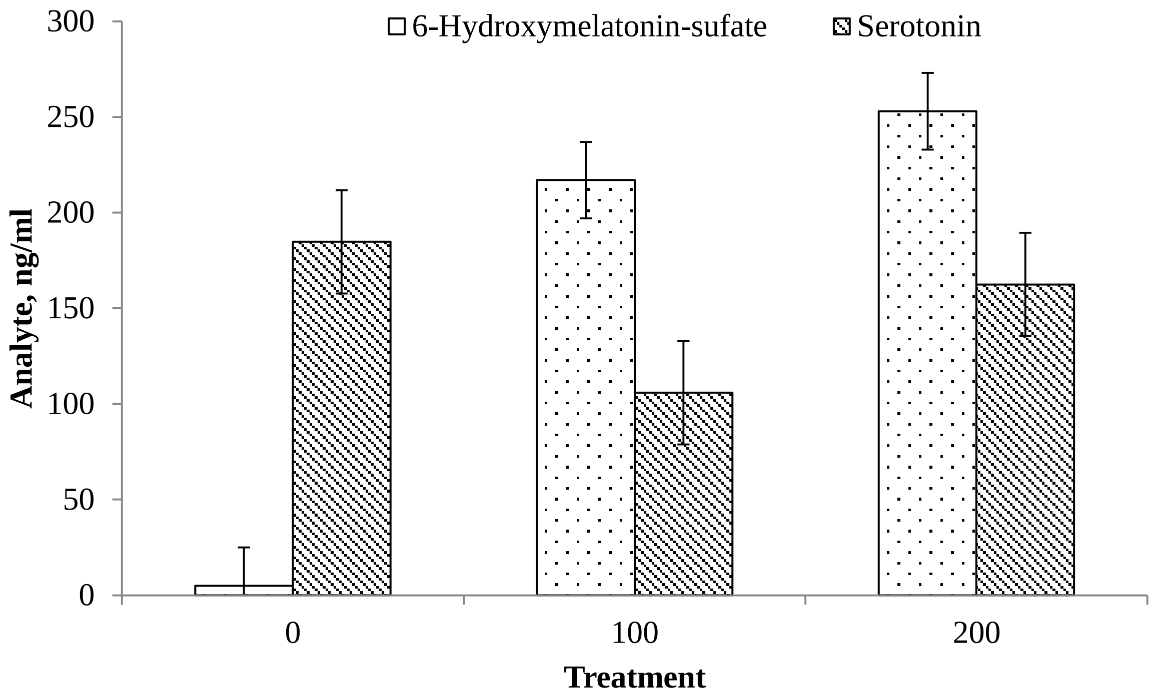

Figure III-5. Urine 6-hydroxymelatonin-sulfate and plasma serotonin levels in steers fed 0, 100, or 200 mg · hd^{-1} · d^{-1} in experiment 4 on d 154 approximately 7 h post feeding. Treatments differ (P = 0.01) and a linear trend was considered significant (P = 0.01, SEM = 20) for 6-hydroxymelatonin-sulfate. Serotonin levels were similar across treatments (P = 0.16, SEM = 27).

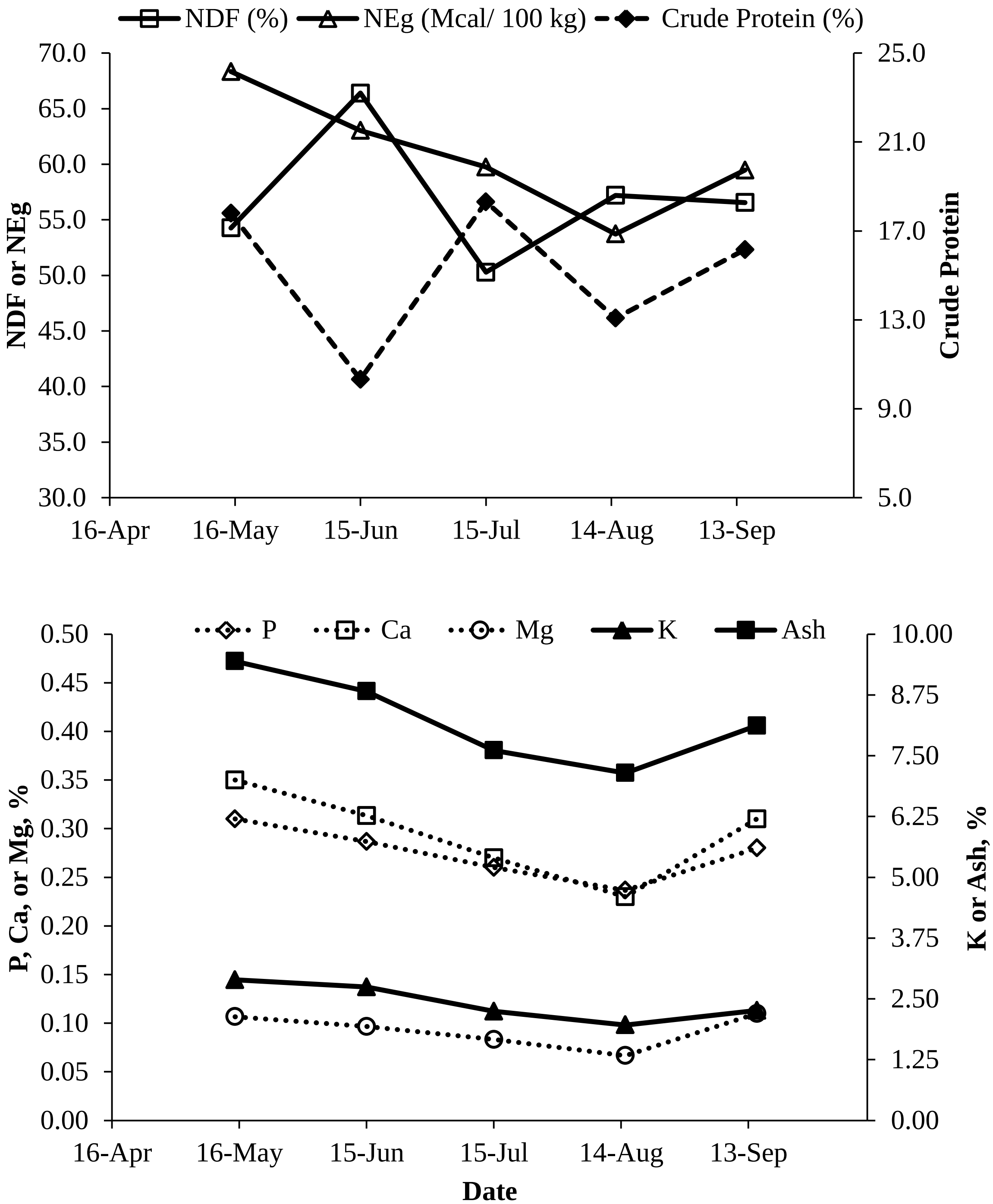

Figure III-6. Chemical composition of available sward structure over the grazing season in experiment 8. No statistical analysis was conducted.

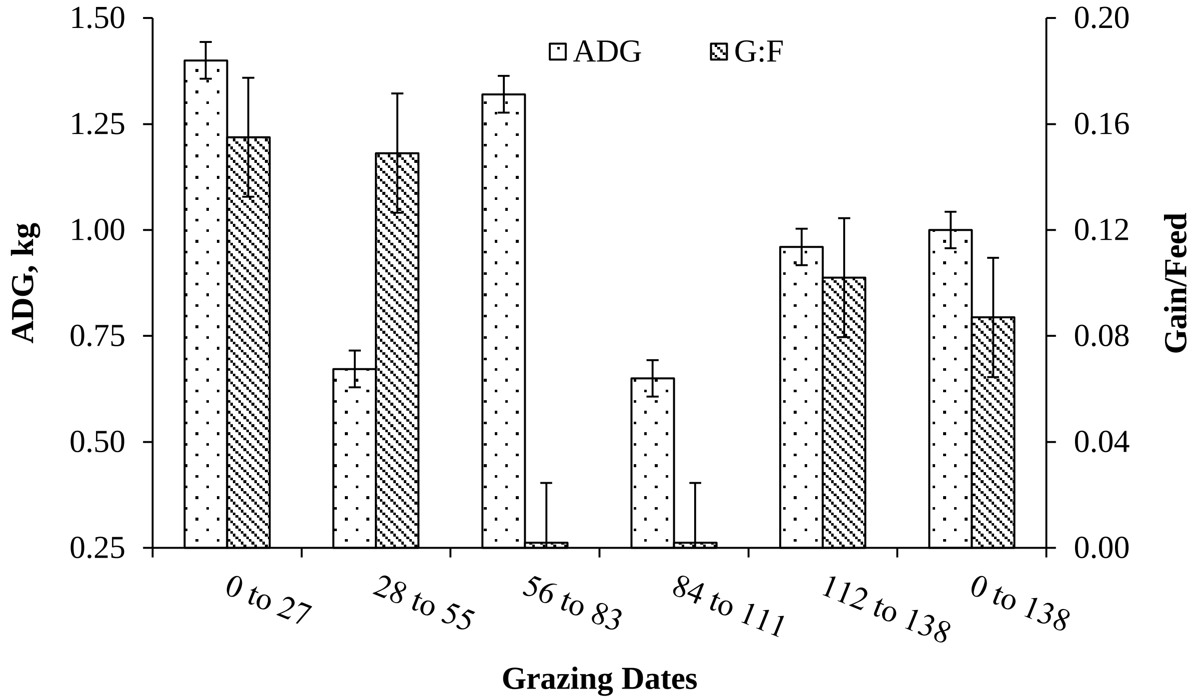

Figure III-7. Stocker heifer performance as grazing season progressed (Exp. 8). No treatment by period interactions were considered significant (*P* > 0.45), and only the main effect of grazing period is shown.

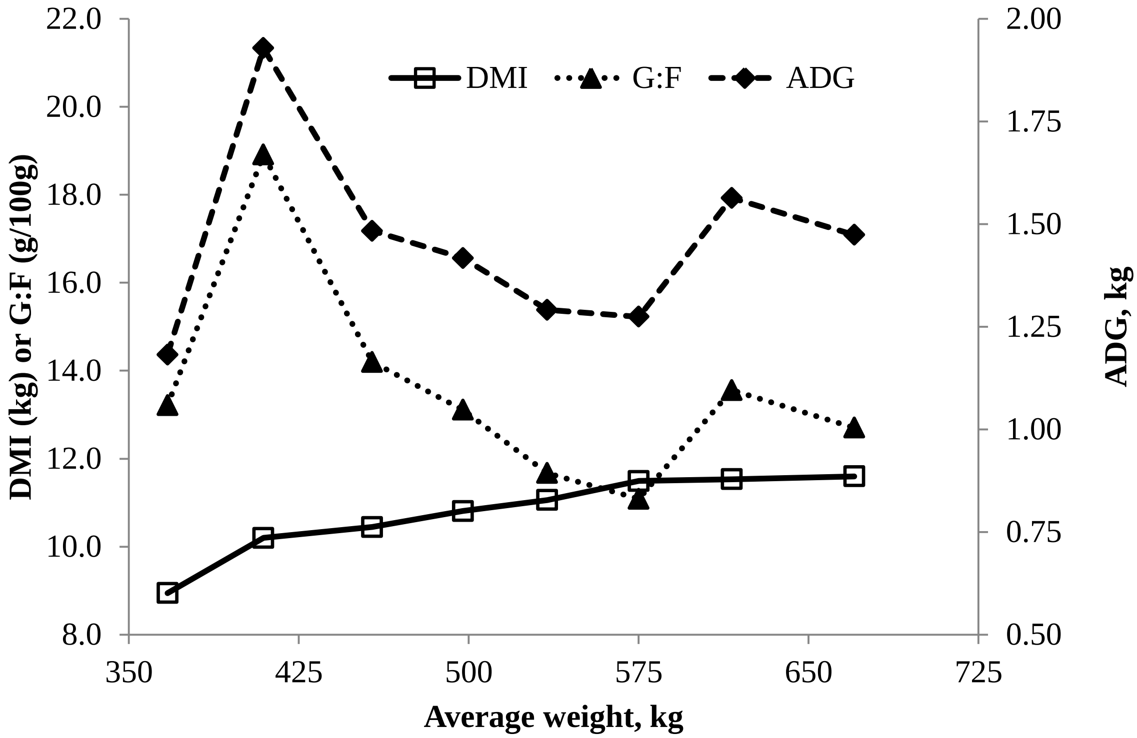

Figure III-8. Holstein steer performance as feeding duration progressed in experiment 9.

