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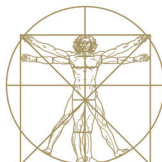
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Immunology and Vaccination of Beef and Dairy Cattle

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Introduction

In order to scientifically choose a vaccine or design a particular vaccination program it is necessary to consider many variables.¹ With the larger herds vaccine program need to be based on more science than ever before. Some of these include:

1. Presence and degree of challenge of the particular diseases on the farm or ranch.
2. Management practices on the facility that lend themselves to or hinder vaccination programs.
3. At what times or ages are the disease problems occurring and are they associated with any stresses.
4. What immune system components are necessary to afford protection against the various disease.
5. Some basic immunology concepts.
6. The information that is available on products being considered and the source and quality of the information.

Challenge

The level of disease challenge and degree of protection are in a continual state of fluctuation. The level of protection is different in every vaccinated animal due to biological variability (level of stress, vaccination history etc.). The same is true with the amount of exposure to a pathogen (addition of animals, weather changes etc.). Overwhelming challenge can override the immunity and lead to disease even in well-vaccinated animals.² Of course, susceptibility to the challenge organism is an important component of the disease process. While stress will be covered later in the section, the potential impact of other factors such as nutrition, environment and housing cannot be overlooked as important pieces of disease prevention and control.

Timing of Disease

Many farms will have consistent, recurring times when certain diseases occur. The timing may give some insight into stresses that are occurring in the management of the cattle. Correcting these stresses can have a positive impact on vaccination and lessen disease susceptibility. Furthermore, this type of a history is helpful to determine the timing of vaccinations. This is a concept that is often underutilized when designing dairy vaccination programs. Knowing when a problem has historically occurred will allow vaccinations to be scheduled when they will give maximum immune responses in preparation for anticipated challenges. The challenge organisms will often change by age of the animal as will the disease syndromes seen. Common diseases for which federally licensed vaccines are available are shown in table 1. Many of these diseases have multiple syndromes (i.e. respiratory and reproductive disease)

Assessing Vaccine Efficacy

Vaccine efficacy can be extremely difficult for the practitioner to assess. Traditionally, serologic data showing pre and post vaccination titers has been equated to protection. For many diseases there is a poor correlation between an antibody being measured and the protection generated by the vaccine in the animal.³ Recently, cell mediated immune function tests have been added to show a more complete stimulation of the immune response after vaccination.⁴ Although this gives more information on the vaccine, it still does not answer the basic question of the how well a vaccine really protects. This can only be answered by well-designed challenge studies. There

are many examples of well designed studies with both viral^{5,6} and bacterial^{7,8} agents. In order to assess a challenge study the following information is needed:

1. Trial design including animal characteristics
2. Statistical analysis of the data
3. Route of administration of the challenge
4. Characteristics of the challenge organism
5. The method for clinical assessment and outcomes measured
6. Publication of the results in a peer reviewed article

Unfortunately, for many of our diseases, the challenge model is not well established.

Field trials are even harder to assess but are valuable at answering the effectiveness (i.e. the efficacy in a particular situation) and efficiency of vaccines (cost effectiveness of a vaccine).⁹ There are several good references on field trial analysis available.^{10,11}

Modified Live Versus Inactivated Vaccines

Each companies' development and manufacture of cattle vaccines is different, thus the composition of the vaccine will vary dramatically among different manufacturers. Outlines of production are proprietary for each manufacturer, however some information can be found in technical and marketing pieces. For example, some viral vaccines are grown on bovine derived kidney cell lines whereas others are grown on porcine derived kidney cells. Some vaccines are grown on only calf serum and some are grown on both calf and fetal calf serum. Differences in times a virus is grown before a vaccine is made (passage) may be found as well. The variability is seen in the following areas;

- a. Strain(s)/agents chosen for the vaccine
- b. Number of viral passages and when bacteria are harvested for vaccine manufacture
- c. Growth medium
- d. Amount of viral or bacterial components in the vaccine

