2021

MONOCYTE ABUNDANCE AND WHITE BLOOD CELL EXPRESSION OF PROLACTIN AND NF-KB PATHWAY GENES IN BEEF STEERS ARE ALTERED BY GRAZING TOXIC ENDOPHYTE-INFECTED TALL FESCUE AND LIPOPOLYSACCHARIDE CHALLENGE

Cecilia Winfrey
University of Kentucky, cwi248@g.uky.edu
Digital Object Identifier: https://doi.org/10.13023/etd.2021.388

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Cecilia Winfrey, Student

Dr. James C. Matthews, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

MONOCYTE ABUNDANCE AND WHITE BLOOD CELL EXPRESSION OF PROLACTIN AND NF-KB PATHWAY GENES IN BEEF STEERS ARE ALTERED BY GRAZING TOXIC ENDOPHYTE-INFECTED TALL FESCUE AND LIPOPOLYSACCHARIDE CHALLENGE

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By
Cecilia Jeanne Winfrey
Lexington, Kentucky
Director: Dr. James C. Matthews, Professor of Animal and Food Sciences
Lexington, Kentucky
2021

ABSTRACT OF THESIS

MONOCYTE ABUNDANCE AND WHITE BLOOD CELL EXPRESSION OF PROLACTIN AND NF-KB PATHWAY GENES IN BEEF STEERS ARE ALTERED BY GRAZING TOXIC ENDOPHYTE-INFECTED TALL FESCUE AND LIPOPOLYSACCHARIDE CHALLENGE

Experiment 1 tested the hypothesis that ad libitum consumption of a 1:1 blend (MIX) of sodium selenite (ISe) and SELPLEX vs solely ISe as selenium (Se) supplements (27 ppm) in a vitamin-mineral mix (V-M) by steers subjected to summer-long grazing of toxic endophyte-infected tall fescue (TE) would ameliorate the negative effects of TE by positively affecting the expression pattern of prolactin (PRL) and NF-kB pathway genes by circulating leukocytes (WBC). Experiment 2 tested the hypothesis that the MIX form of supplemental Se also would ameliorate the negative effects of *in vitro* blood lipopolysaccharide challenge (LPS) on WBC gene expression. The MIX form of Se increased whole blood Se, serum PRL, and red blood cell concentrations; decreased monocyte abundance; but did not affect WBC gene expression in either pathway or experiment. However, Experiment 1 revealed that TE consumption altered expression of 5 PRL pathway genes, and 7 NF-kB genes, suggesting a reduced adaptive immune response in cattle consuming TE. Experiment 2 found that LPS challenge differentially affected expression of WBC PRL receptor (PRLR) isoforms, and altered expression of 4 PRL, and 4 NF-kB pathway genes, indicating an increased inflammatory response in cattle consuming TE with exposure to LPS.

KEYWORDS: Bovine, Fescue Toxicosis, White Blood Cells, Gene Expression, Prolactin, NFκB

Cecilia Jeanne Winfrey

(Name of Student)

09/20/2021

Date

MONOCYTE ABUNDANCE AND WHITE BLOOD CELL EXPRESSION OF PROLACTIN AND NF-KB PATHWAY GENES IN BEEF STEERS ARE ALTERED BY GRAZING TOXIC ENDOPHYTE-INFECTED TALL FESCUE AND LIPOPOLYSACCHARIDE CHALLENGE

By
Cecilia Jeanne Winfrey

Dr. James C. Matthews
Director of Thesis

Dr. David L. Harmon
Director of Graduate Studies

09/20/2021
Date

ACKNOWLEDGMENTS

Throughout the writing of this thesis, I have received a great deal of support and assistance. I would first like to thank my advisor, Dr. James C. Matthews, whose expertise was invaluable in formulating my thesis topic. Your insightful feedback and encouragement allowed me to develop proper talks and writing throughout my journey. Next, I would like to extend my gratitude to Dr. Philip J. Bridges and Dr. James L. Klotz for serving on my advisory committee. I am grateful for each individuals input and valuable insights for the completion of my thesis

I would like to acknowledge the members of my lab for their support and collaboration on ideas. Specifically, Yang Jia for assisting me with research protocols and teaching me new lab techniques. Additionally, Kwangwon Son, Hugo Hamilton, and Susan Hayes for their technical and instrumental support on laboratory assays and sample analysis.

Next, I would like to thank Blair Knight and the crew in UKREC, Princeton for their innumerable support and management of the beef steers used in this thesis. My gratitude is also extended to my friends who have helped me throughout this journey, including those from the graduate program.

Lastly, I am always thankful for my parents and family for their unconditional support and love for me. They have always encouraged me to be successful and further my education. The success of my graduate career would not have been possible without the positive encouragement from my friends and family along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Most tall fescue (*Lolium arundinaceum*) pasture commonly used in livestock grazing systems in the eastern half of the United States is infected with an endophytic fungus *(Epichloë coenophiala*) (Leuchtmann et al., 2014) . The beneficial qualities of tall fescue are directly related to the ergot alkaloids produced by the fungus *Neotyphodium coenophialum* (Siegel and Bush, 1997). Commonly, consumption of ergot alkaloid-infected tall fescue impairs several vascular, metabolic, growth, and reproductive processes in cattle, producing a clinical condition known as "fescue toxicosis" (Strickland et al., 2011). There are over 7.7 million beef cattle grazing endophyte-infected tall fescue pasture (Bussard and Aiken, 2012). The economic loss attributed to fescue toxicosis exceeds one billion dollars per year in the United States (Strickland et al., 2009). A reduction in serum prolactin is a recognized marker of fescue toxicosis (Goetsch et al., 1987; Davenport et al., 1993). Studies have suggested that cattle suffering from fescue toxicosis are more susceptible to disease during transit and in feedlots due to a suppressed immune response. Exposure to ergot alkaloid increases pro-inflammatory cytokine secretion (Filipov et al., 1999a); a recent study (Poole et al., 2019) showed chronic exposure to ergot alkaloids led to increased concentrations of pro-inflammatory cytokines that act to mediate innate immunity.

Aside from fescue toxicosis, another challenge many southeastern United States cattle producers face is selenium (Se) deficiency. Soils in this region are Se-poor, and result in Se-deficient forages requiring the need for supplemental Se (Dargatz and Ross, 1996a). The most common form of Se supplemented in cattle diets is inorganic Se (ISe, sodium selenite), and organic forms of Se (OSe) derived from specially cultivated Saccharomyces

cerevisiae are also available and approved for use in beef cattle. Expression of several genes found to be downregulated in the liver (Liao et al., 2015) of steers grazing high vs. low toxic endophyte-infected forages and consuming ad libitum amounts of vitamin-mineral mixes (VM) containing 35ppm ISe, were upregulated in slow-maturing heifers consuming 3 mg/d Se as a 1:1 blend of ISe:OSe (MIX) vs. ISe (Matthews and Bridges, 2014; Matthews et al., 2014). Additionally, it was determined that steers subjected to summer-long grazing of endophyte-infected pasture and supplemented (3mg/day) as the MIX form had greater serum prolactin concentrations than those consuming the ISe form of supplemental Se (Jia et al., 2018). Se has been shown to impact immune function (Rayman, 2000), and enhance both humoral and cellular immune responses (Reddy and Massaro, 1983). Additionally, in the murine adaptive immune system it was shown that Se played a role in the formation and activity of helper T, cytotoxic T, and natural killer (NK) cells.

Decades of research has characterized the symptoms and physiological effects of fescue toxicosis. However, one area that lacks information is the effect of ergot alkaloids on beef cattle immune response. In addition to our knowledge, studies regarding the effects of forms of Se on immune expression in bovine white blood cells (WBC) have not been reported.

CHAPTER 2. LITERATURE REVIEW

2.1 Tall Fescue

2.1.1 Introduction

Tall fescue (*Lolium arundinaceum)* is a cool season perennial grass, most widely used in the southeastern United States (Steen et al., 1979; Pendlum et al., 1980). Tall fescue mainly originated in Western Europe (Hoveland, 2009), and has been dispersed and cultivated in Australia, Eastern Asia, North and South America, South Africa, and New Zealand (Hannaway et al., 2009). The extensive planting of tall fescue did not occur until the two cultivators Alta and Kentucky 31 were released in the early 1940's (Hoveland, 2009). Kentucky-31 is an ecotype that was found growing in a steep mountain pasture in Eastern Kentucky, and is now the most common cultivator (Hoveland, 1993a). Tall fescue covers an estimated 35 million acres of livestock pasture, hay fields and roadways (Ball et al., 2007). Tall fescue is widely utilized due to its ease of establishment, range of adaptation, extended grazing season, resistance to pests, tolerance to poor management (Stuedemann and Hoveland, 1988), and is the most heat tolerant of cool season grasses (Ball et al., 1991). Previously, according to Hoveland (1993b), 8.5 million cattle and 688,000 thousand horses grazed on tall fescue pastures, whereas it is currently estimated by Bussard and Aiken (2012) that 7.7 million beef cattle are maintained on tall fescue, comprising nearly 26% of the total US beef cattle population.

The beneficial qualities of tall fescue are a direct result of a symbiotic relationship with the fungus *Neotyphodium coenophialum* (Siegel and Bush, 1997). The fungus produces ergot alkaloids which are beneficial to tall fescue, but harmful to animals

consuming *N. coenophialum* infected tall fescue inducing a toxicity syndrome known as "fescue toxicosis" (Strickland et al., 2009). The predominant ergot alkaloid is ergovaline, accounting for 80-97% of the ergot alkaloids produced by the fungus (Arnold et al., 2014), and is recognized as responsible for fescue toxicosis (Guerre, 2015). However there are a variety of alkaloids produced that contribute to ergot alkaloid-induced fescue toxicosis including ergotamine, ergonovine (Guerre, 2015), and ergovalinine (Aiken and Flythe, 2014) (Figure 2.1).

2.1.2 Fescue Toxicosis

Fescue toxicosis is the collective term used to describe a set of biochemical syndromes that affect cattle, horses, and sheep consuming infected tall fescue (Cheeke, 1995). Commonly, consumption of tall fescue causes three syndromes in cattle: fescue foot, fat necrosis, and summer slump. Fescue foot was first described in New Zealand by (Cunningham, 1949) and occurs more commonly in winter weather/colder temperatures (Bush and Buckner, 1973). Fescue foot, similar to foot rot causes cattle to limp, and can lead to gangrene. Typically, fescue foot occurs in rear feet (Thompson and Stuedemann, 1993). Fat necrosis is characterized by formation of necrotic fat that constricts internal organs, with no external signs (Strickland et al., 2009). Summer slump the costliest to producers and is commonly referred to as fescue toxicosis (Schmidt and Osborn, 1993). During the hot summer months, ergot alkaloids cause a constrictive effect on blood vessels leading to the reduced ability to lower body temperature. Clinical signs of fescue toxicosis include rough hair coats during warm weather, elevated core temperatures, labored respiration, and decreased serum concentrations of prolactin (Schmidt and

Osborn, 1993; Strickland et al., 1993; Strickland et al., 2009). Reduction of serum prolactin is recognized as the most consistent physiological hallmark of fescue toxicosis (Goetsch et al., 1987; Davenport et al., 1993). Fescue toxicosis is estimated to result in an annual economic loss in the United states of greater than $1 billion dollars (Strickland et al., 2009).

2.1.3 Immune Response

The symptoms and physiological effects of fescue toxicosis have been researched for decades, however, one area that lacks information is the effect of ergot alkaloid consumption on immune responses. It has been suggested that cattle suffering from fescue toxicosis are more susceptible to disease during transit and in feedlots due to a suppressed immune system. Exposure to ergot alkaloid toxins increases pro-inflammatory cytokine secretion (Filipov et al., 1999a). To combat the increase in pro-inflammatory cytokines, it has been shown that increasing dietary protein can decrease pro-inflammatory cytokine response to endotoxins in beef heifers (Kahl et al., 1997), and improve the humoral immune response in steers during the preconditioning phase (Moriel et al., 2015). A recent study showed that chronic exposure to ergovaline increased concentrations of pro-inflammatory cytokines such as TNF-a, IFN-a, IFN-g, and IL-1-F1 (Poole et al., 2019). These cytokines primarily act to mediate innate immunity (fever response) to combat an infection and stimulate chemokine production triggering the recruitment of leukocytes to the infection site (Graves and Jiang, 1995a). The most recent study by Poole et al. (2019), indicates that further research is needed to determine the specific mechanisms of action of ergovaline on the immune system.

2.2 Immune System

2.2.1 Overview

The primary function of the immune system is to protect its host from foreign antagonists like microbes, viruses, and toxins. The immune system is very complex, but can be simplified into two lines of defense: the innate (non-adaptive), and adaptive immune systems (Warrington et al., 2011). The innate and adaptive immune systems are not mutually exclusive but are complementary in action when safeguarding the host. Adaptive immunity can be obtained by two principal methods: passive or active immunization. The most common form of passive immunization is via maternal passive immunity, whereas active immunization occurs post-exposure to a pathogen, with the most common example being vaccinations (Warrington et al., 2011). The organization of the human immune system is shown in Figure 2.2.1 (Turvey and Broide, 2010).

2.2.2 Innate Immunity

Innate immunity represents the first line of defense to an intruding pathogen. It is an antigen-independent or non-specific defense mechanism that is used immediately or within hours of encountering an antigen by the host (Warrington et al., 2011). There is no immunological memory associated with innate immunity, therefore, it is incapable of recognizing the same pathogen should the body be exposed to it in the future (Warrington et al., 2011). The defining characteristic of the innate immune system is speed: within minutes of pathogen exposure, the innate immune system starts producing a protective inflammatory response (Turvey and Broide, 2010). Innate immunity plays a fundamental role in activating the subsequent adaptive immune response (Turvey and Broide, 2010).

There are three broad strategies used by the innate immune system to recognize invading microorganisms. The first relies on a limited range of germline-encoded receptors to recognize the "microbial nonself", which are conserved molecular structures that are expressed by a large variety of microbes (Turvey and Broide, 2010). These receptors are collectively known as pattern recognition receptors, and the microbial structures recognized by the pattern recognition receptors are known as pathogen associated molecular patterns (PAMPs) (Janeway, 1989). One of the receptor families in this first category are the toll-like receptors (TLRs) which are discussed later in Section 2.3. The second and third approach are not further discussed in this review, but briefly, the second approach used is to detect immunologic danger in the form of damage-associated molecular patterns (DAMPs) (Bianchi, 2007). The third strategy is to detect "missing self" molecules expressed by normal healthy cells, but not by infected cells or microbes (Turvey and Broide, 2010).

2.2.3 Adaptive Immunity

Adaptive immunity is antigen-dependent, and antigen-specific. Therefore, it involves a lag time between exposure to the antigen and maximal response (Warrington et al., 2011). The capacity for memory, which allows the host to mount a more rapid and effective immune response upon successive exposure to the antigen, is the hallmark of the adaptive immune system (Warrington et al., 2011). Adaptive immunity in mammals is characterized by the two types of lymphocytes (T and B cells). T-cells are involved in cell-mediated immune responses, whereas B cells are involved in humoral immune

responses. T and B lymphocytes are the main self-defensive weapons of the adaptive immune system. Adaptive immunity relies on a clonal system, with each T and B cell expressing its own unique receptor. After pathogen exposure, it can take up to 5 days for clonal expansion of these rare antigen-specific T and B cells before the adaptive immune response is sufficiently robust to remove the pathogen (Turvey and Broide, 2010). The adaptive immune has two types: passive and active.

2.2.3.1 Passive Immunity

Passive immunity is acquired via colostrum which provides essential immunological protection. In ruminants no immune factors are exchanged in utero, therefore colostrum provides protection through a high immunoglobulin content (Larson et al., 1980). Without these immunoglobulins, the newborn ruminant would not survive. However, the concentration of immunoglobulins in colostrum, as well as gut permeability rapidly decrease during the first 48 hours after birth (Bush and Staley, 1980; Moore et al., 2005) making the timing of colostrum consumption critical.

2.2.3.2 Active Immunity

Active immunity is the process of exposing the body to an antigen to stimulate an adaptive immune response (Baxter, 2007). Active immunity to a pathogen occurs following exposure which is achieved naturally via wild-type exposure or through vaccination (Grubbs and Kahwaji, 2018). Natural active immunity occurs when an

infection occurs via natural occurrences. After this initial infection B cells create antibodies capable of binding the antigen. Artificial or vaccine-induced active immunity occurs when resistance to a disease is built following immunization using a weakened or dead form of an invasive organism to stimulate a host immune response (Strikas et al., 2018).

2.2.4 White Blood Cell Types

White blood cells (WBC), commonly referred to as leukocytes, are involved in protecting the body against foreign invaders and infectious disease. Most WBC are produced and mature in the bone marrow. However, lymphocytes are produced and mature in lymphoid tissues. Leukocytes are found throughout the body in the lymphatic system and blood. Whole blood component break down is shown in Figure 2.2.4a (Mescher, 2013). WBC are classified by structure (granulocytes or agranulocytes) or cell lineage (myeloid cells or lymphoid cells). Eosinophils, basophils, and neutrophils comprise the granulocytes, whereas monocytes and lymphocytes make up agranulocytes. Myeloid cells are composed of granulocytes and monocytes (Kawamoto and Minato, 2004). The first cells recruited to local sites upon pathogen invasion are granulocytes, providing immediate defense against infections in tissues (Kawamoto and Minato, 2004). Lymphoid cells are composed of lymphocytes; developing into T cells, B cells, and natural killer (NK) cells (Blom and Spits, 2006). The white blood cell differentiation is shown in Figure 2.2.4b.

In cattle, the total number of leukocytes decrease with age (Dirksen, 2006). A newborn calf has more granulocytes than lymphocytes (Jones and Allison, 2007; Wood and Quiroz-Rocha, 2010). Within the first month, calves exhibit a decrease in overall WBC numbers, followed by an increase thereafter (Mohri et al., 2007). Within three months of life, lymphocytes comprise 80% of total circulating WBC population (Roland et al., 2014). In adult cattle, the neutrophil to lymphocyte ratio is approximately 1:2 (Roland et al., 2014).

2.2.5 Granulocytes

2.2.5.1 Neutrophils

Neutrophils are a critical effector cell in humoral and innate immunity. They are the body's first line of defense against microorganisms (Burg and Pillinger, 2001). Neutrophils are polymorphonuclear leukocytes produced daily by the bone marrow (~10^{11} cells/day), and mainly reside in the peripheral vasculature (Chistiakov et al., 2015). Neutrophils are the most abundant leukocyte and predominant phagocyte in circulation, and are the first to arrive at the site of an infection (Lehrer et al., 1988).

Historically, there are three main antimicrobial functions recognized for neutrophils: phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps (NETs) shown in Figure 2.2.5.1 (Rosales, 2018). However, current research has indicated that neutrophils possess a diverse repertoire of functional responses. Neutrophils are transcriptionally active complex cells (Ericson et al., 2014) that contribute to inflammation resolution (Greenlee-Wacker, 2016), produce cytokines and chemokines (Tecchio and Cassatella, 2016), regulate macrophages for

long-term immune responses (Chen et al., 2014), and have a role in innate immunity (Netea et al., 2016).

Phagocytosis is a complex process for the ingestion and elimination of pathogens and apoptotic cells, and is fundamental for tissue homeostasis (Rosales and Uribe-Querol, 2017). In multicellular organisms, phagocytosis is located in phagocytes which include many types of WBC such as neutrophils, monocytes, macrophages, dendritic cells, and eosinophils (Rabinovitch, 1995). Phagocytosis is divided into four main steps: 1) recognition of a target particle, 2) signaling to activate the internalization machinery, 3) phagosome formation, and 4) phagolysosome maturation (Rosales and Uribe-Querol, 2017). Neutrophils are the most efficient of the phagocytes, a single neutrophil can engulf up to 50 bacteria (Lim et al., 2017), often in just a few seconds (Segal et al., 1980)

Neutrophils constitute 40-60 % of total leukocyte population, existing in a resting state to prevent damage to host tissues (Lehrer et al., 1988). Due to the fact that neutrophils circulate, they first need to leave the bloodstream and trans-migrate across the endothelium. This process is termed diapedesis and is required to reach the site of infection (Lim et al., 2017). Chemoattractants are released from either the host or the microorganism at the site of infection and guide the extravasation of neutrophils (Lim et al., 2017). Thus, ensuring phagocytosis of the pathogen. Although neutrophils were originally thought to survive up to 24 hr upon migration into tissue, they can remain present in wound sites for up to 3 days (Selders et al., 2017). The presence of neutrophils at the site of injury is amplified and sustained via neutrophil recruitment either by active neutrophils, or the surrounding resident macrophages (Chistiakov et al., 2015). In

addition, neutrophils can be activated or signaled to migrate by microbial presence, or by activation and recognition of TLRs (see Section 2.3).

2.2.5.2 Basophils

In normal ruminants, basophils are present in small numbers (~0.5-1%) and are often missed on manual differential counts. Basophil levels may be higher in clinically normal calves than in adults (Brun-Hansen et al., 2006), and function in allergic and inflammatory processes by releasing inflammatory mediators. These include heparin and histamine, which are immediate hypersensitivity reactions.

2.2.5.3 Eosinophils

Historically, eosinophils were considered end-stage cells involved in host protection against parasites (Weller, 1997). Although they are a minority granulocyte (~1-4%) (Wen and Rothenberg, 2016) it has been shown that eosinophils can perform several immune functions, including antigen presentation and exacerbation of inflammatory responses (Rothenberg and Hogan, 2006). Eosinophils are pleiotropic multifunctional WBC that modulate the adaptive immune response by directly activating T-cells (Rothenberg and Hogan, 2006). Normally, cattle have higher numbers of eosinophils than other species, and calves typically have lower numbers than adult cattle (Jones and Allison, 2007).

2.2.6 Agranulocytes

2.2.6.1 Monocytes

Monocytes are myeloid cells derived from bone marrow, and have a central role in immunity to infection or injury (van Furth et al., 1972; Auffray et al., 2009). After their production in the bone marrow, monocytes are released into the blood stream and circulate for several days before entering tissues (Hussen and Schuberth, 2017) They are a part of the innate immune system with important effector functions during different phases of inflammation (Hussen and Schuberth, 2017). Functionally, they are characterized by the ability to recognize pathogens, phagocytose microbes, produce cytokines and chemokines, and to present antigens to T cells (Hussen and Schuberth, 2017). In addition, monocytes are precursors for tissue macrophages and dendritic cells.

The functional and phenotypic properties of monocytes were intensively investigated in human and murine as well as bovine (Werling et al., 1998), ovine (Francey et al., 1992), caprine (Werling et al., 1994; Adler et al., 1996), equine (Grünig et al., 1991), and porcine (Summerfield et al., 2003) monocytes and monocyte derived cells. Previously, monocytes were considered as a homogenous population of circulating leukocytes, however, three monocyte subsets were recently identified in bovine peripheral blood according to the surface expression of CD14 and CD16 (Hussen and Schuberth, 2017). The three subsets are: 1) bovine cM (classical) with high CD14 and no CD16 expression, 2) bovine intM (intermediate) with high CD14 and low CD16 expression, and 3) bovine ncM (nonclassical) with high CD16 and no CD14 expression shown in (figure). The majority of total bovine blood monocytes comes from bovine cM (89%), whereas bovine intM and ncM constitute minor proportions (5-10% each)

(Hussen et al., 2013). Bovine cM monocytes have the highest ability to phagocytose bacteria. Bovine intM display an intermediate capacity for phagocytosis, whereas their strong suit is production of reactive oxygen species (ROS) and gene expression levels for inflammatory cytokines (TNF-α, IL-1β). Bovine ncM monocytes show the lowest capacity for phagocytosis and ROS generation (Hussen and Schuberth, 2017).

After monocytes leave the blood stream, they differentiate into tissue monocyte-derived macrophages or dendritic cells. The differentiation of monocytes into macrophages is guided by neutrophil release of cytokines, chemokines, and lipids via neutrophil granules (Hussen et al., 2016). The number of monocytes/macrophages present in a wound directly correlates to the number of neutrophils present (Chistiakov et al., 2015). Macrophages are terminally differentiated cells that phagocytose pathogens or toxins (Chiu and Bharat, 2016). Macrophages are integral components of tissues, contributing to organ development and maintenance of homeostasis through similar mechanisms used to detect pathogens and trigger inflammation during infection (Gordon and Martinez-Pomares, 2017). Macrophages maintain tissue homeostasis through the removal of senescent cells and remodeling/repair of tissues after inflammation (Gordon and Taylor, 2005b). *In vitro* experiments were performed, showing macrophage activation states in Figure 2.2.6.1 (Gordon and Taylor, 2005a). These include classical, alternative, and innate activation. Classical activation is induced by interferon-γ and LPS and is associated with high microbicidal activity, pro-inflammatory cytokine production and cellular immunity. Alternative activation results from a culture in IL-4 or IL-13 and is associated with tissue repair and humoral immunity. In culture, innate activation is

mediated by ligation of receptors (TLRs), and is associated with microbicidal activity and pro-inflammatory cytokine production (Gordon and Taylor, 2005b).

Dendritic cells are considered to be the only cells able to present antigens to naïve T cells and initiate primary immune responses (Howard et al., 1999), making them essential for the induction of the adaptive immune response. Their main function in innate immunity is the ability to sense infections and environmental antigens at the skin and mucosal surface and thus critically influencing decisions about immune activation or tolerance (Summerfield et al., 2015).

2.2.6.2 Lymphocytes

There are two major types of lymphocytes: B cells and T cells. When activated, B cells differentiate into plasma cells that secrete antibodies. T cells have two main classes upon activation: cytotoxic T cells, which kill virally infected cells, and those that differentiate into cells that activate cells such as B cells and macrophages (Janeway et al., 1999). T cells function in cell-mediated and cytotoxic adaptive immunity, and B cells are for humoral, antibody-driven adaptive immunity (Cano and Lopera, 2013). T cells are derived from the thymus, whereas B cells are derived from bone marrow. During antigen presentation, T and B cells recognize specific “non-self” antigens. Mature lymphocytes recirculate continually from the bloodstream though the peripheral or secondary lymphoid organs, returning through lymphatic vessels to the bloodstream. When recirculating T cells recognize its specific antigen on a dendritic cells surface, it triggers an adaptive immune response. Lymphocytes also include natural killer (NK) cells, which function in cell-mediated and cytotoxic innate immunity. Generally considered

components of the innate immune system, NK cells lack antigen-specific cell surface receptors (Vivier et al., 2011).

2.2.7 Age-Dependent Immunity

The development of the immune system in calves' advances in small steps from conception to maturity at approximately 6 months post parturition. As shown in Figure 2.2.7 (Chase et al., 2008a) fetal calves are predominantly protected by the innate immune system. The innate immune response does not fully develop until late gestation, with a decline in functional capacity as gestation approaches due to increased fetal cortisol levels (Barrington and Parish, 2001). The percentage of peripheral blood T cells decreases considerably (from approximately 60 to 30%) beginning 1 month before birth as they traffic and populate lymphoid tissues of fetal calves (Chase et al., 2008b). B cells, however, are present at much lower percentages in developing fetuses (1 % - 2 %) than mature calves (10 % - 20 %) (Senogles et al., 1979; Kampen et al., 2006).

At birth, neonates are immunocompetent and immunonaive due to the dependence on passively acquired maternal immunoglobulins, immune cells, and other protective substances that colostrum provides (Barrington and Parish, 2001). Colostrum ingestion is required for providing immunological protection during the first 2 to 4 weeks of life (Figure 2.2.7). Although all the essential immune components are present in neonates at calving, many of the components are not functional until calves are at least 2 to 4 weeks old and may continue to develop until puberty (Reber et al., 2006). To induce an active immune response via vaccination, the prime window is shown in Figure 2.2.7 to be

anywhere from a few weeks to 8 months of age. The humoral components of the innate system are present in limited quantity in calves and do not function as well as for adults. All of the cellular components of the acquired immune response are present in fetal calves (Wilson et al., 1996).

The number of circulating neutrophils in the newborn calf is approximately 4 times higher than in 3 week-old calves (Chase et al., 2008b). Prior to colostrum ingestion, neonatal neutrophils and macrophages have reduced phagocytic ability (Menge et al., 1998). By the first week of age, neutrophils are functional and able to mount an effective immune response (Kampen et al., 2006). Neutrophil function gradually improves to adult levels by 5 months of age (Hauser et al., 1986). Dendritic cells are lower in neonates and have a limited ability to present antigen to activate the adaptive immune response (Morein et al., 2002). Circulating natural killer cells are lower during the first week of age (approximately 3% of total lymphocytes) versus adults and increase to 10% by 6 to 8 weeks of age (Kampen et al., 2006). B cells represent only 4 % of total lymphocytes at 1 week of age and increase gradually to 20 to 30 % in adults by 6 to 8 weeks of age (Kampen et al., 2006). T-cell subpopulations are present in peripheral blood of calves at levels similar to adults (Kampen et al., 2006). In a study by Kampen et al. (2006), individual calf percentages of T-cell subsets in peripheral blood hardly changed over time, displaying that clinically healthy animals have stable levels of T-cell subpopulations.

2.2.8 Toll-like Receptors

Toll-like receptors (TLR) allow for the recognition and response to diverse microbial epitopes on pathogens, which enables the innate immune system to discriminate among groups of pathogens and to induce an appropriate cascade of effector adaptive responses (Turvey and Broide, 2010). Thus, TLR are critically important for innate immunity. In humans, there are 10 isoforms of the toll-like receptor (TLR) family (Takeda et al., 2003; Akira and Takeda, 2004). Existing as heterodimers or homodimers, individual TLR recognize a distinct but limited range of conserved microbial products. For the purpose of this thesis, TLR4 is the main focus. TLR4 is a well characterized receptor with lipopolysaccharide (LPS) as its ligand pair. As described in Section 2.3, the activation of WBC-expressed TLR4 by binding of LPS initiates activation of several important canonical signaling pathways (ex: NF-kB).

2.3 Lipopolysaccharide-stimulated immune responses

One of the most commonly studied bacterial surface molecules is the glycolipid lipopolysaccharide (LPS), which is produced by most gram-negative bacteria. In the early 1900's, LPS was acknowledged for stimulating the immune system and was referred to as an endotoxin. LPS is a potent inducer of pro-inflammatory cytokines (Harrison et al., 2004). Gram-negative bacteria can cause a wide variety of clinical diseases in humans and animals (Munford, 2008). LPS can be recognized by immune cells as a pathogen-associated molecule through TLR4. Activation of LPS-responsive cells, such as monocytes and macrophages occur rapidly after interaction with TLR4 and CD14 (a glycosylphosphatidylinositol-linked cell surface glycoprotein) (Schletter et al., 1995;

Ulevitch and Tobias, 1995). LPS has been shown to initiate several intracellular signaling events (Sweet and Hume, 1996), including activation of NF-kB, eventually leading to the synthesis and release of proinflammatory mediators such as interleukin-8 (IL-8) and tumor necrosis factor-α (Chow et al., 1999). For laboratory research, LPS derived from *Escherichia coli* and *Salmonella* often is used to simulate a natural infection caused by gram-negative bacteria in an LPS challenge (Needham et al., 2013). Filipov et al. (1999b) was the first to report that following an injection of LPS, steers grazing endophyte-infected tall fescue had increased serum TNF-a concentrations.

2.3.1 *Ex vivo* LPS challenge model

Stressful events such as weaning or bacterial infections, stimulate an acute-phase immune response which can compromise growth performance (Carroll et al., 2009; Arthington et al., 2013). Lipopolysaccharide has proven to be a useful tool in humans (Finch-Arietta and Cochran, 1991), mice and rats (Foster et al., 1993), swine (Carstensen et al., 2005), and cattle (Jahan et al., 2015) to evaluate the capacity of leukocytes to secrete pro-inflammatory cytokines. This is achieved by an *ex vivo* whole blood simulation assay. Typically, whole blood is collected in EDTA-containing collection tubes and split into two, 3-mL samples. One tube serves as the nonstimulated control, and the second is stimulated with *E. coli*-derived LPS (2 μg/mL; Sigma-Aldrich, St. Louis, MO) and both incubated for 2 h at 37 °C as described by Røntved et al. (2005). All tubes were incubated in a shaking water bath (100x/min), stored in -20 °C until RNA extraction.

2.4 Selenium Supplementation in Cattle

2.4.1 Overview

Selenium (Se) was first discovered in 1817 by the Swedish chemist Jöns Jakob Berzelius as a by-product of sulfuric acid production. Selenium is an essential trace element and an antioxidant (Wang et al., 2017). The biological significance of Se was not recognized until it was identified as the toxic component causing death and lameness in livestock grazing certain plants in Wyoming and the Dakotas (Franke, 1934). The chemistry of Se insinuates that in biological systems, its likely present as selenol (selenomercaptan), R-she (e.g., selenocysteine), or as the Se ether similar to/replacing sulfur in the amino acid methionine (Reddy and Massaro, 1983). The therapeutic role of Se as an essential micronutrient was identified by Schwarz and Foltz (1957), who found that rat liver necrosis could be prevented with low dose supplementation of Se. Both inorganic and organically bound forms of selenium are widely distributed to various body tissues and render important biological functions, primarily through the reactiveness of the selenocysteine (Sec) residue of selenoproteins (Kasaikina et al., 2012). Selenoproteins are proteins that contain Se in the form of the amino acid selenocysteine, which is encoded by the codon AUG and synthesized on the $tRNA^{Sec}$ (see below). Currently, 25 selenoprotein genes have been identified for cattle (Mariotti et al., 2012), humans (Labunskyy et al., 2014), and pigs (Chen et al., 2018), and 24 selenoproteins for rodents (Kryukov et al., 2003) and poultry (Li et al., 2018).

Selenoprotein-incorporated Sec plays a role in reducing the accumulation of hydroperoxides from cellular metabolism (Sordillo, 2016), by selenoproteins such as the glutathione peroxidases (GPx) (Brigelius-Flohe and Maiorino, 2013). In 1973 it was

discovered that Se is an integral structural component of GPx (Flohe et al., 1973). GPx are Se-dependent glutathione peroxidases that respond to dietary Se (Hafeman et al., 1974). Since these initial findings, a considerable amount of progress has been made in identifying selenoproteins, and their role in biological processes. So far, the single defining feature of selenoproteins is the fact that they all contain one or more Sec residues in their primary structure. Additionally, with the exception of selenoprotein P, all selenoproteins with known functions in which the Sec residue is located at the catalytic site, appear to have enzymatic activities, where it likely partakes in redox reactions (Moghadaszadeh and Beggs, 2006).

Besides their role in mediating redox capacity, selenoprotein function has been implicated in mammalian development (Köhrle, 2000), immune function (Rayman, 2000), and inhibition of viral expression (Beck et al., 2003). It has been reported that selenium enhances both humoral and cellular immune responses (Reddy and Massaro, 1983). For example, Petrie et al. (1989) showed that in the murine immune system, selenium played a role in the formation and activity of helper T, cytotoxic T and NK cells.

2.4.2 Immune Function

Selenium is essential for ideal endocrine and immune function and moderating the inflammatory response (Beckett and Arthur, 2005). Through the incorporation into selenoproteins, Se plays an important role in commencing “normal” immunity as well as regulating excessive immune responses (Prabhu and Lei, 2016). Though studies using mouse models that lack specific selenoproteins, it has been discovered that selenoproteins

have a role in signaling, calcium flux, oxidative burst, and effector functions, including migration, adherence, cytokine/chemokine production, and phagocytosis (Prabhu and Lei, 2016). Quantitatively selenoprotein P is the major selenoprotein in plasma and has both transport and antioxidant roles (Beckett and Arthur, 2005). Supplementation of Se has been suggested to have preventative and occasional therapeutic roles in immune system function (Kiremidjian-Schumacher and Roy, 2001). It is believed that selenoproteins can also affect cell-signaling molecules such as nuclear factor-κB (Hayashi et al., 1993), and therefore, influence important cellular functions such as cellular growth and gene transcription.