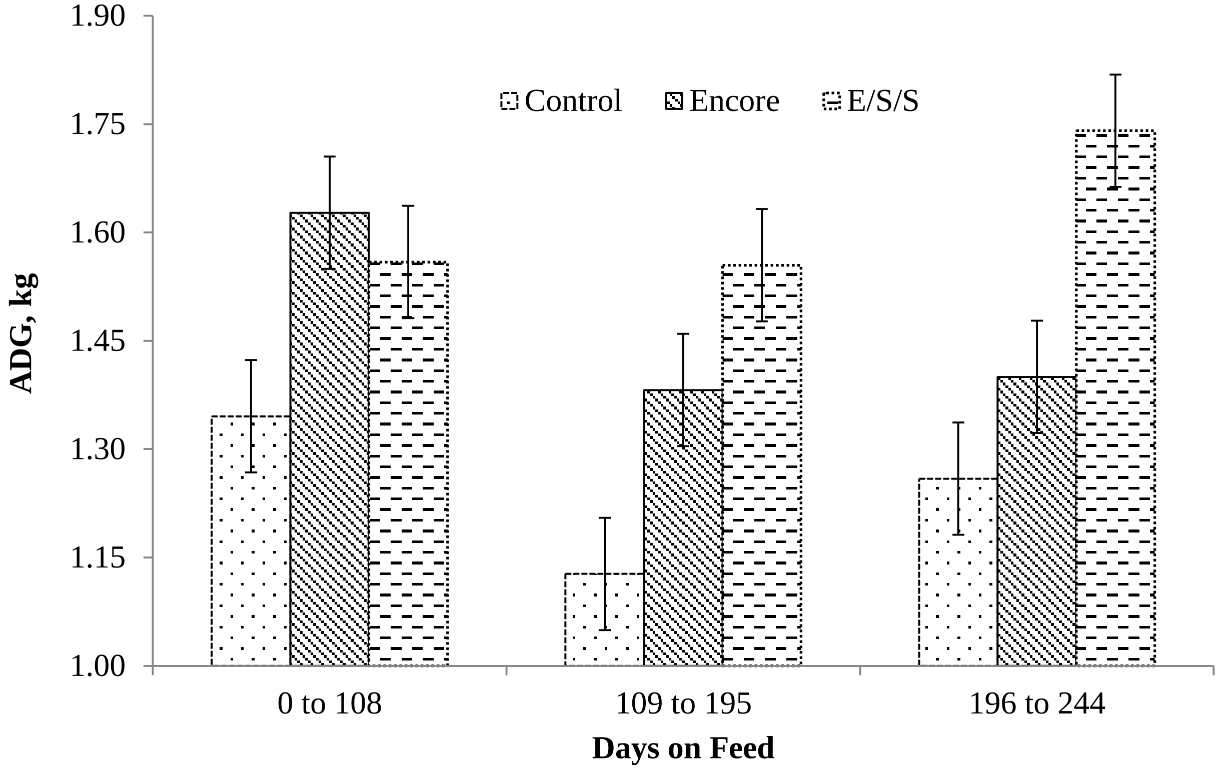

Figure III-9. Interaction of implant regimen and feeding period for Holstein steer ADG in experiment 9. Encore was administered on d 0 for Encore and E/S/S, while E/S/S also received Revalor-S on d 109 and 196. Interaction was significant (P = 0.02), SEM = 0.078.

CHAPTER IV. Feeding antibodies against interleukin 10 to growing ruminants, application for control of gastrointestinal parasites and bovine respiratory disease

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ABSTRACT

Four trials were conducted with growing lambs or cattle to evaluate the effects of feeding antibodies to interleukin-10 (aIL-10) to aid in the control of gastrointestinal (GI) parasites and bovine respiratory disease (BRD). Two trials (1 in cattle and 1 in growing lambs), each using 100 animals and a similar design, were conducted to evaluate aIL-10 vs commercial anthelmintic products. Treatments were 1) negative control (NC), 2) positive control (PC, LongRange or Ivomec for cattle and sheep, respectively), 3) aIL-10 low, 4) aIL-10 medium, and 5) aIL-10 high, in which aIL-10 dosages were fed for the initial 14 d. In both trials, animal ADG was greater ($P < 0.07$) for the aIL-10 high vs the NC, and a positive linear effect between aIL-10 dosage and ADG was observed ($P < 0.10$). The PC animals in both trials grew faster than the NC but only for the cattle trial was this effect considered significant ($P = 0.03$). Fecal egg count (FEC) 14 d post treatment was lower for the PC vs the NC in both trials ($P < 0.05$). Using the above lamb aIL-10 high dosage, a growing lamb trial evaluated the duration of aIL-10 feeding with treatments consisting of 1) NC, 2) PC (Prohibit), 3) aIL-10-7, and 4) aIL-10-14, in which aIL-10 was dosed for the initial 7 or 14 d, respectively. No differences in ADG were detected across treatments ($P = 0.51$). This was probably due to the low parasite challenge as indicated by the initial FEC (100 eggs/g feces). However, the PC did lower FEC vs the NC ($P = 0.05$), but no difference was detected in growth rate ($P = 0.26$) suggesting some tolerance of GI parasites by lambs without affecting growth rate. A total of 113 steers (297 kg) were used to evaluate the effects of feeding aIL-10 for 14 d to newly received steers. Treatments were 1) control (fed control eggs) or 2) aIL-10 (fed eggs containing aIL-10). During the 64 d growing trial, aIL-10 fed steers grew 6.5% faster ($P = 0.13$) and were 6% more efficient ($P = 0.04$) than control fed animals. The percentage of steers requiring two antibiotic treatments to control BRD tended to

be lower (*P* = 0.09) in aIL-10 fed steers. We present evidence here for the use of aIL-10 to aid in the control of GI parasites and as an augmentation to a BRD treatment regimen.

INTRODUCTION

Gastrointestinal (GI) parasite burden is one of the largest health concerns for ruminants worldwide (Armour, 1980). Although there are anthelmintic products to ameliorate the negative effects of parasitism, the efficacy of these products seems to be dwindling. Resistance to anthelmintic drugs by GI nematodes infecting ruminants has been demonstrated throughout the world (Gasbarre, 2014). Problems with GI parasitism in sheep are often classified as production disease (i.e., chronic subclinical condition affecting productivity such as weight loss, reduced weight gain, reproductive inefficiency, etc.) (Miller and Horohov, 2006). Symptoms of parasitic gastroenteritis in cattle are similar to those of sheep and are watery diarrhea, reduced weight gain or weight loss, a dull hair coat, anorexia, and a general loss of body condition (Anderson et al., 1965; Hilderson et al., 1987; Eysker and Ploeger, 2000).

Another significant health concern in the cattle industry is bovine respiratory disease. A 2006 survey of Kansas feedlots suggested a trend for increased death losses over the last decade (Babcock et al., 2006). Bovine respiratory disease (BRD) was the leading cause of morbidity and mortality in a cross-sectional survey sent to 561 feedlots in 21 states (Woolums et al., 2005). In 2014, Rezac et al. observed 13,226 yearling feedlot animals, corresponded pre-harvest and carcass data, and associated the information with lung lesion scores. Across the entire population, 22.5% and 9.8% of the cattle displayed mild or severe pulmonary lesions respectively; however, only 2.1% of these cattle were treated for BRD during the finishing phase. Of cattle treated for BRD during the finishing phase, 44.7% displayed gross pulmonary lesions at slaughter. When pulmonary lesions were mild or severe, there was a significant negative correlation with the animal's growth rate and hot carcass weight; however, lesions did not correlate with the carcass quality grades of Choice or better.

Controlling both GI parasitism and BRD in growing livestock increases the health and welfare of animals and substantial opportunity exists to increase productivity also. Recently, egg antibodies have been used to target intestinal host proteins for the purpose of improving animal growth [see review by Cook and Trott (2010)]. The objective of these trials was to evaluate a new technology involving the oral administration of egg yolk antibodies against interleukin-10 (IL-10). Interleukin-10 is an anti-inflammatory cytokine which inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance (Couper et al., 2008). The following experiments investigated if oral administration of an antibody to an 8 amino acid sequence specific to bovine or ovine IL-10 (aIL-10) can aid in the control of these diseases.

MATERIALS AND METHODS

All animal procedures and protocols were approved by the University of Wisconsin College of Agricultural and Life Sciences Animal Care and Use Committee.

Peptide Selection, Antibody Production and ELISA Analysis. The Universal Protein Resource Knowledgebase (Uniprot KB) was used to access the sequence of human IL-10. The sequence was directly copied into BepiPred 1.0 server to identify antigenic regions. Based upon earlier trials (Sand and Cook, data not published) a peptide sequence with surface exposure was selected for antibody synthesis. An 8 amino acid sequence (VMPQAENH) was selected, and peptide synthesis was conducted by Genscript (Piscataway, NJ). Five milligrams of the peptide sequence were then conjugated to 5 mg bovine gamma globulin (BgG, Sigma Aldrich, St. Louis MO) using glutaraldehyde conjugation. Briefly, 5 mg of the carrier protein BgG was dissolved in 0.1 molar acetate buffer. Five milligrams of the peptide were then added to the buffer (1:1 wt/wt peptide to carrier protein), followed by 0.23 ml of 2 M glutaraldehyde and allowed to conjugate for 3 h at room temperature. The reaction was stopped with 10 mg glycine for 1 h. Dialysis occurred overnight in 1 L phosphate-buffered saline (PBS) at room temperature using 6000-8000 molecular weight dialysis tubing (Bobeck et al., 2012).

Production of antibodies to the peptide sequence was done using mature White Leghorn laying hens. All hens were hyperimmunized on d 0, 14, and 28. Each hen immunization consisted of 0.5 mg conjugated peptide-glutaraldehyde in 500 µl PBS emulsified with 500 µl of Freund complete adjuvant (Becton Dickinson, Sparks, MD). The emulsion was injected intramuscularly into each breast and thigh (0.25 ml at four injection sites). Eggs were collected continuously starting on d 21 after the primary injection and stored at 5°C for no longer than 4 mo. After storage, eggs were homogenized and pasteurized (62.8° C for 5 min) prior to feeding to target animals.

An indirect enzyme-linked immunosorbent assay (ELISA) was utilized to determine specific antibody concentration within the final egg product. Briefly, a 96-well Nunc immunosorbent F-series microplate (Sigma, St. Louis, MO) was coated with 100 µg/plate of peptide-specific ovalbumin (OVA) conjugate (conjugated as described above, except OVA was used as the carrier protein in place of BgG) in sodium carbonate buffer (pH 9.6) overnight (100 *u*L/well) at 4°C. After overnight incubation, the plate was washed 5 times with PBS/0.5% Tween solution, blocked with nonprotein blocking buffer (200 *u*L/well, Pierce Scientific, Rockford, IL), and allowed to incubate at room temperature for at least 1 h. The plate was washed 5 times and then primary antibody samples (standards and egg product samples from trials) were added at 100 uL/well in triplicate at 2-fold serial dilutions starting at 1:20,000 for the standard (1.2 mg peptide specific antibody/ml) and 1:100 for the egg product supernatant, and incubated for 1 h. Primary egg antibodies were produced by diluting freeze dried egg yolk samples or spray dried whole egg at 1:20 or 1:10, respectively, with acidified PBS (pH 5.1), storing overnight at 4^{o}C, and then collecting the supernatant after centrifugation (3,000 × g, 10 min). Purified antibody standards were procured from GeneTel Laboratories LLC (Madison, WI) and used to quantify antibody concentration in samples. After washing the plate 5 times, secondary antibody (HRP-conjugated rabbit anti-chicken antibody, Bethyl Labs, Montgomery, TX) was diluted in blocking buffer 1:1,000, added at 100 uL/well, and incubated for 30 min. After washing, substrate solution (1-Step Ultra TMB-Elisa, Thermo Scientific, Rockford, IL) was added at 100 uL/well and incubated until sufficient blue color development during the linear phase. A 0.5 M sulfuric acid stop solution was added to produce a stable yellow color and then absorbance at 450 nm was determined using a Bio-Tek EL800 plate reader. A standard curve was created based on the absorbance of the standards, and the curve was used to determine antibody concentration of the unknown samples.