There are basically three different technologies available today in cattle viral and bacterial vaccines.^{2,12}

1. Modified live (attenuated) vaccines contain living bacterial or viral organisms. They are usually collected from a field disease and then grown in abnormal host cells (viral) or media (bacterial) to change or attenuate the pathogen. Each time the pathogen is grown through a replication it is called a passage and it is administered back to the animal to see if it is still virulent. After several passages the pathogen will begin to lose virulence factors since it cannot cause "disease" in these unnatural host cells. Once the pathogen can no longer cause "disease" in the target species it is then tested to see if it can confer protection. The final vaccine is usually passed a number of times beyond the passage where virulence is no longer seen. This decreases the risk of reversion to a virulent pathogen. These vaccines usually require good quality control to decrease the risk of a contaminant entering the vaccine.

- b. Inactivated (killed) vaccines are easier to develop since virulence after growth is not a problem. The same pathogen is isolated from a disease outbreak. The pathogen is grown and then chemically or physically killed. The inactivation is usually achieved by either adding a chemical to the pathogens or using ultraviolet rays. The major concern with inactivation is the potential loss of important epitopes. An adjuvant is normally added to inactivated vaccines to heighten the immune response. The vaccine is then tested for efficacy.

- c. Genetically engineered vaccines have been altered genetically usually through a mutation. This mutation may be induced by several different methods but the ensuing bacteria or virus has different properties that may alter virulence or growth characteristics. Most of these vaccines are modified live mutants (temperature sensitive viral vaccines; streptomycin dependent Pasteurellas) but inactivated marker vaccines are also genetically engineered. These vaccines have been engineered to delete a gene and cause an immune response deficient in antibodies to a certain epitope thus allowing diagnostics to differentiate between vaccine and natural exposure responses (gene deleted IBR vaccines).

Once an infectious agent has been chosen for vaccine production and the agent has been altered (modified live or inactivated), then the potential vaccine is put through a series of experiments to determine the minimum antigenic

dose required to give adequate protection. This is called the minimum immunizing dose (MID). In order to obtain shelf life, the MID must be in the vaccine at expiration date so a vaccine will contain more antigen than the MID at time of release of the serial of a vaccine. In effect, a vaccine's efficacy is not determined with the final product used by the veterinarian but at a reduced level of immunogens from the amount contained in the final vaccine.

Designing a Vaccination Program

Vaccination programs in a cowherd need to be custom designed for the particular need(s) of the herd. Vaccination programs in the replacement stock have two specific goals that need to be met. The first is to protect the calf against any pathogens that are prevalent in the calves. The second is to prepare the calf for entry into the adult herd with a good foundation of protection from which to build herd immunity. Although antigens contained in herd vaccination programs may vary, for most dairy herds the minimum vaccination program should be built around the four major viral diseases (Type 1 and 2 BVDV, BHV-1, PI3 and BRSV), the five primary *Leptospira* serovars of cattle and may include the major *Clostridial* agents and *Brucella*. Core endotoxin vaccines against coliform mastitis has shown significant positive economic impacts.¹³ This should be the cornerstone of the program; other pathogens are then optional and are added depending on herd or area problems. At least modified live viral vaccine, containing the viral agents listed above, should be included for replacement animals to establish a strong baseline immunity against BVDV and BHV-^{14,15,15,17,18}

Maternal Antibody Interference Revisited

The belief that maternal antibodies block vaccination is based on the lack of post vaccination titer increases in calves. However, recent studies have shown the formation of B cell memory responses^{19,20,21}. Sero-positive calves vaccinated at a young age with modified live BHV-1, PI3 and/or BRSV have shown higher antibody responses on revaccination than control calves vaccinated only at the second date. These young vaccinates typically do not show increased antibody responses after the first vaccination in the presence of high maternal antibody. Cell mediated immune responses, as indicated by antigen specific T cell blastogenesis, has been demonstrated in the face of high maternal antibody²² when attenuated BRSV and BHV-1 vaccines were used. Similar responses have been reported in laboratory animals.^{23,24,25} One cattle study also demonstrated higher levels of protection when challenged if calves were vaccinated with a modified live BRSV.²⁰ A more recent study demonstrated that modified live BVDV vaccines may be blocked if the existing maternal antibody is high (greater than 1:32-64)²⁶. It is clear from these studies that maternal antibody interference of vaccines is not as absolute as once thought. The immune status of the animal, the specific antigen and presentation of that antigen should be considered when trying to design vaccination programs when maternal antibody may be present.