2.4.3 Nutritional Sources of Se

Selenium is an essential mineral naturally found in soil, water, plants, and animals (Wang et al., 2017). Selenium is a commonly occurring element, and can be found in the atmosphere, lithosphere, biosphere, and hydrosphere of Earth (Reich and Hondal, 2016). Selenium is present in various forms including inorganic forms from dietary supplementation such as selenite and selenate (Kieliszek, 2019). In food, Se is present in organically-bound forms such as Selenocysteine (Sec) and Selenomethionine (SeMet) (Kieliszek, 2019). Selenite and selenate are commonly found in most soils and are typically used as supplemental Se for humans and livestock. Selenium levels in soil varies with soil type, texture, organic matter content and amount of rainfall. On average, soil selenium levels vary from 0.1 to 0.7 mg/kg , up to 4.5 mg/kg in tropical soils, and poor concentrations in volcanic soils and granite (Mehdi et al., 2013). Soil Se levels directly relate to plant Se concentrations. In forages, the normal Se content ranges from

0.1 to 0.5 ppm, and in some places extreme values of 0.006 ppm (Suttle, 2010) to 20,000 ppm (Lebreton et al., 1998) of Se have been found. On a global scale, in the arable layer of soil, the average selenium concentration varies from 0.33 to 2 mg/kg (Mason et al., 2018). Areas with Se deficiencies in the US include the northwest, northeast, and southeast. Forages and grains grown in these regions have low (< 0.05 ppm) to variable (> 0.1 ppm) selenium levels (Ammerman and Miller, 1975).

Because the dietary requirement for Se in cattle is 0.1 ppm, and the typically low (< 0.01 ppm) Se levels in the plants of southeast US, Se supplementation is necessary for cattle raised under forage-based production regimens. Dietary inorganic Se supplements that meet the approved requirement by the FDA of 0.1 ppm for cattle, include sodium selenite or sodium selenate (NRC, 1983). Sodium selenite is the form most commonly supplemented in cattle diets. Southeastern US cattle are typically considered to be marginally or severely deficient in Se (Dargatz and Ross, 1996b) and therefore, require Se supplementation. The organically bound forms of Se can be provided as enriched strains of yeast (*Saccharomyces cerevisiae)* that are available from different manufactures (e.g., SEL-PLEX®, Altech, Inc, Nicholasville, KY). Selenomethionine is a major component from these Se-yeast products, providing over 50% of the total Se (Saha et al., 2016). As shown in Figure 2.4.3, the sulfur in Met and Cys are replaced by Se to form SeMet and Sec, respectively. Yet, there is a central difference between SeMet and Sec regarding their biosynthesis, incorporation into the peptide chain, and whole-body metabolism, which is described later (see Selenium biosynthesis below). Briefly, Sec is encoded into the peptide chain of selenoproteins by the UGA codon, the Sec insertion sequence and after synthesis on $tRNA^{Sec}$ (Suzuki, 2005). Thus, the synthesis and

incorporation of Sec into polypeptide chains is considered to be by the "regulated" pathway for Se incorporation into proteins. In contrast, SeMet is incorporated into proteins by competing with Met for binding to $tRNA^{Met}$, which does not discriminate between SeMet and Met (McConnell and Hoffman, 1972). Thus, the synthesis of SeMet-containing proteins (Se-associated proteins) is by "non-regulated" incorporation of Se into proteins.

2.4.4 Biosynthesis of Seleno-Amino acids

Selenomethionine undergoes unregulated incorporation into the peptide chain by $tRNA^{Met}$. However, the incorporation of Sec into selenoproteins is done so by endogenously synthesizing from Ser and activated Se to Sec $tRNA^{Sec}$ by the intermediates of selenophosphate and serine $tRNA^{Sec}$ (Veres et al., 1992; Glass et al., 1993), as shown in Figure 2.4.4.

There are five known required processes for selenoprotein synthesis (Burk and Hill, 2015). Two of the five are uniquely present in the selenoprotein mRNA structure as a UGA in the open reading frame, and the other is a Sec insertion sequence (SECIS) element (Berry et al., 1993)(Figure 2.4.4). SECIS is a specialized stem-loop structure located in the 3' untranslated region, and is required for the recognition of the in-frame UGA, as the codon for Sec insertion, otherwise UGA is recognized as a stop codon (Burk and Hill, 2015). As shown in Figure 2.4.4, three trans-acting factors: Sec $tRNA^{Sec}$, and a Sec-specific translation elongation factor (eEFsec), and a SECIS-binding protein (SBP2) are identified as playing essential roles in selenoprotein synthesis (Labunskyy et al., 2014). The anticodon for UGA is found in $tRNA^{Sec}$. First, charging of serine on the

specific $tRNA^{Sec}$ by seryl-tRNA synthetase occurs. Then followed by the conversion of Ser-$tRNA^{Sec}$ to Sec-$tRNA^{Sec}$, which is catalyzed by Sec synthase, an enzyme that utilizes monoselenophosphate as an Se donor to generate Sec (Xu et al., 2006). The trans-acting factor eEFsec is responsible for the recruitment of Sec $tRNA^{Sec}$ and SBP2, as well as inserting Sec into nascent protein chains in response to UGA codons (Fagegaltier et al., 2000).

Since the discovery that Sec is encoded by the UGA codon and is the 21st "naturally occurring" amino acid in the genetic code, research and discoveries of the physiological roles and the health benefits of Se has increased tremendously. These findings suggest that Sec proteins containing Sec are largely responsible for the health benefits of Se (Labunskyy et al., 2014).

For many years, the biosynthesis of Sec was unknown in eukaryotes. In recent years, the pathway for the biosynthesis was determined using a combination of genomic, molecular, and structural approaches (Xu et al., 2006; Yuan et al., 2006). Sec is a unique amino acid, in that it is the only amino acid in eukaryotes whose biosynthesis occurs on its own tRNA, Sec $tRNA^{[Ser]Sec}$ (Lee et al., 1989; Hatfield et al., 1994). In order to provide the backbone for Sec biosynthesis, $tRNA^{[Ser]Sec}$ is amino acylated with serine in a reaction catalyzed by seryl-tRNA synthetase (SerRS) to form seryl-$tRNA^{[Ser]Sec}$ (Lee et al., 1989; Leinfelder et al., 1989).

2.4.5 Consumption and absorption

The rate of selenite absorption is 80% in monogastric animals and poultry, whereas it is only 29% in ruminants (Meschy, 2017). In ruminants (ewes), a study by

Galbraith et al. (2016) showed that organically-bound Se as SeMet was incorporated to a larger extent into rumen microorganisms than inorganic Se sources, and resulted in less elemental Se formation. In cattle supplemented with organic Se, total Se concentration of whole blood and tissues was greater compared to those receiving inorganic Se, indicating an improvement in Se availability and tissue Se retention (Juniper et al., 2008). When using sodium selenite for supplementation, Se is distributed in the entire organism. However, most of this absorbed selenium is found in the liver (Herdt et al., 2000). When Se is in excess, selenide is polymethylated and excreted as dimethyl selenide in breath and feces; and as the cation $(CH_3)_3Se^+$ in urine (Meschy, 2017).

2.4.6 Effects of supplementation on cattle

As described above, Se has many biological functions that are carried out by selenoproteins, that function as enzymes. These enzymes include glutathione peroxidase, thioredoxin reductase, and thyroid hormone deiodinase (Labunskyy et al., 2014). The biological roles of these redox enzymes have been comprehensively reviewed in different human and rodent tissues (Rayman, 2012; Avery and Hoffmann, 2018). Some tissues include antioxidant and thyroid hormone metabolism, brain development, spermatogenesis, and the immune system. In cattle, the role and effects of Se supplementation have been reviewed (Mehdi and Dufrasne, 2016). The effect of Se supplementation at various levels and forms in cattle can be evaluated from the perspective of blood/tissue assimilation.

2.4.6.1 Blood and tissue assimilation

Whole-body Se status has been commonly assessed by measuring the concentration of Se in blood/tissue, or by the blood glutathione peroxidase activity. Whole blood Se is comprised of serum (or plasma) Se (active) and Se in glutathione peroxidase in erythrocytes. Whole blood Se has been shown to be quickly responsive and is well correlated with Se intake (Longnecker et al., 1996; Patterson et al., 2013). Despite the research, there is no clear number on Se concentration to determine a deficiency or adequacy in cattle. Regardless, studies have stated that Se is considered adequate when whole blood Se is either greater than 100 ng/mL (Gerloff, 1992), or between 81-161 ng/mL (Dargatz and Ross, 1996a).

2.5 Prolactin

2.5.1 Introduction

As noted above, a reduction of serum prolactin is a hallmark of fescue toxicosis. Prolactin (PRL) is a peptide hormone containing 198 amino acids (Melmed and Jameson, 2014), mainly synthesized and secreted by lactotrophs in the anterior pituitary (Ra, 2016). Mature bovine prolactin is composed of a single chain of 199 amino acids with three intramolecular disulfide bridges between six cysteine residues; sharing 80% of the sequence homology with human prolactin (Wallis, 1974). The synthesis of PRL is not limited to the anterior pituitary or lactotrophs. Numerous extrapituitary tissues also express this protein including: the placenta, testis, ovaries, mammary gland, skin, adipose tissue, endothelial cells, and immune cells (Harvey et al., 2012). Pituitary PRL is

predominantly under inhibitory control exerted by dopamine, but peripheral organs have been shown to inhibit or stimulate PRL secretion (Díaz et al., 2013). In addition, PRL is recognized as an immune cytokine (Ben-Jonathan et al., 1996). Prolactin is a highly versatile cytokine and hormone that displays a wide spectrum of effects in a variety of tissues, with more than 300 actions described in vertebrates (Ben-Jonathan et al., 1996).

2.5.2 Receptor and Isoforms

2.5.2.1 Receptor

The PRL receptor (PRLR) belongs to the class 1 cytokine receptor superfamily (Bazan, 1990a, b). Each PRLR contains an extracellular, transmembrane, and intracellular domain (Bole-Feysot et al., 1998a). The extracellular domain contains two disulfide bridges that are essential for ligand binding. The cytoplasmic domain contains two highly conserved regions among cytokine receptors (Saleem et al., 2018). After PRL binds to its receptor, several signaling pathways can be activated including the Janus kinase-signal transducer and activator of transcription (Jak-Stat), the mitogen-activated protein kinase (MAPK), and the phosphoinositide 3 kinase pathways (PI3K) (Radhakrishnan et al., 2012b). The PRLR is ubiquitously expressed (Nagano and Kelly, 1994; Bakowska and Morrell, 1997; Bole-Feysot et al., 1998b), and facilitates over 300 biological functions.

The human PRLR gene is located on chromosome 5, and alternative splicing has resulted in several different isoforms (Bole-Feysot et al., 1998b). These isoforms, while identical in their extracellular domain, differ within the intracellular portion which can be short, intermediate, or long.

2.5.2.2 Isoforms

In humans, six isoforms have been reported (Diakonova, 2014). In contrast, only two distinct prolactin receptor isoforms have been identified in cattle. (Brym et al., 2005). In mice, three receptor isoforms have been described including short, intermediate, and long forms (Bole-Feysot et al., 1998b). The murine studies have shown that the ratio of isoforms varies based on tissue-type, development stage, and reproductive stage (e.g., estrous cycle, pregnancy, lactation) (Nagano and Kelly, 1994; Bole-Feysot et al., 1998).

The human PRLR long isoform forms a homodimer when PRL binds the two extracellular interaction sites, triggering the downstream intracellular signal transduction. Prolactin signaling can occur via the long isoform activates JAK-2, Src (tyrosine kinase family), and phosphatidylinositol 3-kinase (PI3K/AKT), and mitogen-activated protein kinase (MAPK) 3 (Saleem et al., 2018). There have been two distinct PRL receptor isoforms identified in cattle as a result of alternative splicing events (Bole-Feysot et al., 1998b); a long isoform with 557 amino acids (Scott et al., 1992) and a short isoform with 272 amino acids (Schuler et al., 1997).

2.5.3 Prolactin function and immune system

Prolactin is best known and named for its role in regulating lactation; however, it also affects a wide variety of biological functions. These functions include regulation of reproduction, osmoregulation, immune responses, metabolism, and growth and development.

In the immune system, PRL is believed to act as a locally produced cytokine with significance for immune regulation and modulation of T- and B-cell function. However, the molecular mechanisms regulating PRL expression in the immune system are still not fully understood. Within the immune system, PRL is synthesized by T-cells, B-cells, macrophages, thymocytes, mononuclear and NK cells. (Ben-Jonathan et al., 1996). The binding of PRL to lymphocytes was first demonstrated by Russell et al. (1985), and subsequent studies show that both the long and short forms of the receptor are expressed by rat lymphoid organs, with the long form being more prevalent (Nagano and Kelly, 1994). A wide range of immunological disruptions caused by PRL deficiency was described by Berczi (1986) and Berczi and Nagy (1987). Humoral and cellular immune deficiencies in hypophysectomized rats was restored by exogenous PRL or anterior pituitary transplants (Ben-Jonathan et al., 1996).

Specifically, in pancreatic beta cells, pancreas, and T-lymphocytes, PRL can regulate proliferation (Bole-Feysot et al., 1998b; Freemark et al., 2002), whereas, PRL acts as an antiapoptotic factor in pancreatic beta cells and lymphocytes (LaVoie and Witorsch, 1995). Prolactin has been shown to inhibit apoptosis of lymphocytes (Díaz et al., 2013), as well as to act as a T-cell growth factor (Matera et al., 1997).

2.5.4 Prolactin-receptor and fescue toxicosis

In rats, sheep, and cattle, serum prolactin levels are reduced when consumption of endophyte-infected fescue occurs (Mizinga et al., 1993; Thompson et al., 2001; Gadberry et al., 2003). The decrease in serum prolactin occurs from the binding of ergopeptine alkaloids to dopaminergic receptors (Thompson and Stuedemann, 1993; Oliver, 2005).

Reduced prolactin levels could lead to reduced milk production, reproductive failure, immunosuppression, and a dull and shaggy hair coat (Prasad et al., 1989; Reber, 1993; Regisford and Katz, 1993; Aiken et al., 2006). Intake of infected tall fescue alters blood parameter profiles of livestock, with reduced plasma prolactin being the most frequently observed indicator fescue toxicosis (Strickland et al., 2011). The vasoconstrictive properties of the ergot alkaloid ergonovine also reduces prolactin concentration (Guerre, 2015). Prolactin, like other hormones, is connected to various body systems, and a decline in levels could impact function of these systems.

2.6 Nuclear Factor-Kappa Beta (NF-kB)

2.6.1 Introduction

Nuclear Factor-κβ (NF-kB) represents a family of inducible transcription factors, which regulate a large array of genes involved in different processes of the immune and inflammatory responses (Oeckinghaus and Ghosh, 2009). This family is composed of five structurally related members, including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB and c-Rel which mediates the transcription of target genes by binding to a specific DNA element (Sun et al., 2013). NF-kB proteins are normally sequestered in the cytoplasm by a family of inhibitory proteins, including Iκβ family members (Sun, 2011). Activation of NF-kB involves two major signaling pathways, the canonical and noncanonical (alternative) pathways. Despite differences in their signaling mechanisms, they are both important for regulating immune and inflammatory responses (Vallabhapurapu and Karin, 2009; Sun, 2011). After the discovery of NF-kB, it soon was

identified as a major regulator of the innate and adaptive immunity and inflammatory responses (Bonizzi and Karin, 2004).

2.6.2 Canonical NF-kB pathway

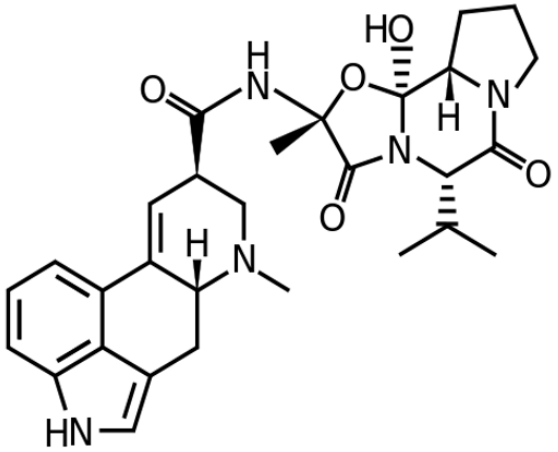
The canonical pathway is based on IKKβ-dependent Iκβ degradation, which is essential for innate immunity. Deficiencies in both RelA and IKKβ result in a marked increase in susceptibility to infections (Bonizzi and Karin, 2004). To date, the most imperative and best studied Iκβ family member is Iκβa. The canonical pathway responds to diverse stimuli, including ligands of various cytokine receptors, pattern-recognition receptors (PRR), TNF receptor (TNFR) superfamily members, as well as T-cell receptor (TCR) and B-cell receptor (BCR) (Zhang and Sun, 2015). The primary mechanism for the canonical activation is the inducible degradation of Iκβa triggered through the site-specific phosphorylation by a multi-subunit Iκβ kinase (IKK) complex (Karin and Delhase, 2000; Oeckinghaus and Ghosh, 2009). Activation of the canonical pathway is responsible for transcriptional induction of pro-inflammatory cytokines, chemokines, and additional inflammatory mediators in different types of innate immune cells (Figure 2.6.2) (Ghosh and Karin, 2002; Hayden and Ghosh, 2011; Sun et al., 2013).

2.6.3 NF-κB and the immune system

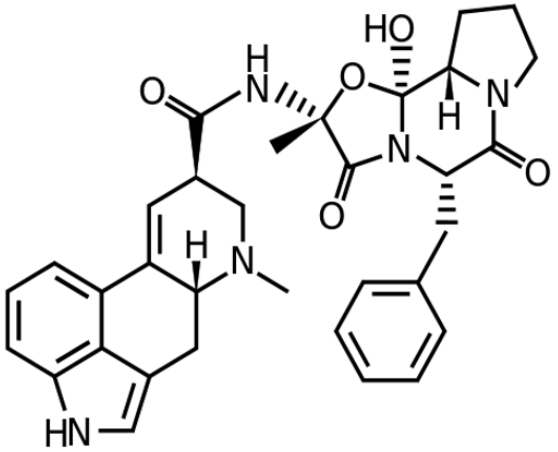
A well-recognized function of NF-kB is regulation of inflammatory responses. The pro-inflammatory function of NF-kB has been greatly studied in macrophages. In addition to mediating various pro-inflammatory genes in innate immune cells, NF-kB regulates the activation, differentiation, and effector function of inflammatory T-cells

(Tak and Firestein, 2001; Lawrence, 2009). Predictably, dysregulated NF-kB activation is a hallmark of chronic inflammatory diseases. Canonical NF-kB members, RelA and c-Rel have a central role in mediating TCR signaling and naïve T-cell activation (Oh and Ghosh, 2013). Aberrant T-cell activation can be caused by deregulated NF-kB activation, which is associated with autoimmune and inflammatory responses (Chang et al., 2011). In addition, NF-kB plays a role in regulating effector function and T-cell differentiation (Liu et al., 2017). In neutrophils, NF-kB has a proapoptotic role during inflammation as described by Lawrence et al. (2001) and Greten et al. (2007), and during acute inflammation it could represent an important anti-inflammatory mechanism for NF-kB. In macrophages, NF-kB has been shown to be an important inhibitor of pathogen-induced apoptosis (*in vitro*) (Park et al., 2005). In this regard, NF-kB could have a pro-inflammatory role by enabling prolonged activation of macrophages.

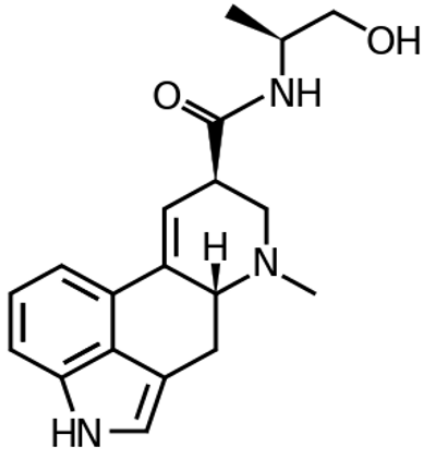
Figure 2.1.1 Chemical structures of selected ergopeptine alkaloids



Ergovaline

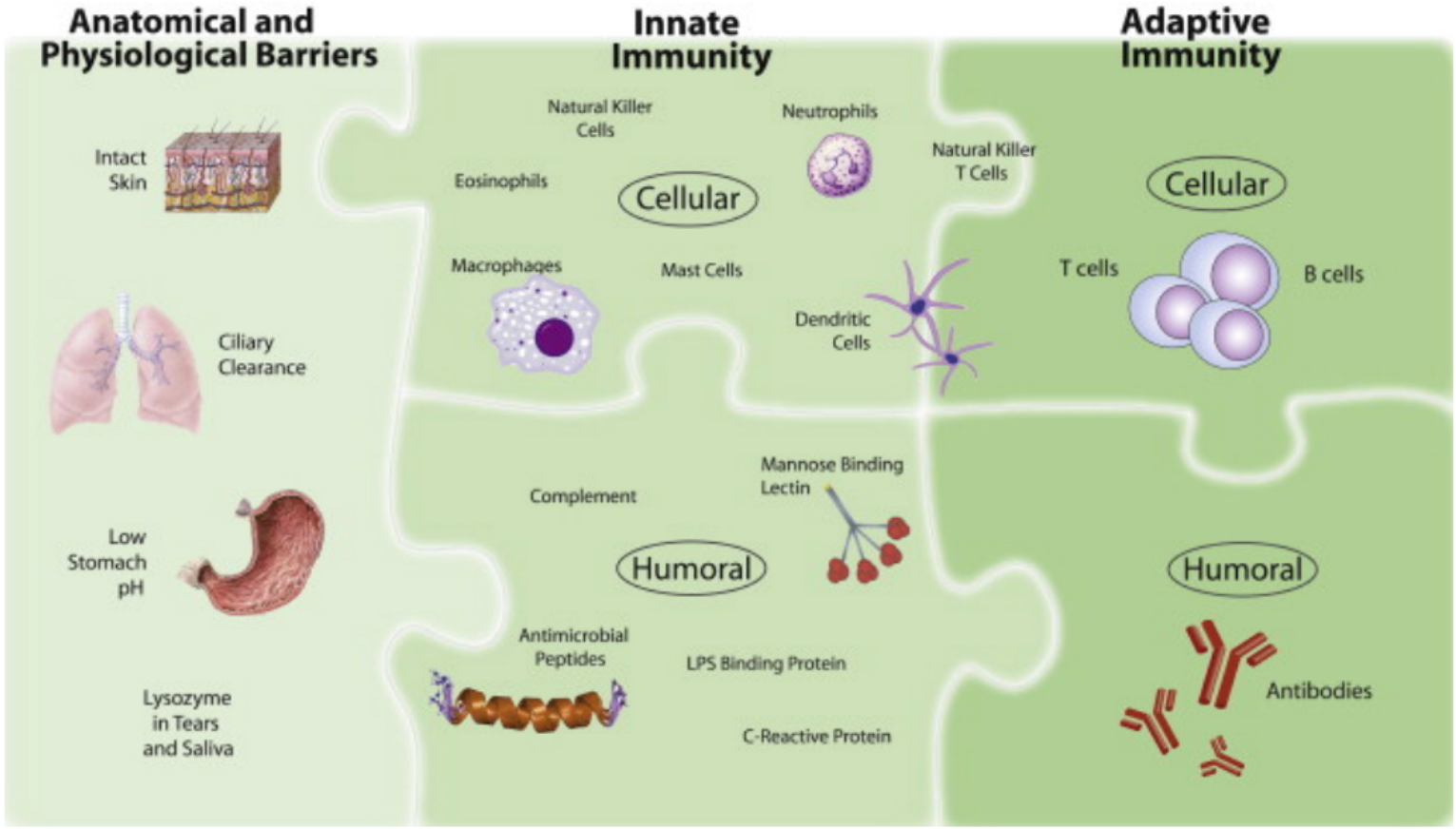


Ergotamine



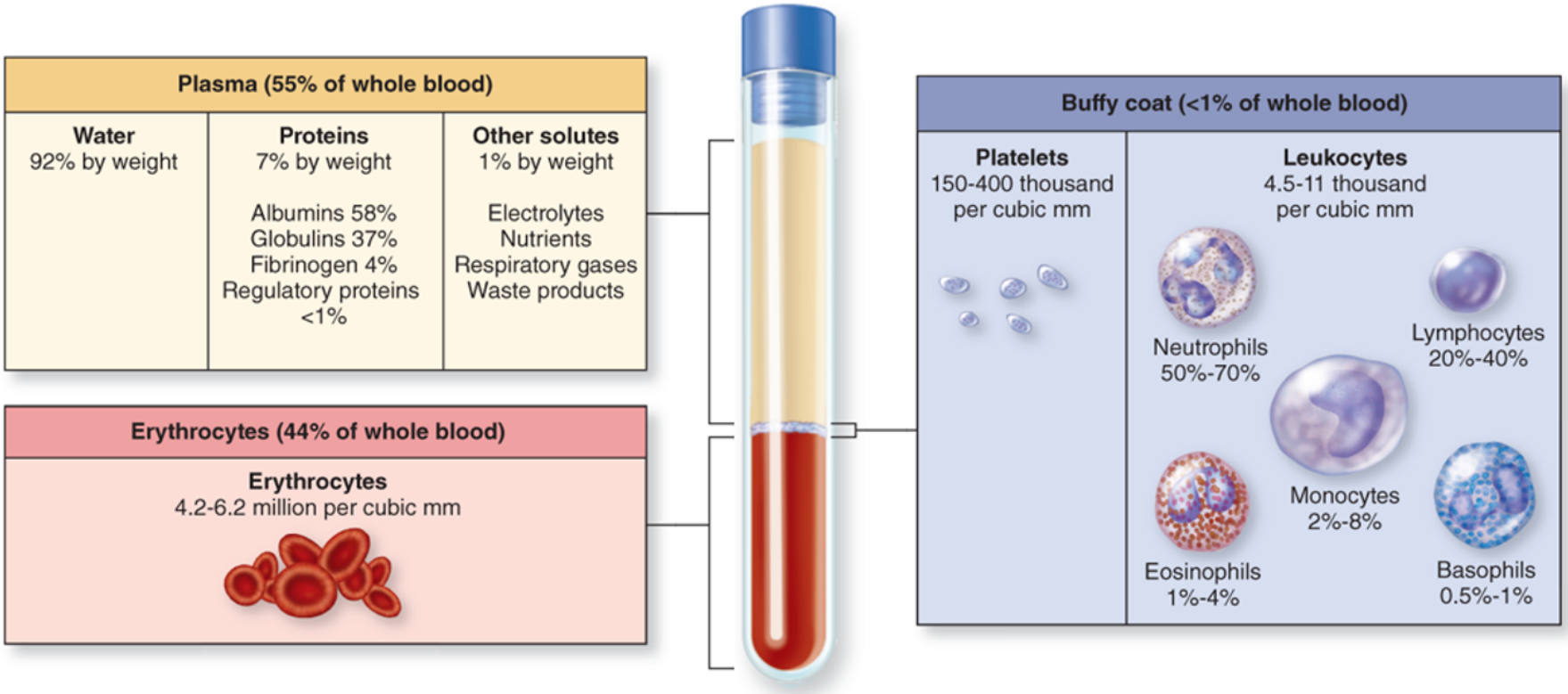
Ergonovine

Figure 2.2.1 Integrated human immune system[1]



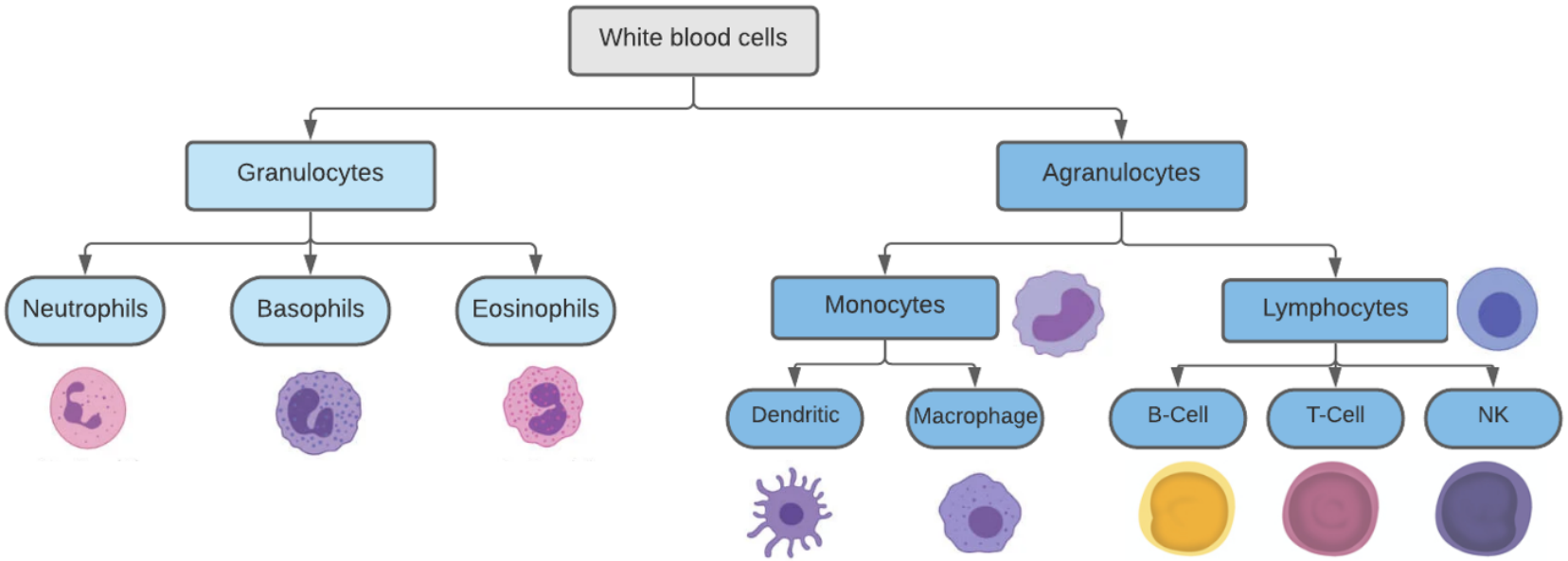
[1] Integrated human immune system. The human microbial defense system can be simplistically viewed as consisting of 3 levels: (1) anatomic and physiologic barriers; (2) innate immunity; and (3) adaptive immunity. In common with many classification systems, some elements are difficult to categorize. (Turvey and Broide, 2010).

Figure 2.2.4a Whole blood composition[1]



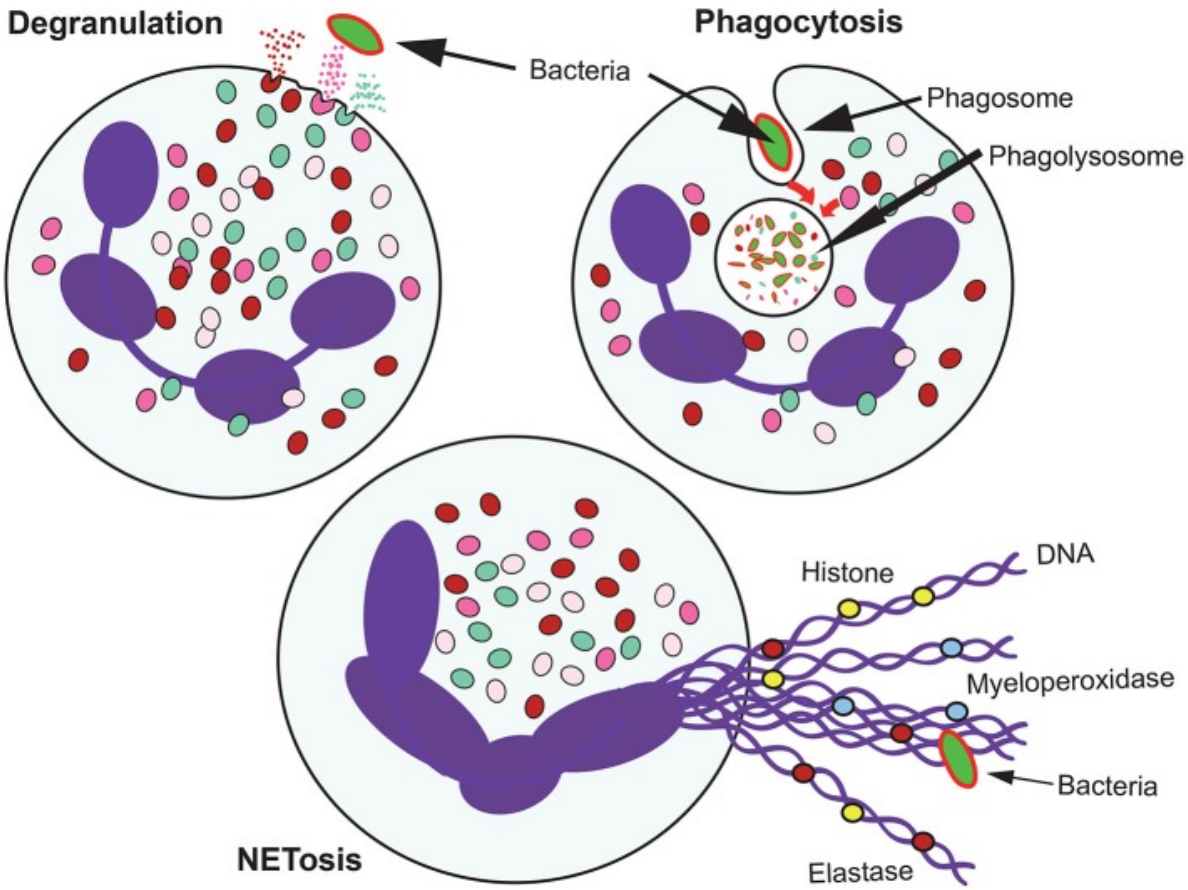
[1]After centrifugation, almost half of the volume is represented by erythrocytes at the bottom of the tube, called the hematocrit. Between the erythrocytes and the supernatant light-colored plasma is a thin layer of leukocytes and platelets called the buffy coat. The concentration ranges of erythrocytes, platelets, and leukocytes in normal blood are included here, along with the percentage range for each type of leukocyte represented in the buffy coat (Mescher, 2013).

Figure 2.2.4b White blood cell taxonomy[1]



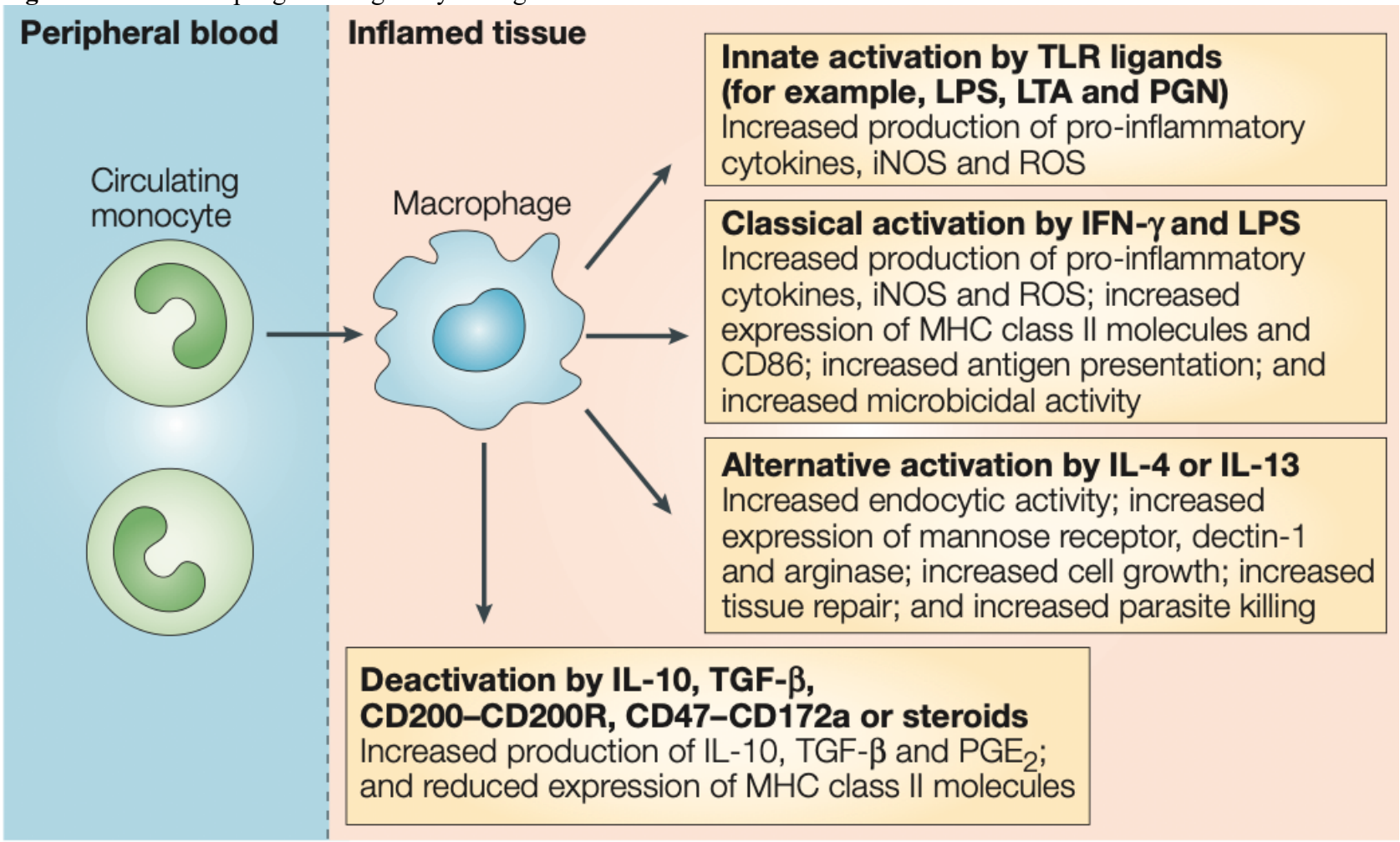
[1]Includes 2 main types (Granulocytes, Agranulocytes) and eight sub-types (neutrophils, basophils, eosinophils, macrophages, dendritic cells, natural killer (NK), B-cell and T-cells.