Experiment 1.

Animals and Experimental Design. One hundred growing dairy type heifers (predominately Holstein genetics, but also Jersey and Holstein × Jersey crossbred) were utilized to assess the effects of feeding an antibody to interleukin-10 (aIL-10) to grazing animals in replacement of a common anthelmintic. The trial was conducted on a collaborating research farm located near Viroqua, WI which had been utilizing rotational grazing for over 30 years. All heifers were given similar veterinary treatment prior to the initiation of the trial. For 29 days, all animals were allowed to graze as a single group, and were rotated through pastures that had been known to carry infective larvae for common GI parasites. Pasture forage species were primarily orchardgrass (*Dactylis glomerata* L.), smooth bromegrass (*Bromus inermis* Leyss.), and red clover (*Trifolium pratense* L.). After this period, a single pre-feeding weight was collected to determine initial BW. Heifers were stratified by breed phenotype and balanced by initial BW across treatments. The 5 treatments assigned were 1) negative control (NC, no anthelmintic given), 2) positive control (PC, injectable parasiticide given at d 0; LongRange, Merial, Duluth, GA) 3) aIL-10 Low, 4) aIL-10 Medium, and 4) aIL-10 High. The aIL-10 levels of Low, Medium and High equaled 175, 350, and 3700 µg aIL-10 $\cdot$ hd^{-1} $\cdot$ d^{-1}. After treatments were assigned to the animals, heifers were sorted into treatment groups and the PC treatment was injected with LongRange at the recommended dosage (1 ml per 50 kg BW). From d 0 to 13, all treatments were fed a similar supplement and were maintained in their respective pasture where forage was not limited. During this 14 d period, all animals were given a common grain supplement (Table IV-1) at 2.3 kg as-fed $\cdot$ hd^{-1} $\cdot$ d^{-1}, while aIL-10 treatments had their appropriate level of aIL-10 included in the supplement, supplied as freeze dried egg yolk. All animals were weighed and a fecal sample was obtained on d 14. After the supplementation period, heifers were returned to one large group and allowed to rotationally graze pastures. A final BW and fecal sample was collected on d 72. During the trial, 4 heifers were removed from the study since each animal had a severe case of pinkeye (infectious bovine keratoconjunctivitis) in both eyes; 1 NC, 1 aIL-10 high, and 2 aIL-10 medium.

Sample collection and analysis. All fecal samples were obtained via a rectal grab collection technique and were recorded with the individual animal identification tag number. Once collected, the samples were chilled in a cooler and sent to an independent consultant for quantification of nematode eggs. Gastrointestinal nematode eggs were extracted using the Wisconsin sugar floatation method (Bliss and Kvasnicka, 1997) using 5 g of feces to determine fecal egg count (FEC). Eggs of nematodes were identified to the family level (e.g., strongyle, Nematodirus) and counted.

Experiment 2.

Animals and Experimental Design. A mixed population of purebred, female, growing lambs (55, 25, 10 and 10 head of Polypay, Hampshire, Rambouillet, and Targhee, respectively) that were born between 3 October, 2013 and 12 March, 2014 were all reared using similar management practices in confinement at the University of Wisconsin-Madison Arlington Agricultural Research Station located near Arlington, WI. Upon initial grazing allowance (June 3), all lambs were allowed to graze as one group for 35 days. Pasture forage species were primarily orchardgrass (*Dactylis glomerata* L.) and alfalfa (*Medicago sativa L.*). On d 0, lambs were weighed, stratified by breed, balanced by weight and sorted into 5 treatments. Because of analysis lag time, no attempt was made to balance the treatments for initial FEC. The 5 treatments were 1) negative control (NC, no anthelmintic given), 2) positive control (PC, ivermectin oral drench on d 0; Ivomec, Merial, Duluth, GA) 3) aIL-10 Low, 4) aIL-10 Medium, and 4) aIL-10 High. The aIL-10 levels of Low, Medium and High equaled 29, 57, and 115 µg aIL-10 $\cdot$ $hd^{-1} \cdot d^{-1}$. The positive control was administered Ivomec at 2× the recommended dose (0.25 ml/ kg BW) for the average weight of the group. From d 0 to 13, all treatments were fed a similar supplement and were maintained in their respective pasture where forage was not limited. In addition to ab libitum forage, ewe lambs were given 0.45 kg as-fed · $hd^{-1} \cdot d^{-1}$ of a common concentrate supplement (Table IV-2), while aIL-10 treatments had their appropriate level of aIL-10, supplied as freeze dried egg yolk, included in the supplement. All animals were weighed, and a fecal sample was obtained on d 14. After the supplementation period, lambs were returned to one large group and allowed to rotationally graze cool season pastures. A final BW and fecal sample was collected on d 35 from each lamb. During the trial, 1 lamb on the aIL-10 Low treatment died from pneumonia, and GI parasites were also observed during the necropsy.

Sample collection and analysis. All fecal samples were obtained via a rectal grab collection technique. Once collected, the samples were chilled in a cooler and sent to Colorado State University Veterinary Diagnostic Laboratories for quantification of nematode eggs. Gastrointestinal parasitic eggs were extracted using the Wisconsin sugar floatation method (Bliss and Kvasnicka, 1997) using 1 g of material. Eggs of nematodes were identified to the family-level and counted.

Experiment 3.

Animals and Experimental Design. A mixed population of one hundred growing, nearly yearling (i.e.,“old crop”) ewe and ram lambs were allowed to graze cool-season pastures without supplementation during the autumn and winter of 2014 at a collaborating ranch near Port Orford, OR. At the start of this trial, lambs were placed in a pasture composed primarily of annual ryegrass (*Lolium rigidum*) that had been tilled and planted the previous autumn. During this pasture exposure period, all lambs presumably ingested infective nematode larvae. Then all animals were individually weighed and a fecal sample was collected on February 3 (d 0). Once weighed, lambs were stratified by sex (ewe or wether) and breed-type (white face or crossbred), and balanced by initial BW across treatments. Treatments administered were 1) negative control (NC, no anthelmintic given), 2) positive control (PC, levamisole hydrochloride oral drench on d 0 and 49; Prohibit, AgriLabs, St. Joseph, MO), 3) aIL-10-7 (lambs were drenched for initial 7 d with 8 mls of solution containing egg yolk aIL-10) and 4) aIL-10-14 (lambs were drenched for initial 14 d with 8 mls of solution containing egg yolk aIL-10). Treatments containing aIL-10 were dosed with 115 µg aIL-10 $\cdot hd^{-1} \cdot d^{-1}$. During the 14 d treatment period (d 0 to 13) all lambs were handled and drenched daily with tap water (8 mls), Prohibit, or aIL-10 according to their assigned treatment.

Lambs were weighed on d 0, 14, 49 and 91 (February 2 and 15, March 24, and May 5, respectively), and a rectal grab sample of greater than 2 g fecal material was obtained for FEC. All lambs were shorn on d 57 and no adjustment was made to lamb BW after shearing due to loss of fleece weight, but the average fleece weight of the 100 lambs was 2.4 kg.

Since FEC were low on March 24, all lambs were moved to a permanent pasture that had been grazed by sheep and cattle in an effort to accentuate the GI parasite challenge to all treatments. This pasture was dominated primarily by perennial ryegrass and all lambs grazed in this pasture for the last 30 d of the trial. Five animals were removed from the data set since 2 died, 1 received antibiotic treatment, 1 NC received parasite control intervention, and 1 had a severe skin irritation at the end of the trial.

Sample collection and analysis. All fecal samples were obtained via a rectal grab collection technique. Once collected, the samples were chilled in a cooler and sent to Colorado State University Veterinary Diagnostic Laboratories for quantification of nematode eggs. Gastrointestinal parasitic eggs were extracted using the Wisconsin sugar floatation method (Bliss and Kvasnicka, 1997) using 1 g of material. Eggs of nematodes were identified to the family-level and they and coccidian oocysts were counted.

Experiment 4.

Animals and Experimental Design. One hundred thirteen beef steers were acquired from a Wisconsin auction barn via 14 lot purchases and transported 160 km to the Beef Nutrition research farm located on the Arlington Agricultural Research Station. Three days after arrival all animals received similar veterinary care and were weighed pre-feeding on day -1 and 0. Veterinary care items included testing for persistently infected bovine viral diarrhea virus, palpation of scrotum to ensure removal of testes, and administration of Bovi-Shield Gold 5 (Zoetis Animal Health, Exton, PA), Vision 7 (Bayer Corporation, Pittsburg, PA), and injectable Dectomax parasiticide (Zoetis Animal Health). After initial BW was determined, steers were blocked by BW and assigned to a confined and sheltered pen each of which held 6 or 7 animals. Pen size and design were similar for all treatments and allowed a minimum of 6.0 m^2 and 0.51 m of linear bunk space per animal. Pen was randomly assigned a treatment of 1) control (fed control egg product) or 2) aIL-10 (fed egg product containing aIL-10). The aIL-10 treatment was designed to deliver 500 μg antibody · hd^{-1} · d^{-1} using whole pasteurized eggs, while the control treatment was fed whole pasteurized eggs that came from chickens only injected with Freunds complete adjuvant and no peptide conjugate. Both egg products were fed for 14 d (d 0 to 13) as part of the total mixed ration (TMR). Feeding of the TMR (composition in Table IV-3) was done with a Rissler TMR cart (New Enterprise, PA) to allow ad libitum intake, and was delivered once daily (0800 hr).

Medication protocol. Animals were visually examined daily and treated for sickness if required. Additionally, on d 7 and 14, a rectal temperature was obtained for each animal, and if temperature was greater than 39.7^{o} C, the animal was considered to be undergoing bovine respiratory disease (BRD) and was treated with an antibiotic. Medication protocol and order of administration consisted of subcutaneous injections of 1) tulathromycin (Draxxin, Zoetis, Florham Park, NJ), 2) florfenicol (Nuflor, Merck Animal Health), and 3) oxytetracycline (LA-200, Zoetis, Florham Park, NJ) all of which were administered at the label dosage. To receive a second or third treatment, a minimum 3 d period following the previous treatment needed to elapse, and the animal was required to be nonresponsive to the previous treatment.

Statistical Analysis

Experiment 1. Data were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Animal was the experimental unit for all analyses. Class variables considered were treatment and breed, while breed was recognized as a random effect. The LSMEANS option was used to generate treatment means. An orthogonal contrast was used to determine if a linear effect of egg yolk aIL-10 inclusion level existed, and used values of 0.772, -0.0156, and -0.616. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 2. Data were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Animal was the experimental unit for all analyses. Class variables considered were treatment and breed, while breed was recognized as a random effect. The LSMEANS option was used to generate individual treatment means. An orthogonal contrast was used to determine if a linear effect of egg yolk aIL-10 inclusion level existed, and used values of 0.772, -0.156, and -0.616. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 3. Analysis of variance, using the PROC MIXED model procedure of SAS version 9.3, was used to test the effects of treatment, sex, breed type and all of their interactions. None of the interactions were found to be significant ($P > 0.05$) and were removed from the final model. Lamb was considered to be the experimental unit for this analysis, which included 95 lambs. The LSMEANS option was used to generate individual treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are reported. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

Experiment 4. Data were analyzed by analysis of variance using the MIXED procedure of SAS, version 9.3 (SAS Inst. Inc., Cary, NC.) for a randomized complete block design. Pen was the experimental unit for all analyses. Class variables considered were treatment and block, and block was recognized as a random effect. The LSMEANS option was used to generate individual treatment means. Significance was declared at $P < 0.05$, and tendencies of $P < 0.10$ are discussed. When the overall F-test was significant ($P \leq 0.05$), treatment means were separated using the PDIFF statement in SAS.