Impact of Stress

Stress impacts the immune system of all cattle. There are several factors that can affect the immune system. The birthing process has a dramatic impact on the newborn's immune system due to corticosteroid release. Furthermore the newborn has an increased number of Suppressor T cells.² These factors, plus others, dramatically decrease systemic immune responses for the first week of life.²⁷ Other stresses should be avoided at vaccination time to maintain immune system integrity. Procedures such as castrations, dehorning, weaning and movement need to be considered as stresses in cattle and all have the potential to temporarily decrease immune system function.^{28,29,30}

Systemic vaccinations during high stress times should be avoided due to these decreased responses and may even have undesired effects (adverse reactions and increased severity of disease).

Booster Importance

It is important to follow the label directions for administering vaccines. Inactivated vaccines and most modified live BRSV vaccines require a booster before protection is complete. The first time an inactivated vaccine is administered, the primary response occurs. This is fairly short-lived, not very strong and is predominantly comprised of Immunoglobulin M. The response seen after a booster vaccination is called the secondary response or anamnestic response. This is much stronger, of longer duration and is primarily comprised of Immunoglobulin

G.^{2,12} If the booster is given too early, the anamnestic response doesn't occur; and if too much time elapses before the booster is given, it acts as an initial dose not as a booster. With most modified live vaccines (with the exception of most BRSV vaccines), the primary vaccination also stimulates the secondary response without needing a booster since the virus or bacteria is replicating in the animal.

Adverse Reactions

Adverse reactions are a potential risk with any vaccination. These reactions fall into three primary types.^{2,4,12,,31.32.33,34,35,36}

1. IgE and the release of granules from basophils and mast cells mediate immediate hypersensitivity. This reaction is seen within minutes of vaccination and often begins with shaking or sweating. The majority of these animals will respond to epinephrine.
2. Delayed hypersensitivity is mediated by an antibody-antigen complex attaching to complement and the ensuing activation of the complement cascade. The resultant reaction may occur locally or systemically. The reaction may be delayed as the complexes form and the cascade begins and subsequently by products begin to exert their effects. The signs are similar to immediate hypersensitivity and the treatment is epinephrine.
3. One of the more common reactions seen in dairy cattle has been associated with the endotoxin and other bacterial components found in most gram negative vaccines. Currently, there are no requirements for monitoring or reporting the amount of endotoxin found in cattle vaccines and the level of endotoxin may vary dramatically between vaccines and serials of the same vaccine. Furthermore, the potency of endotoxin varies among different gram negative bacteria. This is seen primarily in Holsteins due to some genetic predisposition and can be seen following administration of any gram-negative bacterin. The signs seen vary depending on the farm's or individual's sensitivity to gram negative bacterial components. The number or severity of the gram negatives fractions in the vaccination program administered simultaneously are also instrumental in causing these reactions. As a general rule, no more than two gram negative vaccines should be administered on the same day to dairy cattle. These adverse reactions include:
 - a. anorexia and transient decreases in milk production
 - b. early embryonic deaths
 - c. abortions
 - d. gram negative bacterial (endotoxic) shock, requiring flunixin or keprofen, steroids, antihistamines and fluids

The Future

New research is forcing veterinarians to review long established vaccination programs. Areas of active research include;

1. perinatal programming and the potential effects of vaccination on growth during this critical time
2. prime-boost programs that use different vaccines for heterologous boosting and enhanced protection
3. Impact of vaccines on intakes, particularly in stressed cattle.
2. Potential interference of immune responses to vaccines when they are co-administered.