Figure 2.2.5.1 Antimicrobial mechanisms of neutrophils[1]



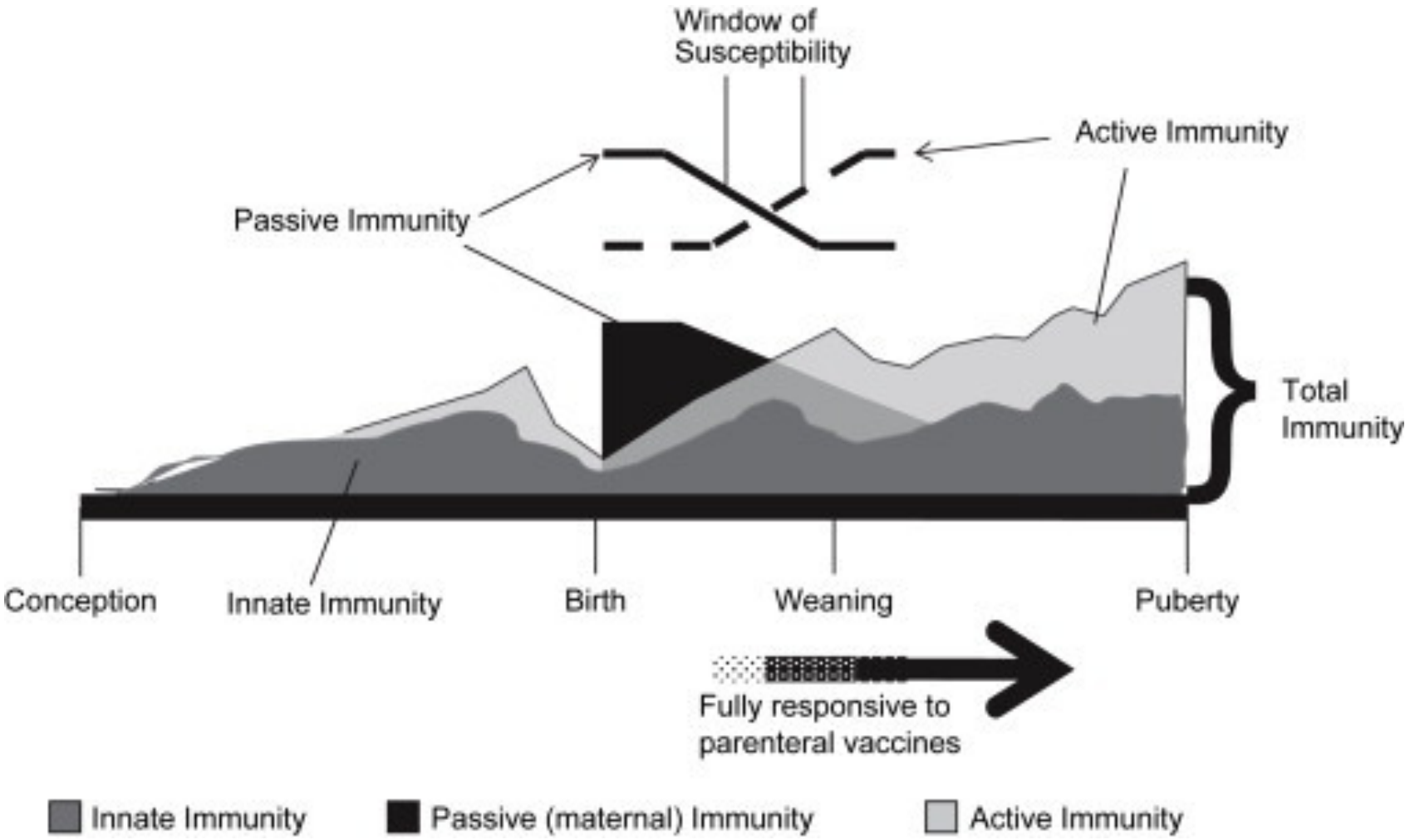
[1]Phagocytosis involves the ingestion of the microorganism into a phagocytic vacuole that upon maturation becomes a phagolysosome. Neutrophils also degranulate and release the contents of their granules to their environment. When the microorganism is too large to be ingested, neutrophil can also produce extracellular traps (NETs) formed by DNA fibers and proteins from the granules (Rosales, 2018).

Figure 2.2.6.1 Macrophage heterogeneity during inflammation[1]



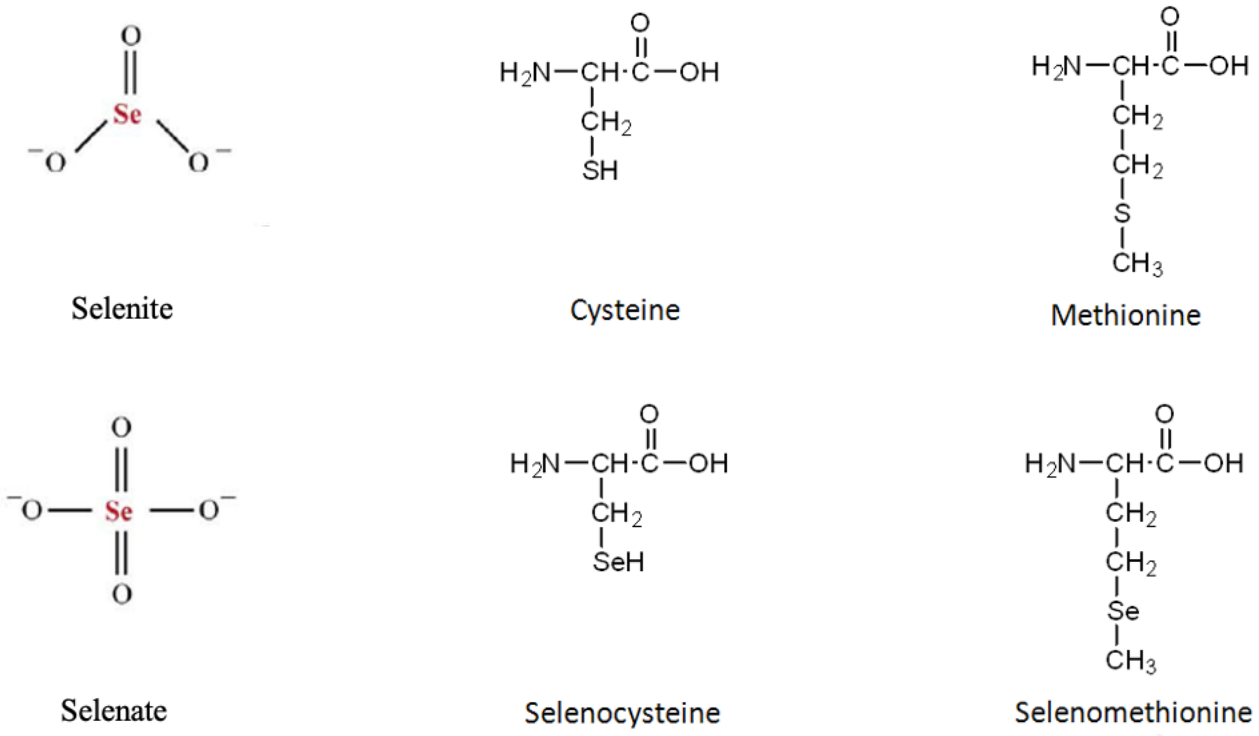
[1]The phenotypes and physiological activities have been observed *in vitro* and shown in this figure. The activation states include the following: classical, alternative, and innate activation (Gordon and Taylor, 2005a).

Figure 2.2.7 Development of the immune response in calves from conception to puberty



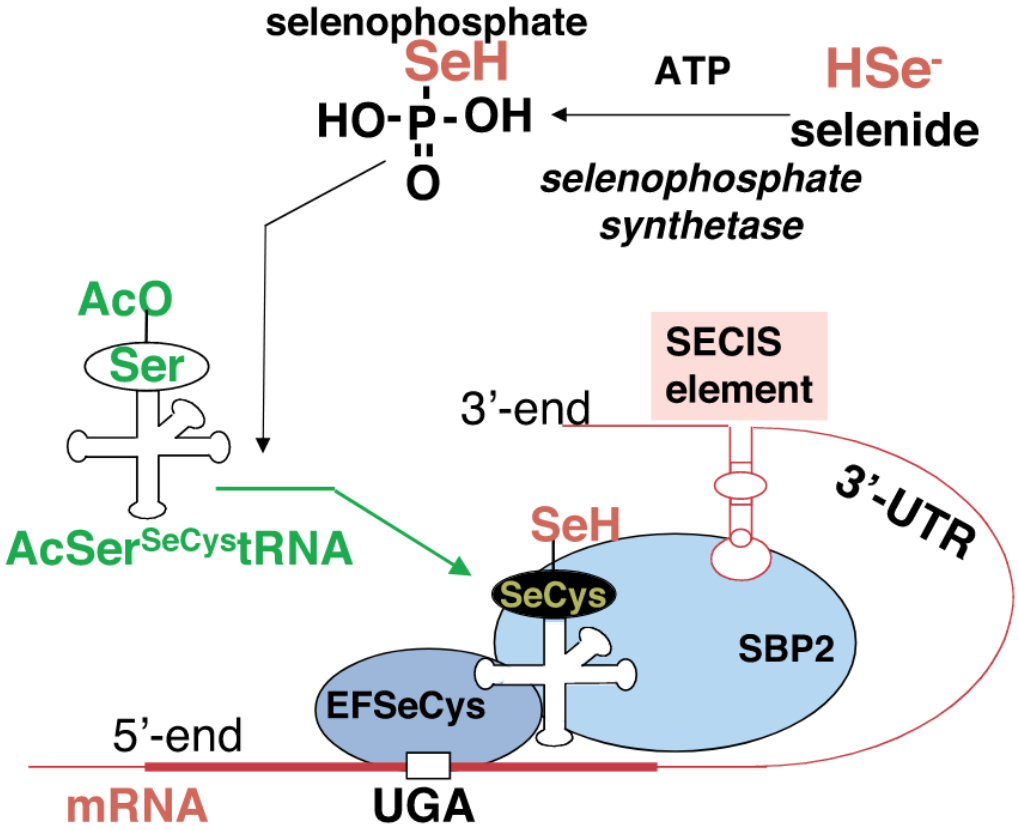
(Chase et al., 2008a)

Figure 2.4.3 Inorganic and Organic forms of selenium[1]



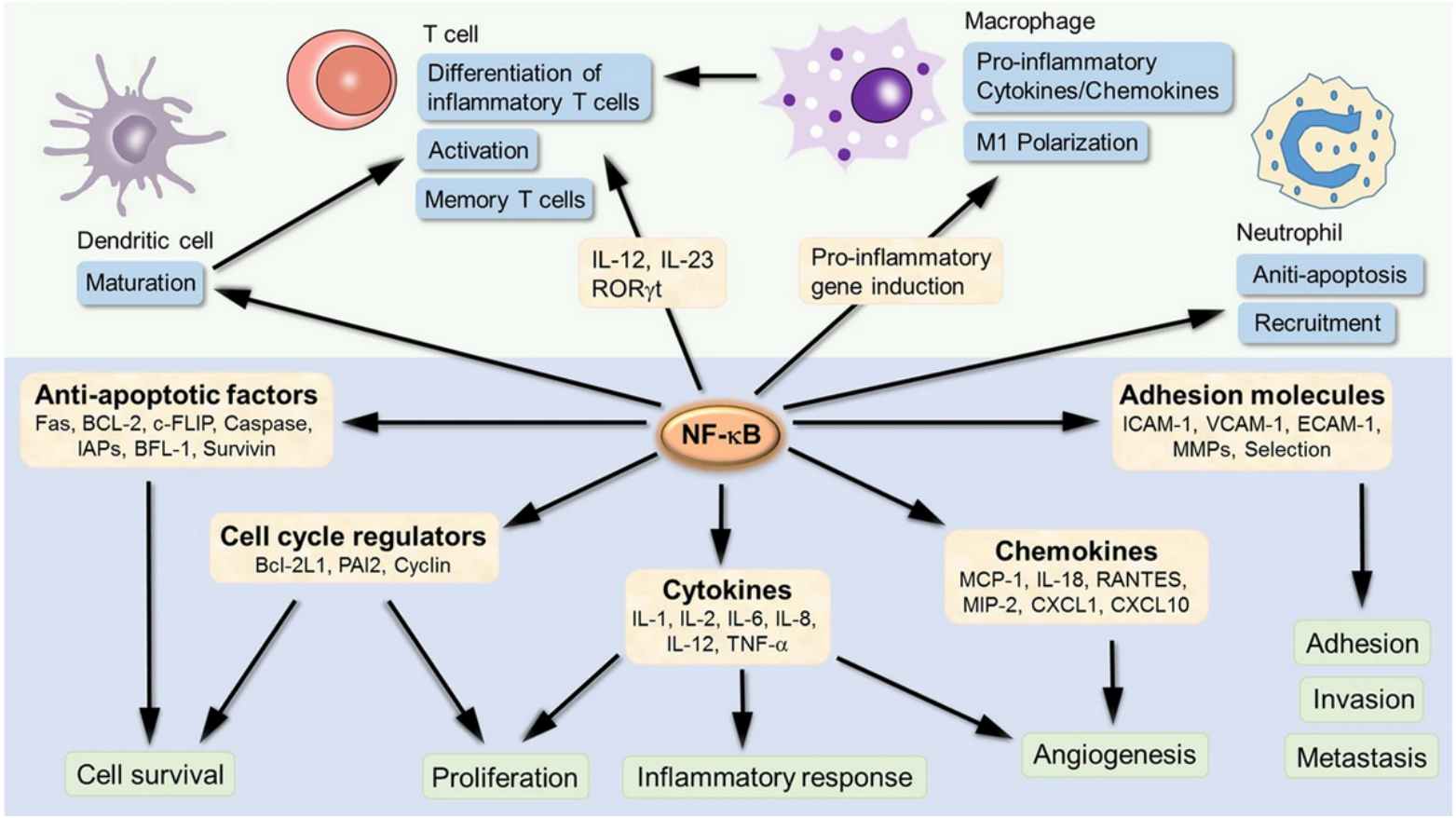
[1]Inorganic forms of selenium: Selenite and Selenate; Organic forms of selenium: Selenocysteine (Sec) and Selenomethionine (SeMet); precursors to the organic forms are shown as: cysteine and methionine, where Se replaces the sulfur in both amino acids. Created by adapting from Fernandes et al. (2012) and (Lindshield and Adhikari, 2011).

Figure 2.4.4 Illustrated representation of the translation mechanism for the synthesis of selenoproteins[1]



[1] Two cis-sequences, a SECIS element in the 3′-untranslated region and a Sec codon (UGA) in the coding region, and three trans-acting factors, a Sec-specific translation elongation factor (EFSeCys), the Sec ^{Sec}tRNA, and a SECIS-binding protein (SBP2) are proposed for the translation of the UGA codon to the Sec sequence. Adapted from Suzuki (2005).

Figure 2.6.2 Schematic illustration of NF-kB in inflammation[1]



[1]NF-kB target genes involved in inflammation development and progression. After its activation, it can activate transcription of various genes and thereby regulate inflammation. NF-kB target inflammation not only directly by increasing the production of inflammatory cytokines, chemokines and adhesion molecules but also regulating the cell proliferation, apoptosis, morphogenesis and differentiation (Liu et al., 2017).

CHAPTER 3. THESIS GOAL/HYPOTHESIS

One challenge to toxic endophyte-infected tall fescue-based beef cattle operations is ergot alkaloid induced fescue toxicosis. A hallmark of fescue toxicosis is a reduction in circulating prolactin (PRL) concentrations. In addition to the classic reduction in prolactin concentrations, a recent study has identified changes in several immune pathways of growing steers afflicted with fescue toxicosis (Liao et al., 2015). In this study, cell-mediated immune response was in the top five gene functional categories, and canonical analysis of differentially expressed genes showed that most of the affected pathways belonged to the cellular immune response category.

A second challenge to Kentucky-based cattle operations is that the forage is typically selenium (Se)-inadequate (below 0.1 ppm), due to Se-poor soils (Ammerman and Miller, 1975). Beef cattle require 0.10 mg Se/kg of diet to meet the daily requirements set by the NRC (National Academies of Sciences and Medicine, 2016). To remedy this, Se is supplemented using free-choice vitamin-mineral mixes. The inorganic (sodium selenite, ISe) form of selenium is the most supplemented. However, the use of organic forms of Se (SEL-PLEX, OSe) in vitamin-mineral mix results in greater blood and tissue Se assimilation (bioavailability) (Gunter et al., 2003; Liao et al., 2011). Feeding a 1:1 blend of ISe:OSe (MIX) of 35 ppm Se results in greater amounts of Se in whole blood, red blood cells, serum, and liver of cattle than when supplemented with only ISe (Brennan et al., 2011; Jia et al., 2018).

Therefore, the overall goal of this thesis was to investigate whether the MIX form of Se in vitamin mineral mixes consumed by growing beef steers grazing toxic-endophyte infected tall fescue pasture would ameliorate negative immune response parameters

associated with fescue toxicosis, and in the presence of an additional stressor. The specific hypotheses to be tested were: 1) a 1:1 blend (MIX treatment) of inorganic (sodium selenite, ISe) and organic (SELPLEX, OSe) forms of supplemental Se in vitamin-mineral mixes consumed by growing beef steers grazing endophyte-infected tall fescue forage would ameliorate the negative immune response parameters associated with fescue toxicosis, including white blood cell (WBC) abundance and gene expression. (Experiment 1, Chapter 4); and 2) the MIX form of supplemental Se would ameliorate the negative effects of an additional *in vitro* stressor (lipopolysaccharide challenge) on WBC gene expression. (Experiment 2, Chapter 4).

CHAPTER 4. MONOCYTE ABUNDANCE AND WHITE BLOOD CELL EXPRESSION OF PROLACTIN AND NF-κB PATHWAY GENES IN BEEF STEERS ARE ALTERED BY GRAZING TOXIC ENDOPHYTE-INFECTED TALL FESCUE AND LIPOPOLYSACCHARIDE CHALLENGE

4.1 Introduction

It has been well demonstrated that cattle consuming tall fescue (*Lolium arundinaceum*) infected with the endophytic fungus *(Epichloë coenophiala*) develop a collectively known syndrome of negatively affected physiological systems, called fescue toxicosis (Strickland et al., 2011). In addition to the classic reduction in prolactin concentrations, a more recent study has identified changes in several immune pathways of growing steers afflicted with fescue toxicosis (Liao et al., 2015). In this study, cell-

mediated immune response was in the top five gene functional categories, and canonical analysis of differentially expressed genes showed that most of the affected pathways belong to the cellular immune response category.

Another challenge to beef cattle operations utilizing toxic endophyte-infected tall fescue (TE) is that the soil is often deficient in Se, necessitating the need to provide supplemental Se (Dargatz and Ross, 1996a). Inorganic Se (ISe, sodium selenite) is the most commonly supplemented Se in cattle diets, while organic forms of Se (OSe) are also approved and available for beef cattle diets. The first goal of this study was to test the hypotheses that a 1:1 blend (MIX treatment) of inorganic (sodium selenite, ISe) and SEL-PLEX forms of supplemental Se in vitamin-mineral mixes consumed by growing beef steers grazing endophyte-infected tall fescue forage would ameliorate the negative immune response parameters associated with fescue toxicosis, including response in white blood cell (WBC) abundance and gene expression. The second goal was to test the hypothesis that the MIX form of supplemental Se would also ameliorate the negative effects of an additional *in vitro* stressor (lipopolysaccharide challenge) on WBC gene expression.

4.2 Materials and Methods

All experimental procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC protocol 1007A2006). All steers had ad libitum access to water, and their respective mineral treatments throughout the study (except during the 14-hr shrink period on the day before, and after, the grazing phase).

4.2.1 *Experimental Model*

This study is a subset of a larger study (Webb et al., July 2021). Sixteen fescue-naïve, predominantly Angus, growing steers (285 ± 21.8 kg) were purchased from a commercial cattle broker and managed under a 154 d, 4-phase (Receiving, Se-depletion, Se-repletion, Grazing), experimental regimen Figure 4.3.1. During the 14-d receiving phase, steers were housed in a feedlot shed in groups of 4 steers/pen. Steers had ad libitum access to a basal vitamin-mineral (BASAL V-M; (Jia et al., 2019); mix that was formulated to contain 27 mg Se/kg as inorganic Se as sodium selenite (ISe V-M mix) and to a standard hay (endophyte-free tall fescue and alfalfa), soyhull, and DGGS-based receiving diet. Next, steers (n = 16, 287 ± 21.8 kg) were subjected to a 21-d Se-depletion phase wherein they had ad libitum access to the BASAL V-M mix that lacked Se and were fed enough of an endophyte-free tall fescue and alfalfa hay and grain diet to achieve 0.57 kg/d BW gain. After the depletion phase, steers (n = 16, 307 ± 21.5 kg) were randomly assigned (n = 8) to have ad libitum access to the BASAL V-M mix that contained either 27 ppm Se as either sodium selenite (ISe; n = 8, 307 ± 19.1 kg) or a 1:1 blend of ISe and organic Se (SEL-PLEX, Alltech Inc, Nicholasville, KY) forms (MIX; n = 8, 307 ± 25.1 kg) for 35 d. During this 35-d Se-repletion phase, all steers were fed enough of the endophyte-free tall fescue and alfalfa hay and grain diet to achieve 0.57 kg BW gain/d. Finally, within their Se-form treatments (ISe = 332 ± 27.7 kg, n = 8; MIX = 328 ± 28.8 kg, n = 8), 2 steers were randomly assigned to graze one of 4, 2-acre endophyte-infected tall fescue paddocks for 84 d (May 30 to August 21, 2019). Throughout this grazing phase, steers had ad libitum access to their respective form of Se supplement V-M mix and identical 2.4 x 2.4 x 2.4 m shade structures. On day -1 and d 84

of the grazing period, steers were denied access to water and feed for 14 h to determine shrunk BW for calculation of ADG throughout the grazing period.

The composition of the BASAL V-M mix (determined by Dairy One Cooperative, Inc., Ithaca, NY) was 11.2% Ca; 6.3% P; 0.91% S; 2.29% Mg; 0.77% K; 10.2% Na; 1,288 ppm Fe; 2,917 ppm Zn; 1,540 ppm Cu; 3,680 ppm Mn; 1.70 ppm Mo; 250,000 IU/kg vitamin A; and 225 IU/kg vitamin E. The ISe V-M mix contained 24.2 ± 0.27 ppm Se, and the MIX contained 29.4 ± 4.8 ppm Se, and did not differ (P = 0.14).

4.2.2 *Forage Analysis*

Forage samples were collected as per Brown et al. (2009) from each of the 8 paddocks (6 sites per paddock) on d -1, 28, 56, and 83 of the 84-d grazing period. Samples were stored on ice during transportation to the laboratory and then frozen and stored at -20°C. Within a paddock, samples were pooled across sampling days. Proximate analysis and mineral content of forage were determined (http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures-Listing-Alphabetical-July-2015.pdf) by Dairy One Cooperative, Inc. (Ithaca, NY). Ergot alkaloid content (ergovaline and ergovalinine) was determined by the laboratory of Lowell Bush (University of Kentucky) using a high-performance liquid chromatography fluorescence procedure (Carter et al., 2010).

4.2.3 *Blood Collection and Analyses*

Individual steer jugular vein blood samples were collected by venipuncture on days 0, 28, 56, and 84 of the grazing phase. For serum prolactin analysis, whole blood

was collected in serum blood collection tubes (Becton Dickinson) lacking an anticoagulant. Serum was recovered after centrifugation at 3,000 × g for 10 min at 4 °C, and stored at -80 °C. Serum prolactin concentrations were quantified by the laboratory of Dr. Lannett Edwards (University of Tennessee), using a double-antibody RIA as described previously (Bernard et al., 1993). Low (5 ng/mL) and high (10 ng/mL) reference samples were included in the RIA. The intra-assay CV was 4.49% and the inter-assay CV was 8.59%.

For preparation of whole blood, blood was collected in sodium heparin-containing tubes (Becton Dickinson, Franklin Lakes, NJ). For complete blood cell count (CBC) analysis, whole blood was collected stored on ice and transported to the Veterinary Laboratory Diagnosticians approved – University of Kentucky Livestock Disease Diagnostic Laboratory (Lexington, KY, USA). The concentration of red blood cells (RBC), white blood cells (WBC), packed cell volume, and hemoglobin in whole blood was determined using a Hemavet HV 950S cell analyzer (Drew Scientific Inc., Miami Lakes, FL, USA). The whole blood concentration of neutrophils, lymphocytes, monocytes, and eosinophils were determined by manual identification and counting of cells (Feldman et al., 2000). For determination of whole blood Se concentration, blood was stored at -20 °C until analysis using an Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (Wahlen et al., 2005) by the University of Kentucky Livestock Disease Diagnostic Laboratory.

To determine the effect of Se form and summer-long grazing of endophyte-infected tall fescue on WBC gene expression (Experiment 1), whole blood was collected into TEMPUS TUBES (Invitrogen Corporation, Carlsbad, CA) and stored at -20 °C. To

determine the effect of in vitro LPS challenge (Experiment 2) on gene expression by WBC, 3 mL of whole blood was collected into heparin-containing tubes and inoculated with 6 µL of PBS or E. coli-derived lipopolysaccharide (LPS; 1mg/mL *E. coli* 0111:B4, MilliporeSigma, Temecula, CA), incubated in a water bath at 37° C for 2 h, and then loaded into TEMPUS TUBES and stored at -20 °C.

4.2.4 *Real-Time RT-PCR analysis*

For both Experiment 1 and Experiment 2, total RNA was extracted using the TEMPUS Spin RNA isolation kit (Invitrogen) following the manufacturer's instructions. The purity and concentration of total RNA samples was analyzed using a NanoDrop ND-100 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples had an average concentration of 90.22 µg/µL. Specifically, in Experiment 1, all samples had an average concentration of 96.15 ± 36.37 µg/µL, a high purity with 260/280 absorbance ratios of 2.01 to 2.16 and 260/230 absorbance ratios of 2.12 to 2.79. For Experiment 2, all samples had an average concentration of 83.21 ± 29.54 µg/µL, a high purity with 260/280 absorbance ratios of 2.05 to 2.18 and 260/230 absorbance ratios of 2.17 to 2.61. The integrity of total RNA was accessed by gel electrophoresis using an Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA) at the University of Kentucky Microarray Core Facility. Visualization of gel images and electropherograms showed that all RNA samples were of high quality. Specifically, Experiment 1 mRNA had an average 28S/18S rRNA absorbance ratio of 1.65 ± 0.20, and RNA integrity numbers (RIN) of 9.40 ± 0.30. Experiment 2 had an average 28S/18S rRNA absorbance ratio of 1.59 ± 0.23, and RIN values of 9.28 ± 0.37.

Primer sets **(Table 4.3.4)** for genes of interest and candidate calibrator genes were designed using the NCBI Primer- BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) based on their respective RefSeq sequence (https://www.ncbi.nlm.nih.gov/refseq/, accessed February to December 2019). All real-time RT-PCR cDNA products were validated by DNA sequencing and were 99% to 100% identical with their RefSeq (https://www.ncbi. nlm.nih.gov/refseq/) sequences (**Figures 4.3.4a** (prolactin pathway), **4.3.4b** (NF-kB pathway), **4.3.4c** (Candidate Calibration Genes)). Briefly, the PCR-amplified cDNA products were purified using the PureLink Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA), then sequenced by Eurofins Genomics (Eurofins MWG Operon LLC, Louisville, KY). The resulting sequences (**Figures 4.3.4a to 4.3.4c**) were compared to the NCBI RefSeq mRNA sequences used as templates for primer pair set design. From 9 candidate calibrator genes (ACTB, GAPDH, SDHA, UBC, YWHAZ, HPRT1, PPIA, TBP), 3 were determined to be constitutively-expressed based on their having the lowest average stability M value (geNorm software v3.5, (Vandesompele et al., 2002); 0.23, YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; 0.27, UBC, ubiquitin C; 0.23, SDHA, succinate dehydrogenase complex subunit A flavoprotein).

The relative quantification of mRNA content of genes of interest was performed using standard procedures in our laboratory, as previously described (Li et al., 2017). Briefly, 1 μg of each steers whole blood RNA was reversely transcribed to cDNA using the SUPERSCRIPT IV VILO (SSIV VILO) Master Mix (Invitrogen). Real-time RT-PCR was performed using an Eppendorf Mastercycler ep realplex2 system (Eppendorf, Hamburg, Germany) with iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). A total

volume of 25 µL was used in each real-time RT-PCR reaction containing 5 µL of cDNA, 1 µL of a 10 µM stock of each primer (forward and reverse), 12.5 µL of 2× SYBR Green PCR Master Mix, and 5.5 µL of nuclease-free water. The relative amount of each transcript was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001), calibrating mRNA content to the geometric mean of the 3 constitutively expressed genes and then normalizing calibrated values to day 0 ISe supplemented steers for Experiment 1 (Table 4.4.4a, Table 4.4.4b). For Experiment 2 (Table 4.4.4c, Table 4.4.4d), constitutively expressed gene-calibrated values were normalized to day 0, non-LPS-challenged ISe supplemented steers.

For the RT-PCR analysis in Experiment 1, n = 12 and 14 for ISe and MIX treatments, respectively. For Experiment 2, n = 12 and 10 for ISe and MIX treatments, respectively. All RT-PCR reactions were conducted in triplicate.

4.2.5 *Statistical Analysis*

Data are presented as least square means (± SD). Paddocks were the experimental units with 4 paddocks per Se form (n = 4). Comparison of proximate, mineral, and ergot alkaloid content between paddocks grazed by ISe vs. MIX steers was evaluated by ANOVA, using the GLM procedure of SAS (v 9.4, SAS Inst. Inc., Cary, NC). The effect of form of Se supplementation (ISe vs. MIX) and length of grazing endophyte-infected tall fescue (time, day 0 vs. day 84) on all other experimental parameters was evaluated by ANOVA, using the MIXED procedure of SAS. The statistical model included Se form, time, and their interaction as fixed effects. Class variables were Se supplementation and steer, with steer included in the random statement. The Kenward-Roger adjustment was

used to calculate the denominator of df (Kenward and Roger, 1997), and the PROC GLIMMIX procedure of SAS was used to verify the PROC MIXED results. Because no interactions between form of Se supplementation and time of grazing endophyte-infected tall fescue, the interaction term was removed and the model re-run. For all analyses, statistical significance was declared when $P \leq 0.06$ and the F-stat ≥ 4.0. For serum prolactin, data were analyzed after $\log_{10}$ transformation to stabilize variation between d 0 and 84 means.

4.3 Results

4.3.1 *Nutrient and Ergot Alkaloid Profiles of Paddock Forage*

The composited means of proximate, mineral, and alkaloid analysis of pasture samples are presented in **Table 4.4.1**. The mean (n = 4) TDN (59.56, 60.00%), CP (10.86, 11.79%), NEgain (0.29, 0.30 Mcal/lb.) did not ($P > 0.532$) differ. Likewise, the mean (n = 4) ergovaline content (μg/g) between the ISe (0.277 ± 0.143) and MIX (0.280 ± 0.092) steer paddocks did not differ ($P = 0.971$), nor ($P = 0.090$) did the content of ergovalinine (ISe, 0.189 ± 0.030; MIX, 0.149 ± 0.025).

4.3.2 *Whole Blood Se and Serum Prolactin concentrations*

The concentration of Se in whole blood was greater ($P = 0.001$) for MIX than for ISe steers, and lesser ($P < 0.001$) after 84 days of grazing E+ forage (**Table 4.4.2**). No interaction between Se treatment and day effects was found ($P = 0.74$). Across days, MIX steers had 24% greater whole blood Se concentrations than did ISe steers. Across Se treatments, d 84 Se concentrations were 35% lesser than at d 0.

The mean serum prolactin concentration decreased ($P < 0.01$) from day 0 to day 84 and a day x treatment interaction reflected a 98% greater ($P = 0.06$) prolactin concentration for MIX than ISe steers at day 84.

4.3.3 *Blood Cell Types and Parameters*

MIX steers averaged 9.8% more ($P = 0.01$) RBC across d 0 and 84 than did ISe steers (**Table 4.4.3**). Whereas the number of total of WBC, and individual counts of neutrophils, lymphocytes, and eosinophils, were not affected ($P \geq 0.14$) by Se treatment, 84 d of grazing TE, or their interaction. The number of circulating monocytes across both d was 33% less ($P = 0.04$) in MIX than ISe steers and 48% less ($P = 0.004$) at d 84 than 0 across both MIX and ISe steers.

The percent hematocrit and MCV numbers were not affected ($P \geq 0.14$) by Se treatment, 84 d of grazing TE, or their interaction. There was no ($P \geq 0.17$) Se treatment effect, or treatment*day interaction for MCH and hemoglobin, however there was an 8% ($P \leq 0.004$) decrease in MCH, and a 9% ($P = 0.005$) decrease in hemoglobin after the 84-d grazing of TE. The number of MCHC across both d was 3% greater ($P = 0.009$) in MIX than ISe steers and 10% less ($P < 0.001$) at day 84 than 0 across both MIX and ISe steers.

4.3.4 *WBC Gene Expression*

The template sequences, primer sequences used to generate RT-PCR amplicons, and amplicon length, % identity, and GenBank accession number of sequence-validated amplicons used to evaluate the effect of Se form and summer-long grazing of endophyte-infected tall fescue on the relative mRNA content of selected genes in the prolactin

pathway and NF-kB pathway, and calibrating genes are presented (**Table 4.3.4**). The sequences of the amplicons are presented in **Figures 4.3.4a (prolactin pathway), 4.3.4b (NF-kB pathway), and 4.3.4c (Candidate Calibration Genes)**. All amplicons had at least 99% identity with their template sequences, thus validating the use of these primers for RT-PCR analysis of gene expression.

4.3.4.1 Experiment 1

Prolactin pathway (**Table 4.4.4a**). There was no ($P \geq 0.11$) effect of form of Se on relative mRNA content in whole blood for any gene in the prolactin pathway. In contrast, there was a day effect ($P \leq 0.05$) on expression of 5 of 10 selected genes. For CISH, there was ($P < 0.01$) a 76% increase ([1.78 + 1.59]/ [1.07 + 0.85] in expression after summer-long (84 d) grazing of endophyte-infected tall fescue. Similarly, there was a 20% increase in expression STAT5B. In contrast, there was a decrease ($P \leq 0.05$) in expression for PPIB (21%), AGAP2 (31%), and SPRLR (44%) after steers grazed TE for 84 d.