RESULTS

Experiment 1. At the end of the 72 d grazing period no differences were detected in heifer final BW (*P* ≥ 0.85, Table IV-4). However, average daily gain (ADG) of the PC treatment, which received LongRange on d 0, was greater than that of the NC which received no anthelmintic treatment (0.61 vs 0.46 kg/d, *P* = 0.01). A positive linear tendency (*P* = 0.10) was observed between aIL-10 level and ADG for Low, Medium and High levels (0.47, 0.50, and 0.56 kg/d, respectively). Additionally, growth rate of aIL-10 High (0.56 kg/d) was greater (*P* = 0.06) than the NC (0.46 kg/d).

At the initiation of the trial (d 0), strongyle FEC was similar among all treatments (*P* = 0.57, Table IV-5). On d 14, a difference (*P* = 0.05) between the NC and PC treatments (14 vs 2 strongylid eggs/5 g feces, respectively) was observed, which affirmed the efficacy of LongRange to control GI parasitism. However, by d 72 no difference was observed between the NC and PC treatments (8 vs 1 eggs/5 g feces), but the PC did have a lower (*P* < 0.05) FEC than the aIL-10 Low treatment (1 vs 17 eggs/5 g feces).

Experiment 2. Lamb treatment groups were allowed to graze cool season pastures for 34 d and did not differ (*P* ≥ 0.95, Table IV-6) in initial or final BW. A comparison of the NC to the PC revealed a numerically greater (*P* = 0.13) ADG (114 vs 68 g/d) for the PC treatment. The aIL-10 High treatment gained BW faster (*P* = 0.03) than the NC (138 vs 68 g/d), and a positive linear effect between aIL-10 level and growth rate was found to exist (*P* = 0.03).

During the initial 35 d inoculation period, a natural infection of GI parasites took place as represented by day 0 FEC in Table IV-7. Fecal egg shedding at the start of the trial was not different (*P* = 0.49) across treatments. Typical of the overdispersion of strongylid egg shedding in animals, individual ewes ranged between 0 and 3,900 eggs per gram (EPG) with over 75% of lambs shedding strongyle type eggs (Fig. IV-1). On d 14 of the trial, FEC was similar across all treatments ($P = 0.21$), but the PC was lower than the NC (40 vs 490 EPG). Fecal samples obtained on d 35 suggest both the PC and aIL-10 High had lower FEC than the NC ($P < 0.03$; 40 and 80, vs 590 EPG, respectively). Once again the FEC range was large for the entire population and ranged from 0 to 4,300 EPG.

Experiment 3. Initial BW was similar at the start of the trial for all treatments ($P = 0.96$), but ewe lambs were lighter than wether lambs ($P = 0.001$; 42.5 vs 47.0 kg). Final BW and growth rate for the 91 d trial were not different across treatments ($P = 0.51$), and wether lamb final BW was heavier, and had greater ADG than ewe lambs ($P = 0.01$).

Strongyle FEC were similar across all treatments ($P = 0.55$, Table IV-9) at the start of the trial, but white-faced lambs had lower FEC than crossbred lambs ($P = 0.01$; 64 vs 132 EPG). On d 14, strongyle FEC was lower ($P = 0.01$) for the PC than the NC (12 vs 77 EPG) which indicates efficacy of the anthelmintic used. The final FEC on d 91 showed a tendency ($P = 0.10$) for a difference in strongyle FEC across treatments, and the PC was lower than the NC ($P = 0.05$; 24 vs 107 EPG). There was a tendency for the PC coccidian oocyst score to exceed the score for NC coccidian oocyst score on d 14. Levamisole is not considered to suppress coccidial oocyst shedding.

Experiment 4. After receiving a growing diet for 64 d, final BW of steers receiving control or aIL-10-containing eggs was not different ($P = 0.14$, Table IV-10). Additionally, steer growth rate was not different ($P = 0.13$), but numerically aIL-fed steers grew 6.5% faster than the controls (1.58 vs 1.48 kg/d). Feed intake was similar for both treatments (*P* = 0.84) and was typical of the type and origin of the calves (8.2 kg/d). Analysis of feed efficiency (BW gain/feed DM consumed) showed steers fed aIL-10 were 6% more (P = 0.04) efficient than control fed animals.

Health records were recorded over the duration of the experiment and analysis of BRD treatments can be seen in Table IV-10. A similar proportion of the population (16%) was treated once for BRD across treatments and was not different (*P* = 0.97) due to treatment. However there was a tendency for more control steers to require two treatments for BRD control (7 vs 0%, *P* = 0.09) compared to the aIL-10 fed steers.

DISCUSSION

Experiment 1. Growth rates for average heifer performance (0.52 kg/d) and also the PC treatment (0.61 kg/d) were lower than previously observed on the collaborating stocker operation (0.77 kg/d). Approximately 70 d after grazing turn-out (d 42 of experiment), it was determined that the trace mineral supplement was being offered in feed bunks in a manner that provided less than 50% of the recommended exposure. No attempt was made to diagnose a mineral deficiency which may have accounted for the lower performance. However, the trace mineral supplement was then offered at the recommended rate per head daily until the end of the trial.

Initial body weights were not different between treatments. Over the course of the 72 d grazing trial, growth rate of the PC (0.61 kg/d) was greater than the NC (0.46 kg/d), which is consistent with the trial design and has been observed previously (Kunkle et al., 2013). A positive linear tendency between aIL-10 feeding level and growth rate was observed.

The dosage of aIL-10 should be re-evaluated to more fully characterize the dose-response relationship. Growth rate was similar between the PC and the aIL-10 High treatment suggesting that 700 µg aIL-10 per head daily may enable growth to occur at a rate equivalent to that of anthelmintic-treated cattle and despite the presence of a FEC equal to non-anthelmintic-treated cattle. This aIL-10 technology may represent a novel concept for control of GI parasitism.

The pre- and recurrent post-treatment FEC substantiate that the animals were infected pre- treatment and that continuous pasture contamination and animal exposure to infective larvae occurred throughout the 72 d trial. Geometric mean FEC prior to treatment was low (9 eggs per 5 g feces), and remained low in the NC for the duration of the study. Assessment of efficacy of the anthelmintic used to establish the PC (eprinomectin; LongRange) followed the recommendations of Wood et al., (1995) and Gasbarre, (2014). Reduction of FEC occurred in the PC compared to the NC after 14 d. Reduced fecal shedding of strongyle eggs would result in reduced larval contamination of pasture (Stromberg and Averbeck, 1999). It should be noted that all heifers grazed common pastures, thus all cattle had the same opportunity for ingestion of larvae and NC cattle provided a continuous source of pasture contamination. Feeding aIL-10 at 175 to 700 µg did not alter FEC at any time during the trial. Interestingly, ADG of aIL-10 High tended to be greater than the NC, but FEC was similar throughout the trial. This result demonstrates the need for a greater understanding of the aIL-10 mechanism.

Experiment 2. Weight gain results in this trial confirm the contribution of GI parasites to reduced productivity in growing lambs, as the PC lambs grew 67% faster than the non-treated NC lambs ($P = 0.13$). Feeding aIL-10 increased weight gain in a positive dose dependent fashion (linear = 0.03), and aIL-10 High grew faster (P = 0.03) than the NC, and similar to the PC treatment, which is in agreement with the results reported for experiment 1.

Fecal egg count data confirm the PC lambs contributed fewer nematode eggs to the pasture than the NC, all of which could potentially re-inoculate other lambs later in the grazing season. Treatment consisting of aIL-10 High also reduced fecal egg shedding on d 35, but did not affect shedding on d 14. This suggests that the host response to aIL-10 feeding for 14 d is manifested after d 14. An infective parasite is benchmarked by its ability to actively skew the host immune system to prevent elimination and development of immunity (Loukas and Prociv, 2001). This immune skewing results in the induction of a "modified Th2" response (White and Artavanis-Tsakonas, 2012) characterized by expansion of Th2 cells, alternatively activated macrophages, inducible regulatory T cells expressing Foxp3, increased levels of IL-10, and consequent suppression of the Th1/Th17 axis (McSorley and Loukas, 2010). Feeding aIL-10 is proposed to bind luminal IL-10, thereby limiting its effect on $CD4^+$ cells, and allowing unimpaired action by IFN-γ, IL-2, IL-4, and IL-5 to rid the parasite infection. Jacobs et al. (2015) demonstrated, in response to a *Haemonchus contortus* infection, that parasite-resistant sheep have a greater serum IL-4 concentration, which was associated with a marked FEC reduction. However, complete removal of IL-10 and its signaling during infection can lead to the onset of severe, often fatal immunopathology in IL-10 knockout mice (Hunter et al., 1997).

Experiment 3. Over the 91 d duration of the experiment, no differences were observed for lamb growth rate across treatments, except wether lambs grew faster than ewe lambs. Generally, the FEC of the NC lambs was low at d 0 and did not increase over the duration of the trial. This possibly was due to dry environmental conditions and/or increased immunity due to lambs being older at the trial initiation. There was a reduction in FEC due to de-worming in the PC lambs. Despite this reduction in FEC, no advantage was observed in lamb growth rate. This illustrates the natural resilience of lambs to GI parasites. A FEC threshold above which farmers should de-worm lambs is not easily defined. Multiple factors, as reported by Vercruysse and Claerebout (2001), can contribute to the level of parasitism animals can withstand. The definition of therapeutic, production-based, or preventative thresholds need to be addressed. In experiment 2, the mean FEC was approximately 500 EPG at d 0, and a positive response was observed for growth rate following ivermectin de-worming. For experiment 3, the mean FEC at d 0 was approximately 100 EPG and no advantage was observed for levamisole de-worming. The production-based threshold for lamb responsiveness to anthelmintic treatment presumably lies somewhere between 100 and 500 EPG.

Experiment 4. Feeding aIL-10 for the initial 14 d of the 64-d growing period increased steer feed efficiency 6% ($P = 0.04$). The percentage of steers that received only one treatment for BRD was similar between Control and aIL-10; however, more control animals required two treatments to control BRD. Further research is needed to explain how the fed aIL-10 could impart a resistance to disease in the respiratory tract. No steers fed aIL-10 required two or three antibiotic treatments which suggests the fed antibodies aided in the control of BRD. Gardner et al. (1999) reported steers that received antibiotic treatment for the control of BRD grew slower, and had a lower hot carcass weight than steers which had never been treated. Sowell et al. (1997) observed that steers treated for clinical disease spent 23% less time eating and made fewer trips to the feed bunk during a 32 d receiving period. However, their trial did not detect a difference in DMI over the duration of the trial. The respiratory and gastrointestinal tracts have many structural similarities (Mestecky, 1987) and lymphoid tissue, which is present in both tracts, regulates antigen sampling, lymphocyte trafficking and mucosal host defense (Holt, 1993). It is suggested that the similar inflammatory and immune components of these organs account for the overlap in pathological changes during respiratory and intestinal mucosal diseases (Keely et al., 2012). Zuercher et al. (2002) suggest that cross-protection occurs in different organs because the mucosal immune system is common to the digestive and respiratory tracts and it mediates trafficking of B cells and effector memory cytotoxic T cells. More research is needed to assess if a similar mechanism occurs in the bovine animal.

CONCLUSIONS

Feeding aIL-10 was shown to accelerate both cattle and lamb growth rate compared to non-dewormed animals when an adequate GI parasite challenge was present in pastured animals. Suppression of fecal egg shedding was evident in the aIL-10 fed lambs when initial FEC was greater than 500 EPG. Steers that consumed aIL-10 for the first 14 d after entering the feedlot had better gain efficiency and required fewer antibiotic treatments compared to control animals receiving current best management practices. These results encourage further exploration of dietary aIL-10 administration since it could be a novel technology for control of GI nematodes in lambs and calves and BRD in young cattle.