Summary

Designing a vaccination program involves a good history of the individual farm as well as a basic understanding of the immune system. The vaccines chosen should have good solid efficacy studies (as well as effectiveness and efficiency studies if possible) to ensure that the product can fulfill the needs of the farm or ranch. Management decisions may be made that do not maximize the potential of the product chosen and realistic expectations of all products should be well explained to the producer before they are used. The owner should be involved in the vaccine decision making process and all of the information on the product should be shared.

The establishment of good baseline immunity in the replacement heifers and the foundation vaccination program in the cows can have dramatic effects on the health and profitability of the herd and needs to be well planned.

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Common cattle disease for which USDA licensed vaccines are available³⁷

System	Pathogen class	Infectious Agent
Intestinal (diarrhea)	Virus	Rotavirus
	Virus	Coronavirus
	Virus	Bovine Viral Diarrhea virus
	<i>Bacteria</i>	<i>Clostridium perfringens</i>
	<i>Bacteria</i>	<i>E.coli</i>
	<i>Bacteria</i>	<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i> (Johne's disease)
Respiratory	<i>Bacteria</i>	<i>Salmonella</i> various species
	Virus	Bovine Herpesvirus-1
	Virus	Bovine Respiratory Syncytial virus
	Virus	Bovine Viral Diarrhea virus type 1 and 2
	Virus	Parainfluenza-3
	Bacteria	<i>Mycoplasma bovis</i>
	Bacteria	<i>Mannheimia hemolytica</i>

	Bacteria	<i>Pasteurella multocida</i>
	Bacteria	<i>Hemophilus somnus</i>
Reproductive Diseases	Bacterial	<i>Brucella abortus</i>
	Bacterial	Campylobacter foetus subspecies venerealis (vibrio)
	Bacteria	<i>Leptospira borgpetersenii</i> serovar Hardjo
	Bacteria	<i>Leptospira interrogans</i> serovar Hardjo <i>Leptospira</i>
	Protozoa	<i>Tritrichomonas foetus</i>
Miscellaneous diseases	Virus	Rabies
	Virus	viral warts (papillomas).
	Bacteria	<i>Salmonella dublin</i>
	Bacteria	<i>Moraxella bovis</i> (pinkeye)
	Bacteria	<i>Bacillus anthracis</i> (anthrax)
	Bacteria	<i>Clostridium hemolyticum</i>
	Bacteria	<i>Clostridium chauvoei</i>
	Bacteria	<i>Clostridium septicum</i>
	Bacteria	<i>Clostridium novyi</i>
	Bacteria	<i>Clostridium sordellii</i>
	Bacteria	<i>Clostridium tetani</i>
	Bacteria	<i>Moraxella bovis</i> (pinkeye)
	Bacteria	<i>Leptospira kirschneri</i> serovar Grippotyphosa)
	Bacteria	<i>Staphylococcus aureus</i>
	Bacteria	<i>Streptococcus uberis</i> .
	Bacteria	<i>L interrogans</i> serovar Pomona, ,
	Bacteria	<i>L interrogans</i> serovar Canicola
	Bacteria	<i>L interrogans</i> serovar Icterohemorrhagiae
	Bacteria	Core endotoxin vaccines (<i>Salmonella</i> or <i>E.coli</i> based)

Selenium Supplementation Using Se-Biofortified Forages Improves Cattle Health and Performance

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Background

Selenium (Se) has been recognized for years as an essential trace element for ruminant animals. Selenium deficiencies have been described in many species, including cattle, sheep goats, horse, swine, white-tailed deer, and elk. In general, the majority of livestock raised in low-Se regions do not receive sufficient dietary Se for optimum health. Severe Se deficiency in ruminants results in nutritional myodegeneration known as “white muscle disease” [1], whereas insufficient Se intake has been implicated as the cause of a group of Se-responsive disorders including unthriftiness, reduced weight gain, and immunosuppression [2]. The Se status of plants, animals, and humans varies markedly around the world as a result of different geological conditions. Animal health is affected by Se deficiency in the diet, which depends on the amount of bioavailable Se taken up by plants. In the US, a survey of state veterinarians and state veterinary diagnostic labs revealed that Se-deficiency diseases were diagnosed in 46 states and were reported to be an important livestock problem in regions of 37 states [3].