NF-kB pathway (**Table 4.4.4b**). There was no ($P \geq 0.07$) effect of form of Se on relative mRNA content in whole blood for any gene in NF-kB pathway. In contrast, there was a day effect ($P \leq 0.06$) on 7 of 10 selected genes. After consumption of TE for 84 days, the relative content of IL-8 (258%), RELA (10%), NFKBIA (126%), TLR4 (74%) mRNA increased ($P \leq 0.04$). In contrast, the relative mRNA content decreased ($P \leq 0.06$) for IKBKG (22%), ZAP70 (20%), and LCK (33%).

4.3.4.2 Experiment 2

Prolactin pathway (**Table 4.4.4c**). There was no ($P \geq 0.19$) effect of form of Se on relative mRNA content in whole blood of any of the 10 selected genes in the prolactin pathway after a 2-h in vitro challenge exposure to LPS. In contrast, there was a day effect ($P \leq 0.03$) on 4 of 10 selected genes. After consumption of TE for 84 days, the relative content of CISH (57%) increased. In contrast, the relative mRNA content decreased ($P \leq 0.01$) for PPIB (29%), LPRLR (27%), and SPRLR (37%).

NF-kB pathway (**Table 4.4.4d**). There was no ($P \geq 0.18$) effect of form of Se on relative mRNA content in whole blood for any of the selected genes in the NF-kB pathway after a 2-h in vitro challenge exposure to LPS. In contrast, there was a day effect ($P \leq 0.05$) on expression of 4 of 10 selected genes. After 84 d of grazing TE the mRNA content of IL-8 (76%), TNFa (42%), and TLR4 (51%) increased ($P \leq 0.05$). Whereas the relative mRNA content decreased ($P \leq 0.03$) for LCK (27%).

4.4 Discussion

4.4.1 *Animal Model*

The current study assessed the effect of ad libitum intake of a basal vitamin-mineral mix that contained 27 mg/kg (27 ppm) of supplemental Se as either the ISe or MIX forms of Se to growing fescue-naïve beef steers purchased as a group from MN. Because of their unknown mineral status, all steers were initially managed under a common mineral management regimen. Upon receipt, all steers had ad libitum access to the ISe form of Se supplement for 14 d, followed by a 21-d Se-depletion phase consisting of ad libitum access to the basal vitamin-mineral mix that lacked any form of Se.

Previous research has shown this regimen results in stabilized concentrations of whole blood and liver Se (Brennan et al., 2011; Liao et al., 2011). Then, steers had ad libitum access to their respective ISe or MIX Se form treatments throughout the subsequent 35-d pre-grazing repletion period, 84-d grazing of TE paddocks period, and 3- to 23-d grazing of TE while awaiting slaughter.

Within the grazing and slaughter periods, 2 steers grazed each 2-acre TE paddock, and steers from 1 paddock of each Se form treatment were killed on any given slaughter day. There was no statistical difference in the mean concentration of ergot alkaloids (ergovaline plus ergovalinine) between ISe (0.466 ppm) and MIX (0.429 ppm) paddocks, and the ergot alkaloid concentrations were stable throughout the grazing and slaughter periods (Webb et al., July 2021).

Whole-animal Se status is commonly determined by measuring the concentration of Se, or glutathione peroxidase activity, in whole blood, serum, or tissues. Whole blood Se concentrations are well correlated with Se intake (Hall et al., 2013). In growing cattle, whole blood Se concentrations are a better proxy for liver Se concentrations than either serum or red blood cell Se concentrations (Brennan et al., 2011). Although there is no consensus as to what concentrations of whole blood Se constitute a deficient or sufficient status, studies by Gerloff (1992) and Dargatz and Ross (1996a) reported that concentrations greater than 100 ng/mL, or between 81-160 ng/mL are adequate, respectively. In the current study, across-periods mean whole blood Se concentration ranged from 147 to 239 and 196 to 281ng/mL in ISe and MIX steers respectively, indicating that all steers had adequate Se.

Therefore, as described above, an appropriate animal model was successfully established to evaluate the potential effect of different forms of supplemental Se treatments on physiological parameters of Se-adequate beef steers subjected to summer-long grazing of TE pastures.

4.4.2 *MIX form of Se supplementation resulted in greater whole blood Se than ISe*

In the current study with growing beef steers having ad libitum access to vitamin-mineral mixes that contained 27 ppm Se as ISe or MIX forms, the whole blood Se concentration of MIX steers was 27% greater than for ISe steers. This difference is consistent with previous studies that imposed a controlled intake of vitamin-mineral mixes that contained 35 ppm ISe of MIX and was consumed in a predetermined amount to deliver 3 mg Se/d in slow-maturing beef heifers (Brennan et al., 2011) or growing beef steers grazing TE pastures (Jia et al., 2018). Thus, an important understanding gained from the current study is that whole blood Se concentrations can be increased by ad libitum consumption of industry-typical regimen of ad libitum consumption of vitamin-mineral mixes containing supplemental Se in the MIX form.

4.4.3 *Serum prolactin concentrations*

One of the physiological hallmarks of fescue toxicosis is the reduction in serum prolactin of cattle consuming endophyte-infected tall fescue (Goetsch et al., 1987; Davenport et al., 1993). For example, serum prolactin concentrations in growing beef steers subjected to summer-long grazing of high endophyte-infected tall fescue were

decreased 85% to 90% relative to steers grazing low endophyte-infected forage (Brown et al., 2009; Jackson et al., 2015). Of note, and as typical for the beef industry, the steers had ad libitum access to a vitamin-mineral mix that contained the ISe form of supplemental Se.

The potential differential effects of Se forms on serum prolactin and other indicators of fescue toxicosis in growing beef steers were recently studied by our lab (Jia et al., 2018; Jia et al., 2019). Using a controlled consumption of 3 mg Se per day as 100% ISe, 100% OSe, or MIX (50:50, ISE, MIX) while grazing of TE pasture, implemented using in-pasture Calan gates, the study found that OSe and MIX steers had 59% and 52% more serum prolactin than ISe steers, respectively. The intake of supplemental Se was set at 3 mg per day because that is the maximal legal limit for daily supplemental Se to cattle allowed by the Food and Drug Administration, and is considered adequate to achieve the NRC (National Academies of Sciences and Medicine, 2016) recommendation of 0.1 mg/kg of diet.

In the current summer-long grazing study (conducted June to August of 2019) grazing study, where steers exercised ad libitum intake of Se-containing vitamin-mineral mixes of 27 ppm ISe or MIX form of Se, serum prolactin concentrations decreased (88%) from d 0 to d 84 regardless of Se form treatment. This result is consistent with the known inverse relationship between length of photoperiod and concentration of circulating prolactin in several species, including cattle (Dahl et al., 2000). In addition to photoperiod effects, previous research (Aldrich et al., 1993; Jackson et al., 2015; Jia et al., 2018) has shown that serum prolactin concentrations decrease rapidly after consumption of TE forages, a decrease that can be partially ameliorated by consumption

of MIX versus ISe supplemental Se (Jia et al., 2018). Similarly, in the current study with ad libitum intake of vitamin-mineral mix, serum prolactin was 98% greater for MIX than ISe steers, after 84 days of grazing TE forage. Thus, in two summer-long grazing studies, one with a controlled intake of 3 mg per d as ISe or MIX (Jia et al., 2018), and ad libitum intake of ISe or MIX supplemental Se in vitamin-mineral mixes, the intake of MIX form of Se ameliorated the negative effects of consuming TE forages containing about 0.50 ppm ergovaline plus ergovalinine.

4.4.4 *Circulating Monocyte Abundance Was Decreased by Ergot Alkaloid Consumption*

In the current study, there was no change in levels of total WBC, neutrophils, lymphocytes, or eosinophils with regards to Se treatment or consumption of endophyte-infected tall fescue for 84 d (Table 4.4.3). In contrast, the abundance of circulating monocytes was decreased. These findings are consistent with another summer-long evaluation of the effect of consuming TE forage on circulating WBC (Jackson et al., 2015) in which it was found that monocyte abundance decreased after 36 d of grazing TE forage. Because monocytes are an important source of circulating IL-8, the physiological significance of reduced monocyte abundance is that the capacity to recruit neutrophils to sites of infection may have been reduced by TE consumption (Remick, 2005).

Although neutrophil abundance statistically did not differ in response to TE consumption, LPS exposure, or their interaction, it is important to note that IL-8 recruits neutrophils to sites of infection (Remick, 2005). This would be an area for future research regarding the organs negatively affected by endophyte-infected tall fescue. In a

transgenic murine study, high levels of human IL-8 led to neutrophil accumulation in vascular beds of a few organs including the liver (Simonet et al., 1994). As mentioned above, a previous study by Liao et al. (2015), reported that in the liver, most of the affected pathways belong to the cellular immune response category. Therefore, examining WBC levels in liver samples of cattle consuming endophyte infected tall fescue would further enhance the research done in the current as well as previous studies (Liao et al., 2015; Jia et al., 2018).

4.4.5 *Experiment 1*

4.4.5.1 Se form effect on WBC gene expression

The first goal of the present study was to test the specific hypothesis that negative immune response would be ameliorated by the forms of Se in steers. As described above, serum prolactin decreased 88% by d 84 and was 98% greater ($P < 0.01$) for MIX steers on d 84. However, expression of prolactin pathway (Figures 4.5.5a, b) genes by WBC did not differ between MIX and ISe steers (Table 4.4.4a). Similarly, expression of genes of the NF-kB pathway (Figures 4.5.5c, d) (Table 4.4.4b) did not differ between MIX and ISe steers. Therefore, our initial hypothesis is rejected. However, gene expression in the prolactin and NF-kB pathways did change after summer-long grazing of endophyte-infected tall fescue, regardless of supplemented Se form.

4.4.5.2 Consumption of TE forage effect on WBC gene expression

4.4.5.2.1 PROLACTIN PATHWAY

Eighty-four-d consumption of TE forages increased the expression of CISH and STAT5B, and decreased the expression of PPIB, AGAP2, and the short form of the prolactin receptor (SPRLR) (Table 4.4.4a; Figure 4.5.5e). In a study in mice, it has been shown that STAT5A is critical in PRL signaling (Liu et al., 1997), whereas STAT5B is necessary to maintain liver gene expression (Udy et al., 1997), IL-2 mediated T-cell proliferation, and NK cell development (Imada et al., 1998; Moriggl et al., 1999). Relative expression of CISH and STAT5B were found to be upregulated after summer-long grazing of endophyte infected tall fescue. CISH and STAT5B are a part of the JAK-STAT pathway, and cellular signals from the JAK-STAT pathway are involved in immunity and inflammation (Kaplan, 2013). CISH has been shown to be critical for T-cell proliferation and survival in response to infection (Niu et al., 2017). In the current study, upregulation of CISH in WBC during consumption of endophyte-infected tall fescue along with unremarkable changes in lymphocyte values (Table 4.4.3) appear to agree with previous research regarding T-cell survival. However, more information is needed to determine if there is a cause and effect with regards to the JAK-STAT pathway and the adaptive immune response. STAT5 has been shown to induce CISH expression (Matsumoto et al., 1997), however CISH is a negative modulator of STAT5, therefore indicating another gene may be responsible for the upregulation of STAT5B in the current study. The CISH family of proteins are feedback inhibitors for cytokine signaling and function to limit the duration and/or magnitude of cytokine signaling. CISH expression can prevent hyper-activation of immune responses, thereby bringing the

system back to a homeostatic state (Linossi et al., 2018) and obviating dysregulation associated with chronic inflammation (Yoshimura et al., 2012).

The relative expression of PPIB, AGAP2, and SPRLR genes were found to be downregulated (Figure 4.5.5e) after consumption of endophyte-infected tall fescue for 84 d. PPIB is a chaperone protein involved in modulating the host immune response (Pandey et al., 2017). Studies have reported that PPIB might be contributing factor during inflammation (Bukrinsky, 2002), and suggest that PPIB might recruit T-cell populations to tissue *in vivo* therefore regulating inflammatory response (Allain et al., 2002). Thus, a decrease in circulating PPIB could be due to higher levels of expression in other tissues being affected by fescue toxicosis. However, further research is needed to validate this supposition.

Also known as PIKE, AGAP2 has been linked to STAT5A and the PRLR expression. To date, PIKE has had three forms characterized, with PIKE-A being expressed by a variety of tissues, whereas PIKE-L and PIKE-S expression is brain specific (Ahn and Ye, 2005). However, northern blot analysis shows that PIKE-A only has small mRNA expression in various non-brain organs including the liver, spleen, thymus, small intestine, and periphery blood leukocytes (Qi and Ye, 2013). AGAP2 has been designated as PIKE-A (Ahn and Ye, 2005). Importantly, PIKE-A has been implicated in regulating the activity of STAT5 in the PRL-stimulated JAK-STAT cascade (Chan et al., 2010). Moreover, bovine pituitary LPRLR mRNA content is decreased by endophyte consumption, whereas SPRLR mRNA content was not (Li et al., 2017). In contrast, in the current study, WBC LPRLR was not affected by endophyte consumption, whereas SPRLR mRNA was reduced. The reduction in SPRLR mimics the reduction in

circulating PRL after consumption of endophyte infected fescue. However, due to increasing photoperiod time, PRLR could be reduced due to photoperiod effects. Although more research is needed to determine the full effects of TE consumption on this pathway, it is a promising area for future research on different tissue types. Circulating PRL levels are highly relevant to mRNA levels of PRLR in both the pituitary gland and hypothalamus, indicating that PRL positively regulates PRLR expression (Leclerc et al., 2007). It has been well documented that circulating PRL levels are reduced during long photoperiods, as well as when exposed to endophyte-infected tall fescue. Therefore, it's likely the reduction of SPRLR is due to the reduction of circulating PRLR in WBC. However, it is known that PRL cannot activate the JAK/STAT pathway through the SPRLR (Binart et al., 2010), therefore suggesting an alternative gene is involved in the activation of this pathway. However, due to the lack of change in LPRLR, the activation of JAK/STAT could have stemmed from this gene, as it's been shown that PRL activates this pathway through the LPRLR (Goffin et al., 2002).

4.4.5.2.2 NF-KB PATHWAY

The relative expression of IL-8, RELA, NFKBIA, and TLR4 were upregulated after summer-long grazing of endophyte infected tall fescue (Table 4.4.4b; Figure 4.5.5f). In humans, IL-8 has been implicated in several inflammatory diseases. The primary function of IL-8, a potent pro-inflammatory chemokine is to recruit and activate inflammatory cells, mainly neutrophils to sites of infection (Remick, 2005). As mentioned previously, monocytes represent an important source of IL-8, however, it can also be produced via T-lymphocytes and neutrophils (Gessler et al., 2003). High levels of

IL-8 have been reported after either septic shock or systemic ingestion of endotoxins (Martich et al., 1991). IL-8 is increased after ingestion of TE, and due to decreasing monocyte levels, it's likely the production of IL-8 came from another leucocyte or tissue type. In addition to monocytes, it can be produced from macrophages, smooth muscle cells, and endothelial cells that recruit neutrophils, T-cells, and basophils to sites of infection (Mitchell et al., 2003). Transcription of IL-8 is NF-kB dependent, and acts as a potent chemoattractant and activator of neutrophils (Sen, 2000).

Many key cellular processes such as cell survival, proliferation, and immunity are regulated through NF-kB dependent transcription. Dysregulation of NF-kB pathways result in severe diseases including immunodeficiency (Oeckinghaus and Ghosh, 2009), and chronic inflammation (Chen and Greene, 2004). In resting cells, NF-kB proteins are kept in association with IkB proteins including NFKBIA which is the most abundant (Gupta et al., 2010). In the current study, NFKBIA mRNA content was increased after consumption of TE (Table 4.4.4b). NFKBIA is primarily believed to inhibit p65 containing complexes (REL A). However the knowledge about the mechanisms leading to NF-kB activation far exceeds what is known about the regulatory mechanisms that determine its inactivation (Oeckinghaus and Ghosh, 2009). The best studied and well accepted mechanism for the inactivation of NF-kB response involves the resynthesis of IKB proteins that are induced by activated NF-kB (Pahl, 1999). REL A signaling pathway has been a pivotal point for drug discovery, as disproportionate increase in activated REL A is integral to many chronic diseases (Giridharan and Srinivasan, 2018). REL A is subjected to phosphorylation, and like NFKBIA, its increase in mRNA expression may be due to phosphorylated versions of the gene. This could explain the

reason why NFKBIA levels increased, as phosphorylated REL A has a lower affinity for its negative regulator, NFKBIA (Bohuslav et al., 2004). More NFKBIA would be expected to be produced in a negative feedback loop if the target gene was less responsive in the phosphorylated form. Activation of NF-kB has been reported to require the phosphorylation, polyubiquitination, and subsequent degradation of NFKBIA (Gupta et al., 2010). However, in the current study NFKBIA mRNA content is increased about 100%. Although this expression has increased, it could be due to the phosphorylated levels of NFKBIA, and further testing is needed to distinguish between the phosphorylated and unphosphorylated versions.

Whereas TLR4 is typically upregulated in response to LPS exposure, TLR4 mRNA content was increased after consumption of endophyte-infected tall fescue, without the presence of LPS (Table 4.4.4b). A study using bromocriptine in place of ergot alkaloids showed that TLR4 expression was upregulated in the top 30 regulators for both the intestinal epithelium and mesenteric adipose tissue (McLean et al., 2020). The current study showcases an increase in TLR4 expression by WBC in response to consumption of endophyte infected fescue.

In contrast to the aforementioned NF-kB pathway genes, the expression of IKBKG, ZAP70, and LCK genes were decreased (Table 4.4.4b; Figure 4.5.5f) after summer-long grazing of endophyte infected tall fescue. IKBKG encodes the regulatory subunit of the inhibitor of kappaB kinase (IKK) complex, which activates NF-kB, resulting in the downstream activation of genes involved in inflammation, immunity, cell survival, and other pathways. The classical NF-kB pathway depends on IKK complex consisting of IKKα, IKKβ, IKKγ (IKBKG) and the inhibitory subunit IκBs (Gupta et al.,

2010). Although it is known that IKBKG (a.k.a. NEMO) is required for the formation of a high molecular weight IKK complex, the exact role of this in IKK activation remains unclear (Tang et al., 2003). It has been proposed that IKBKG may link upstream activators to the IKK complex, due to its interaction with a variety of signaling molecules (Tang et al., 2003). NF-kB is involved in both the adaptive and innate immune responses. Immunodeficiency associated with IKBKG mutations results from an impaired NF-kB activation after stimulation by various receptors such as TLR (Fusco et al., 2015). However, it has also been reported that ubiquitin binding by IKBKG is essential for IKK activation (Chen and Chen, 2013), which could be the reason for the decreased mRNA expression found in the current study. Determination of the length of IKBKG that was being expressed due to polyubiquitination would help understand the reasoning behind this finding.

LCK is a lymphocyte-specific protein tyrosine kinase. Whereas genes in the same family as LCK have been implicated in many intracellular signaling pathways in macrophages, LCK expression is initiated by a diverse set of receptors, including the TLR family. However, it has been difficult to implicate any given LCK family member in any specific pathway (Abram and Lowell, 2008). LCK tends to be more restricted to cells of hematopoietic origin. In the current study, LCK expression was decreased after consumption of infected tall fescue (Table 4.4.4.b). In T-cells, LCK is critical to the early propagation and modulation of T-cell receptor (TCR) signaling. LCK protein is expressed at a fairly constant level throughout T-cell development and is largely constitutively active in resting T lymphocytes (Rossy et al., 2012). TCR signaling relies on the phosphorylation by LCK of receptor complex at immunoreceptor tyrosine-based

activation motif (ITAM) consensus sites (Rossy et al., 2012). ZAP70 is then recruited by ITAMs and requires additional phosphorylation by LCK to be activated (Wange and Samelson, 1996; Palacios and Weiss, 2004). Subsequently, ZAP70 phosphorylates other proteins in the TCR cascade, leading to T-cell activation. The current study showed a decrease in both LCK and ZAP70 mRNA after consumption of infected tall fescue. This finding suggests a reduction in the adaptive immune response in cattle grazing endophyte-infected tall fescue due to the decreased expression of both TCR-activating genes. While TCR activation has no impact on LCK activity, it is not unreasonable that while lymphocyte levels did not change LCK expression decreased. Given that IL-8 can be produced from T-lymphocytes, and the reduction in both TCR activating genes LCK and ZAP70, these findings suggest that neutrophils are the source of IL-8 production during the consumption of endophyte-infected fescue.

Statistically ($P = 0.07$), TNFα mRNA content was not affected by consumption of TE forages (Table 4.4.4b). Quantitatively, however, TNFα mRNA content increased by 81%. Consistent with this quantitative increase, TNFa expression is typically upregulated in cattle grazing endophyte-infected fescue, a phenomenon most recently reported by (Poole et al., 2019).

4.4.6 *Experiment 2*

4.4.6.1 Se form effect on WBC gene expression after in vitro LPS challenge

The second goal of the present study was to test the specific hypothesis that the MIX Se form treatment would ameliorate the effects of an additional stressor, LPS, in addition to the negative immune response in cattle grazing endophyte-infected tall fescue.

Cytokines are produced by leukocytes in response to exposure to bacterial toxins (Qazi et al., 2011). To test this hypothesis, the blood of ISe and MIX steers was inoculated with LPS after collection, at day 0 and day 84. Selenium form did not affect expression of prolactin (Table 4.4.4c) nor NF-kB (Table 4.4.4d) pathway genes after in vitro LPS challenge. Along with the findings of Tables 4.4.4a and 4.4.4b, these results indicate that total WBC gene expression is not affected by the form of ad libitum-accessed supplemental Se consumed by Se-adequate beef steers grazing TE forages.

4.4.6.2 Consumption of TE forage effects on WBC gene expression after *in vitro* LPS challenge

4.4.6.2.1 PROLACTIN PATHWAY

LPS is used as a substitute to induce physical stress. The combination of a LPS-induced physical stress in conjunction with consumption to ergot alkaloids leads to poor growth performance and could potentially compromise expression of genes important to optimal immune function in steers, including an acute-phase immune response, compromising growth performance (Carroll et al., 2009; Arthington et al., 2013).

The expression of CISH was increased (Figure 4.5.6a) after consumption of endophyte-infected tall fescue with LPS as an additional stressor. CISH is part of the JAK-STAT pathway, and cellular signals from this pathway are involved in immunity and inflammation (Kaplan, 2013). CISH has been shown to be critical for T-cell proliferation and survival in response to infection (Niu et al., 2017). In the current study, upregulation of CISH in WBC during consumption of endophyte-infected tall fescue along with unremarkable changes in lymphocyte values (Table 4.4.3) appear to agree with previous research regarding T-cell survival. However, more information is needed to

determine if there is a cause and effect with regards to the JAK-STAT pathway and the adaptive immune response. CISH is a negative modulator of STAT5, therefore suggesting a role in the unremarkable expression levels of STAT5A and STAT5B after exposure to LPS. A study by Jiang et al. (2020) showed that in carp hepatocytes, incubation with LPS induced rises in CISH mRNA levels. With CISH being a feedback inhibitor for cytokine signaling, their inhibitory effects are mediated through the JAK/STAT pathway (Yoshimura et al., 2018).

In contrast to CISH, the expression of PPIB, LPRLR, and SPRLR genes were decreased (Figure 4.5.6a) after exposure to LPS. PPIB is a chaperone protein involved in modulating the host immune response (Pandey et al., 2017). Studies have reported that PPIB might be contributing factor during inflammation (Bukrinsky, 2002), and suggest that PPIB might recruit T-cell populations to tissue *in vivo* therefore regulating inflammatory response (Allain et al., 2002). A decrease in circulating PPIB could be due to higher levels of expression in other tissues being affected by fescue toxicosis and LPS, however, further research is needed. PPIB also is known as cyclophilin B (CypB). It has been shown that LPS-stimulated macrophages did not have a modified response to CypB when added simultaneously. However, when added prior to LPS exposure, production of pro-inflammatory cytokines decreased (Marcant et al., 2012). Although these findings by Marcant et al. (2012) suggest that CypB may act as an anti-inflammatory factor and mediate tolerance to cells exposed to proinflammatory stimuli, the current study indicates that consumption of infected tall fescue reduces the expression of PPIB, thereby indicating an increase in inflammatory responses in cells exposed to LPS, an indication strengthened by an increase in CISH, TNFα, and IL-8.

A murine study showed that expression of L-PRLR mRNA was predominantly in adrenal glands, pituitary, thymus, spleen, skin, heart, and skeletal muscle, whereas SPRLR was expressed to a greater extent in both the kidney and lung (Ouhtit et al., 1993). In the current study, the detection of both SPRLR and LPRLR mRNA (Tables 4.5.5e and 4.5.6a) demonstrates that both the short and long form of SPRLR and LPRLR are expressed by bovine WBC. Because SPRLR was decreased after 84 d of grazing in both Experiment 1 (Table 4.5.5e) and Experiment 2 (Table 4.5.6a) the SPRLR gene may be insensitive to LPS challenge. In contrast, however, because LPRLR was not affected by consumption of ergot alkaloids (Table 4.5.5e) but was after the additional stress of LPS challenge, LPRLR expression appears sensitive to LPS challenge. This conclusion is consistent with the demonstration of decreased mouse LPRLR mRNA and protein after LPS challenge (Corbacho et al., 2004). Thus, a salient understanding from the current study is that the regulation of bovine PRLR isoforms may be differentially sensitive to physiological stressors in at least WBC.

4.4.6.2.2 NF-κB Pathway

The expression of IL-8, TLR4, and TNFα genes was increased (Figure 4.5.6b) after consumption of endophyte-infected tall fescue with LPS as an additional stressor. In contrast the content of LCK mRNA was decreased (Figure 4.5.6b).

In a previous study, it was reported that chronic exposure to ergot alkaloids suggests a hyperactive innate immune response like inflammation, which may lead to immune-compromised animals having greater susceptibility to disease (Poole et al., 2019). In the current study, summer-long grazing of endophyte infected tall fescue

increased concentrations of proinflammatory cytokine and chemokine TNFα and IL-8 respectively. Primarily proinflammatory cytokines act to mediate innate immune response and mediators such as TNFα induce a family of chemokines including IL-8 (Graves and Jiang, 1995b). IL-8 represents an important event in the inflammatory cascade (Graves and Jiang, 1995a). IL-8 production is induced by pro-inflammatory agents such as TNF and LPS (Barnes et al., 1992; Tamura et al., 1992). IL-8 is critical for recruitment of leukocytes to the site of infection and is upregulated in response to LPS via the TLR4 pathway (Dentener et al., 1993). Given these understanding, IL-8 and TLR4 expression would be expected to increase after a LPS challenge. Consistently, both IL-8 and TLR4 mRNA content was increased in response to the in vitro LPS challenge (Table 4.5.5f).

In healthy tissues, IL-8 is scarcely detectable, but upon response to proinflammatory cytokines or bacterial products, it is rapidly induced up to 100-fold (Hoffmann et al., 2002). TNFα expression was also increased in the current study, and has been shown to help enhance IL-8 secretion in the presence of LPS (Sohn et al., 2007). It is likely that both TNFα and LPS were key mediators in IL-8 expression in this experiment. The current findings of increased TNFα expression in the presence of LPS are also supported by a previous study showing increased serum TNFα concentrations in cattle grazing endophyte infected tall fescue following LPS administration (Filipov et al., 1999b).

TLR dependent activation of monocyte/macrophage release of TNFα is considered a key driver of subsequent inflammation (Marcant et al., 2012). Recognition of LPS by immune system cells is mediated through TLR4 (Hoshino et al., 1999). Primarily, these

receptors are expressed by macrophages, and generate an initial inflammatory response by binding LPS. LPS binds to TLR4 in a complex that results in phosphorylation of NF-kB components, leading to the production of pro-inflammatory cytokines such as TNFα, and chemokines such as IL-8 (Bromfield and Sheldon, 2011). In the present study, TLR4 was found to be increased after exposure to LPS and TE-infected fescue, corroborating previous findings. Cytoplasmic tyrosine kinase Src family members (LCK) have been implicated in many intracellular signaling pathways in macrophages, initiated by a diverse set of receptors including the toll-like receptors (Abram and Lowell, 2008). Amongst some of the family members, LCK tends to be more restricted to cells of hematopoietic origin. In the current study, LCK expression was found to be decreased after consumption of infected tall fescue after exposure to LPS. Although LCK is required for ZAP70 activation, it is interesting that there was no significant change in ZAP70 expression after LPS exposure.

4.5 Summary

In summary, fescue naïve steers subjected to summer-long grazing of toxic endophyte-infected tall fescue supplemented with MIX form of Se had higher whole blood Se, serum prolactin, and red blood cell concentrations than ISe-supplemented steers. In contrast, the form of Se supplementation did not affect WBC gene expression in either the prolactin or NF-kB pathways, in both Experiments 1 and 2. However, for Experiment 1, consumption of toxic-infected tall fescue altered WBC gene expression in both the prolactin and NF-kB pathways. In the prolactin pathway, mRNA expression of CISH and STAT5B increased, whereas PPIB, AGAP2, and SPRLR mRNA expression

decreased. In the NF-kB pathway, IL-8, RelA, NFKBIA, and TLR4 mRNA expression increased, whereas IKBKG, Zap70, and LCK expression decreased. This suggests a reduced adaptive immune response in steers consuming TE pasture.

For Experiment 2, consumption of TE with *in vitro* LPS resulted in an increased inflammatory response to LPS. In the prolactin pathway, CISH mRNA expression increased, whereas PPIB, LPRLR, and SPRLR mRNA expression decreased thus, indicating that bovine PRLR isoforms are differentially sensitive to physiological stressors in WBC when exposed to LPS. In the NF-kB pathway, IL-8, TNFa, and TLR4 mRNA expression was increased by LPS challenge, whereas LCK mRNA expression was decreased. The increase in CISH, IL-8, and TNF-a indicate an increased inflammatory response in cattle consuming TE when exposed to LPS.

4.6 Future Research

Additional research is needed on the WBC components: neutrophils, lymphocytes, basophils, eosinophils, and monocytes. Research on genes in both the NF-kB and prolactin pathways necessary for gene function that are not indicated by the current pathways is needed to enhance the overall impact of TE consumption and additional stress on cattle. For example, interleukin 2 (IL2) determines immediate T-cell response, maturation of macrophages, as well as proliferation of B cells and natural killer cells (Lin and Leonard, 1997); and its function is linked to CISH. Further research is also needed on the same pathways studied in this paper in liver cells to determine the significance of their function in conjunction with a previous corresponding paper by Liao et al. (2015). Additionally, due to increased IL-8 expression, measuring neutrophil

concentration, as well as other WBC components in other tissues such as kidney, liver, and SI would be an area for future research. Furthermore, looking at macrophage levels in addition to monocytes could show why circulating monocyte levels decrease during exposure to TE. Other forms of research including nanostring, microarray, and immunohistochemistry would be useful to complement the research done in this study.

Table 4.3.4. Primer sets used for quantitative real-time RT-PCR analysis of the selected target genes in the prolactin and NF-kB pathway.

Gene	Gene name[1]	Primer & Accession number[2]	Sequence (5' to 3' direction)	Amplicon length (bp)	Product identity (%)[3]	NCBI submission/ accession
ZAP70	Zeta chain of T cell receptor associated protein kinase 70	*NM_001193017.3* Forward Reverse	 TCTTCTACGGCAGCATCTCG CGTCGCGAAGGTTGTCAAAT	333	100%	2425986/ MW574631
LCK	LCK proto-oncogene, Src family tyrosine kinase	*NM_001034334.1* Forward Reverse	 CCACTGGATGGCAAGACCAC CGCTTTGGCCACAAAGTTGA	273	100%	2425986/ MW574624
TRAF6	TNF receptor associated factor 6	*NM_001034661.2* Forward Reverse	 TACTCCGTGGCTGAAAACCC CAGCTGTGAGGCAAAACCAC	234	100%	2425986/ MW574634
RELA	RELA proto-oncogene, NF-kB subunit	*NM_001080242.2* Forward Reverse	 TGTATTTCACGGGACCAGGC GGTGCTGAGAGATGGCGTAA	395	100%	2322006/ MT180971
NFKBIA	NF-kB inhibitor alpha	*NM_001045868.1* Forward Reverse	 TGCAGGCCACCAACTACAAT CGGCAGTGTCTGAAGGTTCT	317	100%	2322006/ MT180972
NFKB1	Nuclear factor kappa B subunit 1	*NM_001076409.1* Forward Reverse	 CCCCACGTATGGCGGAATTA TTCACGTCTCCTGTCACTGC	336	100%	2322006/ MT180974

Table 4.3.4 (continued)

IKBKG	Inhibitor of nuclear factor kappa B kinase regulatory subunit gamma	*NM_174354.3* Forward Reverse	 TCCACTGTGTGAGATGGTGC CTTGTCCTCAGCCATCTGCT	394	100%	2322006/ MT180973
TLR4	Toll like receptor 4	*NM_174198.6* Forward Reverse	 CAACCAAGATGCTGGACCTG AAAAGGCTCCCCAGGCTAAA	204	100%	2425986/ MW574637
TNFα	Tumor necrosis factor alpha	*NM_173966.3* Forward Reverse	 AAGTAACAAGCCGGTAGCCC ACTGAGGCGATCTCCCTTCT	376	100%	2425986/ MW574635
IL-8	C-X-C motif chemokine ligand 8 (CXCL8)	*NM_173925.2* Forward Reverse	 CCAATGGAAACGAGGTCTGC CACACAGAACATGAGGCACTG	206	99%	2425986/ MW574636
s-PRLR	Short prolactin receptor	*NM_174155.3* Forward Reverse	 TTGTGCAGATTCGCTGCAAG TAGACACAAGGCGAGAAGGC	276	100%	2425986/ MW574627
l-PRLR	Long prolactin receptor	*NM_001039726.2* Forward Reverse	 GGAGTCAGAAGGCTGCAGTT ACAGAGTCAGGTTTTGCGCT	266	100%	2425986/ MW574625
AGAP2	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2	*NM_001192039.1* Forward Reverse	 GCCAAGCGCAAAATGTGGAA CACGCAGATTGAGTTCCCCT	285	100%	2425986/ MW574632
PPIB	Peptidylprolyl isomerase B	*NM_174152.2* Forward Reverse	 AGGTGTACTTTGACCTGCGA GCTTGAAGTTCTCATCGGGGA	255	100%	2425986/ MW574626

Table 4.3.4 (continued)

STAT5A	Signal transducer and activator of transcription 5A	*NM_001012673.1*		296	100%	2425986/ MW574630
		Forward	CGGATACGTGAAGCCACAGA			
		Reverse	CTCTGCCGGGGGTAAAGAGA			
STAT5B	Signal transducer and activator of transcription 5B	*NM_174617.4*		240	100%	2425986/ MW574633
		Forward	CCACAGATCAAGCAGGTGGT			
		Reverse	CCACTGACTGTCCATGGGTC			
SOCS2	Suppressor of cytokine signaling 2	*NM_177523.2*		294	100%	2425986/ MW574628
		Forward	CGGGAACTCAGTCACACAGG			
		Reverse	TGTCCGCTTATCCTTGCACA			
SOCS3	Suppressor of cytokine signaling 3	*NM_174466.2*		318	100%	2425986/ MW574629
		Forward	TGGTCACCCACAGCAAGTTT			
		Reverse	CGCTCTGGAGAGAGAAGCTG			
IRF1	Interferon regulatory factor 1	*NM_001191261.2*		377	100%	2425986/ MW574623
		Forward	TATGGGGAATACAGCCCCGA			
		Reverse	TGGGCCCCAGGTTCATTAAG			
CISH	Cytokine inducible SH2 containing protein	*NM_001046586.1*		285	100%	2425986/ MW574622
		Forward	GAGCTGCCCCAGCAAGTTAT			
		Reverse	GGTCTTGACGGACAGTGTGA			
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	*NM_174178.2*		185	99%	NA
		Forward	GCAGAACCTGATGCTTTGTG			
		Reverse	CGTAGGAGAGCGTGTGCTT			

Table 4.3.4 (continued)

YWHAZ	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein zeta	*NM_174814.2*		208	99%	NA/ MK396254
		Forward	TTGATCCCCAACGCTTCACA			
		Reverse	AGTTAAGGGCCAGACCCAGT			
UBC	Ubiquitin C	*NM_001206307.1*		258	100%	NA
		Forward	TAGGGGTGGGTTAGAGTTCAAG			
		Reverse	ACCACCTCCCTGCTGGTATT			

[1]Each gene was retrieved using the NCBI RefSeq database for Bos Taurus

[2]The contents in this column associated with each gene symbol are the accession numbers of the sequences retrieved from the NCBI RefSeq database and are used as templates for designing primers.