CHAPTER IV REFERENCES

Anderson, N., J. Armour, W.F.H. Jarrett, F.W. Jennings, J.S.D. Ritchie, and G.M. Urquhart. 1965. A field study of parasitic gastritis in cattle. Vet. Rec. 41:196–204.

Armour, J. 1980. The epidemiology of helminth disease in farm animals. Vet. Parasitol. 6:7-46.

Babcock, A., R. Jones, and M. Langemeier. 2006. Examining death loss in Kansas feedlots. Pages 46-52. In Beef Cattle Research-2006, Report of Prog. 959, Kansas State Univ., Manhattan. http://www.asi.k-state.edu/species/beef/research-and-extension/2006CD.pdf Accessed 13 July, 2015.

Bobeck, E.A., K.S. Burgess, T.R. Jarmes, M.L. Piccione, and M.E. Cook. 2012. Maternally-derived antibody to fibroblast growth factor-23 reduced dietary phosphate requirements in growing chicks. Biochem. Biophys. Res. Commun. 420:666-670.

Bliss, D.H., and W. G. Kvasnicka. 1997. The fecal examination: A missing link in food animal practice. Compend. Contin. Educ. Pract. Vet. 19 (Suppl. 4):S104-S109.

Cook, M.E., and D.L. Trott. 2010. IgY-Immune component of eggs as a source of passive immunity for animals and humans. World's Poul. Sci. J. 66:215-226.

Couper, K.N., D.G. Blount, and E.M. Riley. 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180:5771-5777.

Eysker, M., and H.W. Ploeger. 2000. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology 120:S109–S119.

Gardner, B. A., H. G. Dolezal, L. K. Bryant, F. N. Owens, and R. A. Smith. 1999. Health of finishing steers: Effects on performance, carcass traits, and meat tenderness. J. Anim. Sci. 77:3168–3175.

Gasbarre, L.C. 2014. Anthelmintic resistance in cattle nematodes in the US. Vet. Parasitol. 204:3-11.

Hilderson, H., P. Berghen, P. Dorny, and J. Vercruysse. 1987. Bovine ostertagiosis. Part 1: a review. Vlaams Diergeneeskd. Tijdschr. 56:269–295.

Holt, P.G. 1993. Development of bronchus associated lymphoid tissue (BALT) in human lung disease: a normal host defense mechanism awaiting therapeutic exploitation? Thorax. 48:1097-1098.

Hunter, C.A, L.A. Ellis-Neyes, T. Slifer, S. Kanaly, G. Grunig, M. Fort, D. Rennick, and F.G. Araujo. 1997. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. J. Immunol. 158:3311–3316.

Jacobs, J.R., S.P. Greiger, and S.A. Bowdride. 2015. Serum interleukin-4 (IL-4) production is associated with lower fecal egg count in parasite-resistant sheep. Vet. Parasitol. 211:102-105.

Keely, S., N.J. Talley, and P.M. Hansbro. 2012. Pulmonary-intestinal cross talk in mucosal inflammatory disease. Mucosal Immunol. 5:7-18.

Kunkle, B.N., Williams, J.C., Johnson, E.G., Stromberg, B.E., Yazwinski, T.A., Smith, L.L., Yoon, S., Cramer, L.G., 2013. Therapeutic and persistent (120 day) duration of efficacy, acceptability, and productivity of extended-release injectable eprinomectin when used under field conditions. Vet. Parasitol. 192:332–337.

Loukas, A., and P. Prociv 2001. Immune responses in hookworm infections. Clin. Microbiol. Rev. 2001: 14:689-703.

McSorley, H.J., and A. Loukas. 2010. The immunology of human hookworm infections. Parasite Immunol. 32:549–559.

Mestecky. J. 1987. The common mucosal immune system and current strategies for induction of immune response in external secretions. J. Clin. Immunol. 7:265-276.

Miller, J.E., and D.W. Horohov. 2006. Immunological aspects of nematode parasite control in sheep. 84(Suppl.):E124-E132.

Rezac, D.J., D.U. Thomson, S.J. Bartle, J.B. Osterstock, F.L. Prouty, and C.D. Reinhardt. 2014. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. J. Anim. Sci. 92:2595-2602

SAS Institute. 2008. SAS for Windows. Release Version 9.3. SAS inst., Cary, NC.

Sowell, B. F., J.G.P. Bowman, C. Huisma, M. E. Branine, and M. E. Hubbert. 1997. Feeding behavior of feedlot cattle. Proc. Southwest Nutrition and Management Conference, Phoenix, AZ.

Stromberg, B.E. and Averbeck, G.A., 1999. The role of parasite epidemiology in the management of grazing cattle. Int. J. Parasitol. 29:33–39.

Vercruysse, J. and E. Claerebout. 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Vet. Parasitol. 98:195-214.

White, R. R., and K. Artavanis-Tsakonas. 2012. How helminths use excretory secretory fractions to modulate dendritic cells. Virulence. 3:668–677.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, Y., Malone J.B.Jr., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine) Vet. Parasitol. 58, 181–213.

Woolums, A.R., G.H. Loneragan, L.L. Hawkins, and S.W. Williams. 2005. Baseline management practices and animal health data reported by US feedlots responding to a survey regarding acute interstitial pneumonia. Bovine Pract. 39:116-124.

Zuercher, A.W., H. Jiang, M.C. Thurnheer, C.F. Cuff, and J.J. Cebra. 2002. Distinct mechanisms for cross-protection of the upper versus lower respiratory tract through intestinal priming. J. Immunol. 169:3920-3925.

Table IV-1. Ingredient composition of supplement offered during the 14-d supplementation period to growing dairy type heifers while grazing cool season pastures in experiment 1.

Ingredient	Percentage, As-Fed basis
Cracked corn	32.8
Oats	30.8
DDGS[a]	29.2
Liquid molasses	7.2
Total[b]	100.0

[a]Offered to heifers at 2.3 kg as-fed · $hd^{-1} \cdot d^{-1}$

[b]aIL-10 treatments had their respective amount of dried egg yolk mixed in the above supplement.

Table IV-2. Ingredient composition of supplement offered during 14-d supplementation period to growing ewe lambs while grazing cool season pastures in experiment 2.

Ingredient	Percentage, As-Fed basis
Whole corn	72.50
Lamb pellet[a]	13.75
Oats	7.50
Liquid molasses	5.00
Ammonium chloride	0.50
Limestone	0.75
Total[b]	100.0

[a]Guaranteed analysis: minimum levels of crude protein 39%, crude fat 1.2%, P 0.5%, Se 0.7 ppm, and vitamin A 5,000 IU/lb, and maximum levels of crude fiber 4.0%, Ca 3.7%, salt 2.3%, Cu 17 ppm, and contained 63 g/ton Decoquinate.

[b]Offered to lambs at 0.45 kg as-fed · hd^{-1} · d^{-1}, and aIL-10 treatments had their respective amount of dried egg yolk mixed in the above supplement.

Table IV-3. Ingredient composition of the growing diet used when feeding pasteurized whole egg treatments to growing steers in experiment 4.

Ingredient	Percentage, DM basis
Cracked corn	15.91
Corn silage	70.00
DDGS[a]	5.84
Wheat midds[a]	5.00
Urea[a]	1.00
Limestone[a]	1.42
Salt[a]	0.20
Calcium sulfate[a]	0.09
Micro ingredients[ab]	0.63
Chemical Composition	
CP	12.0
NDF	33.9
Fat	2.72
Calcium	0.74
Phosphorus	0.36
Potassium	0.89
Ash	6.22
NEm, Mcal/kg	1.75
NEg, Mcal/kg	1.13

[a]Ingredients mixed together as one supplement
[b]Designed to meet NRC requirements for micro minerals and also provide 2790, 390, and 25 IU/kg diet DM of vitamins A, D, and E, respectively; also provided 22 g monensin and 11 g tylosin /1,000 g diet DM.

Table IV-4. Dairy heifer performance when allowed to graze cool season pastures for 72 days (experiment 1).

Treatment[1]	Initial BW, kg	Final BW, kg	ADG, kg
Negative Control (NC)	215	248	0.46[b]
Positive Control (PC)	213	256	0.61[a]
aIL-10 Low	215	249	0.47[b]
aIL-10 Medium	213	248	0.50[b]
aIL-10 High	210	250	0.56[ab]
SEM	12	13	0.06
P-Value			
Treatment	0.98	0.94	0.01
Linear	0.61	0.92	0.10
NC vs PC	0.79	0.46	0.01
NC vs High	0.61	0.85	0.06

[1]Positive control received LongRange on d 0. aIL-10 Low, Medium and High contained 175, 350, and 700 µg peptide specific antibody · $hd^{-1} \cdot d^{-1}$, respectively.

[a, b]Within a column, means without a common letter differ ($P < 0.05$).

Table IV-5. Fecal shedding of strongyle type eggs (per 5 grams feces) from dairy heifers allowed to graze cool season pastures for 72 days (experiment 1).

Treatment[1]	Day 0	Day 14	Day 72
Negative control (NC)	10	14	8[ab]
Positive control (PC)	5	2	1[b]
aIL-10 Low	6	20	17[a]
aIL-10 Medium	2	16	9[ab]
aIL-10 High	9	15	11[ab]
SEM	5.3	4.4	3.6
P-Value			
Treatment	0.57	0.06	0.04
Linear	0.74	0.39	0.14
NC vs PC	0.34	0.05	0.16
NC vs High	0.90	0.92	0.66

[1]Positive control received LongRange on d 0. aIL-10 Low, Medium and High contained 175, 350, and 700 µg peptide specific ab $\cdot hd^{-1} \cdot d^{-1}$, respectively.

[a, b]Within a column, means without a common letter differ ($P < 0.05$).

Table IV-6. Growing ewe performance when allowed to graze cool season pastures for 35 days in experiment 2.

Treatment[1]	Initial BW, kg	Final BW, kg	ADG, g
Negative control (NC)	48.4	50.8	68
Positive control (PC)	47.5	51.4	114
aIL-10 Low	48.1	50.4	68
aIL-10 Medium	47.0	50.3	97
aIL-10 High	47.7	52.5	138
SEM	2.6	3.0	35
P-Value			
Treatment	0.98	0.95	0.10
Linear	0.95	0.45	0.03
NC vs PC	0.71	0.82	0.13
NC vs High	0.79	0.56	0.03

[1]Positive control received Ivomec on d 0. aIL-10 Low, Medium and High contained 29, 57, and 115 µg peptide specific antibody · hd^{-1} · d^{-1}, respectively.

Table IV-7. Fecal egg shedding (eggs per gram of feces) of strongyle type eggs from ewe lambs allowed to graze cool season pastures for 35 days in experiment 2.

Treatment[1]	Day 0	Day 14	Day 35
Negative control (NC)	700	490	590
Positive control (PC)	370	40	40
aIL-10 Low	420	220	310
aIL-10 Medium	570	120	120
aIL-10 High	270	40	80
SEM	260	146	168
P-Value			
Treatment	0.49	0.21	0.10
Linear	0.41	0.52	0.34
NC vs PC	0.21	0.03	0.02
NC vs High	0.10	0.39	0.03

[1]Positive control received Ivomec on d 0. aIL-10 Low, Medium and High contained 29, 57, and 115 µg peptide specific antibody · $hd^{-1} \cdot d^{-1}$ respectively.

Table IV-8. Effect of parasite control treatment, sex, or breed type on growth performance of grazed growing lambs in experiment 3.