Livestock fed Se-deficient forages must receive Se supplements to ensure optimum health. In the United States, Se was approved as a feed additive in 1979 by the Food and Drug Administration (FDA) to respond to the documented deficiency of Se in animal feeds, first at a concentration of 0.1 mg/kg DM, and then after April 1987 at a concentration of 0.3 mg/kg DM [4]. For cattle, salt can be added to feed up to 120 ppm, but must not exceed a maximum intake of 3 mg Se/head daily (equivalent to 1.5 oz salt/head daily). For sheep, salt mineral mixtures can be added to feed up to 90 ppm, but must not exceed a maximum intake of 0.7 mg Se/head daily (equivalent to 0.25-0.33 oz salt/head daily). These amounts are for supplemental Se and do not take into account natural Se concentrations already present in feed.

There has been a significant decline in nutritional myodegeneration cases in ruminant species using the current FDA-allowed Se-supplementation regulations. Selenium supplementation is credited with improving animal health, e.g., reduced prevalence of retained fetal membranes, decreased severity and prevalence of clinical mastitis, decreased somatic cell counts in milk, decreased calf mortality, and increased mobilization and killing capacity of neutrophils (reviewed in [5-9]). Nonetheless, we have observed suboptimal blood-Se concentrations in ruminants offered Se supplements [10]. Reasons for this may include inadequate Se intake, or problems with Se bioavailability. Selenium bioavailability can be limited by dietary factors. For example, increasing dietary sulfur decreases Se bioavailability, and the presence of cyanogenic glycosides in certain legumes are also antagonistic to Se (reviewed in [11]).

There is also large variation in oral bioavailability between different chemical forms of Se. Most Se is supplemented in the form of inorganic sodium salts in mineral mixtures that are commonly left outside for several weeks for sheep or cattle to consume *ad libitum*. The primary form of Se in these supplements is inorganic sodium selenite because it is easier to purchase than sodium selenate, although sodium selenate is more stable than sodium selenite and has greater small intestinal absorption in nonruminants (reviewed in [12]). Selenium can also be supplemented as organic selenomethionine (SeMet). In the United States, organic Se was approved as a feed additive in 2003 by the FDA at the same supplementation rates as inorganic Se forms, even though there is a documented increase in bioavailability.

Rumen microorganisms (RMO) can also decrease Se bioavailability by reducing selenite into non-absorbable elemental Se, which is then excreted in the feces [13-15]. It is known that absorption of Se by ruminants is less compared with non-ruminants [16]. For example, researchers have shown that absorption of orally administered Se was 34% in sheep compared with 85% in swine [11]. Although enrichment of Se occurs in RMO compared with dietary levels [17,18], smaller amounts of inorganic sodium selenite or sodium selenate are incorporated into

RMO than SeMet, which is the primary form of Se found in Se-yeast [19,20] and high-Se grains and forages [12,21].

We previously showed that ewes receiving sodium selenite or sodium selenate by weekly oral drenching had decreased whole-blood and serum-Se concentrations compared with ewes receiving the same dosage of SeMet as Se-yeast [22]. To determine if RMO were responsible in part for these findings, we conducted an *ex vivo* experiment to evaluate the effect of sodium selenite, sodium selenate, and SeMet on Se uptake and elemental Se formation by RMO. Our results showed that organic Se as SeMet was incorporated to a greater extent into RMO than inorganic Se sources and resulted in less elemental Se formation. Thus, decreased bioavailability of inorganic Se compared with Se-yeast noted in our whole animal studies may be explained by these *ex-vivo* results showing increased elemental Se formation and decreased microbial incorporation of inorganic Se. In ruminants, the improved bioavailability of SeMet compared with inorganic Se may be the result of rumen-based reactions (increased incorporation of SeMet into RMO and decreased formation of elemental Se) rather than at the level of the small intestine (increased absorption efficiency). Consumption of Se-fertilized forage [23,24] as a source of organic Se provides an attractive alternative to inorganic Se supplements, because organic SeMet in forage is better incorporated into RMO and results in less elemental Se formation.