[3]All real-time RT-PCR products were validated by sequencing. The identity values (%) presented are the base-pair ratios between the number of identical base pairs and total amplicon length

Table 4.4.1. Proximate, mineral, and alkaloid analysis of composited endophyte-infected tall fescue paddock samples (DM basis) grazed by steers with ad libitum access to free-choice vitamin-mineral mixes containing either inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of Se[1]

Item	ISe	SD	MIX	SD	P-value
Proximate analysis					
DM, %	27.68	2.19	25.74	0.90	0.153
TDN, %	59.56	0.87	60.00	1.55	0.675
CP, %	10.86	2.93	11.79	3.04	0.532
ADF, %	38.39	2.29	36.31	3.40	0.350
NDF, %	62.52	1.99	61.43	4.70	0.683
NEgain, Mcal/lb.	0.29	0.01	0.30	0.02	0.731
Mineral analysis					
Ca, %	0.49	0.10	0.50	0.13	0.880
P, %	0.39	0.05	0.40	0.08	0.795
Mg, %	0.26	0.06	0.26	0.06	0.939
K, %	2.38	0.23	2.03	0.44	0.781
Na, %	0.02	0.007	0.02	0.008	0.837
Fe, ppm	360	120	451	137	0.352
Zn, ppm	18.6	4.78	20.9	5.47	0.588
Cu, ppm	6.88	2.63	7.31	2.21	0.808
Mn, ppm	52.4	8.28	53.2	9.31	0.900
Mb, ppm	1.71	0.63	1.87	0.66	0.744
Se, ppm	0.05	0.02	0.04	0.01	0.609
Ergot alkaloid analysis					
Ergovaline, µg/g	0.277	0.143	0.280	0.092	0.971
Ergovalinine, µg/g	0.189	0.030	0.149	0.025	0.086

[1]Values are the least square means (± SD) of pooled (d -1, 28, 56, and 83) forage sample from ISe (n = 4) and MIX (n = 4) paddocks. Samples were obtained systematically from 6 sites • paddock^{-1}·d^{-1}.

Table 4.4.2. Whole blood selenium and serum prolactin concentrations of steers grazing endophyte-infected tall fescue and with ad libitum access to free-choice vitamin-mineral mixes containing either inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium[1]

Item, ng/mL	Se Trt	Day 0	SD	Day 84	SD	p-value		
						TRT	DAY	DAY*TRT[3]
Whole blood Se, ng/mL	ISe	239	16.4	147	22.3	0.001	<0.001	0.74
	MIX	281	25.6	196	20.4			
Serum prolactin[2], ng/mL	ISe	131	67.9	9.75	4.52	0.31	<0.0001	0.06
	MIX	105	62.4	19.3	4.31			

[1]Values are the least square means (n = 4, ± SD) of ISe and MIX treatments.

[2]Whereas actual means and SD are reported, data were analyzed after $\log_{10}$ transformation.

[3]F-value is = 4.15.

Table 4.4.3. Blood cell types and parameters of steers grazing endophyte-infected tall fescue and with ad libitum access to free-choice vitamin-mineral mixes containing either inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium[1]

Item	Se Trt	Day 0	SD	Day 84	SD	p-value		
						TRT	DAY	DAY*TRT
Red blood cells 1×10^6/uL	ISe	7.30	0.25	7.13	0.58	0.01	0.70	0.69
	MIX	7.85	0.37	7.85	0.39			
White blood cells, 1×10^3/uL	ISe	8.85	1.35	8.36	1.43	0.32	0.55	0.19
	MIX	8.63	0.36	9.86	1.41			
Segmented neutrophils, 1×10^3/uL	ISe	3.64	0.95	3.26	1.43	0.15	0.57	0.21
	MIX	3.75	0.33	4.71	1.01			
Lymphocytes, 1×10^3/uL	ISe	5.35	0.96	5.67	1.48	0.14	0.62	0.26
	MIX	5.14	0.34	4.30	0.85			
Monocytes, 1×10^3/uL	ISe	0.84	0.10	0.48	0.13	0.04	0.004	0.85
	MIX	0.60	0.32	0.28	0.13			
Eosinophils, 1×10^3/uL	ISe	0.26	0.23	0.61	0.30	0.15	0.17	0.30
	MIX	0.63	0.34	0.68	0.20			
Hematocrit, %	ISe	0.29	0.02	0.29	0.03	0.24	0.78	0.58
	MIX	0.30	0.03	0.31	0.03			
Hemoglobin, g/dL	ISe	11.94	0.80	10.73	1.14	0.17	0.05	0.89
	MIX	12.64	1.10	11.58	1.11			
MCV[2], fL	ISe	39.84	1.12	40.26	1.20	0.14	0.29	0.56
	MIX	38.01	1.84	39.45	2.30			
MCH[2], pg	ISe	16.36	0.60	15.04	0.52	0.44	0.004	0.96
	MIX	16.09	0.78	14.73	0.98			
MCHC[2], g/dL	ISe	41.03	0.40	37.36	0.27	0.009	<0.001	0.01
	MIX	42.31	0.19	37.38	0.66			

[1]Values are the least square means (n = 4, ± SD) of blood samples collected from ISe (n = 4) and MIX (n = 4) steers.

[2]MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

Table 4.4.4a. Analysis of genes in the prolactin pathway in Experiment 1[1]

Gene	Se Trt	Day 0	SD	Day 84	SD	p-value	
						TRT	DAY
Experiment 1							
CISH	ISe	1.07	0.30	1.78	0.53	0.35	<0.01
	MIX	0.85	0.08	1.59	0.57		
IRF1	ISe	1.03	0.18	1.11	0.19	0.96	0.17
	MIX	0.98	0.19	1.15	0.06		
SOCS2	ISe	1.00	0.38	1.30	0.45	0.97	0.36
	MIX	1.16	0.17	1.16	0.08		
SOCS3	ISe	1.04	0.26	1.21	0.31	0.83	0.13
	MIX	0.94	0.37	1.39	0.53		
STAT5A	ISe	1.01	0.14	1.02	0.11	0.85	0.67
	MIX	1.00	0.07	1.05	0.12		
STAT5B	ISe	1.03	0.13	1.14	0.06	0.83	0.01
	MIX	0.93	0.07	1.21	0.20		
PPIB	ISe	1.03	0.10	0.82	0.08	0.81	<0.01
	MIX	1.05	0.08	0.82	0.06		
AGAP2	ISe	1.18	0.42	0.79	0.26	0.11	0.05
	MIX	0.85	0.23	0.61	0.23		
LPRLR	ISe	1.05	0.13	1.07	0.18	0.92	0.86
	MIX	1.06	0.12	1.07	0.12		
SPRLR	ISe	1.09	0.22	0.57	0.14	0.65	<0.01
	MIX	1.10	0.20	0.66	0.17		

[1]Values are the means (n = 4, ± SD) relative to ISe day 0, steer expression of prolactin pathway genes analyzed via blood samples collected on d 0 and 84 from predominately Angus, fescue-naïve steers supplemented with inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium in free-choice vitamin-mineral mixes.

Table 4.4.4b. Analysis of genes in the NF-kB pathway in Experiment 1[1]

Gene	Se Trt	Day 0	SD	Day 84	SD	p-value	
						TRT	DAY
Experiment 1							
IL-8	ISe	1.13	0.58	5.14	1.36	0.86	<0.01
	MIX	1.66	1.11	4.87	2.53		
TNFα	ISe	1.12	0.83	1.81	0.70	0.99	0.07
	MIX	1.22	0.30	1.71	0.46		
REL A	ISe	1.03	0.11	1.11	0.13	0.28	0.04
	MIX	1.05	0.06	1.19	0.02		
NFKB1	ISe	1.2	0.11	1.06	0.30	0.39	0.91
	MIX	1.14	0.18	1.12	0.18		
IKBKG	ISe	1.06	0.18	0.81	0.23	0.92	0.01
	MIX	1.03	0.06	0.83	0.05		
NFKBIA	ISe	1.02	0.41	2.06	0.63	0.15	<0.01
	MIX	1.18	0.23	2.91	1.03		
TRAF6	ISe	1.04	0.14	1.10	0.13	0.07	0.15
	MIX	0.92	0.03	1.02	0.06		
TLR4	ISe	1.12	0.32	1.89	0.42	0.75	<0.01
	MIX	1.04	0.14	1.87	0.23		
ZAP70	ISe	1.12	0.30	0.90	0.27	0.71	0.06
	MIX	1.17	0.15	0.93	0.11		
LCK	ISe	1.08	0.26	0.69	0.21	0.30	<0.01
	MIX	0.91	0.06	0.65	0.15		

[1]Values are the means (n = 4, ± SD) relative to ISe day 0, steer expression of NF-kB pathway genes analyzed via blood samples collected on d 0 and 84 from predominately Angus, fescue-naïve steers supplemented with inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium in free-choice vitamin-mineral mixes.

Table 4.4.4c. Analysis of genes in the prolactin pathway in Experiment 2[1]

Gene	Se Trt	Day 0	SD	Day 84	SD	p-value	
						TRT	DAY
Experiment 2							
CISH	ISe	6.34	1.48	8.75	1.06	0.34	0.03
	MIX	4.53	0.85	8.31	4.25		
IRF1	ISe	1.76	0.14	1.48	0.10	0.90	0.13
	MIX	1.74	0.47	1.54	0.34		
SOCS2	ISe	1.02	0.38	1.05	0.43	0.93	0.49
	MIX	1.19	0.06	0.91	0.24		
SOCS3	ISe	11.93	3.45	10.11	2.98	0.89	0.76
	MIX	10.39	3.06	11.16	3.24		
STAT5A	ISe	2.10	0.18	2.05	0.17	0.26	0.47
	MIX	2.10	0.39	1.86	0.20		
STAT5B	ISe	1.22	0.08	1.36	0.02	0.60	0.09
	MIX	1.13	0.22	1.35	0.29		
PPIB	ISe	0.96	0.10	0.70	0.07	0.53	<0.01
	MIX	1.02	0.09	0.70	0.10		
AGAP2	ISe	0.90	0.71	0.91	0.36	0.68	0.94
	MIX	0.82	0.21	0.78	0.33		
LPRLR	ISe	1.07	0.07	0.85	0.21	0.89	<0.01
	MIX	0.17	0.08	0.78	0.17		
SPRLR	ISe	0.84	0.18	0.51	0.16	0.19	0.01
	MIX	1.01	0.31	0.65	0.23		

[1]Values are the means (n = 4, ± SD) relative to ISe day 0, non-LPS-challenged steer expression of prolactin pathway genes analyzed via blood samples collected on d 0 and 84 from predominately Angus, fescue-naïve steers supplemented with inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium in free-choice vitamin-mineral mixes.

Table 4.4.4d. Analysis of genes in the NF-kB pathway in Experiment 2[1]

Gene	Se Trt	Day 0	SD	Day 84	SD	p-value	
						TRT	DAY
Experiment 2							
IL-8	ISe	12.96	5.85	20.69	2.43	0.95	0.02
	MIX	11.21	5.89	21.98	9.20		
TNFα	ISe	4.20	1.64	5.40	0.24	0.32	0.02
	MIX	3.26	0.16	5.16	1.25		
REL A	ISe	1.26	0.08	1.32	0.10	0.18	0.12
	MIX	1.30	0.01	1.44	0.16		
NFKB1	ISe	3.86	0.87	4.70	0.79	0.32	0.09
	MIX	4.32	1.00	5.19	0.84		
IKBKG	ISe	0.84	0.19	0.72	0.18	0.52	0.08
	MIX	0.90	0.04	0.75	0.05		
NFKBIA	ISe	5.83	1.58	6.05	0.15	0.92	0.22
	MIX	5.31	0.58	6.69	1.54		
TRAF6	ISe	0.99	0.12	1.01	0.18	0.27	0.76
	MIX	0.92	0.03	0.94	0.10		
TLR4	ISe	2.20	1.14	2.88	0.40	0.85	0.05
	MIX	1.93	0.44	3.34	1.24		
ZAP70	ISe	0.99	0.24	0.95	0.35	0.78	0.09
	MIX	1.13	0.13	0.89	0.15		
LCK	ISe	0.82	0.18	0.57	0.18	0.68	0.03
	MIX	0.74	0.06	0.57	0.18		

[1]Values are the means (n = 4, ± SD) relative to ISe day 0, non-LPS-challenged steer expression of NF-kB pathway genes analyzed via blood samples collected on d 0 and 84 from predominately Angus, fescue-naïve steers supplemented with inorganic (ISe) or a 1:1 blend of ISe and organic (MIX) forms of selenium in free-choice vitamin-mineral mixes.

Figure 4.3.1 Flow chart of experimental regimen and steer assignment

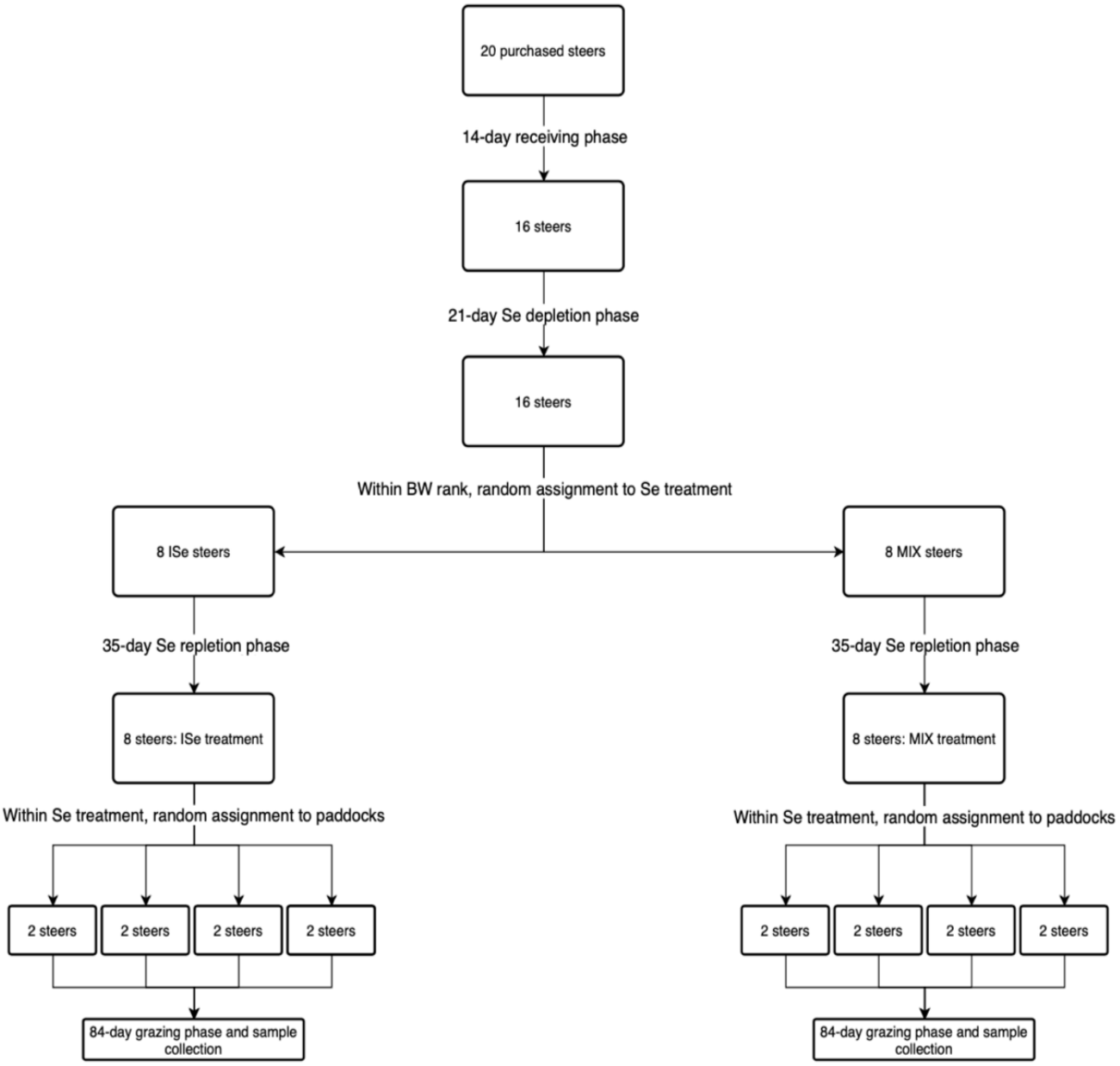
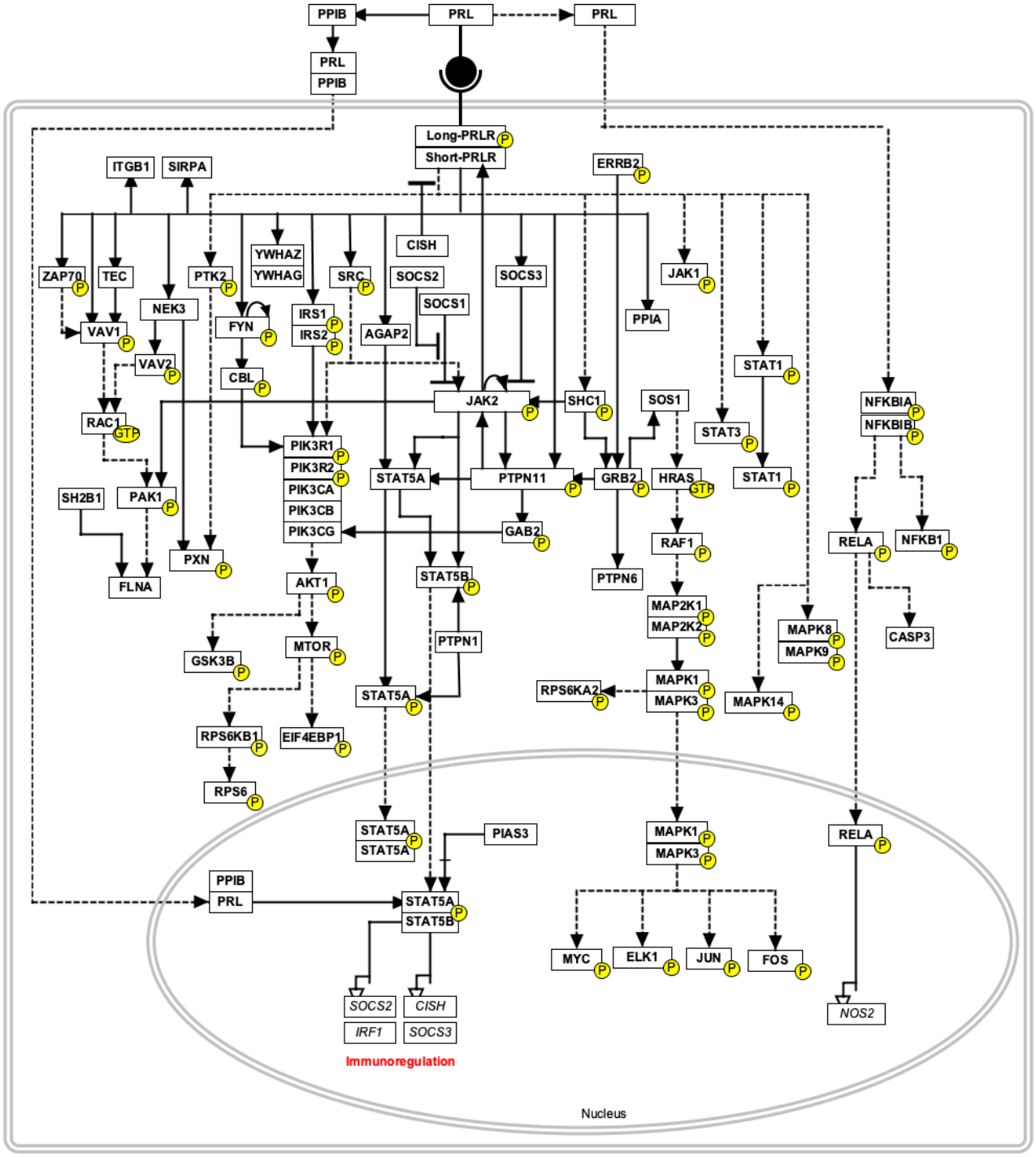
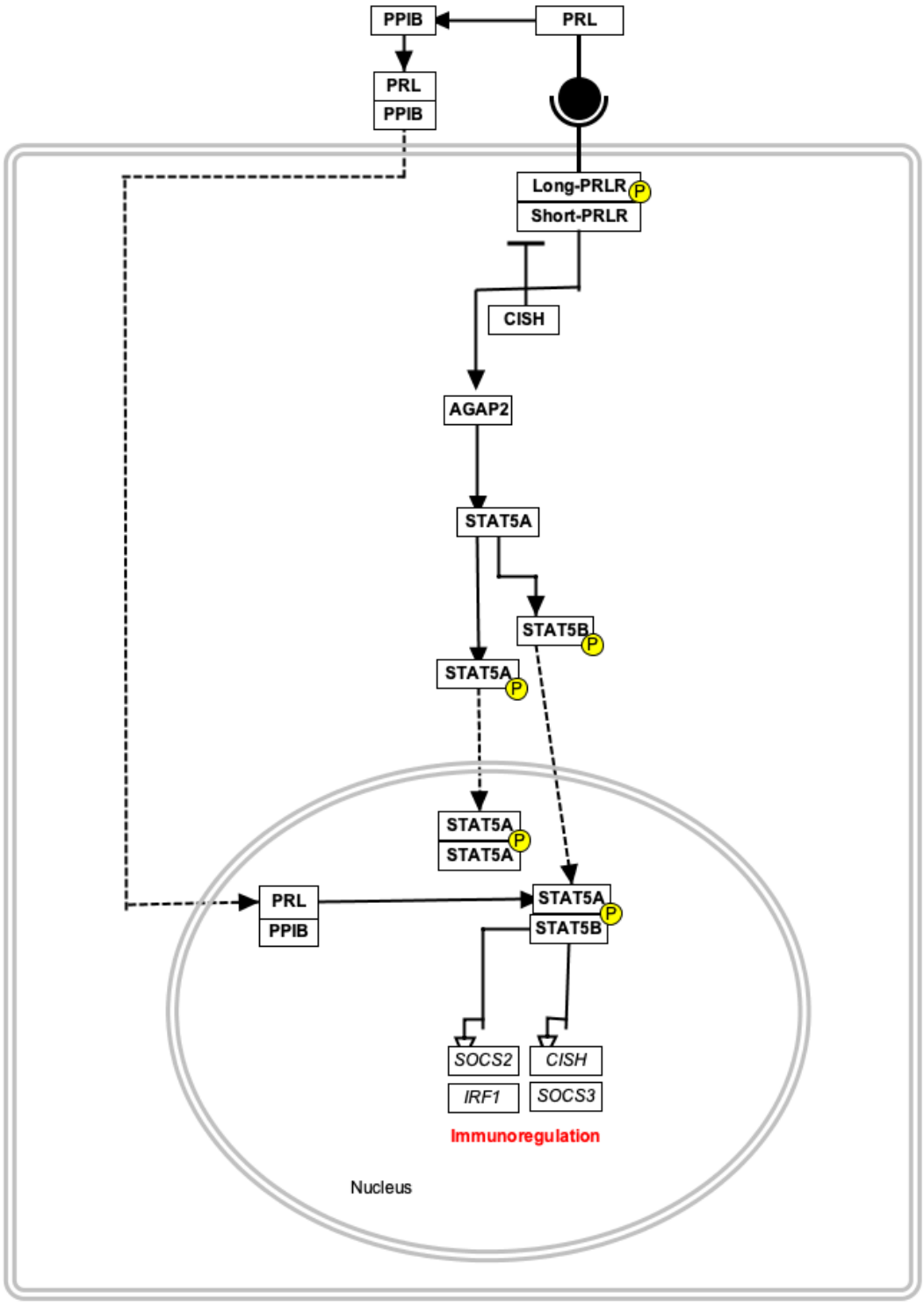


Figure 4.5.5a Prolactin pathway overview[1]



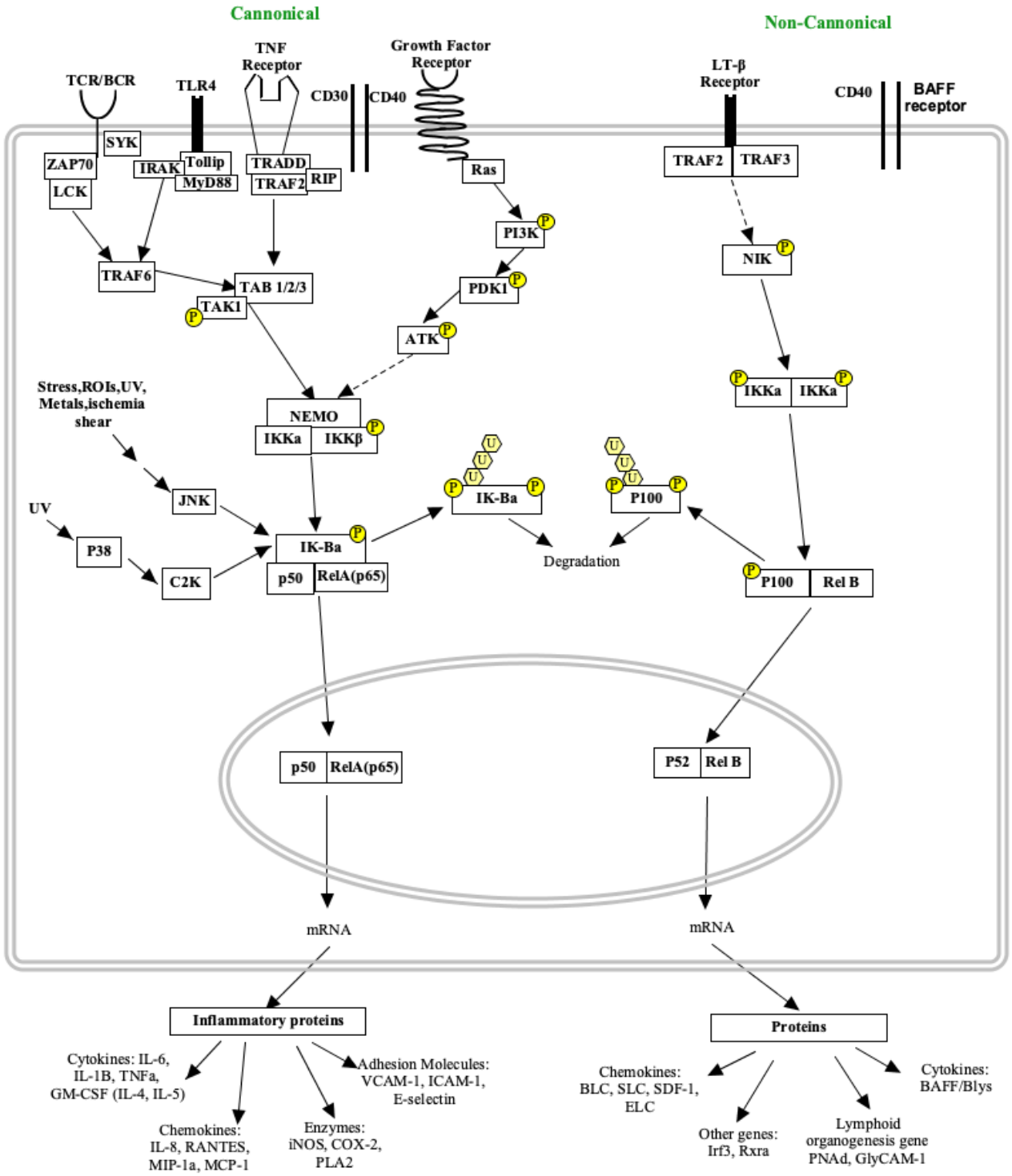
[1]Large overview of the prolactin pathway created with PathVisio, adapted from (Radhakrishnan et al., 2012a)

Figure 4.5.5b Prolactin pathway with selected genes[1]



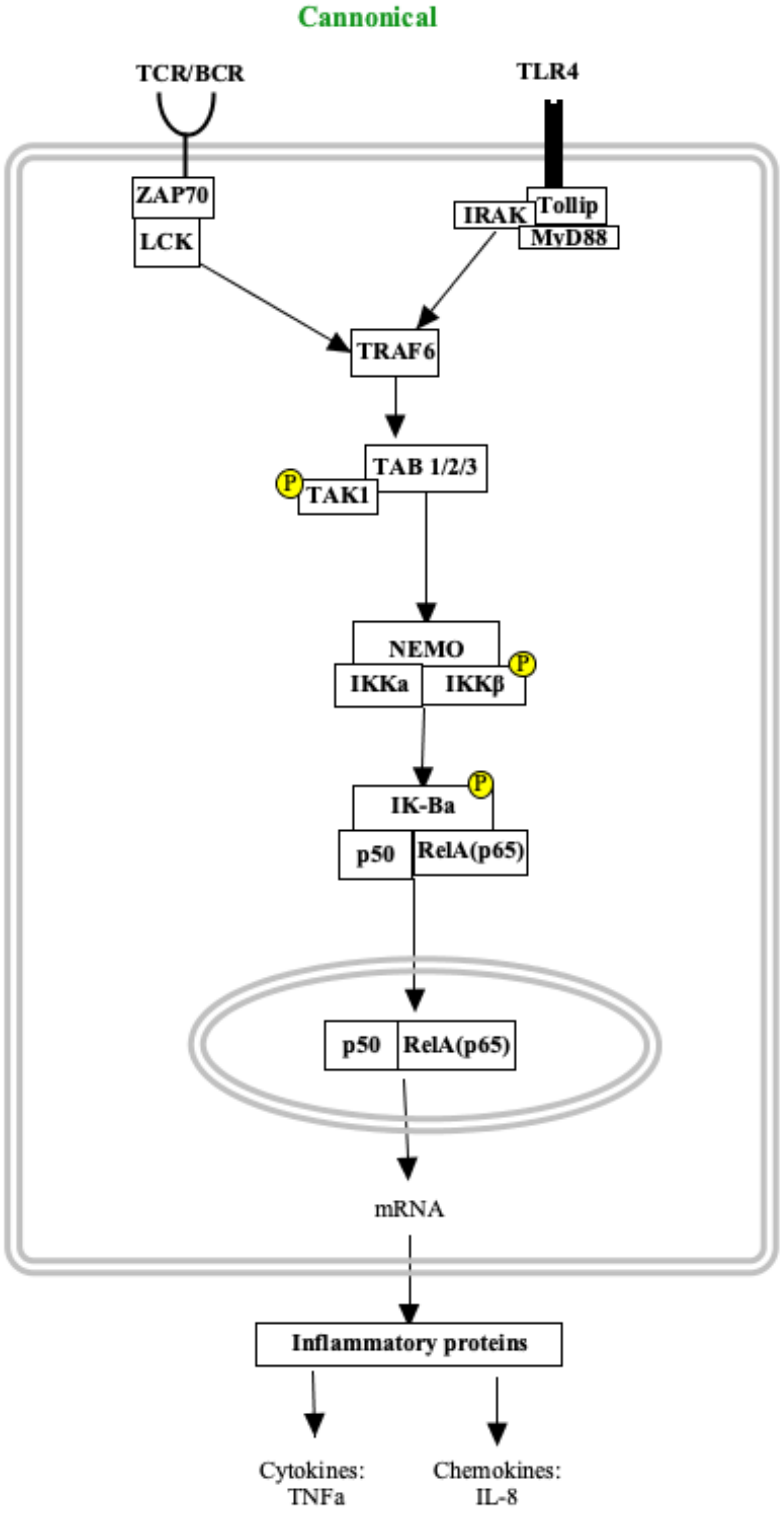
[1]Selected genes in the prolactin pathway created with PathVisio, adapted from (Radhakrishnan et al., 2012a)

Figure 4.5.5c NF-kB pathway overview[1]



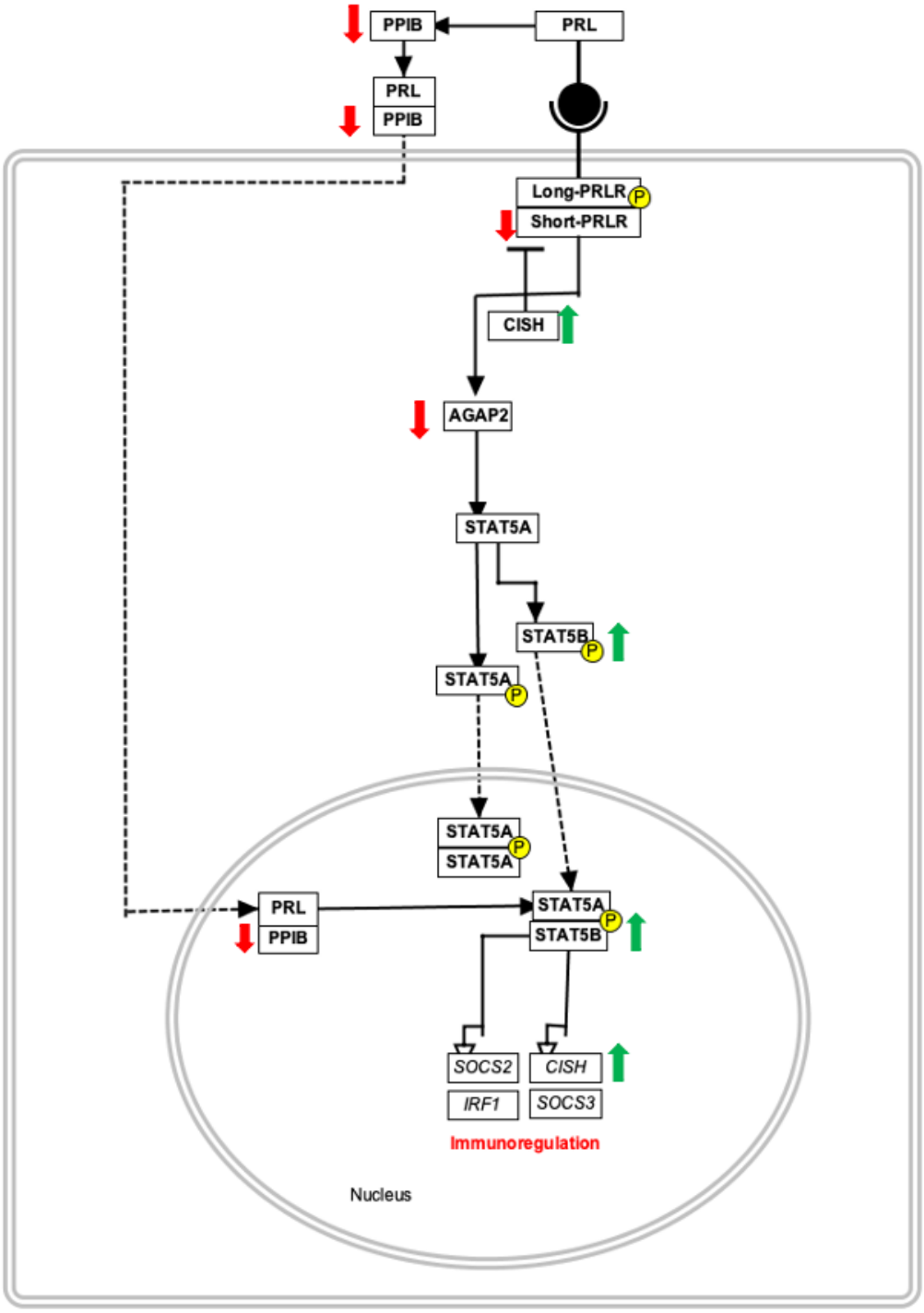
[1]Large overview of the NF-kB pathway created with PathVisio, adapted from Creative Diagnostics

Figure 4.5.5d NF-kB pathway with selected genes[1]