	Comparison[1]	n	Initial weight, kg	Final BW, kg	Overall ADG, g
Treatment	NC	24	44.9	51.8	73
	PC	25	45.1	50.3	55
	aIL-10-7	23	44.3	51.2	77
	aIL-10-14	24	44.8	51.8	77
	SEM	-	1.13	1.50	11.3
Sex	Ewe	46	42.5[b]	47.6[b]	55[b]
	Wether	49	47.0[a]	54.9[a]	86[a]
Breed	White face	67	45.1	50.9	64
	Crossbred	28	44.5	51.6	77
	P-value				
	Treatment	-	0.96	0.86	0.51
	NC vs PC	-	0.91	0.47	0.26
	Sex	-	0.001	0.001	0.01
	Breed	-	0.62	0.65	0.24

[1]NC= Negative control, PC= Positive control which was administered Prohibit on d 0 and 49. aIL-10 contained 115 µg peptide specific antibody · $hd^{-1} \cdot d^{-1}$ and was fed for 7 or 14 d.
[a, b]Within a column and category, means without a common letter differ ($P < 0.05$).

Table IV-9. Effect of parasite control treatment, sex, or breed type on strongyle eggs per gram of feces and coccidia oocyst score in experiment 3.

Comparison[1]		Day 0		Day 14		Day 91	
		Strongyle	Coccidia[2]	Strongyle	Coccidia	Strongyle	Coccidia
Treatment	NC	86	1.8	77	1.4	107	1.6
	PC	86	1.8	12	1.8	24	1.5
	aIL-10-7	125	1.9	-	-	96	1.5
	aIL-10-14	95	1.9	-	-	111	1.5
	SEM	23.2	0.12	13	0.14	29	0.16
Sex	Ewe	91	1.9	43	1.3	65	1.4
	Wether	105	1.9	41	1.4	104	1.7
Breed	White face	64[b]	1.8	24	1.4	80	1.5
	Crossbred	132[a]	2.0	60	1.3	90	1.5
	P-value						
	Treatment	0.55	0.77	0.01	0.06	0.10	0.99
	NC vs PC	0.98	0.76	0.01	0.06	0.05	0.76
	Sex	0.51	0.64	0.93	0.48	0.18	0.07
	Breed	0.01	0.21	0.08	0.63	0.75	0.81

[1]NC= Negative control, PC= Positive control which was administered Prohibit on d 0 and 49. aIL-10 contained 115 µg peptide specific antibody · hd^{-1} · d^{-1} and was fed for 7 or 14 d.
[2]Coccidia counts are coded as follows: 1= 1-10 oocysts per gram feces on the coverslip; 2= average of 1-10 oocysts/10X field; 3 = average of 11-100 oocysts/ 10X field.
[a, b]Within a column and category, means without a common letter differ ($P < 0.05$)

Table IV-10. Performance of newly arrived steers when fed control eggs or eggs containing antibodies to interleukin-10 (experiment 4).

	Treatment			*P*-Value
Item	Control	aIL-10[1]	SEM	Treatment
Performance				
No. of pens (steers)	9 (56)	9 (57)		
Initial BW, kg	297	297	7.55	0.77
Final BW, kg	391	397	8.7	0.14
ADG, kg	1.48	1.58	0.050	0.13
DMI, kg	8.1	8.2	0.20	0.84
G:F	0.182[b]	0.193[a]	0.0032	0.04
BRD treatment[2], %				
1X	16	16	5.4	0.97
2X	7	0	2.6	0.09
3X	2	0	1.1	0.35
Total[3]	35	16	8.4	0.15

[1]aIL-10 = 500 µg of antibody fed · $hd^{-1} \cdot d^{-1}$ for the initial 14 d.
[2]BRD= bovine respiratory disease. Decision to treat cattle was based on observed symptoms of BRD and/or rectal temperature in excess of 39.7^{o} C.
[3]Sum of total BRD treatments divided by total population.
[a, b]Within a row, means without a common letter differ ($P < 0.05$)

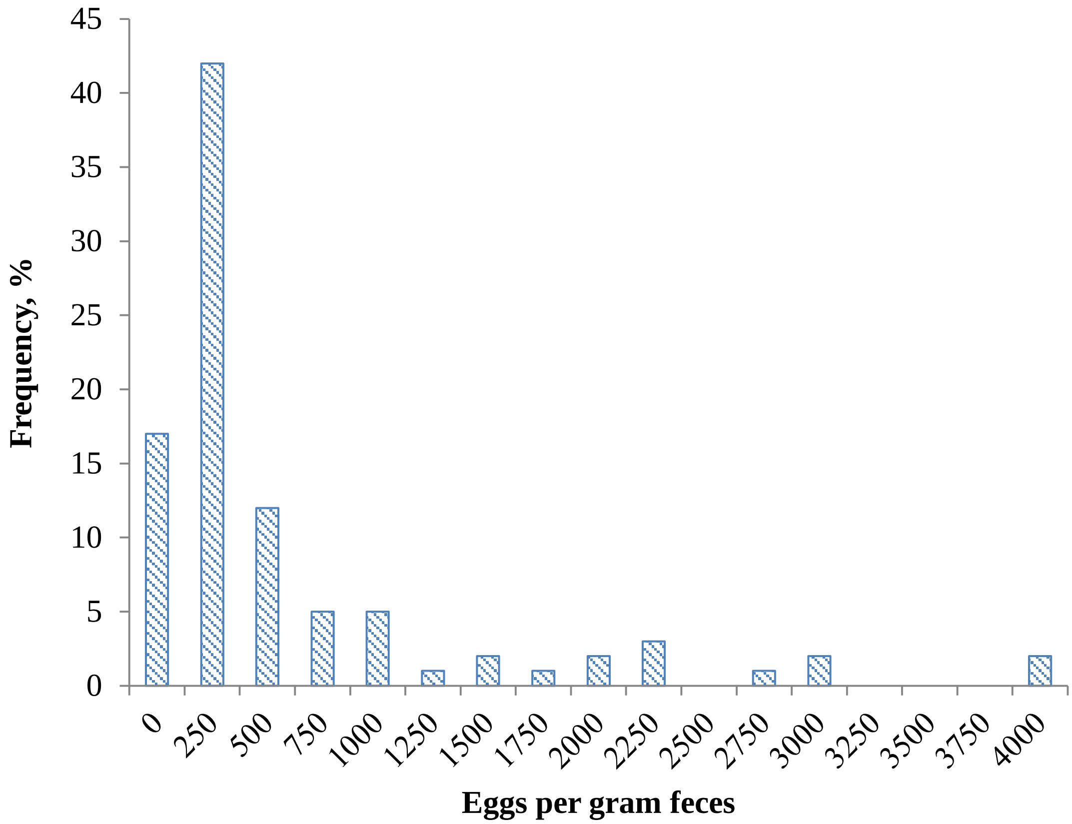

Figure IV-1. Histogram of fecal egg counts from 100 ewe lambs on d 0 of experiment 2.

APPENDIX A

Validation of an analytical technique to quantify IgY antibody concentration in various egg products and stability in dry feedstuffs

Introduction

Avian species, not being mammals and consuming colostrum, and with development that occurs in ovo without placental antibody transfer, rely on passive immunity which is acquired by maternal transfer of protective antibodies (IgY) during the formation of the egg. Antibodies deposited in the avian egg have been found to be a platform for the production of a diverse array of safe commercial products for improving animal health. Cook and Trott (2010) recently reviewed the efficiency of egg yolk antibody production, as well as storage, stabilization and use of egg yolk antibodies. Vaccinating laying hens against a single, or multiple organic molecule(s) permits researchers to produce antibodies that can be readily acquired without invasive procedures conducted on the animal. In total, one egg will contain approximately 150 mg IgY, and the amount of antigen-specific (poly-epitope) antibodies can near 10% of the total IgY (Tini et al., 2002). Quantification of the amount of antigen-specific IgY in egg products is needed, as IgY titer can vary between vaccination protocols and over time depending on the booster schedule.

Three main types of non-purified egg products can be used to provide IgY to the target species and are whole liquid egg, whole spray- or freeze- dried egg, and spray- or freeze-dried yolk. Each product has pros and cons, as whole liquid egg is the cheapest to manufacture, but shelf life is limited due to potential mold growth, while dried products increase production cost but have increased shelf life (Cook and Trott, 2010). A potential way to inhibit mold growth on whole liquid egg could be to mix whole liquid egg with another dry feed product to decrease the average moisture content of the mixture to less than 15% water. The objectives of the observational trials reported here are to validate an ELISA procedure for determining egg IgY content; and to test the hypothesis that mixing whole egg with dry feedstuffs can inhibit mold growth without losing antibody binding activity.

Materials and Methods

Egg products. Thirty eggs were procured on the same day from laying hens hyperimmunized to an 8 amino acid sequence. The peptide sequence and immunization protocol can be found in Chapter IV. Ten eggs were randomly sorted into the following treatment groups: 1) whole liquid egg (WLE), 2) whole freeze dried egg (WFE), and 3) yolk freeze dried (YFE). Eggs in the WLE treatment were cracked and mixed together to create a homogenous mixture, while WFE followed the same procedure but were then poured into a pan, and lyophilized for 2 d. Eggs in the YFE treatment were cracked, the egg yolk and albumen were separated, the yolks were mixed together, and then they were poured into a pan and lyophilized for 2 d. After lyophilization, each product was crushed using a mortar and pestle, and mixed.

Diluting whole egg with dry feedstuffs. Six eggs were collected from similar hens on a given day as described above. All 6 eggs were mixed together to create a WLE product, and 2 feedstuffs were used as the primary diluent. Mixtures contained 367 g pelleted wheat middlings (92% DM), 45 g salt, and 47 g WLE (PELLET), and the other contained 367 g alfalfa meal (91 % DM), 45 g salt, and 47 g WLE (HAY). For each, ingredients were added to a plastic zip-lock bag and mixed for several minutes until mixture appeared homogenous. A third bag which contained WLE was also used to confirm the environment was favorable for mold growth, and can be seen in Fig. A-1. All 3 bags were opened, and set out in the Poultry Research Laboratory so natural contamination could occur, much like could be experienced in the field. After 16 d, the bags were closed, re-mixed, and taken to the lab for analysis.

ELISA procedure. Two 96 well plates were coated with 100 µg/plate of peptide-specific ovalbumin conjugate in sodium carbonate buffer, and were allowed to sit over night at 4^{o}C. At a similar time, each product (WLE, WFE, YFE) was diluted in acidified PBS (aPBS) in a 15 ml centrifuge tube: 2.4 mls of WLE was added to 3.6 mls aPBS, 0.6g WFE was added to 5.4 mls aPBS, and 0.3g YFE was added to 5.7 ml aPBS. All were vortexed for at least 15 seconds, and incubated at 4^{o}C overnight. Ten grams of each treatment (PELLET or HAY) was added to 40 mls aPBS, vortexed, and allowed to incubate overnight at 4^{o}C. The remainder of the ELISA procedure was followed as previously outlined in Chapter IV, except when HAY or PELLET was analyzed, the supernatant was only diluted at 1:2 instead of the typical 1:100.

Results and Discussion

Figures A-2 and A-3 show the standard curves used to predict specific antibody concentration in the unknown egg product samples, and were considered acceptable (R^2 = 0.99). The products were found to have the following specific antibody concentrations: WLE = 2.5 µg /g WLE, WFE = 23.0 µg /g WFE, and YFE = 25 µg /g YFE. Mountney (1976) suggests the average egg weighs 50 g as-is, contains 74% water, and has 8.3 g dried yolk. Multiplying the antibody concentration of each product by their amount per egg shows a total specific antibody content of 125, 300 and 207 µg/egg for WLE, WFE, and YFE, respectively. The total antibody content per egg is quite low, as eggs from the same hens approximately 150 d prior contained 1,250 µg specific antibody/egg. No statistical analysis was conducted as each product had only 1 replicate. These data highlight the drastic change in antibody titer over time when hens were not re-boostered and that using the described ELISA procedure reports numerically similar antibody levels for each product.