Methods of Se Supplementation

Several means of administering Se to deficient livestock are available. For example, there are a number of injectable preparations, which often include vitamin E. Selenium can also be added to feed, mineral, and protein supplements. Sustained-release boluses with a life of several months may be used. Because of their weight, these boluses stay in the rumen whereby they gradually release Se. Selenium supplemented by these methods is usually inorganic sodium selenite or selenate. One limitation of supplementing with inorganic Se in salt or feed is the apparent short duration of Se storage in the animal. If Se is removed from the diet, blood Se concentrations may become deficient if they were initially in the lower part of the normal reference interval. Seasonal grazing practices may result in limited access to Se-containing salt-mineral mixes for extended periods of time, and therefore, livestock may be Se deficient by the end of the grazing season.

Agronomic biofortification is defined as increasing the bioavailable concentrations of essential elements in edible portions of crop plants through the use of fertilizers. The potential for using Se-containing fertilizers to increase crop Se concentrations and, thus, dietary Se intake has been demonstrated in Finland, New Zealand, and Australia where it has been proven to be both effective and safe [25-27].

Functions of Selenoproteins

Selenium is incorporated into selenoproteins whose functions range from antioxidant, anti-inflammatory, and detoxification to thyroid hormone activation [28]. Evidence suggests that Se exerts its effects in part by enhancing innate and adaptive immune responses [29]. This is not surprising given that many selenocysteine (SeCys)-containing proteins are involved in regulating redox reactions, in removal of reactive oxygen species (ROS), and in other important cellular reactions (e.g., cell growth, apoptosis, and regulation of transcription) in a variety of tissues [29,28]. Levels of ROS influence inflammatory gene expression; thus selenoproteins can affect inflammatory responses by regulating the oxidative state of immune cells [30]. The selenoproteins involved in controlling oxidative stress include glutathione peroxidases (GPX) and thioredoxin reductases (TXNRD) [31]. In the GPX family, GPX1 acts as an antioxidant and uses glutathione as a cofactor to reduce hydroperoxides to their corresponding alcohols [32]. Hydroperoxides are non-radical ROS. The phospholipid hydroperoxide GPX4 is the major antioxidant enzyme that directly reduces phospholipid hydroperoxides within membranes and lipoproteins [32]. The TXNRD use NADPH in thioredoxin-dependent antioxidant pathways to reduce oxidized thioredoxins that are formed when oxidized proteins and lipids are detoxified [30]. Selenoprotein P (SELENOP) is important in the transport of Se to tissues, but also has antioxidant activity [33], as does selenoprotein W (SELENOW) [34]. Transcription factors such as FBJ murine osteosarcoma viral oncogene homolog (FOS; also a member of the activator protein-1 family) and nuclear factor kappa B (NFκB) are activated by ROS, and removal of these from cells by selenoproteins prevents induction and activation of pro-inflammatory signaling cascades (reviewed in

[35]). For example, overexpression of GPX4 inhibits the expression of NF κ B target genes, and IL-1- dependent signaling of leukotriene and prostanoic acid biosynthesis is reduced [32]. Specific selenoproteins also have ROS-independent roles in modulating inflammatory responses [30]. Selenoprotein S (SELENOS) plays a role in the transcription of genes encoding pro-inflammatory cytokines [36,37]. The iodothyronine deiodinases (DIO) regulate the bioactivity of thyroid hormones by controlling levels of thyroxine (T4) and the active hormone, 3,3',5-triiodo L-thyronine (T3). Whereas DIO2 is responsible for the conversion of T4 to the active hormone T3, DIO3 catalyzes the inactivation of T4 and T3. Although the role of thyroid hormones in inflammatory responses remains unclear, suppression of DIO2 has recently been shown to result in increased expression of inflammatory cytokine mediators [38]. Thus, several mechanisms are hypothesized for how Se affects immune responses, including protection against oxidative damage resulting in decreased inflammation and transcriptional regulation of genes encoding pro-inflammatory cytokines.