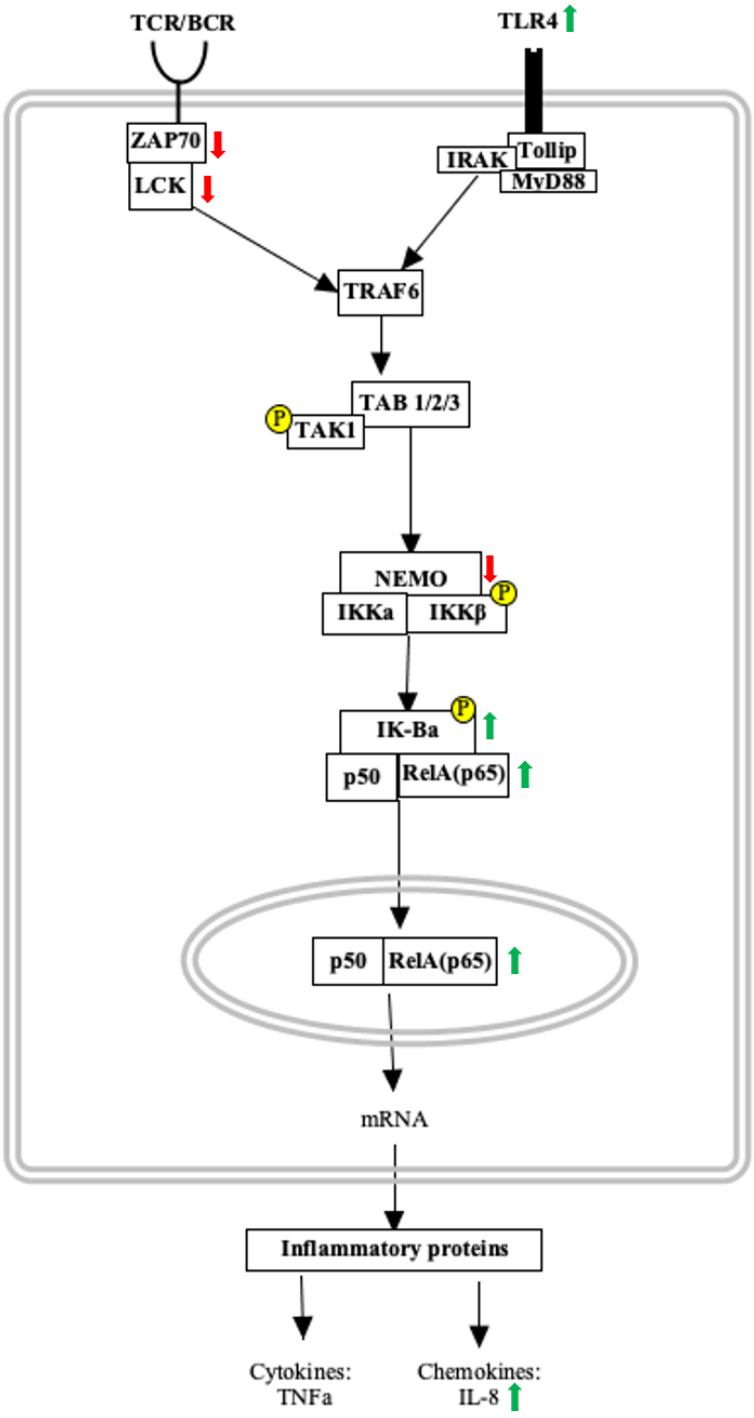
[1] Selected genes in the NF-kB pathway created with PathVisio, adapted from creative diagnostics

Figure 4.5.5e Prolactin pathway gene expression Experiment 1[1]



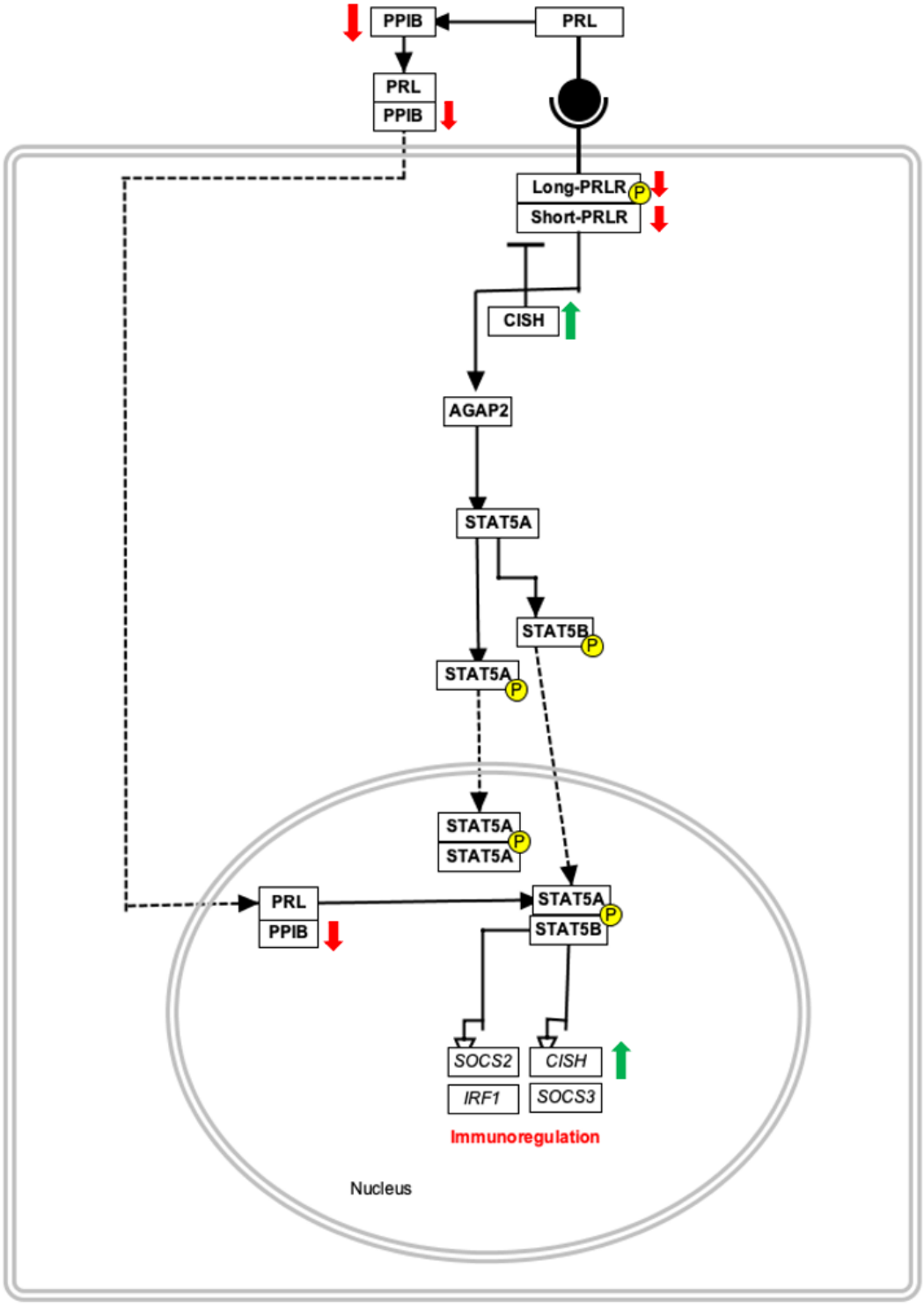
[1] WBC mRNA abundance of PRL pathway genes in Experiment 1

Figure 4.5.5f NF-kB pathway gene expression Experiment 1 [1]



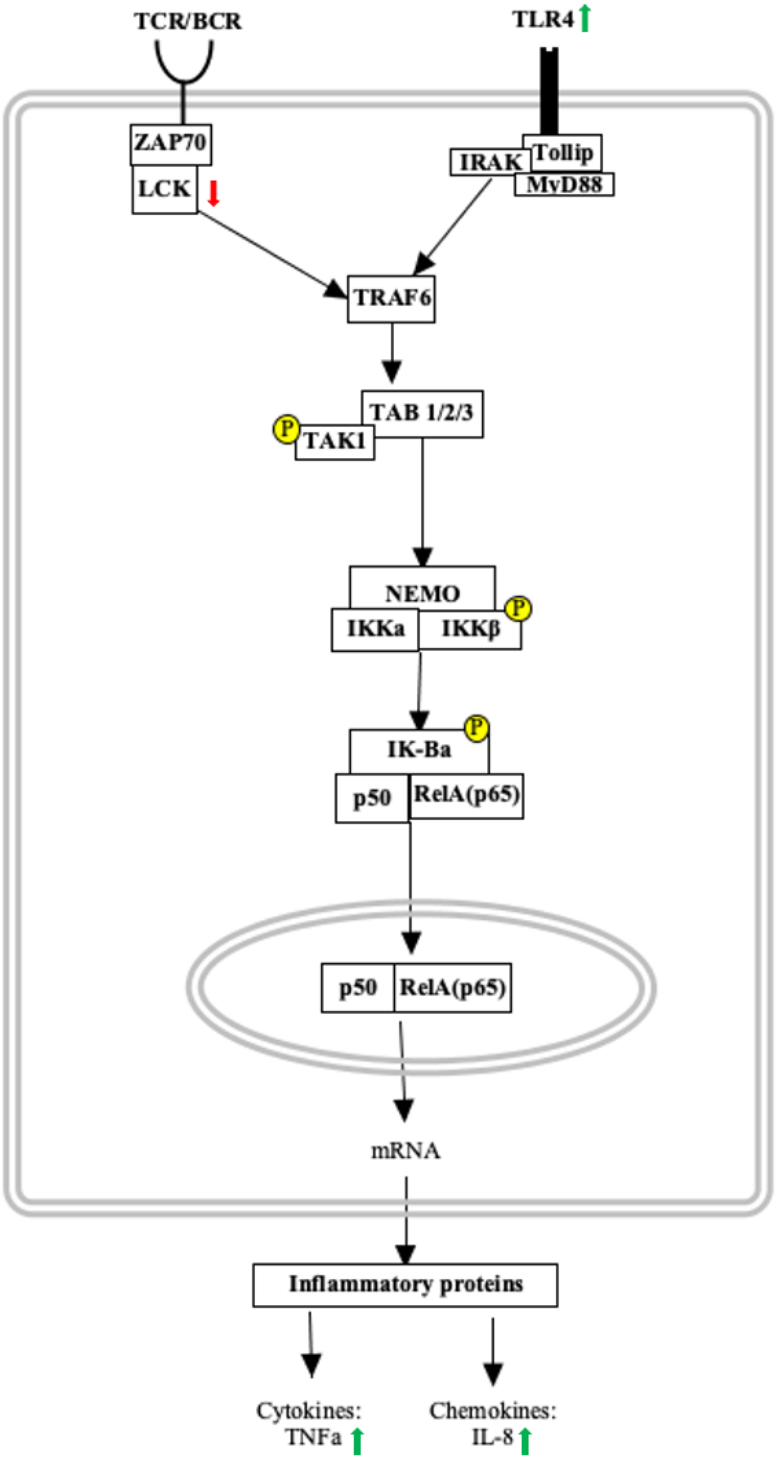
[1] WBC mRNA abundance of NF-kB pathway gene expression in Experiment 1

Figure 4.5.6a Prolactin pathway gene expression Experiment 2[1]



[1]WBC mRNA abundance of PRL pathway gene expression in Experiment 2

Figure 4.5.6b NF-kB pathway gene expression Experiment 2[1]



[1] WBC mRNA abundance of NF-kB gene expression in Experiment 2

Figure 4.3.4a Sequences of the real-time RT-PCR products (5'-3' orientation) of the target genes in the prolactin pathway. Within a sequence, underlined nucleotides indicate the forward and reverse primer positions, respectively.

CISH:

GGTCTTGACGGACAGTGTGAACAGGTAGCTGGGGTGGGTGCTGTCACGTACC
AGGAAGGTGCCCTCTGGCATCTTCTGAAGGTGTTGCCGGGCCTCACTGGCCGT
AATGGAACCCCAGTACCAGCCAGATTCCCGAAGGTAGGAGAAGGTCTTGGCT
ATGCACAGCAGGTCCTCCTCGGGGTCCACCACCTTGGGCTCCCTCTCTGGCTG
GGCTGGGGACTCCTCTACTGCCTCCTCCAGGAAGGCGCCAGCAGACAAAGGC
TGCATAACTTGCTGGGGCAGCTC

IRF1:

TGGGCCCCAGGTTCATTAAGCAGATACCCCTTCCCATCCACGCTTGTCTGCTG
CCACCCTGTTTGCTCCAAGAGCTTCATGATGTCCTCAGTTAATTTCCCTTCCTC
GTCCTCATCTGTTGCAGCTTCAGAGGTGGAGGGCATGGGCGACACCTGAAAG
TTGTACAGGTCGCTGGTGCTGTCTGGCACAATTTCCACCGGAATGGACCAGTT
AGGGAGAGTGCTACTGACGCCACACGGTGACAGCGCTGGAGTGAGAGTCCGT
TCGATGTCCAGGTCCTGCCCCATGTAGCTGCGAACTGTGTAGTTGCTGTGGTC
ATCAGGCAGGGTGGAGCTACTGAGTCCATCAGAGAAGGTATCGGGGCTGTAT
TCCCCATA

SOCS2:

TGTCCGCTTATCCTTGCACATCTGAACATAGTAGTCGATCAGATGAACCACAC
TGTCAAATTGTTTAAGCTTGGACTTGACACATATGATAGAGTCCAATCTAAAT
TTCCCATCTTGGTATTCGATGCGAAGATTAGTTGGTCCAGCTGATGTCTTAAC
AGATATTGTTAGTAGGTAGTCTGAATGCGAACTATCTCTAATCAAGAAAGTTC
CTTCTGGTGCCTCTTTTAACTTCTCTTTGGCTTCATTAACAGTCATACTTCCCC
AGTACCAACCTGTGTGACTGAGTTCCCG

SOCS3:

CGCTCTGGAGAGAGAAGCTGCCCCCCTCGCACTGGATGCGCAGGTTCTTGGT
CCCCGACTGGGTCTTGACGCTGAGGGTGAAAAAGTGGCGCTGGTCCGAGCTG
TCGCGGATGAGGAAGGTGCCCGCGGGCTCGGCGCTCAGCAGCAAGTTCGCTT
CGCCGCCCGTCACGGTGCTCCAGTAAAAGCCGCTCTCCTGCAGCTTGCGCACT
GCGTTCACCACCAGCTGGTACTCGCTCTTGGAGCTGAAGGTCTTGAGGCGCA
GGCTGGTGTCCAGGGGGCGGCTCATCCCGGCGGCGGGAAACTTGCTGTGGGT
GACCA

STAT5A:

CTCTGCCGGGGGTAAAGAGAACCAGTGGGCGGGGGGAGAGAGGGCTCCAGAC
TGTCCATTGGGCGGCGGAGGAGTTCTTCCACGTGCCGGGCCACATCCATGGT
CTCGTCCAGGTCGAATTCTCCATCCTGGTCGAGCACCGGGTCAGGGTTCTGTG
GGTACATGTTATAGTGAGGCTGGGGGCACACGGCTGGGGAAGGAGCCTGGTC

CATGTAGGTGGCGTTGCTTCCAGCAGAGTCTGCAGAGGCGCTCACAAACTCA
GGGACCACTTGCTTGATCTGTGGCTTCACGTATCCG

STAT5B:

CCACTGACTGTCCATGGGTCGGCCTAGGAGCTCCTCCACACGCCGCGCCACG
TCAATCGTGTCGTCCAGATCGAAGTCACCATCGTTGTCAAGGACGGGGTCGG
GGTTCTGTGGGTACATGTTATAGTGAGGCTGGGGGCACACGGCTGGGGAGGG
AGCCTGGTCCATGTAGGTGGCACTGCCGCCGGCAGAGTCTGCAGAGGCGCTC
ACAAACTCAGGGACCACCTGCTTGATCTGTGG

PPIB:

GCTTGAAGTTCTCATCGGGGAAGCGTTCACCGTAGATGCTCTTACCTCCAGTG
CCGTCTCCCCGGGTGAAATCTCCACCCTGGATCATGAAGTCCTTGATCACACG
ATGGAATTTGCTGTCTTTGTAGCCAAATCCTTTCTCTCCTGTAGCCAAGGCCA
CAAAATTATCCACTGTTTTTGGAACAGTCTTTCCAAAGAGACCGATGACCACC
CGGCCTATATCTTCATCTCCAATTCGCAGGTCAAAGTACACCT

AGAP2:

CACGCAGATTGAGTTCCCCTTGGCATTCCGGATCGCCTGGATGGCCACGGCTT
CGCTTTGGCTGTCTGTGCGCAGCTTGACCTTGCTGCTCTCACAGCACTGGAGA
CTGGCTAGGATCTGACTTTCGATGGCCTGAACCCAGGCGTCCCGCTCCTCAAA
ACTGGCTGCCTCAAAGTGCCATGTCTGACCAGTGCTGGACACGATCAGGAAC
TCGAAGTTTTCTTCTGCTTTATAAATATTTCTTAAACTACCAAAGGATTTTAGT
TTCCACATTTTGCGCTTGGC

LPRLR:

GGAGTCAGAAGGCTGCAGTTCCAAGCCTGACCAAGACACGGTGTGGCCACG
ACCCCAAGACAAAACCCCCTTGATCTCTGCTAAACCCTTGGAATACGTGGAG
ATCCACAAGGTCAGCCAAGATGGAGTGCTGGCTCTGTTCCCAAAACAAAACG
AGAAGTTTGGCGCCCCTGAAGCCAGCAAGGAGTACTCAAAGGTGTCCCGGGT
GACAGATAGCAACATCCTGGTATTGGTGCCGGATCCGCAAGCGCAAAACCTG
ACTCTGT

SPRLR:

TAGACACAAGGCGAGAAGGCTGTGATATCTCCAGCAGATGAACATCAAATCC
TTTTATTTTTGGCCCTGGAACTGGTGGGAGGATGCAGGTCACCATGCTATAGC
CCTTCAAAGCCACTGCCCAGACCATAATCAAACAGATGACAGCAGAAAGGAT
GGCCACAAAGATCCACATGCTTGTGTCCTTCACTGGGAAGTCATTAGGTATCT
GGATGGAGCTCTCTGGGCTCCACTCACTCCAGTATCCATGGTCTGGCTTGCAG
CGAATCTGCACAA

Figure 4.3.4b Sequences of the real-time RT-PCR products (5'-3' orientation) of the target genes in the NF-kB pathway. Within a sequence, underlined nucleotides indicate the forward and reverse primer positions, respectively.

IL-8:

CCAATGGAAACGAGGTCTGCTTAAACCCCAAGGAAAAGTGGGTGCAGAAGG
TTGTGCAGGTATTTGTGAAGAGAGCTGAGAAGCAAGATCCATGAAAAAGAA
AAAACCACCAAAAATCCTTTTTCCATTGCTTCTAAGAATTCCTCAGTAAAGAT
GCCAATGAAACTTCAAAACAAATCTACTTCAGTGCCTCATGTTCTGTGTG

TNFα:

AAGTAACAAGCCGGTAGCCCACGTTGTAGCCGACATCAACTCTCCGGGGCAG
CTCCGGTGGTGGGACTCGTATGCCAATGCCCTCATGGCCAACGGTGTGAAGC
TGGAAGACAACCAGCTGGTGGTGCCTGCTGACGGGCTTTACCTCATCTACTCA
CAGGTCCTCTTCAGGGGCCAAGGCTGCCCTTCCACCCCCTTGTTCCTCACCCA
CACCATCAGCCGCATTGCAGTCTCCTACCAGACCAAGGTCAACATCCTGTCTG
CCATCAAGAGCCCTTGCCACAGGGAGACCCCAGAGTGGGCTGAGGCCAAGC
CCTGGTATGAACCCATCTACCAGGGAGGAGTCTTCCAGCTGGAGAAGGGAGA
TCGCCTCAGT

RELA:

TGTATTTCACGGGACCAGGCTGGGAGGCCCGAGGCTCTTTTTCACAAGCTGA
CGTGCACCGGCAAGTGGCCATCGTGTTCCGGACGCCGCCCTACGCGGACCCC
GGCCTGCAGGCCCCTGTGCGCGTCTCCATGCAGCTGCGGCGGCCTTCCGATC
GGGAGCTCAGTGAGCCCATGGAATTCCAGTACTTGCCAGACACAGACGATCG
TCACCGGATTGAGGAGAAGCGCAAAAGGACGTACGAGACCTTCAAGAGCAT
CATGAAAAAGAGCCCTTTCAATGGACCCACCGACCCCCGGCCTCCAACCCGG
CGCATCGCTGTGCCTAACCGCGGCTCAGCCTCCATCCCCAAGCCAGCTCCCCA
GCCCTATTCCTTTACGCCATCTCTCAGCACC

NFKB1:

CCCCACGTATGGCGGAATTAACCTTCCATACTGGAACCACTAAATCTAATGCTG
GGATGAAGCATGGAACCATAGACACCCCATCTAAAAATGACCCTGAAAGTTG
TGGCAAGAGTGATGACAGAGAGGTTGTAAATCTCTCTGAGCAAGTAACAGAA
ACCACAGAACAAGATGGAGGATCCTGCAAGAGGGGCACTGCAGCTAACCTG
ACGTGCTCCGTAGGAGTAAAGGAAGAGAACCGCAGCTTCCAGGATAACCTCT
TTCTGGAGAAGGCTATGCAGCTGGCCAAGCAGCACGCCAACGCCCTCTTTGA
CTACGCAGTGACAGGAGACGTGAA

IKBKG:

TCCACTGTGTGAGATGGTGCAGCCGAGTGGCAGTCCGGCAGGGGACCAGGAC
ATGCTGGGTGAAGAGTCTTCTCTGGGGAAGCCAGCCATGCTCCACGTGCCTTC
AGAGCAGGGCACCCCTGAGACCTTCCAGCGCTGTCTGGAGGAGAATCAAGA
GCTCCGAGACGCCATCCGCCAGAGCAACCAGATGCTGCGCGAGCGCTGTGAG
GAGCTGCAGCATTTCCAGGGCAACCAGAGGGAGGAGAAGGCCTTCCTCATGC

AGAAGTTCCAGGAGGCCCGGGACTTGGTGGTGAGGCTGAGCCTGGAGAAGC
GTGAACTGCGCCAGCAGAGGGAGCAGGCCCTGAAGGAGGTGGAGCGCCTGA
AGACGTGCCAGCAGCAGATGGCTGAGGACAAG

NFKBIA:
TGCAGGCCACCAACTACAATGGCCACACGTGTCTGCACTTGGCCTCTATTCAT
GGCTACCTGGGCATCGTGGAGCTGTTGGTGTCCTTGGGCGCTGATGTCAACGC
TCAGGAGCCCTGCAATGGCCGAACTGCCCTCCATCTGGCAGTGGACCTGCAG
AATCCCGACCTGGTGTCACTCCTATTGAAGTGTGGGGCTGATGTCAACAGAG
TCACCTACCAGGGCTACTCCCCGTACCAGCTCACGTGGGGCCGCCCCAGCAC
CCGGATACAGCAGCAGCTGGGCCAGCTGACCCTAGAGAACCTTCAGACACTG
CCG

TRAF6:
TACTCCGTGGCTGAAAACCCTCCCTCCGAAGACTGCCCATGTCGAAGCGGGT
GGAGACCTCACAGCGTACTAATAACGTGTCATCCTTGATGAAGGTTCTTTGTC
TGAGGGCTTCCAGATGCATAAAAGTCACGTAGCCAAAACCCTTGGGGTTCCG
GGGGATGGTGGGTCTCTGAAAGGCCAGGAGCTCTGGTTTGGCGTCCATTATTT
CTTCGTGGTTTTGCCTCACAGCTG

TLR4:
CAACCAAGATGCTGGACCTGAGCTTTAACTACCTGAGACATTTAGGCAGCCA
TAACTTCCTCCAGCTTCCCCAGAACTGCAAGTGCTGGATTTATCCAGATGTGA
AATTAAGATTATTGAAGACGACACATTTCAGGGCCTAAACCACCTCTCCACCT
TGATACTGACGGGAAACCCTATCCAGAGTTTAGCCTGGGGAGCCTTTT

ZAP70:
CGTCGCGAAGGTTGTCAAATACCCCGGGCTGCGGCTCGAGCCCCGACGGCCG
GTTGCACGGCTTGCGCAGGTTGCAGGGCAGCCCGTCGGGGTCGCGTGAATAG
AACTCGCAGAGCTCAGCCGGGCCGCAGTGCGCCTTTCCTCCCGCGATAGCGT
AGGTGCCGTTGAGCTGGCGCTCGATGGGGAAGTGGTGGAAGCGCACTTCGTG
CACGAGCGACAGCACATAGCCGCCCAGCGAGCGCAGGCACTGCCGCAGCAG
GAAGAGCCCGTCGGCCATGCCCGCCAGCTTCAGGTGCTCCTCGGCCTCGGCG
CGCGAGATGCTGCCGTAGAAGA

LCK:
CGCTTTGGCCACAAAGTTGAAGGGGATGAAACCTTCCTGGCCCGTGGTCAGG
GACTGCGCCTTCCACCACTCGCCGTTCTGCTCCAGGATACGGAGCTGTTCACC
CTTCTCGAAGCCCAGGTCTCCGTCGTGAGAAGGCTCATAGCTGTGCAGGGCG
ATAACCAGGTTGTCTTGCAGTGGAGAAGCTGGGGGGTTGGATCCCTCGTAGG
TGACCAGTGGATCACGCACTTCAGAGCCATTCCGCATGGGCAGCGTGGTCTT
GCCATCCAGTGG

Figure 4.3.4c Sequences of the real-time RT-PCR products (5'-3' orientation) of the candidate calibration genes. Within a sequence, underlined nucleotides indicate the forward and reverse primer positions, respectively.

SDHA:
CGTAGGAGAGCGTGTGCTTCCTCCAGTGCTGCTCAAAGGGCTTCTTCTGCTGC
CCCTGGATGGGCTTGGAGTAATCGTACTCGTCAACCCTCTCCTTGAAGTCCTC
CCTGGCGTGGGCGCCGCGCGACTCCTtCCGGGCCTCCGCTCCGTAGATGGTCT
GCAGAGCACAAAGCATCAGGTTCTGC

UBC:
TAGGGGTGGGTTAGAGTTCAAGGTTTTTGTTCTACCAGATGTTTTAGTAGTAA
TCTGGAGGTAAGAAATGTCAAGAAAACATGGCCTTAATTAGAACTGTAGTGG
GTGAGTATAAATAAAAAATTTGGAGGTTGTAGTTAGAATTCTCCATATGTAC
ACTCATATGTAGATCTACTTATAAGCTACTGATTTTTAAAAGCACACGTTTGG
GAGTTGTGCTTAAGAGTGGGAAAGTTTCTGGAATACCAGCAGGGAGGT

YWHAZ:
TTGATCCCCAACGCTTCACAAGCAGAGAGCAAAGTCTTCTATTTGAAAATGA
AGGAGACTACTACCGCTACTTGGCTGAGGTTGCAGCTGGTGATGACAAGAAA
GGGATTGTGGACCAGTCACAGCAAGCATACCAAGAAGCTTTGAAATCAGCAA
AAAGGAAATGCAACCAACACATCCTATCAGACTGGGTCTGGCCCTTAACT

GAPDH:
ACATCAATGGGGTGATGCTGGTGCTGAGTATGTGGTGGAGTCCACTGGGGTC
TTCACTACCATGGAGAAGGCTGGGGCTCACTTGAAGGGTGGCGCCAAGAGGG
TCATCATCTCTGCACCTTCTGCCGATGCCCCCATGTTGTGATGGGCGTGAACC
ACGAGAAGTATAACAACACCCTCAAGATTGTCAGCAATGCC

HPRT1:
GCCAGCCGGCTACGTTATGGCGGCCCGCAGCCCCAGCGTGGTGATTAGCGAT
GATGAACCAGGTTATGACCTAAATTTATTTTGTATACCCAATCATTATGCTGA
GGATTTGGAGAAGGTGTTTATTCCTCATGGACTAATTATGGACAGGACCGAA
CGGCTGGCTCGAGATGTGATGAAGGAGATGGGTGGCCATCACATTGTGGCCC
TCTGTGTGCTCAAGGGGGGCTATAAGTTCTTTGCCGACCTGTTGGAT

ACTB:
GAGCGGGAAATCGTCCGTGACATCAAGGAGAAGCTCTGCTACGTGGCCCTGG
ACTTCGAGCAGGAGATGGCCACCGCGGCCTCCAGCTCCTCCCTGGAGAAGAG
CTACGAGCTTCCTGACGGGCAGGTCATCACCATCGGCAATGAGCGGTTCCGC
TGCCCTGAGGCTCTCTTCCAGCCTTCCTTCCTGGGCATGGAATCCTGCGGCAT
TCACGAAACTACCTTCAATTCCATCATGAAGTGTGACGTCGACATCCGCAAG
GACCTCTACACCAACAC

PPIA:

GGCAAGTCCAATTATGGCGAGAAATTTGATGATGAGAATTTCATTTTGAAGC
ATACAGGTCCTGGCATCTTGTCCATGGCAAATGCTGGCCCCAACACAAATGG
TTCCCAGTTTTTCATTTGCACTGCCAAGACTGAGTGGTTGGATGGCAAGCACG
TGGTCTTTGGCAAG
GTGAAAGAGGGCATGAATATTGTGGAAGCCATGGAGCGCTTTGGGTCCAGGA
ATGGCAAGACCAGCAA

TBP:

CCGCTGTTTCTTGGTGTGCACAAAGATAACCCACAGAGCCGAGCAAGTCGCC
AAGAATAGTGTGCTGGGGATGCTCTAGGAAAAGACTGAATGCTGAGGCAATT
TCCAGTCCAGAGGTTTTTGATTTGCCAAGAAGGTGAACGTCATGGATCAGAA
CAACAGCCTCCCACCCTATGCCCAGGGCCTGGCCTCCCCTCAGGGTGCCATG
ACTCCTGGAATCCCTATCTTTAGTCCAATGATGCCTTACGGCA

4.7 APPENDIX 1. PROC GLM SAS CODE

Analysis of mRNA expression of DM in forage samples of toxic endophyte-infected tall fescue (Chapter 5)

1. Example of SAS programming language using the PROC GLM procedure

```
PROC GLM data=Month plots=all;
class Trt;
model DM CP TDN ADF NDF NEG Ca P Mg K Na Fe Zn Cu Mn Mb Se Ergovaline
Ergovalanine TotalEA =Trt/ss3;
lsmeans Trt/pdiff lines;
lsmeans Trt/stderr;
RUN; QUIT;
```

The SAS System

The GLM Procedure

Class Level Information		
Class	Levels	Values
Trt	4	"NTE,lse "NTE,MIX "TE,lse" "TE,MIX"

Number of Observations Read	16
Number of Observations Used	16

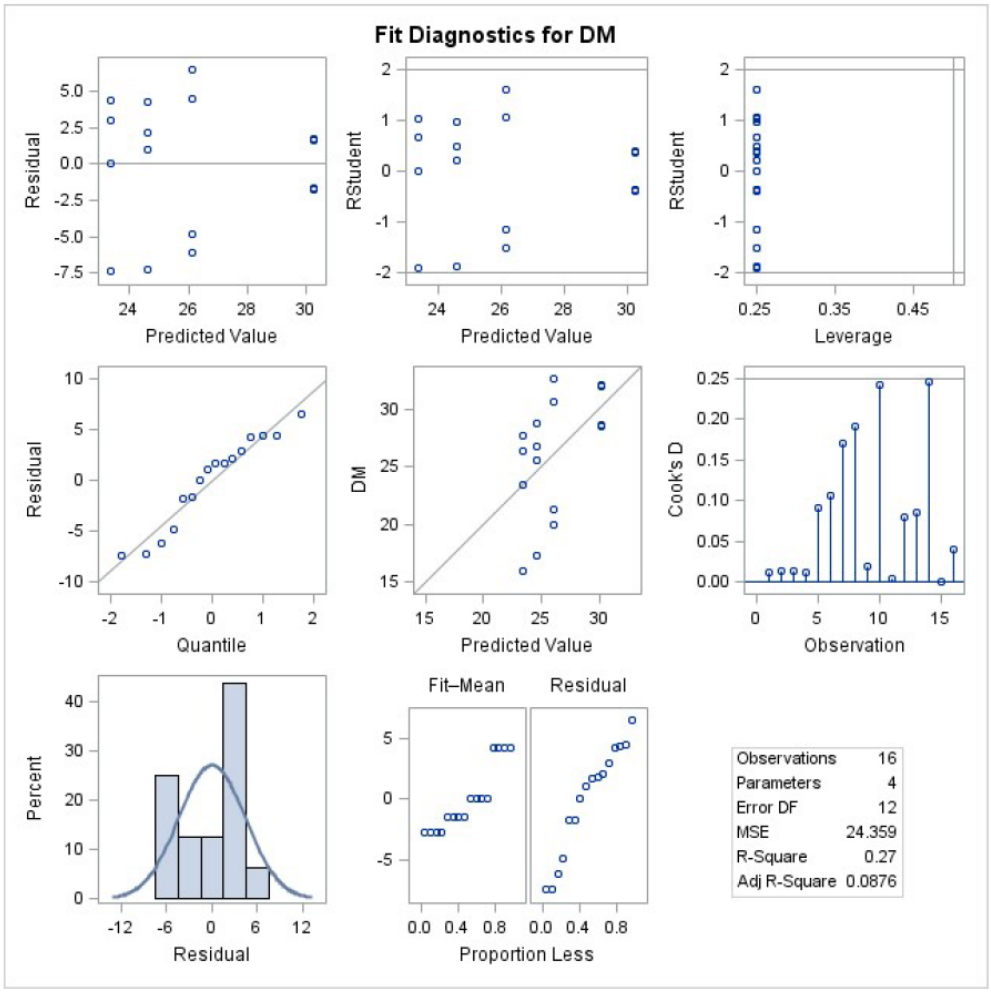
The GLM Procedure

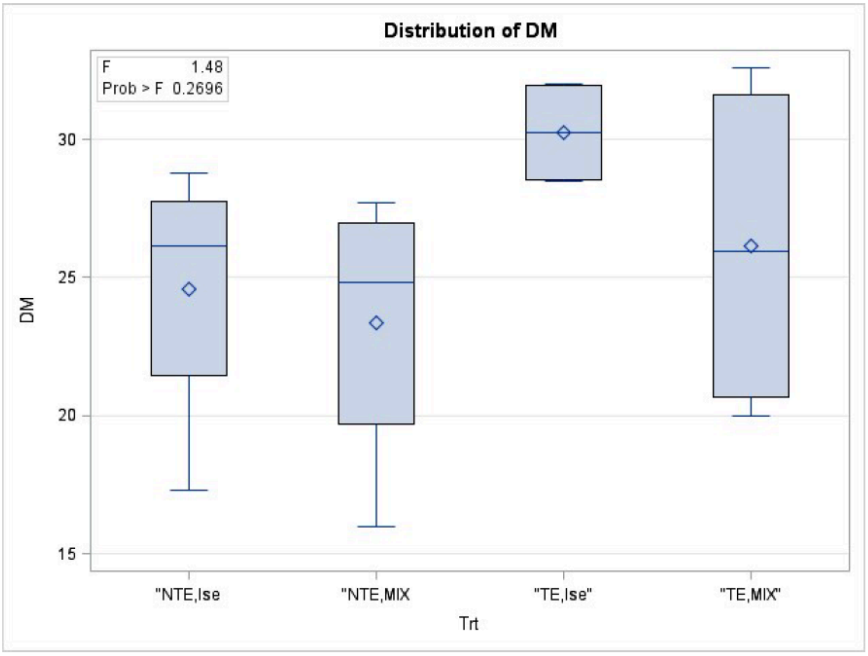
Dependent Variable: DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	108.1368750	36.0456250	1.48	0.2696
Error	12	292.3075000	24.3589583		
Corrected Total	15	400.4443750			

R-Square	Coeff Var	Root MSE	DM Mean
0.270042	18.92348	4.935480	26.08125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	3	108.1368750	36.0456250	1.48	0.2696