Visual results suggest that leaving whole mixed egg exposed to a natural environment can allow for substantial mold growth within 10 d (Fig. A-1). However when whole egg was mixed with salt and a dry feed product, mold growth was inhibited (visual determination) for greater than 21 d. When feed products were assayed for specific antibody content, HAY contained 175 ng antibody/g mixture, and PELLET contained 411 ng/g mixture. No replicate ELISA analysis was made for either mixture, but it appeared the HAY sample was more variable in its sample consistency as clumps of product were observed. Mixing whole egg with dry feedstuffs appears to be a viable way to limit mold growth while maintaining antibody binding activity. Antibody recovery for the HAY was 68%, while PELLET recovery was 160%.

Figure A-1. Photos of the same bag over time containing whole mixed egg showing the development of mold and spores when exposed to the environment. Photos were taken 3, 5, and 10 d after exposure started

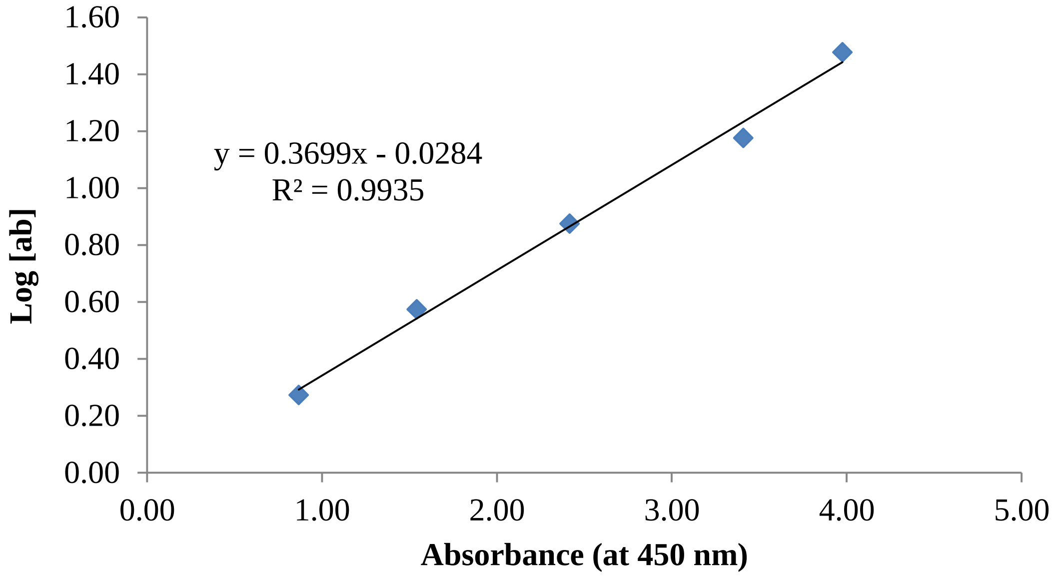

Figure A-2. Standard curve using a known amount of IgY antibody (1.9 to 30 ng/ml) against an 8 amino acid peptide sequence.

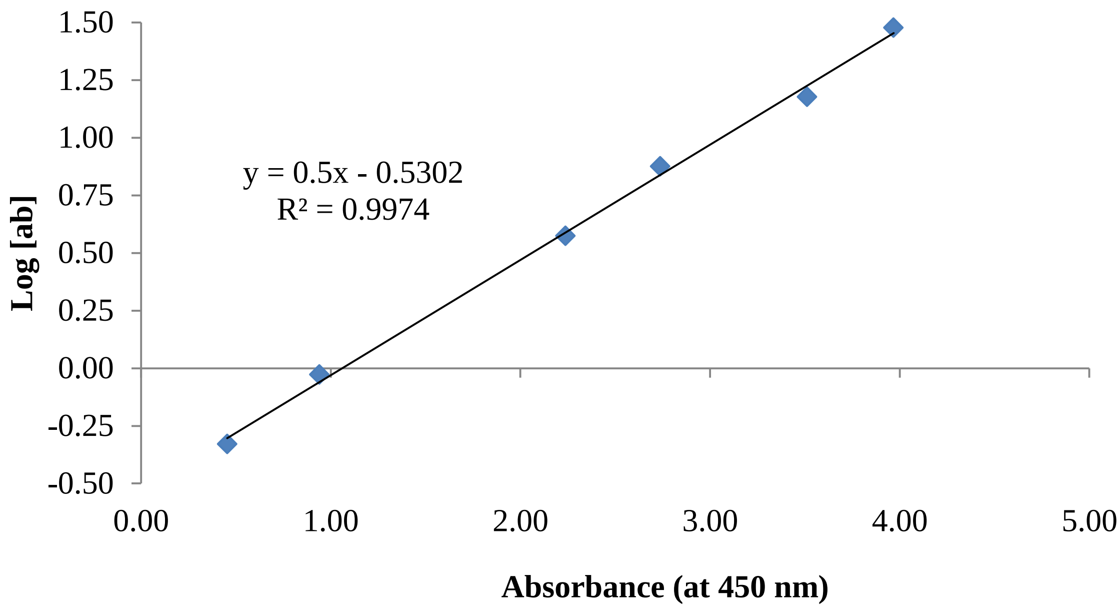

Figure A-3. Standard curve using a known amount of antibody (1.9 to 30 ng/ml) against an 8 amino acid peptide sequence.

APPENDIX B

Animal growth rate when fed various levels of anionic salts during pasture or confinement rearing

Introduction

To be profitable, a stocker operation requires dependable animal growth rates during the grazing season without negatively affecting forage yield. Enhancing average daily gain (ADG) can be accomplished by sustaining animal health, reducing parasite (external and internal) burden, feeding supplemental energy, implementing available technologies, good forage management, and more.

Maximizing feed efficiency in the feedlot is undoubtedly a key profit driver when feeding cattle. Determining feed efficiency on pasture however, is labor intensive and is seldom done. Several feedlot trials have fed intake limiters (such as anionic salts) as a potential way to increase feed efficiency, but few have been conducted with pastured cattle. Intake limiters are thought to either cause a physiological change within the animal therefore suppressing appetite, or to deter consumption by factors such as taste or smell. The goals of this grazing trial were to 1) supplement a palatable energy source to increase animal performance on pasture and 2) deliver increasing levels of anionic salts within the above supplement. The hypothesis was that calves will readily ingest the supplement and increase ADG, and as anionic salt inclusion increases, voluntary forage intake will decrease. The objective of the follow-up feedlot trial was to observe voluntary feed intake of a grass-legume diet and animal performance when fed differing levels of anionic salts in a supplement

.

Materials and Methods

Pasture trial. This study was conducted in 2013 at the Lancaster Agricultural Research Station with 45 non-implanted crossbred beef steers. Prior to turnout, steers were trained to use individual Calan gates, de-wormed, and weighed on two consecutive days prior to feeding. Once individual BW was determined, animals were balanced across 3 replicates in which initial BW averaged 287 ± 7 kg. Treatments consisted of an un-supplemented control (GRAZE) and 4 supplemented (SUPP) groups with increasing levels of anionic salts (0, 1, 2, and 3). Each replicate was considered a grazing unit that contained 15 steers (12 SUPP, and 3 GRAZE). Supplement treatments were fed at 1 kg supplement dry matter (DM) per 0.65% BW, and composition can be found in Table B-1. Body weights were taken every 28 d and feeding amounts were adjusted the following day. The trial lasted 113 d, starting on May 15, 2013 and ending September 5.

Pastures consisted of mixed cool season grasses and legumes. Animals were rotated every 3-4 d and forage allowance was similar across replicates. No attempt was made to determine individual animal forage intake during the grazing season. Feed efficiency was determined for each SUPP steer within each weighing period as follows:
[animal weight change – average weight change of 3 GRAZE animals within each replicate] / amount of SUPP DM fed.

Feedlot Trial. One hundred four calves (steers and heifers) which were born at the Lancaster Agricultural Research Station were used for a 70 d growing trial. Animal pre-fed weights were collected on two consecutive days to determine initial BW. Animals were balanced by weight and sex into 8 semi confined bedded pens. Four treatments were imposed and replicated twice, with treatments being 0, 1, 2, and 3. Each pen was fed to ad libitum intake of a grass-legume silage that was ensiled in the spring of 2013, which was similar in composition as the pasture trial swards. Additionally, each pen was fed a fixed amount of their respective supplement (Table B-1) at 0.65% BW, which was similar to the pasture trial. Daily feed deliveries were recorded and used to determine total DM intake and G:F efficiency. Final pre-feeding BW was collected on d 68 and 69.

Results and Discussion

Pasture Trial. No differences among SUPP treatments were detected for ADG or G:F (Table B-1), therefore only the main effect of SUPP vs GRAZE will be discussed. Animal performance was greater for SUPP steers in all periods except for period 4, and tended to be similar in period 3 as can be seen in Fig. B-1. Figure B-2 displays the 3 replicated pastures, and how G:F varied across replicates and grazing period. Feed efficiency ratios were very favorable in the early grazing season but decreased as the season progressed. In replicate 1, no positive effect was found after the second grazing period. It is worthy to note the variation across replicates and time.

Across the four various anionic salt inclusion levels; no differences in supplement intake or growth rate were detected. This suggests the levels of anionic salts fed did not suppress voluntary supplement intake, but raises the question if forage intake was suppressed by high levels. Overall supplement feed efficiency values were favorable for reps 2 and 3, but not for rep 1 (Table B-2). It should be noted that animal performance for the GRAZE treatments in reps 2 and 3 was low, revealing supplemental feed may be more advantageous in lower quality swards to increase ADG. Other WI grazing research (Schaefer et al., 2014) has reported stocker ADG values of > 1.0 kg/d without supplemental feeding. The ability to manage swards to increase

ADG (through species selection or grazing management) is thought to be a lower cost alternative than supplemental feeding.

This trial suggests that feeding an energy supplement is able to increase stocker ADG, especially when animals are gaining less than 0.70 kg/d. This is probably due to the drastic difference in energy content of a low quality sward vs the supplement (reps 2 and 3), but higher quality swards approach energy content of some supplements (rep 1). It still may be advantageous to supplement high quality swards where little increase in ADG would be expected, but supplementation may increase pasture stocking rate or grazing days.

Feedlot Trial. Over the 70 d feeding trial no differences ($P \geq 0.35$) were observed for any growth or feed intake measurement. This trial utilized similar feedstuffs and calves with initial BW similar to the pasture trial. Similar growth rates were observed. In the feedlot trial, voluntary intake of the grass-legume silage was similar and not affected by level of anionic salts in the supplement. Therefore it is unlikely that supplementing anionic salts will lower voluntary forage intake in a pasture setting.

Table B-1. Supplement composition fed to grazing or confined growing calves on a DM basis (%), and season long ADG and G:F ratio of stocker steers.

	SUPP Treatment			
	0	1	2	3
Supplement Composition				
Ground corn	47.67	47.12	46.64	46.13
DDG	48.23	47.65	47.12	46.67
Limestone	2.12	2.12	2.12	2.12
Ca Sulfate	0	0.82	0.82	0.82
NH4Cl	0	0.31	1.33	2.28
Molasses	1.98	1.98	1.98	1.98
Animal Performance[1]				
ADG, kg	1.05	0.94	1.00	1.02
G:F ratio	0.189	0.110	0.141	0.151

[1]Animal performance of the pasture trial. No difference was detected across treatments ($P > 0.10$).

Table B-2. Overall stocker steer performance across treatments and within replicates.

		Overall Performance, kg		
Replicate	Treatment	ADG	Supplement DM Intake	G:F
Rep 1	SUPP	1.07	237	0.080
	GRAZE	0.90	0	-
Rep 2	SUPP	0.99	241	0.189
	GRAZE	0.60	0	-
Rep 3	SUPP	0.94	227	0.157
	GRAZE	0.63	0	-
SEM		0.05	4	0.022
P-Value				
Treatment		0.001	-	-
Replicate		0.01	0.07	0.01

Table B-3. Animal growth performance when fed various levels of anionic salts during a feedlot growing trial.

		Item, kg				
Treatment	No. of pens (hd)	Initial BW	Final BW	ADG	DMI	G:F
0	2 (13)	270	334	0.91	6.9	0.130
1	2 (13)	271	333	0.89	6.9	0.128
2	2 (13)	270	331	0.86	6.9	0.124
3	2 (13)	271	333	0.88	6.9	0.128
SEM		2	2	0.02	0.1	0.004
P-Value						
Treatment		0.95	0.64	0.35	0.93	0.51
Linear		0.63	0.74	0.26	0.77	0.36

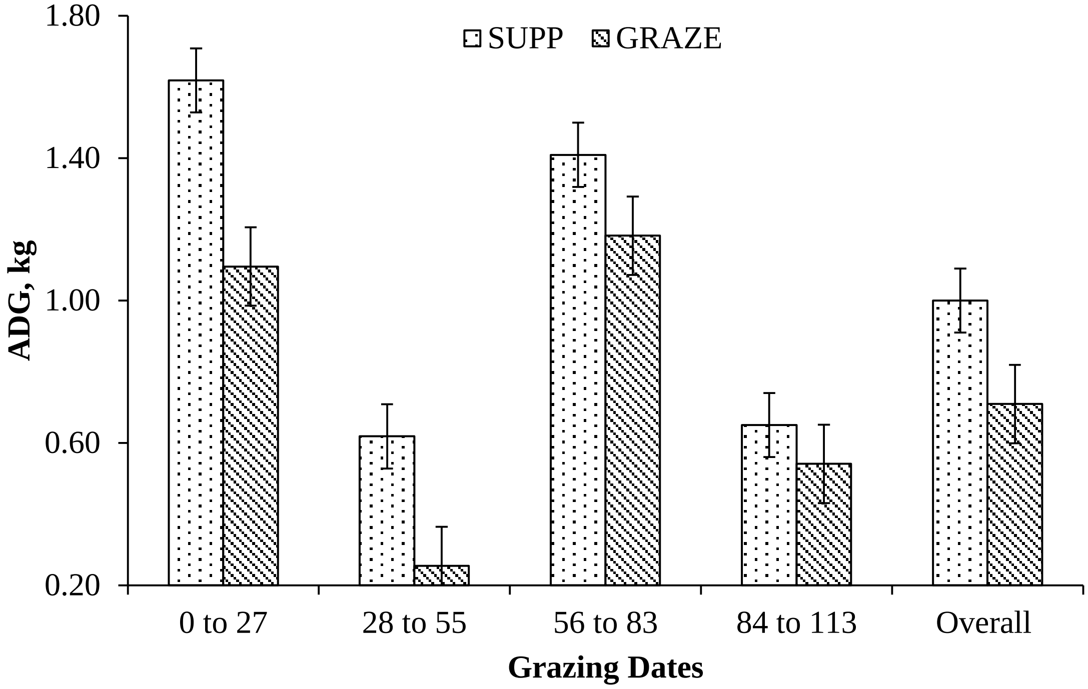

Figure B-1. Stocker steer performance when supplemented at 0.65% BW or only allowed to graze cool season pastures.

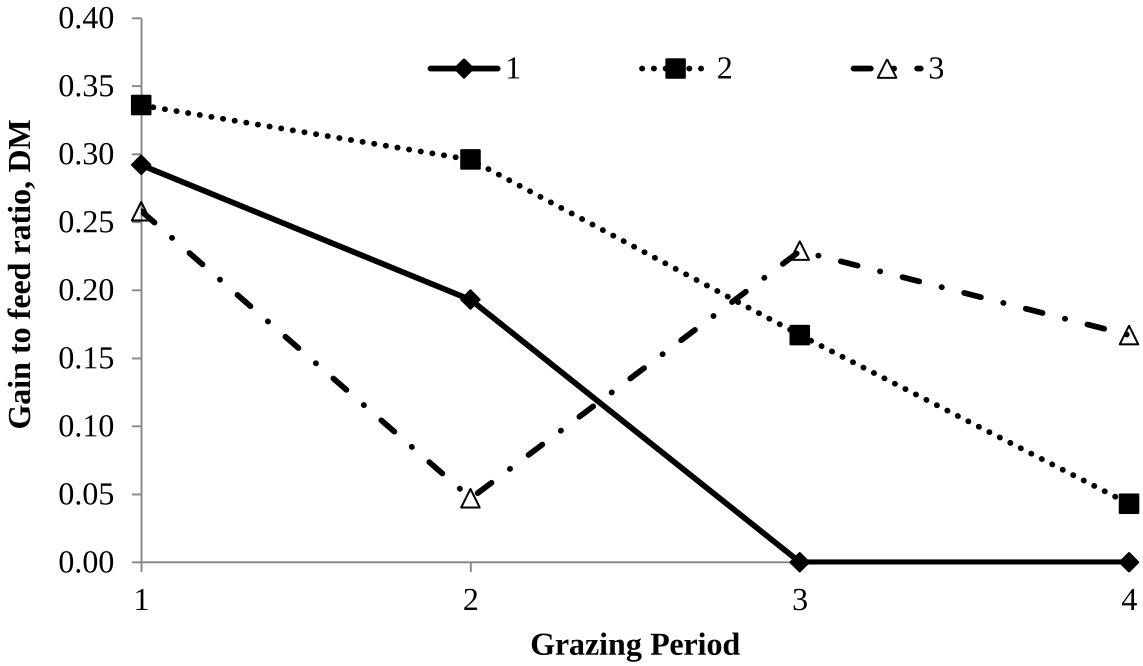

Figure B-2. Stocker steer gain to feed ratio as grazing season progressed across 3 replicates.

APPENDIX C

Heavy feedlot steer growth responses when fed various levels of rumen degradable protein

Introduction

Adequate metabolizable protein (MP) intake is necessary for growth and many other physiological demands when raising livestock. It has been estimated that steers implanted with a medium to high potency implant require 8.04 g MP/kg BW$^{0.75}$ (DiCostanzo and Zehnder, 1999). Protein supplementation is required in corn based diets, as corn grain or corn silage alone cannot provide enough protein to allow for maximal gains. The quantity and quality of MP supplied by rumen microbes and the basal feedstuffs need to be sufficient when energy is not limiting. Wagner et al. (2010) reported a linear increase in ADG (5.9%) and DMI (5.8%) when feeding more rumen degradable protein (as well as more crude protein), but G:F was not altered. The dilution of maintenance concept argues that G:F should have increased as DMI increased in the trial. The objective of the current trial was to evaluate steer growth responses when fed various levels of degradable intake protein (DIP) at a fixed DMI allowance across iso-nitrogenous diets.

Materials and methods

One feedlot trial was conducted with 60 crossbred beef steers procured from a local Wisconsin auction barn. All steers received similar veterinary care, including an implant of Synovex-S and were weighed on 2 consecutive d prior to feeding. Once initial BW was determined, animals were blocked by BW and randomly assigned to a confined pen under roof (5 hd/pen). Four treatments of DIP level (4.75, 5.75, 6.75 and 7.75% DM) were designed (Table C-1) and randomly assigned to pen within a block. Steers were sorted into 3 weight blocks and fed for 59, 80, and 95 d. Final weights were taken on 2 consecutive d prior to feeding, and no carcass data was collected. Data were analyzed using ANOVA via SAS and pen was the experimental unit for all analyses.

Results and discussion

Diets were fed at a fixed percentage of live BW within block. Steer DMI was similar across treatments by trial design. ADG (P = 0.11) and G:F (P = 0.08) tended to decrease with higher DIP levels (Table C-2). Observed performance and performance predicted by the NRC level 2 model were compared. The NRC model suggested metabolizable energy (ME) to be limiting growth for the 4.75, 5.75, and 6.75 treatments, but MP to be growth-limiting in the 7.75 % DIP treatment. Observed performance divided by the NRC suggested limiting nutrient (ME or MP) was 99, 104, 102, and 114% for 4.75, 5.75, 6.75 and 7.75, respectively. This research highlights the need to balance ME and MP, and the potential over-estimation of protein requirement by the NRC model, however the NRC was quite accurate in predicting steer performance when ME was the limiting nutrient.

Table C-1. Diet composition when various levels of rumen degradable intake protein (DIP) were fed to heavy yearling steers, dry matter basis.

	Treatment, % DIP			
	4.75	5.75	6.75	7.7
Cracked corn	83.03	84.04	85.05	86.06
Corn silage	10.00	10.00	10.00	10.00
Urea[a]	0.01	0.47	0.93	1.39
Blood meal[a]	4.67	3.18	1.69	0.21
Limestone[a]	1.44	1.44	1.43	1.43
Salt[a]	0.20	0.20	0.20	0.20
Ca sulfate[a]	0.10	0.13	0.15	0.18
Vitamins[a]	0.23	0.23	0.23	0.23
Tylan 10 and Monensin[ab]	0.26	0.26	0.26	0.26
Trace minerals[ab]	0.05	0.05	0.05	0.05
Predicted chemical composition				
Crude protein	12.0	12.0	12.0	12.0
Degradable intake protein	4.75	5.75	6.75	7.75
NDF	11.81	11.89	11.96	12.04
Fat	3.87	3.89	3.91	3.93
Ca	0.60	0.60	0.60	0.60
P	0.30	0.30	0.30	0.30
NEg, Mcal/kg DM	1.42	1.42	1.42	1.42

[a]Ingredients mixed together as one pelleted supplement
[b]Designed to provide NRC requirements for micro minerals and provide 2790, 390, and 25 IU/kg diet DM vitamins A, D, and E, respectively; and provide 22 g monensin and 11 g tylosin /1,000 kg diet DM.

Table C-2. Heavy feedlot steer growth performance when fed various levels of rumen degradable intake protein at fixed intakes.

	Treatment, % DIP					*P*- Value		
	4.75	5.75	6.75	7.75	SEM	Trt	Linear	Quad.
No of pens (steers)	3 (15)	3 (15)	3 (15)	3 (15)				
Initial BW, kg	453	454	453	455	1	0.38	0.36	0.49
Final BW, kg	586	591	584	578	5	0.34	0.18	0.28
ADG, kg	1.72	1.79	1.73	1.58	0.06	0.17	0.11	0.11
DMI, kg	10.0	10.0	10.0	10.0	0.1	0.21	0.14	0.98
G:F	0.173	0.178	0.173	0.157	0.006	0.14	0.08	0.10
Theoretical models								
ME allowable ADG[1]	1.76	1.74	1.71	1.69				
MP allowable ADG[1]	2.60	2.22	1.85	1.48				
MP from Bacteria, g/d[1]	536	540	543	546				
MP from UIP[1]	482	386	290	195				
Total MP supply[1]	1018	926	833	741				
MP requirement[2]	791	791	791	791				

[1]Predicted using the NRC level 2 model for a 12 mo. old Angus × Hereford steer given the assigned diets and feed intake. ME = metabolizable energy, MP = metabolizable protein, UIP = undegradable intake protein.
[2]Calculated using the equation by Dicostanzo and Zehnder (1999).

APPENDIX REFERENCES

Cook, M.E., and D.L. Trott. 2010. IgY-Immune component of eggs as a source of passive immunity for animals and humans. World's Poul. Sci. J. 66:215-226.

DiCostanzo, A., and C. M. Zehnder. 1999. Estimation of protein requirements of feedlot steers. Prof. Anim. Sci. 15:116–123.

Mountney, G.J. 1976. Poultry products technology, second edition. Food Products Press. Binghamton, NY.

National Research Council. 2000. Nutrient requirements of beef cattle. 7th revised ed. Natl. Academy Press, Washington, DC.

Schaefer, M.R., K.A. Albrecht, and D.M. Schaefer. 2014. Stocker steer performance on tall fescue or meadow fescue alone or in binary mixture with white clover. Agron. J. 106:1902-1910.

Tini, M., U.R. Jewell, G. Camenisch, D. Chilov, and M. Gassman. 2002. Generation and application of chicken egg-yolk antibodies. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 131: 569-574.

Wagner, J.J., T.E. Engle, and T.C. Bryant. 2010. The effect of rumen degradable and rumen undegradable intake protein on feedlot performance and carcass merit in heavy yearling steers. J. Anim. Sci. 88:1073-1081.