Fit Diagnostics for DM
Residual
Predicted Value
RStudent
Predicted Value
RStudent
Leverage
Residual
Quantile
DM
Predicted Value
Cook's D
Observation
Percent
Residual
Fit–Mean
Residual
Proportion Less
Observations 16
Parameters 4
Error DF 12
MSE 24.359
R-Square 0.27
Adj R-Square 0.0876


Distribution of DM
F 1.48
Prob > F 0.2696
DM
"NTE,lse
"NTE,MIX
"TE,lse"
"TE,MIX"
Trt

4.8 APPENDIX 2. PROC MIXED SAS CODE

Analysis of mRNA expression of prolactin pathway CISH in WBC tissue of steers grazing toxic endophyte-infected tall fescue and supplemented with ad libitum Se in vitamin-mineral mixes as either ISe, or MIX (Chapter 4)

1. Example of SAS programming language using the PROC MIXED procedure

```
proc mixed data=WORK.EXP1_PASTURE;
class trt Day;
model           CISH   = trt Day trt * Day ;
Repeated Day / subject=ID type=ar (1);
Contrast 'Day' Day 1 -1 ;
run; quit;
```

Model Information	
Data Set	WORK.EXP1_PASTURE
Dependent Variable	CISH
Covariance Structure	Autoregressive
Subject Effect	ID
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information		
Class	**Levels**	**Values**
trt	2	ISe MIX
Day	2	0 84

Dimensions	
Covariance Parameters	2
Columns in X	9
Columns in Z	0
Subjects	16
Max Obs per Subject	1

Number of Observations	
Number of Observations Read	26
Number of Observations Used	16
Number of Observations Not Used	10

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	18.79541322	
1	1	18.79541322	0.00000000

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
AR(1)	ID	0
Residual		0.1766

Fit Statistics	
-2 Res Log Likelihood	18.8
AIC (Smaller is Better)	22.8
AICC (Smaller is Better)	24.1
BIC (Smaller is Better)	24.3

Null Model Likelihood Ratio Test		
DF	Chi-Square	Pr > ChiSq
1	0.00	1.0000

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	1	12	0.94	0.3519
Day	1	12	11.89	0.0048
trt*Day	1	12	0.01	0.9421

Contrasts				
Label	Num DF	Den DF	F Value	Pr > F
day	1	12	11.89	0.0048

4.9 APPENDIX 3. PROC GLIMMIX SAS CODE

Analysis of mRNA expression of CISH in WBC tissue of steers grazing toxic endophyte-infected tall fescue and supplemented with ad libitum Se in vitamin-mineral mixes as either ISe or MIX (Chapter 4)

1. Example of SAS programming language using the PROC GLIMMIX procedure

```
proc glimmix data = WORK.EXP1_PASTURE;
class trt Day;
model CISH     = trt |Day/ddfm=kr;
random Day/sub= CISH         (trt) type=ar(1) residual;
lsmeans trt Day/pdiff lines;
run; quit;
```

Model Information	
Data Set	WORK.EXP1_PASTURE
Response Variable	CISH
Response Distribution	Gaussian
Link Function	Identity
Variance Function	Default
Variance Matrix Blocked By	CISH(trt)
Estimation Technique	Restricted Maximum Likelihood
Degrees of Freedom Method	Kenward-Roger
Fixed Effects SE Adjustment	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
trt	2	ISe MIX
Day	2	0 84

Number of Observations Read	26
Number of Observations Used	16

Dimensions	
R-side Cov. Parameters	2
Columns in X	9
Columns in Z per Subject	0
Subjects (Blocks in V)	16
Max Obs per Subject	1

Optimization Information	
Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	1
Lower Boundaries	1
Upper Boundaries	1
Fixed Effects	Profiled
Residual Variance	Profiled
Starting From	Data

Iteration History					
Iteration	**Restarts**	**Evaluations**	**Objective Function**	**Change**	**Max Gradient**
0	**0**	4	18.795413222	.	0

Convergence criterion

Fit Statistics	
-2 Res Log Likelihood	18.80
AIC (smaller is better)	22.80
AICC (smaller is better)	24.13
BIC (smaller is better)	24.34
CAIC (smaller is better)	26.34
HQIC (smaller is better)	22.87
Generalized Chi-Square	2.12
Gener. Chi-Square / DF	0.18

Covariance Parameter Estimates			
Cov Parm	**Subject**	**Estimate**	**Standard Error**
AR(1)	CISH(trt)	0	.
Residual		0.1766	0.07211

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
trt	1	12	0.94	0.3519
Day	1	12	11.89	0.0048
trt*Day	1	12	0.01	0.9421

trt Least Squares Means					
trt	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
ISe	1.4276	0.1486	12	9.61	<.0001
MIX	1.2240	0.1486	12	8.24	<.0001

Differences of trt Least Squares Means						
trt	**trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
ISe	MIX	0.2035	0.2101	12	0.97	0.3519

T Grouping for trt Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
trt	**Estimate**	
ISe	1.4276	A
		A
MIX	1.2240	A

Day Least Squares Means					
Day	Estimate	Standard Error	DF	t Value	Pr > |t|
0	0.9634	0.1486	12	6.48	<.0001
84	1.6881	0.1486	12	11.36	<.0001

Differences of Day Least Squares Means						
Day	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
0	84	-0.7247	0.2101	12	-3.45	0.0048

T Grouping for Day Least Squares Means (Alpha=0.05)		
LS-means with the same letter are not significantly different.		
Day	Estimate	
84	1.6881	A
0	0.9634	B

REFERENCES

Abram, C. L., and C. A. Lowell. 2008. The diverse functions of Src family kinases in macrophages. Front. Biosci. 13:4426-4450. doi: 10.2741/3015

Adler, H., B. Adler, P. Peveri, E. R. Werner, H. Wachter, E. Peterhans, and T. W. Jungi. 1996. Differential regulation of inducible nitric oxide synthase production in bovine and caprine macrophages. J. Infect. Dis. 173(4):971-978.

Ahn, J.-Y., and K. Ye. 2005. PIKE GTPase signaling and function. Int. J. Biol. Sci. 1(2):44-50. doi: 10.7150/ijbs.1.44

Aiken, G., M. Looper, S. Tabler, D. Brauer, J. Strickland, and F. Schrick. 2006. Influence of stocking rate and steroidal implants on growth rate of steers grazing toxic tall fescue and subsequent physiological responses. J. Anim. Sci. 84(6):1626-1632.

Aiken, G. E., and J. R. Strickland. 2013. Forages and pastures symposium: managing the tall fescue-fungal endophyte symbiosis for optimum forage-animal production. J. Anim. Sci. 91(5):2369-2378. doi: 10.2527/jas.2012-5948

Akira, S., and K. Takeda. 2004. Toll-like receptor signalling. Nat. Rev. Immunol. 4(7):499-511.

Aldrich, C. G., J. A. Paterson, J. L. Tate, and M. S. Kerley. 1993. The effects of endophyte-infected tall fescue consumption on diet utilization and thermal regulation in cattle. J. Anim. Sci. 71(1):164-170. doi: 10.2527/1993.711164x

Allain, F., C. Vanpouille, M. Carpentier, M.-C. Slomianny, S. Durieux, and G. Spik. 2002. Interaction with glycosaminoglycans is required for cyclophilin B to trigger integrin-mediated adhesion of peripheral blood T lymphocytes to extracellular matrix. Proc. Natl. Acad. Sci. U.S.A. 99(5):2714-2719.

Ammerman, C., and S. Miller. 1975. Selenium in ruminant nutrition: a review. J. Dairy Sci. 58(10):1561-1577.

Arnold, M., C. Gaskill, and S. Smith. 2014. Fescue Toxicosis.

Arthington, J. D., R. F. Cooke, T. D. Maddock, D. B. Araujo, P. Moriel, N. Dilorenzo, and G. C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91(4):1831-1837. doi: 10.2527/jas.2012-5724

Auffray, C., M. H. Sieweke, and F. Geissmann. 2009. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu. Rev. Immunol. 27

Avery, J. C., and P. R. Hoffmann. 2018. Selenium, selenoproteins, and immunity. Nutrients 10(9):1203.

Bakowska, J. C., and J. I. Morrell. 1997. Atlas of the neurons that express mRNA for the long form of the prolactin receptor in the forebrain of the female rat. J. Comp. Neurol. 386(2):161-177.

Ball, D., G. D. Lacefield, and C. S. Hoveland. 1991. The Tall Fescue Endophyte. Agric. Nat. Resour. 33

Ball, D. M., G. D. Lacefield, and C. S. Hoveland. 2007. Cool season grasses, Southern forages. International Plant Nutrition Institute, Norcross, GA.

Barnes, P. F., D. Chatterjee, J. S. Abrams, S. Lu, E. Wang, M. Yamamura, P. J. Brennan, and R. L. Modlin. 1992. Cytokine production induced by Mycobacterium tuberculosis lipoarabinomannan. Relationship to chemical structure. J. Immunol. 149(2):541-547.

Barrington, G. M., and S. M. Parish. 2001. Bovine Neonatal Immunology. Vet. Clin. North Am. Food Anim. 17(3):463-476. doi: https://doi.org/10.1016/S0749-0720(15)30001-3

Baxter, D. 2007. Active and passive immunity, vaccine types, excipients and licensing. Occup. Med. 57(8):552-556. doi: 10.1093/occmed/kqm110

Bazan, J. F. 1990a. Haemopoietic receptors and helical cytokines. Immunol. Today 11(10):350-354. doi: 10.1016/0167-5699(90)90139-z

Bazan, J. F. 1990b. Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. U.S.A. 87(18):6934-6938. doi: 10.1073/pnas.87.18.6934

Beck, M. A., O. A. Levander, and J. Handy. 2003. Selenium deficiency and viral infection. J. Nutr. 133(5):1463S-1467S.

Beckett, G. J., and J. R. Arthur. 2005. Selenium and endocrine systems. J. Endocrinol. 184(3):455-465. doi: 10.1677/joe.1.05971

Ben-Jonathan, N., J. L. Mershon, D. L. Allen, and R. W. Steinmetz. 1996. Extrapituitary Prolactin: Distribution, Regulation, Functions, and Clinical Aspects*. Endocr. Rev. 17(6):639-669. doi: 10.1210/edrv-17-6-639

Berczi, I. 1986. Pituitary function and immunity. CRC Press.

Berczi, I., and E. Nagy. 1987. The effect of prolactin and growth hormone on hemolymphopoietic tissue and immune function, Hormones and immunity. MTP press Lancaster. p. 145-171.

Bernard, J., A. Chestnut, B. Erickson, and F. Kelly. 1993. Effects of prepartum consumption of endophyte-infested tall fescue on serum prolactin and subsequent milk production of Holstein cows. J. Dairy Sci. 76(7):1928-1933.

Berry, M. J., L. Banu, J. W. Harney, and P. R. Larsen. 1993. Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. EMBO J. 12(8):3315-3322.

Bianchi, M. E. 2007. DAMPs, PAMPs and alarmins: all we need to know about danger. J. Leukoc. Biol. 81(1):1-5.

Binart, N., A. Bachelot, and J. Bouilly. 2010. Impact of prolactin receptor isoforms on reproduction. Trends in Endocrinology & Metabolism 21(6):362-368. doi: https://doi.org/10.1016/j.tem.2010.01.008

Blom, B., and H. Spits. 2006. DEVELOPMENT OF HUMAN LYMPHOID CELLS. Annu. Rev. Immunol. 24(1):287-320. doi: 10.1146/annurev.immunol.24.021605.090612

Bohuslav, J., L.-f. Chen, H. Kwon, Y. Mu, and W. C. Greene. 2004. p53 induces NF-κB activation by an IκB kinase-independent mechanism involving phosphorylation of p65 by ribosomal S6 kinase 1. J. Biol. Chem. 279(25):26115-26125.

Bole-Feysot, C., V. Goffin, M. Edery, N. Binart, and P. A. Kelly. 1998a. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 19(3):225-268. doi: 10.1210/edrv.19.3.0334

Bole-Feysot, C., V. Goffin, M. Edery, N. Binart, and P. A. Kelly. 1998b. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev. 19(3):225-268. doi: 10.1210/edrv.19.3.0334

Bonizzi, G., and M. Karin. 2004. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 25(6):280-288. doi: 10.1016/j.it.2004.03.008

Brennan, K. M., W. R. Burris, J. A. Boling, and J. C. Matthews. 2011. Selenium content in blood fractions and liver of beef heifers is greater with a mix of inorganic/organic or organic versus inorganic supplemental selenium but the time required for maximal assimilation is tissue-specific. Biol. Trace Elem. Res. 144(1-3):504-516. doi: 10.1007/s12011-011-9069-y

Brigelius-Flohe, R., and M. Maiorino. 2013. Glutathione peroxidases. Biochim. Biophys. Acta. 1830(5):3289-3303. doi: 10.1016/j.bbagen.2012.11.020

Bromfield, J. J., and I. M. Sheldon. 2011. Lipopolysaccharide initiates inflammation in bovine granulosa cells via the TLR4 pathway and perturbs oocyte meiotic progression in vitro. Endocrinology 152(12):5029-5040. doi: 10.1210/en.2011-1124

Brown, K. R., G. A. Anderson, K. Son, G. Rentfrow, L. P. Bush, J. L. Klotz, J. R. Strickland, J. A. Boling, and J. C. Matthews. 2009. Growing steers grazing high versus low endophyte (Neotyphodium coenophialum)-infected tall fescue have reduced serum enzymes, increased hepatic glucogenic enzymes, and reduced liver and carcass mass1. J. Anim. Sci. 87(2):748-760. doi: 10.2527/jas.2008-1108

Brun-Hansen, H. C., A. H. Kampen, and A. Lund. 2006. Hematologic values in calves during the first 6 months of life. Vet. Clin. Pathol. 35(2):182-187.

Bukrinsky, M. I. 2002. Cyclophilins: unexpected messengers in intercellular communications. Trends Immunol. 23(7):323-325. doi: https://doi.org/10.1016/S1471-4906(02)02237-8

Burg, N. D., and M. H. Pillinger. 2001. The Neutrophil: Function and Regulation in Innate and Humoral Immunity. Clinical Immunology 99(1):7-17. doi: https://doi.org/10.1006/clim.2001.5007

Burk, R. F., and K. E. Hill. 2015. Regulation of selenium metabolism and transport. Annu. Rev. Nutr. 35:109-134.

Bush, L., and R. Buckner. 1973. Tall fescue toxicity.

Bush, L. J., and T. E. Staley. 1980. Absorption of Colostral Immunoglobulins in Newborn Calves1. J. Dairy Sci. 63(4):672-680. doi: https://doi.org/10.3168/jds.S0022-0302(80)82989-4

Bussard, J., and G. Aiken. 2012. Number of beef cows exposed toxic tall fescue: small or large? American Forage Grassland Conference Proceedings

Cano, R. L. E., and H. D. E. Lopera. 2013. Introduction to T and B lymphocytes, Autoimmunity: From Bench to Bedside [Internet]. El Rosario University Press.

Carroll, J. A., J. D. Arthington, and C. C. Chase, Jr. 2009. Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves. J. Anim. Sci. 87(12):4167-4172. doi: 10.2527/jas.2009-2016

Carstensen, L., C. M. Røntved, and J. P. Nielsen. 2005. Determination of tumor necrosis factor-α responsiveness in piglets around weaning using an ex vivo whole blood stimulation assay. Vet. Immunol. Immunopathol. 105(1-2):59-66.

Carter, J. M., G. E. Aiken, C. T. Dougherty, and F. N. Schrick. 2010. Steer responses to feeding soybean hulls and steroid hormone implantation on toxic tall fescue pasture1. J. Anim. Sci. 88(11):3759-3766. doi: 10.2527/jas.2009-2536

Chan, C. B., X. Liu, M. A. Ensslin, D. L. Dillehay, C. J. Ormandy, P. Sohn, R. Serra, and K. Ye. 2010. PIKE-A is required for prolactin-mediated STAT5a activation in mammary gland development. EMBO J. 29(5):956-968. doi: 10.1038/emboj.2009.406

Chang, M., W. Jin, J.-H. Chang, Y. Xiao, G. C. Brittain, J. Yu, X. Zhou, Y.-H. Wang, X. Cheng, and P. Li. 2011. The ubiquitin ligase Peli1 negatively regulates T cell activation and prevents autoimmunity. Nat. Immunol. 12(10):1002-1009.

Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008a. Development of the immune response in the calf: from conception to puberty. (Data from Morein B, Abusugra I, Blomqvist G. Immunity in neonates. Vet Immunol Immunopath 2002;87:207–13; and Butler JE, Sinkora M, Wertz N, et al. Development of the neonatal B and T cell repertoire in swine: implications for comparative and veterinary immunology. Vet Res 2006;37:419.).

Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008b. Neonatal Immune Development in the Calf and Its Impact on Vaccine Response. Vet. Clin. North Am. Food Anim. 24(1):87-104. doi: https://doi.org/10.1016/j.cvfa.2007.11.001

Cheeke, P. R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. J. Anim. Sci. 73(3):909-918. doi: 10.2527/1995.733909x

Chen, F., W. Wu, A. Millman, J. F. Craft, E. Chen, N. Patel, J. L. Boucher, J. F. Urban, Jr., C. C. Kim, and W. C. Gause. 2014. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat. Immunol. 15(10):938-946. doi: 10.1038/ni.2984

Chen, J., and Z. J. Chen. 2013. Regulation of NF-κB by ubiquitination. Curr Opin Immunol 25(1):4-12. doi: 10.1016/j.coi.2012.12.005

Chen, L. F., and W. C. Greene. 2004. Shaping the nuclear action of NF-kappaB. Nat. Rev. Mol. Cell Biol. 5(5):392-401. doi: 10.1038/nrm1368

Chen, X.-D., Z.-P. Zhao, J.-C. Zhou, and X. G. Lei. 2018. Evolution, regulation, and function of porcine selenogenome. Free Radical Biol. Med. 127:116-123.

Chistiakov, D. A., Y. V. Bobryshev, and A. N. Orekhov. 2015. Neutrophil's weapons in atherosclerosis. Exp. Mol. Pathol. 99(3):663-671. doi: https://doi.org/10.1016/j.yexmp.2015.11.011

Chiu, S., and A. Bharat. 2016. Role of monocytes and macrophages in regulating immune response following lung transplantation. Curr. Opin. Organ Transplant. 21(3):239-245. doi: 10.1097/MOT.0000000000000313

Chow, J. C., D. W. Young, D. T. Golenbock, W. J. Christ, and F. Gusovsky. 1999. Toll-like Receptor-4 Mediates Lipopolysaccharide-induced Signal Transduction. J. Biol. Chem. 274(16):10689-10692. doi: 10.1074/jbc.274.16.10689

Corbacho, A. M., G. Valacchi, L. Kubala, E. Olano-Martín, B. C. Schock, T. P. Kenny, and C. E. Cross. 2004. Tissue-specific gene expression of prolactin receptor in the acute-phase response induced by lipopolysaccharides. Am. J. Physiol. Endocrinol. Metab. 287(4):E750-E757. doi: 10.1152/ajpendo.00522.2003

Cunningham, I. 1949. A note on the cause of tall fescue lameness in cattle. Aust. Vet. J. 25:27-28.

Dahl, G. E., B. A. Buchanan, and H. A. Tucker. 2000. Photoperiodic Effects on Dairy Cattle: A Review1. J. Allergy Clin. Immunol. 83(4):885-893. doi: https://doi.org/10.3168/jds.S0022-0302(00)74952-6

Dargatz, D. A., and P. F. Ross. 1996a. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. 74(12):2891-2895. doi: 10.2527/1996.74122891x

Dargatz, D. A., and P. F. Ross. 1996b. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. Journal of Animal Science 74(12):2891-2895. doi: 10.2527/1996.74122891x

Davenport, G., J. Boling, and C. Rahe. 1993. Growth and endocrine responses of cattle to implantation of estradiol-17 beta during continuous or discontinuous grazing of high-and low-endophyte-infected tall fescue. J. Anim. Sci. 71(3):757-764.

Dentener, M. A., V. Bazil, E. J. Von Asmuth, M. Ceska, and W. A. Buurman. 1993. Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-alpha, IL-6 and IL-8 release by human monocytes and alveolar macrophages. J. Immunol. 150(7):2885-2891.

Díaz, L., M. D. Muñoz, L. González, S. Lira-Albarrán, F. Larrea, and I. Méndez. 2013. Prolactin in the immune system.

Dirksen, G. 2006. Innere Medizin und Chirurgie des Rindes. Georg Thieme Verlag.

Ericson, J. A., P. Duffau, K. Yasuda, A. Ortiz-Lopez, K. Rothamel, I. R. Rifkin, P. A. Monach, and C. ImmGen. 2014. Gene expression during the generation and activation of mouse neutrophils: implication of novel functional and regulatory pathways. PLoS One 9(10):e108553-e108553. doi: 10.1371/journal.pone.0108553

Fagegaltier, D., N. Hubert, K. Yamada, T. Mizutani, P. Carbon, and A. Krol. 2000. Characterization of mSelB, a novel mammalian elongation factor for selenoprotein translation. EMBO J. 19(17):4796-4805.

Feldman, B. F., J. G. Zinkl, N. C. Jain, and O. W. Schalm. 2000. Schalm's veterinary hematology. Lippincott Williams & Wilkins, Philadelphia.

Fernandes, A. P., M. Wallenberg, V. Gandin, S. Misra, F. Tisato, C. Marzano, M. P. Rigobello, S. Kumar, and M. Björnstedt. 2012. Methylselenol Formed by Spontaneous Methylation of Selenide Is a Superior Selenium Substrate to the Thioredoxin and Glutaredoxin Systems. PLOS ONE 7(11):e50727. doi: 10.1371/journal.pone.0050727

Filipov, N. M., F. N. Thompson, R. P. Sharma, and R. R. Dugyala. 1999a. Increased proinflammatory cytokines production by ergotamine in male BALB/c mice. J. Toxicol. Environ. Health A. 58(3):145-155. doi: 10.1080/009841099157359

Filipov, N. M., F. N. Thompson, J. A. Stuedemann, T. H. Elsasser, S. Kahl, R. P. Sharma, C. R. Young, L. H. Stanker, and C. K. Smith. 1999b. Increased responsiveness to intravenous lipopolysaccharide challenge in steers grazing endophyte-infected tall fescue compared with steers grazing endophyte-free tall fescue. J. Endocrinol. 163(2):213-220. doi: 10.1677/joe.0.1630213

Finch-Arietta, M. B., and F. R. Cochran. 1991. Cytokine production in whole bloodex vivo. Agents Actions 34(1):49-52. doi: 10.1007/BF01993235

Flohe, L., W. A. Gunzler, and H. H. Schock. 1973. Glutathione peroxidase: a selenoenzyme. FEBS Lett. 32(1):132-134. doi: 10.1016/0014-5793(73)80755-0

Foster, S. J., L. M. McCormick, B. A. Ntolosi, and D. Campbell. 1993. Production of TNFα by LPS-stimulated murine, rat and human blood and its pharmacological modulation. Agents Actions 38(2):C77-C79. doi: 10.1007/BF01991143

Francey, T., L. Schalch, M. Brcic, E. Peterhans, and T. Jungi. 1992. Generation and functional characterization of ovine bone marrow-derived macrophages. Vet. Immunol. Immunopathol. 32(3-4):281-301.

Franke, K. W. 1934. A new toxicant occurring naturally in certain samples of plant foodstuffs. 1. Results obtained in preliminary feeding trials. J. Nutr. 8:597-608.

Freemark, M., I. Avril, D. Fleenor, P. Driscoll, A. Petro, E. Opara, W. Kendall, J. Oden, S. Bridges, N. Binart, B. Breant, and P. A. Kelly. 2002. Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. Endocrinology 143(4):1378-1385. doi: 10.1210/endo.143.4.8722

Fusco, F., A. Pescatore, M. I. Conte, P. Mirabelli, M. Paciolla, E. Esposito, M. B. Lioi, and M. V. Ursini. 2015. EDA-ID and IP, two faces of the same coin: how the same IKBKG/NEMO mutation affecting the NF-κB pathway can cause immunodeficiency and/or inflammation. Int. Rev. Immunol. 34(6):445-459. doi: 10.3109/08830185.2015.1055331

Gadberry, M., T. Denard, D. Spiers, and E. Piper. 2003. Effects of feeding ergovaline on lamb performance in a heat stress environment. J. Anim. Sci. 81(6):1538-1545.

Galbraith, M., W. Vorachek, C. Estill, P. Whanger, G. Bobe, T. Davis, and J. Hall. 2016. Rumen microorganisms decrease bioavailability of inorganic selenium supplements. Biol. Trace Elem. Res. 171(2):338-343.

Gerloff, B. J. 1992. Effect of selenium supplementation on dairy cattle. J. Anim. Sci. 70(12):3934-3940. doi: 10.2527/1992.70123934x

Gessler, P., J. Pfenninger, J.-P. Pfammatter, T. Carrel, O. Baenziger, and C. Dahinden. 2003. Plasma levels of interleukin-8 and expression of interleukin-8 receptors on circulating neutrophils and monocytes after cardiopulmonary bypass in children. J. Thorac. Cardiovasc. Surg. 126(3):718-725. doi: https://doi.org/10.1016/S0022-5223(03)00685-8

Ghosh, S., and M. Karin. 2002. Missing pieces in the NF-κB puzzle. Cell 109(2):S81-S96.

Giridharan, S., and M. Srinivasan. 2018. Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. J. Inflamm. Res. 11:407-419. doi: 10.2147/JIR.S140188

Glass, R. S., W. P. Singh, W. Jung, Z. Veres, T. D. Scholz, and T. Stadtman. 1993. Monoselenophosphate: synthesis, characterization, and identity with the prokaryotic biological selenium donor, compound SePX. Biochem. 32(47):12555-12559.

Goetsch, A., A. Jones, S. Stokes, K. Beers, and E. Piper. 1987. Intake, digestion, passage rate and serum prolactin in growing dairy steers fed endophyte-infected fescue with noninfected fescue, clover or wheat straw. J. Anim. Sci. 64(6):1759-1768.

Goffin, V., N. Binart, P. Touraine, and P. A. Kelly. 2002. Prolactin: the new biology of an old hormone. Annual review of physiology 64(1):47-67.

Gordon, S., and L. Martinez-Pomares. 2017. Physiological roles of macrophages. Pflug. Arch. Eur. J. Physiol. 469(3-4):365-374. doi: 10.1007/s00424-017-1945-7

Gordon, S., and P. R. Taylor. 2005a. Macrophage heterogeneity during inflammation.

Gordon, S., and P. R. Taylor. 2005b. Monocyte and macrophage heterogeneity. Nat. Rev. Immunol. 5(12):953-964.

Graves, D. T., and Y. Jiang. 1995a. Chemokines, a family of chemotactic cytokines. Crit. Rev. Oral Biol. Med. 6(2):109-118. doi: 10.1177/10454411950060020101

Graves, D. T., and Y. Jiang. 1995b. Chemokines, a family of chemotactic cytokines. Crit Rev Oral Biol Med 6(2):109-118. doi: 10.1177/10454411950060020101

Greenlee-Wacker, M. C. 2016. Clearance of apoptotic neutrophils and resolution of inflammation. Immunol. Rev. 273(1):357-370. doi: 10.1111/imr.12453

Greten, F. R., M. C. Arkan, J. Bollrath, L. C. Hsu, J. Goode, C. Miething, S. I. Göktuna, M. Neuenhahn, J. Fierer, S. Paxian, N. Van Rooijen, Y. Xu, T. O'Cain, B. B. Jaffee, D. H. Busch, J. Duyster, R. M. Schmid, L. Eckmann, and M. Karin. 2007. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. Cell 130(5):918-931. doi: 10.1016/j.cell.2007.07.009

Grubbs, H., and C. I. Kahwaji. 2018. Physiology, Active Immunity

Grünig, G., C. Hulliger, C. Winder, M. Hermann, T. Jungi, and R. Von Fellenberg. 1991. Spontaneous and lipopolysaccharide-induced expression of procoagulant activity by equine lung macrophages in comparison with blood monocytes and blood neutrophils. Vet. Immunol. Immunopathol. 29(3-4):295-311.

Guerre, P. 2015. Ergot alkaloids produced by endophytic fungi of the genus Epichloë. Toxins (Basel) 7(3):773-790. doi: 10.3390/toxins7030773

Gunter, S. A., P. A. Beck, and J. M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves1. J. Anim. Sci. 81(4):856-864. doi: 10.2527/2003.814856x

Gupta, S. C., C. Sundaram, S. Reuter, and B. B. Aggarwal. 2010. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. Biochim. Biophys. Acta. 1799(10-12):775-787. doi: 10.1016/j.bbagrm.2010.05.004

Hafeman, D. G., R. A. Sunde, and W. G. Hoekstra. 1974. Effect of Dietary Selenium on Erythrocyte and Liver Glutathione Peroxidase in the Rat. J. Nutr. 104(5):580-587. doi: 10.1093/jn/104.5.580

Hall, J. A., G. Bobe, J. K. Hunter, W. R. Vorachek, W. C. Stewart, J. A. Vanegas, C. T. Estill, W. D. Mosher, and G. J. Pirelli. 2013. Effect of feeding selenium-fertilized alfalfa hay on performance of weaned beef calves. PLoS One 8(3):e58188-e58188. doi: 10.1371/journal.pone.0058188

Hannaway, D., C. Daly, M. D. Halbleib, D. James, C. P. West, J. Volenec, D. Chapman, X. Li, W. Cao, J. Shen, X. Shi, and S. Johnson. 2009. Development of Suitability Maps with Examples for the United States and China No. 53. p. 33-47.

Harrison, L. M., W. C. E. van Haaften, and V. L. Tesh. 2004. Regulation of proinflammatory cytokine expression by Shiga toxin 1 and/or lipopolysaccharides in the human monocytic cell line THP-1. Infect. Immun. 72(5):2618-2627. doi: 10.1128/IAI.72.5.2618-2627.2004

Harvey, S., C. Aramburo, and E. Sanders. 2012. Extrapituitary production of anterior pituitary hormones: an overview. Endocr. 41(1):19-30.

Hatfield, D. L., I. S. Choi, T. Ohama, J.-E. Jung, and A. M. Diamond. 1994. Selenocysteine tRNA [Ser] Sec isoacceptors as central components in selenoprotein biosynthesis in eukaryotes, Selenium in Biology and Human Health. Springer. p. 25-44.

Hauser, M., M. Koob, and J. Roth. 1986. Variation of neutrophil function with age in calves. Am. J. Vet. Res. 47(1):152-153.

Hayashi, T., Y. Ueno, and T. Okamoto. 1993. Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin. J. Biol. Chem. 268(15):11380-11388.
Hayden, M. S., and S. Ghosh. 2011. NF-κB in immunobiology. Cell Res. 21(2):223-244.
Herdt, T. H., W. Rumbeiha, and W. E. Braselton. 2000. The use of blood analyses to evaluate mineral status in livestock. Vet. Clin. North Am. Food Anim. 16(3):423-444. doi: 10.1016/s0749-0720(15)30078-5
Hoffmann, E., O. Dittrich-Breiholz, H. Holtmann, and M. Kracht. 2002. Multiple control of interleukin-8 gene expression. J. Leukoc. Biol. 72(5):847-855.
Hoshino, K., O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J. Immunol. 162(7):3749-3752.
Hoveland, C. S. 1993a. Importance and economic significance of the Acremonium endophytes to performance of animals and grass plant. Agriculture, Ecosystems & Environment 44(1):3-12. doi: https://doi.org/10.1016/0167-8809(93)90036-O
Hoveland, C. S. 1993b. Importance and economic significance of the Acremonium endophytes to performance of animals and grass plant. Agric. Ecosyst. Environ. 44(1):3-12. doi: https://doi.org/10.1016/0167-8809(93)90036-O
Hoveland, C. S. 2009. Origin and History. In: H. A. Fribourg, D. B. Hannaway and C. P. West, editors, Tall Fescue for the Twenty-first Century. Agronomy Monographs. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 3-10.
Howard, C. J., G. P. Brooke, D. Werling, P. Sopp, J. C. Hope, K. R. Parsons, and R. A. Collins. 1999. Dendritic cells in cattle: phenotype and function. Vet. Immunol. Immunopathol. 72(1):119-124. doi: https://doi.org/10.1016/S0165-2427(99)00124-5
Hussen, J., A. Düvel, O. Sandra, D. Smith, I. M. Sheldon, P. Zieger, and H.-J. Schuberth. 2013. Phenotypic and functional heterogeneity of bovine blood monocytes. PLoS One 8(8):e71502.
Hussen, J., M. Koy, W. Petzl, and H.-J. Schuberth. 2016. Neutrophil degranulation differentially modulates phenotype and function of bovine monocyte subsets. Innate Immun. 22(2):124-137.
Hussen, J., and H.-J. Schuberth. 2017. Heterogeneity of Bovine Peripheral Blood Monocytes. Front. Immunol. 8:1875-1875. doi: 10.3389/fimmu.2017.01875
Imada, K., E. T. Bloom, H. Nakajima, J. A. Horvath-Arcidiacono, G. B. Udy, H. W. Davey, and W. J. Leonard. 1998. Stat5b is essential for natural killer cell–mediated proliferation and cytolytic activity. J. Exp. Med. 188(11):2067-2074.
Jackson, J. J., M. D. Lindemann, J. A. Boling, and J. C. Matthews. 2015. Summer-long grazing of high vs. low endophyte (Neotyphodium coenophialum)-infected tall fescue by growing beef steers results in distinct temporal blood analyte response patterns, with poor correlation to serum prolactin levels. Front. Vet. Sci. 2:77.
Jahan, N., A. Minuti, and E. Trevisi. 2015. Assessment of immune response in periparturient dairy cows using ex vivo whole blood stimulation assay with lipopolysaccharides and carrageenan skin test. Vet. Immunol. Immunopathol. 165(3):119-126. doi: https://doi.org/10.1016/j.vetimm.2015.04.003

Janeway, C. A. 1989. Approaching the asymptote? Evolution and revolution in immunology. In: Cold Spring Harb. Symp. Quant. Biol. p 1-13.

Janeway, C. A., J. D. Capra, P. Travers, and M. Walport. 1999. Immunobiology: the immune system in health and disease.

Jia, Y., Q. Li, W. R. Burris, G. E. Aiken, P. J. Bridges, and J. C. Matthews. 2018. Forms of selenium in vitamin-mineral mixes differentially affect serum prolactin concentration and hepatic glutamine synthetase activity of steers grazing endophyte-infected tall fescue. J. Anim. Sci. 96(2):715-727. doi: 10.1093/jas/skx068

Jia, Y., K. Son, W. R. Burris, P. J. Bridges, and J. C. Matthews. 2019. Forms of selenium in vitamin-mineral mixes differentially affect serum alkaline phosphatase activity, and serum albumin and blood urea nitrogen concentrations, of steers grazing endophyte-infected tall fescue. J. Anim. Sci. 97(6):2569-2582. doi: 10.1093/jas/skz109

Jiang, X., M. He, J. Bai, C. B. Chan, and A. O. L. Wong. 2020. Signal Transduction for TNFα-Induced Type II SOCS Expression and Its Functional Implication in Growth Hormone Resistance in Carp Hepatocytes. Front. Endocrinol. 11:20-20. doi: 10.3389/fendo.2020.00020

Jones, M. L., and R. W. Allison. 2007. Evaluation of the ruminant complete blood cell count. Vet. Clin. North Am. Food Anim. 23(3):377-402.

Juniper, D. T., R. H. Phipps, E. Ramos-Morales, and G. Bertin. 2008. Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in beef cattle. J. Anim. Sci. 86(11):3100-3109. doi: 10.2527/jas.2007-0595

Kahl, S., T. H. Elsasser, and J. W. Blum. 1997. Nutritional regulation of plasma tumor necrosis factor-alpha and plasma and urinary nitrite/nitrate responses to endotoxin in cattle. Proc Soc Exp Biol Med 215(4):370-376. doi: 10.3181/00379727-215-44146

Kampen, A. H., I. Olsen, T. Tollersrud, A. K. Storset, and A. Lund. 2006. Lymphocyte subpopulations and neutrophil function in calves during the first 6 months of life. Vet. Immunol. Immunopathol. 113(1):53-63. doi: https://doi.org/10.1016/j.vetimm.2006.04.001

Kaplan, M. H. 2013. STAT signaling in inflammation. Taylor & Francis.

Karin, M., and M. Delhase. 2000. The IκB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. In: Semin Immunol. p 85-98.

Kasaikina, M. V., D. L. Hatfield, and V. N. Gladyshev. 2012. Understanding selenoprotein function and regulation through the use of rodent models. Biochim. Biophys. Acta. 1823(9):1633-1642. doi: 10.1016/j.bbamcr.2012.02.018

Kawamoto, H., and N. Minato. 2004. Myeloid cells. Int. J. Biochem. Cell Biol. 36(8):1374-1379. doi: 10.1016/j.biocel.2004.01.020

Kieliszek, M. 2019. Selenium–Fascinating Microelement, Properties and Sources in Food. Molecules 24(7):1298. doi: 10.3390/molecules24071298

Kiremidjian-Schumacher, L., and M. Roy. 2001. Effect of selenium on the immunocompetence of patients with head and neck cancer and on adoptive immunotherapy of early and established lesions. Biofactors 14(1-4):161-168. doi: 10.1002/biof.5520140121

Köhrle, J. 2000. The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell. Mol. Life Sci. 57(13-14):1853-1863.
Kryukov, G. V., S. Castellano, S. V. Novoselov, A. V. Lobanov, O. Zehtab, R. Guigó, and V. N. Gladyshev. 2003. Characterization of Mammalian Selenoproteomes. Science 300(5624):1439. doi: 10.1126/science.1083516
Labunskyy, V. M., D. L. Hatfield, and V. N. Gladyshev. 2014. Selenoproteins: molecular pathways and physiological roles. Physiol. Rev. 94(3):739-777.
Larson, B. L., H. L. Heary, Jr., and J. E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. J. Dairy Sci. 63(4):665-671. doi: 10.3168/jds.S0022-0302(80)82988-2
LaVoie, H. A., and R. J. Witorsch. 1995. Investigation of intracellular signals mediating the anti-apoptotic action of prolactin in Nb2 lymphoma cells. Proc. Soc. Exp. Biol. Med. 209(3):257-269. doi: 10.3181/00379727-209-43901
Lawrence, T. 2009. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harb. Perspect. Biol. 1(6):a001651.
Lawrence, T., D. W. Gilroy, P. R. Colville-Nash, and D. A. Willoughby. 2001. Possible new role for NF-kappaB in the resolution of inflammation. Nat. Med. 7(12):1291-1297. doi: 10.1038/nm1201-1291
Lebreton, P., O. Salat, and J. Nicol. 1998. Un point sur le sélénium. Bull. Tech. GTV 5:47-35.
Leclerc, B., D. Zadworny, G. Bédécarrats, and U. Kuhnlein. 2007. Development of a real-time (Q) PCR assay to measure variation in expression of prolactin receptor mRNA in the hypothalamus and pituitary gland during late embryogenesis in turkeys and chickens. Gen. Comp. Endocrinol. 150(2):319-325. doi: 10.1016/j.ygcen.2006.08.007
Lee, B. J., P. J. Worland, J. N. Davis, T. C. Stadtman, and D. L. Hatfield. 1989. Identification of a selenocysteyl-tRNA (Ser) in mammalian cells that recognizes the nonsense codon, UGA. J. Biol. Chem. 264(17):9724-9727.
Lehrer, R. I., T. Ganz, M. E. Selsted, B. M. Babior, and J. T. Curnutte. 1988. Neutrophils and Host Defense. Annals of Internal Medicine 109(2):127-142. doi: 10.7326/0003-4819-109-2-127
Leinfelder, W., T. Stadtman, and A. Böck. 1989. Occurrence in vivo of selenocysteyl-tRNA (SERUCA) in Escherichia coli. Effect of sel mutations. J. Biol. Chem. 264(17):9720-9723.
Leuchtmann, A., C. W. Bacon, C. L. Schardl, J. F. White Jr, and M. Tadych. 2014. Nomenclatural realignment of Neotyphodium species with genus Epichloë. Mycologia 106(2):202-215.
Li, Q., R. Hegge, P. J. Bridges, and J. C. Matthews. 2017. Pituitary genomic expression profiles of steers are altered by grazing of high vs. low endophyte-infected tall fescue forages. PLoS One 12(9):e0184612.
Li, S., F. Gao, J. Huang, Y. Wu, S. Wu, and X. G. Lei. 2018. Regulation and function of avian selenogenome. Biochim. Biophys. Acta. Gen. Subj. doi: 10.1016/j.bbagen.2018.03.029
Liao, S. F., J. A. Boling, and J. C. Matthews. 2015. Gene expression profiling indicates an increased capacity for proline, serine, and ATP synthesis and mitochondrial mass

by the liver of steers grazing high vs. low endophyte-infected tall fescue1. J. Anim. Sci. 93(12):5659-5671. doi: 10.2527/jas.2015-9193

Liao, S. F., K. R. Brown, A. J. Stromberg, W. R. Burris, J. A. Boling, and J. C. Matthews. 2011. Dietary supplementation of selenium in inorganic and organic forms differentially and commonly alters blood and liver selenium concentrations and liver gene expression profiles of growing beef heifers. Biol. Trace Elem. Res. 140(2):151-169. doi: 10.1007/s12011-010-8685-2

Lim, J. J., S. Grinstein, and Z. Roth. 2017. Diversity and Versatility of Phagocytosis: Roles in Innate Immunity, Tissue Remodeling, and Homeostasis. Front Cell Infect Microbiol 7:191-191. doi: 10.3389/fcimb.2017.00191

Lin, J. X., and W. J. Leonard. 1997. Signaling from the IL-2 receptor to the nucleus. Cytokine Growth Factor Rev. 8(4):313-332. doi: 10.1016/s1359-6101(97)00021-x

Lindshield, B. L., and K. Adhikari. 2011. The Kansas State University Human Nutrition (HN 400) Flexbook, Educause Quarterly No. 34.

Linossi, E. M., D. J. Calleja, and S. E. Nicholson. 2018. Understanding SOCS protein specificity. Growth Factors 36(3-4):104-117.

Liu, T., L. Zhang, D. Joo, and S.-C. Sun. 2017. NF-κB signaling in inflammation. Signal Transduct. Target. Ther. 2(1):17023. doi: 10.1038/sigtrans.2017.23

Liu, X., G. W. Robinson, K.-U. Wagner, L. Garrett, A. Wynshaw-Boris, and L. Hennighausen. 1997. Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. 11(2):179-186.

Livak, K., and T. Schmittgen. 2001. Analysis of relative gene expression data using real-time quanti tative PCR and the 2 (-MC (T)) Method. Methods 25:402-408.

Longnecker, M. P., D. O. Stram, P. R. Taylor, O. A. Levander, M. Howe, C. Veillon, P. A. McAdam, K. Y. Patterson, J. M. Holden, J. S. Morris, C. A. Swanson, and W. C. Willett. 1996. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology 7(4):384-390. doi: 10.1097/00001648-199607000-00008

Marcant, A., A. Denys, A. Melchior, P. Martinez, A. Deligny, M. Carpentier, and F. Allain. 2012. Cyclophilin B Attenuates the Expression of TNF-α in Lipopolysaccharide-Stimulated Macrophages through the Induction of B Cell Lymphoma-3. J. Immunol. 189(4):2023-2032. doi: 10.4049/jimmunol.1102803

Mariotti, M., P. G. Ridge, Y. Zhang, A. V. Lobanov, T. H. Pringle, R. Guigo, D. L. Hatfield, and V. N. Gladyshev. 2012. Composition and evolution of the vertebrate and mammalian selenoproteomes. PLoS One 7(3):e33066-e33066. doi: 10.1371/journal.pone.0033066

Martich, G. D., R. L. Danner, M. Ceska, and A. F. Suffredini. 1991. Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. J. Exp. Med. 173(4):1021-1024. doi: 10.1084/jem.173.4.1021

Mason, R. P., A. L. Soerensen, B. P. DiMento, and P. H. Balcom. 2018. The global marine selenium cycle: Insights from measurements and modeling. Global Biogeochem. Cycles 32(12):1720-1737.

Matera, L., M. Cutufia, M. Geuna, M. Contarini, S. Buttiglieri, S. Galin, A. Fazzari, and C. Cavaliere. 1997. Prolactin is an autocrine growth factor for the Jurkat human T-leukemic cell line. J. Neuroimmunol. 79(1):12-21.

Matsumoto, A., M. Masuhara, K. Mitsui, M. Yokouchi, M. Ohtsubo, H. Misawa, A. Miyajima, and A. Yoshimura. 1997. CIS, a Cytokine Inducible SH2 Protein, Is a Target of the JAK-STAT5 Pathway and Modulates STAT5 Activation. Blood 89(9):3148-3154. doi: 10.1182/blood.V89.9.3148

Matthews, J. C., and P. J. Bridges. 2014. NutriPhysioGenomics applications to identify adaptations of cattle to consumption of ergot alkaloids and inorganic versus organic forms of selenium: altered nutritional, physiological and health states? Anim. Prod. Sci. 54(10):1594-1604.

Matthews, J. C., Z. Zhang, J. D. Patterson, P. J. Bridges, A. J. Stromberg, and J. A. Boling. 2014. Hepatic Transcriptome Profiles Differ Among Maturing Beef Heifers Supplemented with Inorganic, Organic, or Mixed (50 % Inorganic:50 % Organic) Forms of Dietary Selenium. Biol. Trace Elem. Res. 160(3):321-339. doi: 10.1007/s12011-014-0050-4

McConnell, K., and J. Hoffman. 1972. Methionine-selenomethionine parallels in rat liver polypeptide chain synthesis. FEBS Lett. 24(1):60-62.

McLean, K. J., R. L. Baldwin, C.-J. Li, J. L. Klotz, J. L. Edwards, and K. R. McLeod. 2020. Synthetic Alkaloid Treatment Influences the Intestinal Epithelium and Mesenteric Adipose Transcriptome in Holstein Steers. Front. Vet. Sci. 7:615-615. doi: 10.3389/fvets.2020.00615

Mehdi, Y., and I. Dufrasne. 2016. Selenium in Cattle: A Review. Molecules 21(4):545-545. doi: 10.3390/molecules21040545

Mehdi, Y., J. L. Hornick, L. Istasse, and I. Dufrasne. 2013. Selenium in the environment, metabolism and involvement in body functions. Molecules 18(3):3292-3311. doi: 10.3390/molecules18033292

Melmed, J., and J. Jameson. 2014. Anterior pituitary: physiology of pituitary hormones.

Menge, C., B. Neufeld, W. Hirt, N. Schmeer, R. Bauerfeind, G. Baljer, and L. Wieler. 1998. Compensation of preliminary blood phagocyte immaturity in the newborn calf. Vet. Immunol. Immunopathol. 62(4):309-321.

Mescher, A. L. 2013. Junqueira's basic histology: text and atlas. McGraw-Hill Medical, New York.

Meschy, F. 2017. Nutrition minérale des ruminants. Quae.

Mitchell, G. B., B. N. Albright, and J. L. Caswell. 2003. Effect of interleukin-8 and granulocyte colony-stimulating factor on priming and activation of bovine neutrophils. Infect. Immun. 71(4):1643-1649.

Mizinga, K., F. Thompson, J. Stuedemann, and G. Edwards. 1993. Neural dopamine D2 receptors in rats fed endophyte-infected fescue seed. Drug Chem. Toxicol. 16(3):307-319.

Moghadaszadeh, B., and A. H. Beggs. 2006. Selenoproteins and their impact on human health through diverse physiological pathways. Physiology (Bethesda) 21:307-315. doi: 10.1152/physiol.00021.2006

Mohri, M., K. Sharifi, and S. Eidi. 2007. Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. Res. Vet. Sci. 83(1):30-39.

Moore, M., J. W. Tyler, M. Chigerwe, M. E. Dawes, and J. R. Middleton. 2005. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. J. Am. Vet. Med. Assoc. 226(8):1375-1377. doi: 10.2460/javma.2005.226.1375

Morein, B., I. Abusugra, and G. Blomqvist. 2002. Immunity in neonates. Vet. Immunol. Immunopathol. 87(3-4):207-213.
Moriel, P., L. F. Artioli, M. H. Poore, A. W. Confer, R. S. Marques, and R. F. Cooke. 2015. Increasing the metabolizable protein supply enhanced growth performance and led to variable results on innate and humoral immune response of preconditioning beef steers. J. Anim. Sci. 93(9):4473-4485. doi: 10.2527/jas.2015-9238
Moriggl, R., D. J. Topham, S. Teglund, V. Sexl, C. McKay, D. Wang, A. Hoffmeyer, J. Van Deursen, M. Y. Sangster, and K. D. Bunting. 1999. Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. Immunity 10(2):249-259.
Munford, R. S. 2008. Sensing Gram-Negative Bacterial Lipopolysaccharides: a Human Disease Determinant? Infect. Immun. 76(2):454-465. doi: 10.1128/iai.00939-07
Nagano, M., and P. A. Kelly. 1994. Tissue distribution and regulation of rat prolactin receptor gene expression. Quantitative analysis by polymerase chain reaction. J. Biol. Chem. 269(18):13337-13345.
National Academies of Sciences, E., and Medicine. 2016. Nutrient Requirements of Beef Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.
Needham, B. D., S. M. Carroll, D. K. Giles, G. Georgiou, M. Whiteley, and M. S. Trent. 2013. Modulating the innate immune response by combinatorial engineering of endotoxin. Proc. Natl. Acad. Sci. U.S.A. 110(4):1464-1469. doi: 10.1073/pnas.1218080110
Netea, M. G., L. A. B. Joosten, E. Latz, K. H. G. Mills, G. Natoli, H. G. Stunnenberg, L. A. J. O'Neill, and R. J. Xavier. 2016. Trained immunity: A program of innate immune memory in health and disease. Science 352(6284):aaf1098-aaf1098. doi: 10.1126/science.aaf1098
Niu, B., D. Guo, Z. Liu, X. Han, and X. Wang. 2017. Characterization of porcine cytokine inducible SH2-containing protein gene and its association with piglet diarrhea traits. Asian-australas. J. Anim. Sci. 30(12):1689-1695. doi: 10.5713/ajas.16.0169
NRC. 1983. Selenium in Nutrition: Revised Edition. National Academies Press.
Oeckinghaus, A., and S. Ghosh. 2009. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb. Perspect. Biol. 1(4):a000034-a000034. doi: 10.1101/cshperspect.a000034
Oh, H., and S. Ghosh. 2013. NF-κB: roles and regulation in different CD 4+ T-cell subsets. Immunol. Rev. 252(1):41-51.
Oliver, J. 2005. Pathophysiologic response to endophyte toxins. In 'Neotyphodium in cool-season grasses'.(Eds CA Roberts, CP West, DE Spiers) pp. 291–304. Blackwell Publishing: Ames, IA.
Ouhtit, A., G. Morel, and P. A. Kelly. 1993. Visualization of gene expression of short and long forms of prolactin receptor in the rat. Endocrinology 133(1):135-144. doi: 10.1210/endo.133.1.8319561
Pahl, H. L. 1999. Activators and target genes of Rel/NF-κB transcription factors. Oncogene 18(49):6853-6866.
Palacios, E. H., and A. Weiss. 2004. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene 23(48):7990-8000. doi: 10.1038/sj.onc.1208074
Pandey, S., D. Tripathi, M. Khubaib, A. Kumar, J. A. Sheikh, G. Sumanlatha, N. Z. Ehtesham, and S. E. Hasnain. 2017. Mycobacterium tuberculosis Peptidyl-Prolyl

Isomerases Are Immunogenic, Alter Cytokine Profile and Aid in Intracellular Survival. Front. Cell. Infect. Microbiol. 7:38. doi: 10.3389/fcimb.2017.00038

Park, J. M., F. R. Greten, A. Wong, R. J. Westrick, J. S. Arthur, K. Otsu, A. Hoffmann, M. Montminy, and M. Karin. 2005. Signaling pathways and genes that inhibit pathogen-induced macrophage apoptosis--CREB and NF-kappaB as key regulators. Immunity 23(3):319-329. doi: 10.1016/j.immuni.2005.08.010

Patterson, J. D., W. R. Burris, J. A. Boling, and J. C. Matthews. 2013. Individual Intake of Free-Choice Mineral Mix by Grazing Beef Cows May Be Less Than Typical Formulation Assumptions and Form of Selenium in Mineral Mix Affects Blood Se Concentrations of Cows and Their Suckling Calves. Biol. Trace Elem. Res. 155(1):38-48. doi: 10.1007/s12011-013-9768-7

Pendlum, L., J. Boling, L. Bush, and R. Buckner. 1980. Digestibility and metabolism of Kenhy tall fescue harvested at three stages of physiological maturity. J. Anim. Sci. 51(3):704-711.

Petrie, H. T., L. W. Klassen, P. S. Klassen, J. R. O'Dell, and H. D. Kay. 1989. Selenium and the immune response: 2. Enhancement of murine cytotoxic T-lymphocyte and natural killer cell cytotoxicity in vivo. J. Leukoc. Biol. 45(3):215-220. doi: 10.1002/jlb.45.3.215

Poole, R. K., A. R. Brown, M. H. Poore, C. L. Pickworth, and D. H. Poole. 2019. Effects of endophyte-infected tall fescue seed and protein supplementation on stocker steers: II. Adaptive and innate immune function. J. Anim. Sci. 97(10):4160-4170. doi: 10.1093/jas/skz250

Prabhu, K. S., and X. G. Lei. 2016. Selenium. Adv. Nutr. 7(2):415-417. doi: 10.3945/an.115.010785

Prasad, M., G. U. Devi, and S. Govindappa. 1989. Effect of Hypo and Hyperprolactinemia on the Testicular Maturation of Wistar Rats During Puberal Transition. Arch. Int. Physiol. Biochim. 97(5):347-353.

Qazi, B. S., K. Tang, and A. Qazi. 2011. Recent advances in underlying pathologies provide insight into interleukin-8 expression-mediated inflammation and angiogenesis. Int. J. Inflamm. 2011:908468-908468. doi: 10.4061/2011/908468

Qi, Q., and K. Ye. 2013. The roles of PIKE in tumorigenesis. Acta Pharmacol Sin 34(8):991-997. doi: 10.1038/aps.2013.71

Ra, L. 2016. Hyperprolactinemia, galactorrhea, and pituitary adenomas: etiology, differential diagnosis, natural history, and management. Comp. Gynecol.:853-864.

Rabinovitch, M. 1995. Professional and non-professional phagocytes: an introduction. Trends Cell Biol 5(3):85-87. doi: 10.1016/s0962-8924(00)88955-2

Radhakrishnan, A., R. Raju, N. Tuladhar, T. Subbannayya, J. K. Thomas, R. Goel, D. Telikicherla, S. M. Palapetta, B. A. Rahiman, D. D. Venkatesh, K.-K. Urmila, H. C. Harsha, P. P. Mathur, T. S. K. Prasad, A. Pandey, C. Shemanko, and A. Chatterjee. 2012a. A pathway map of prolactin signaling. J. Cell Commun. Signal. 6(3):169-173. doi: 10.1007/s12079-012-0168-0

Radhakrishnan, A., R. Raju, N. Tuladhar, T. Subbannayya, J. K. Thomas, R. Goel, D. Telikicherla, S. M. Palapetta, B. A. Rahiman, D. D. Venkatesh, K.-K. Urmila, H. C. Harsha, P. P. Mathur, T. S. K. Prasad, A. Pandey, C. Shemanko, and A. Chatterjee. 2012b. A pathway map of prolactin signaling. J Cell Commun Signal 6(3):169-173. doi: 10.1007/s12079-012-0168-0

Rayman, M. P. 2000. The importance of selenium to human health. Lancet 356(9225):233-241.
Rayman, M. P. 2012. Selenium and human health. Lancet 379(9822):1256-1268. doi: https://doi.org/10.1016/S0140-6736(11)61452-9
Reber, A., A. Lockwood, A. Hippen, and D. Hurley. 2006. Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. Vet. Immunol. Immunopathol. 109(1-2):139-150.
Reber, P. M. 1993. Prolactin and immunomodulation. Am. J. Med. 95(6):637-644.
Reddy, C. C., and E. J. Massaro. 1983. Biochemistry of Selenium: A Brief Overview. Toxicological Sciences 3(5):431-436. doi: 10.1093/toxsci/3.5.431
Regisford, E. G., and L. S. Katz. 1993. Effects of bromocriptine-induced hypoprolactinaemia on gonadotrophin secretion and testicular function in rams (Ovis aries) during two seasons. J. Reprod. Fertil. 99(2):529-537. doi: 10.1530/jrf.0.0990529
Reich, H. J., and R. J. Hondal. 2016. Why Nature Chose Selenium. ACS Chem. Biol. 11(4):821-841. doi: 10.1021/acschembio.6b00031
Remick, D. G. 2005. Interleukin-8. Crit. Care Med. 33(12 Suppl):S466-467. doi: 10.1097/01.ccm.0000186783.34908.18
Roland, L., M. Drillich, and M. Iwersen. 2014. Hematology as a diagnostic tool in bovine medicine. J. Vet. Diagn. Investig. 26(5):592-598. doi: 10.1177/1040638714546490
Røntved, C., J. B. Andersen, J. Dernfalk, and K. Ingvartsen. 2005. Effects of diet energy density and milking frequency in early lactation on tumor necrosis factor-alpha responsiveness in dairy cows. Vet. Immunol. Immunopathol. 104:171-181. doi: 10.1016/j.vetimm.2004.11.001
Rosales, C. 2018. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? Front. Physiol. 9:113-113. doi: 10.3389/fphys.2018.00113
Rosales, C., and E. Uribe-Querol. 2017. Phagocytosis: A Fundamental Process in Immunity. Biomed Res Int 2017:9042851-9042851. doi: 10.1155/2017/9042851
Rossy, J., D. Williamson, and K. Gaus. 2012. How does the kinase Lck phosphorylate the T cell receptor? Spatial organization as a regulatory mechanism. Front. Immunol. 3(167)(Mini Review) doi: 10.3389/fimmu.2012.00167
Rothenberg, M. E., and S. P. Hogan. 2006. THE EOSINOPHIL. Annu. Rev. Immunol. 24(1):147-174. doi: 10.1146/annurev.immunol.24.021605.090720
Russell, D. H., R. Kibler, L. Matrisian, D. F. Larson, B. Poulos, and B. Magun. 1985. Prolactin receptors on human T and B lymphocytes: antagonism of prolactin binding by cyclosporine. J. Immunol. 134(5):3027-3031.
Saha, U., A. Fayiga, D. Hancock, and L. Sonon. 2016. Selenium in animal nutrition: Deficiencies in soils and forages, requirements, supplementation and toxicity. Int. J. Appl. Agric. Sci 2(6):112-125.
Saleem, M., H. Martin, and P. Coates. 2018. Prolactin Biology and Laboratory Measurement: An Update on Physiology and Current Analytical Issues. Clin. Biochem. Rev. 39(1):3-16.
Schletter, J., H. Heine, A. J. Ulmer, and E. T. Rietschel. 1995. Molecular mechanisms of endotoxin activity. Arch. Microbiol. 164(6):383-389.

Schmidt, S. P., and T. G. Osborn. 1993. Effects of endophyte-infected tall fescue on animal performance. Agric. Ecosyst. Environ. 44(1):233-262. doi: https://doi.org/10.1016/0167-8809(93)90049-U

Schuler, L. A., R. J. Nagel, J. Gao, N. D. Horseman, and M. A. Kessler. 1997. Prolactin Receptor Heterogeneity in Bovine Fetal and Maternal Tissues*. Endocrinology 138(8):3187-3194. doi: 10.1210/endo.138.8.5292

Schwarz, K., and C. M. Foltz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration J. Am. Chem. Soc. 79(12):3292-3293. doi: 10.1021/ja01569a087

Scott, P., M. A. Kessler, and L. A. Schuler. 1992. Molecular cloning of the bovine prolactin receptor and distribution of prolactin and growth hormone receptor transcripts in fetal and utero-placental tissues. Mol. Cell Endocrinol. 89(1-2):47-58. doi: 10.1016/0303-7207(92)90210-w

Segal, A. W., J. Dorling, and S. Coade. 1980. Kinetics of fusion of the cytoplasmic granules with phagocytic vacuoles in human polymorphonuclear leukocytes. Biochemical and morphological studies. J Cell Biol 85(1):42-59. doi: 10.1083/jcb.85.1.42

Selders, G. S., A. E. Fetz, M. Z. Radic, and G. L. Bowlin. 2017. An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. Regen. Biomater. 4(1):55-68. doi: 10.1093/rb/rbw041

Sen, C. K. 2000. Cellular thiols and redox-regulated signal transduction. Curr. Top. Cell. Regul. 36:1-30. doi: 10.1016/s0070-2137(01)80001-7

Senogles, D., P. Paul, D. Johnson, and C. C. Muscoplat. 1979. Ontogeny of T cells, B cells and monocytes in the bovine foetus. Clin. Exp. Immunol. 36(2):299.

Siegel, M. R., and L. P. Bush. 1997. Toxin Production in Grass/Endophyte Associations. In: G. C. Carroll and P. Tudzynski, editors, Plant Relationships: Part A. Springer Berlin Heidelberg, Berlin, Heidelberg. p. 185-207.

Simonet, W. S., T. M. Hughes, H. Q. Nguyen, L. D. Trebasky, D. M. Danilenko, and E. S. Medlock. 1994. Long-term impaired neutrophil migration in mice overexpressing human interleukin-8. J. Clin. Invest. 94(3):1310-1319. doi: 10.1172/jci117450

Sohn, E. J., M. J. Paape, E. E. Connor, D. D. Bannerman, R. H. Fetterer, and R. R. Peters. 2007. Bacterial lipopolysaccharide stimulates bovine neutrophil production of TNF-alpha, IL-1beta, IL-12 and IFN-gamma. Vet. Res. 38(6):809-818. doi: 10.1051/vetres:2007033

Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci. 99(6):4967-4982. doi: 10.3168/jds.2015-10354

Steen, W. W., N. Gay, J. Boling, R. Buckner, L. Bush, and G. Lacefield. 1979. Evaluation of Kentucky 31, G1-306, G1-307 and Kenhy Tall Fescue as Pasture for Yearling Steers II. Growth, Physiological Response and Plasma Constituents of Yearling Steers. J. Anim. Sci. 48(3):618-623.

Strickland, J., G. E. Aiken, D. Spiers, L. R. Fletcher, and J. Oliver. 2009. Physiological Basis of Fescue Toxicosis.

Strickland, J. R., M. L. Looper, J. C. Matthews, C. F. Rosenkrans, Jr., M. D. Flythe, and K. R. Brown. 2011. BOARD-INVITED REVIEW: St. Anthony's Fire in livestock: Causes, mechanisms, and potential solutions1,2. J. Anim. Sci. 89(5):1603-1626. doi: 10.2527/jas.2010-3478

Strickland, J. R., J. W. Oliver, and D. L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. Vet. Hum. Toxicol. 35(5):454-464.
Strikas, R. A., A. C. Mawle, L. K. Pickering, and W. A. Orenstein. 2018. 6 - Active Immunization. In: S. S. Long, C. G. Prober and M. Fischer, editors, Principles and Practice of Pediatric Infectious Diseases (Fifth Edition). Elsevier. p. 43-71.e44.
Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue Endophyte: History and Impact on Animal Agriculture. J. Prod. Agric. 1(1):39-44. doi: 10.2134/jpa1988.0039
Summerfield, A., G. Auray, and M. Ricklin. 2015. Comparative Dendritic Cell Biology of Veterinary Mammals. Annu. Rev. Anim. Biosci. 3(1):533-557. doi: 10.1146/annurev-animal-022114-111009
Summerfield, A., L. Guzylack-Piriou, A. Schaub, C. P. Carrasco, V. Tâche, B. Charley, and K. C. McCullough. 2003. Porcine peripheral blood dendritic cells and natural interferon-producing cells. Immunology 110(4):440-449.
Sun, S.-C. 2011. Non-canonical NF-κB signaling pathway. Cell Res. 21(1):71-85.
Sun, S.-C., J.-H. Chang, and J. Jin. 2013. Regulation of nuclear factor-κB in autoimmunity. Trends Immunol. 34(6):282-289.
Suttle, N. F. 2010. Mineral nutrition of livestock. Cabi.
Suzuki, K. T. 2005. Metabolomics of Selenium: Se Metabolites Based on Speciation Studies. J. Health Sci. 51(2):107-114. doi: 10.1248/jhs.51.107
Sweet, M. J., and D. A. Hume. 1996. Endotoxin signal transduction in macrophages. J. Leukoc. Biol. 60(1):8-26. doi: 10.1002/jlb.60.1.8
Tak, P. P., and G. S. Firestein. 2001. NF-κB: a key role in inflammatory diseases. J. Clin. Invest. 107(1):7-11.
Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. Annu. Rev. Immunol. 21(1):335-376.
Tamura, M., M. Tokuda, S. Nagaoka, and H. Takada. 1992. Lipopolysaccharides of Bacteroides intermedius (Prevotella intermedia) and Bacteroides (Porphyromonas) gingivalis induce interleukin-8 gene expression in human gingival fibroblast cultures. Infect. Immun. 60(11):4932-4937. doi: 10.1128/iai.60.11.4932-4937.1992
Tang, E. D., C.-Y. Wang, Y. Xiong, and K.-L. Guan. 2003. A Role for NF-κB Essential Modifier/IκB Kinase-γ (NEMO/IKKγ) Ubiquitination in the Activation of the IκB Kinase Complex by Tumor Necrosis Factor-α *. J. Biol. Chem. 278(39):37297-37305. doi: 10.1074/jbc.M303389200
Tecchio, C., and M. A. Cassatella. 2016. Neutrophil-derived chemokines on the road to immunity. Semin. Immunol. 28(2):119-128. doi: 10.1016/j.smim.2016.04.003
Thompson, F., and J. Stuedemann. 1993. Pathophysiology of fescue toxicosis. Agric. Ecosyst. Environ. 44(1-4):263-281.
Thompson, F., J. Stuedemann, and N. Hill. 2001. Anti-quality factors associated with alkaloids in eastern temperate pasture. J. Range Manag.:474-489.
Turvey, S. E., and D. H. Broide. 2010. Innate immunity. J. Allergy Clin. Immunol. 125(2):S24-S32. doi: 10.1016/j.jaci.2009.07.016
Udy, G. B., R. P. Towers, R. G. Snell, R. J. Wilkins, S.-H. Park, P. A. Ram, D. J. Waxman, and H. W. Davey. 1997. Requirement of STAT5b for sexual dimorphism of body

growth rates and liver gene expression. Proc. Natl. Acad. Sci. U.S.A. 94(14):7239-7244.
Ulevitch, R., and P. Tobias. 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu. Rev. Immunol. 13(1):437-457.
Vallabhapurapu, S., and M. Karin. 2009. Regulation and function of NF-κB transcription factors in the immune system. Annu. Rev. Immunol. 27:693-733.
van Furth, R., Z. Cohn, J. Hirsch, J. Humphrey, W. Spector, and H. Langevoort. 1972. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull. World Health Organ. 46(6):845.
Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3(7):1-12.
Veres, Z., L. Tsai, T. D. Scholz, M. Politino, R. S. Balaban, and T. C. Stadtman. 1992. Synthesis of 5-methylaminomethyl-2-selenouridine in tRNAs: 31P NMR studies show the labile selenium donor synthesized by the selD gene product contains selenium bonded to phosphorus. Proc. Natl. Acad. Sci. U.S.A. 89(7):2975-2979.
Vivier, E., D. H. Raulet, A. Moretta, M. A. Caligiuri, L. Zitvogel, L. L. Lanier, W. M. Yokoyama, and S. Ugolini. 2011. Innate or adaptive immunity? The example of natural killer cells. Science 331(6013):44-49. doi: 10.1126/science.1198687
Wahlen, R., L. Evans, J. Turner, and R. Hearn. 2005. The use of collision/reaction cell ICP-MS for the determination of elements in blood and serum samples. Spectrosc.
Wallis, M. 1974. The primary structure of bovine prolactin. FEBS Letters 44(2):205-208. (https://doi.org/10.1016/0014-5793(74)80726-X) doi: https://doi.org/10.1016/0014-5793(74)80726-X
Wang, N., H.-Y. Tan, S. Li, Y. Xu, W. Guo, and Y. Feng. 2017. Supplementation of Micronutrient Selenium in Metabolic Diseases: Its Role as an Antioxidant. Oxid. Med. Cell. Longev. 2017:7478523-7478523. doi: 10.1155/2017/7478523
Wange, R. L., and L. E. Samelson. 1996. Complex Complexes: Signaling at the TCR. Immunity 5(3):197-205. doi: 10.1016/S1074-7613(00)80315-5
Warrington, R., W. Watson, H. L. Kim, and F. R. Antonetti. 2011. An introduction to immunology and immunopathology. Allergy Asthma Clin. Immunol. 7(1):S1. doi: 10.1186/1710-1492-7-S1-S1
Webb, K., R. J. Trotta, P. Bridges, and J. C. Matthews. July 2021. Ad libitum consumption of a 1:1 blend of inorganic:organic Se by steers grazing endopyte-infected tall fescue increases serum prolactin and alkaline phosphatase activity, but not average daily gain. ASAS-CSAS Annual Meetings & Trade Show, Louisville, KY.
Weller, P. F. 1997. Human eosinophils. J. Allergy Clin. Immunol. 100(3):283-287. doi: https://doi.org/10.1016/S0091-6749(97)70237-9
Wen, T., and M. E. Rothenberg. 2016. The Regulatory Function of Eosinophils. Microbiol. Spectr. 4(5):10.1128/microbiolspec.MCHD-0020-2015. doi: 10.1128/microbiolspec.MCHD-0020-2015
Werling, D., C. Howard, E. Niederer, O. Straub, A. Saalmüller, and W. Langhans. 1998. Analysis of the phenotype and phagocytic activity of monocytes/macrophages from cattle infected with the bovine leukaemia virus. Vet. Immunol. Immunopathol. 62(3):185-195.

Werling, D., W. Langhans, and N. Geary. 1994. Caprine arthritis encephalitis virus infection changes caprine blood monocyte responsiveness to lipopolysaccharide stimulation in vitro. Vet. Immunol. Immunopathol. 43(4):401-411.

Wilson, R. A., A. Zolnai, P. Rudas, and L. V. Frenyo. 1996. T-cell subsets in blood and lymphoid tissues obtained from fetal calves, maturing calves, and adult bovine. Vet. Immunol. Immunopathol. 53(1-2):49-60.

Wood, D., and G. Quiroz-Rocha. 2010. Schalm's veterinary hematology. J. Vet. Diagn. Invest.

Xu, X.-M., B. A. Carlson, H. Mix, Y. Zhang, K. Saira, R. S. Glass, M. J. Berry, V. N. Gladyshev, and D. L. Hatfield. 2006. Biosynthesis of selenocysteine on its tRNA in eukaryotes. PLoS Biol. 5(1):e4.

Yoshimura, A., M. Ito, S. Chikuma, T. Akanuma, and H. Nakatsukasa. 2018. Negative Regulation of Cytokine Signaling in Immunity. Cold Spring Harb. Perspect. Biol. 10(7)doi: 10.1101/cshperspect.a028571

Yoshimura, A., M. Suzuki, R. Sakaguchi, T. Hanada, and H. Yasukawa. 2012. SOCS, inflammation, and autoimmunity. Front. Immunol. 3:20.

Yuan, J., S. Palioura, J. C. Salazar, D. Su, P. O'Donoghue, M. J. Hohn, A. M. Cardoso, W. B. Whitman, and D. Söll. 2006. RNA-dependent conversion of phosphoserine forms selenocysteine in eukaryotes and archaea. Proc. Natl. Acad. Sci. U.S.A. 103(50):18923-18927.

Zhang, H., and S.-C. Sun. 2015. NF-κB in inflammation and renal diseases. Cell Biosci. 5(1):1-12.

VITA

Education

M.S. Candidate- University of Kentucky, College of Agriculture, Food and Environment, Lexington, KY. 2018-2021. Animal and Food Sciences (Ruminant Nutritional Physiology).

B.S-University of Kentucky, College of Agriculture, Food and Environment, Lexington, KY. 2013-2017. Animal Sciences (pre-Professional)

B.S-University of Kentucky, College of Agriculture, Food and Environment, Lexington, KY. 2013-2017. Agricultural and Medical Biotechnology (Biological Sciences)