Se Metabolism: Fate of Se in the Body after Uptake from the Diet

Regulation of Se metabolism is controlled by its availability. When the supply of Se is greater than needed for selenoprotein synthesis, excess Se is excreted. There are several cellular mechanisms that regulate Se use for synthesis of specific selenoproteins (reviewed in [39]). Some selenoproteins are favored over others for Se incorporation, creating a cellular hierarchy. For example, SELENOP is higher in the liver cellular hierarchy than GPX1. The liver secretes SELENOP into the plasma to supply other tissue with Se. SELENOP binds to the endocytic receptor apo ER2. Apo ER2 varies among tissues, creating an organ hierarchy for Se in SELENOP uptake. This is how the liver Se can maintain selenoproteins in other parts of the body. Human plasma contains most of its Se in two selenoproteins: GPX3, which originates in the kidney, and SELENOP, which originates in the liver. Plasma also contains unregulated SeMet in all of its methionine-containing proteins. Thus, plasma Se does not accurately mirror the regulated Se pool. Plasma GPX3 reflects kidney Se and SELENOP reflects liver Se. Plasma SELENOP may be the most accurate indicator of whole-body Se [39].

Selenium-Biofortification: Forms and Methods of Se Application, Uptake and Metabolism by Plants.

For over 20 years, research at Oregon State University has demonstrated the potential for using Se as a fertilizer to increase Se concentrations in forage for livestock feeds. Research in New Zealand, confirmed by OSU trials, found that sodium selenate is the form of Se most efficiently taken up by plants. The recommended level of application is 5 to 10 grams of actual Se per acre to achieve adequate levels of Se in forage. Sodium selenate is 41% Se. An application rate of 12 to 24 grams of sodium selenate per acre will provide the recommended 5 to 10 grams of actual Se per acre.

A pelleted material from New Zealand (called Selcote Ultra) contains sodium selenite that is 4.5 grams of actual Se per pound. This product is approved for use in Oregon by the Oregon Department of Agriculture and can only be mixed with fertilizer by a licensed fertilizer dealer. Recommended application rates of Selcote Ultra are 1 to 2 pounds per acre. Late winter or early spring applications are most effective; however, there is some evidence that a fall application will provide sufficient Se for plant uptake in the spring. Hay produced from Se-fertilized forage is another excellent source of organic Se.

Selenium is not an essential element for plants, and excess Se accumulation is toxic to most plants, most likely because the indiscriminate incorporation of SeCys and SeMet into proteins impairs their function [40].

Recent Studies that Show a Benefit for Feeding Se-Biofortified Hay

Hall JA, Harwell AM, Van Saun RJ, Vorachek WR, Stewart WC, Galbraith ML, Hooper KJ, Hunter JK, Mosher WD, Pirelli GJ. **Agronomic biofortification with selenium: Effects on whole blood selenium and humoral immunity in beef cattle.** *Animal Feed Science and Technology* 2011; 164:184-190.

The purpose of this study [24] was to evaluate Se supplementation strategies in mature beef cattle by measuring changes in whole-blood Se (WB-Se) status and humoral immune response to vaccination. Mature beef cows (n =

45) were balanced by age and randomly assigned to 1 of 3 supplementation groups that received different chemical forms of Se or Se dosages compared to a standard (control) Se treatment. Supplementation treatment groups were provided limited access (6 weeks) to either sodium selenite (200 mg/kg Se; LSe) or Se-fertilized forage (FSe) and subsequently had no additional Se in their mineral supplement for the study duration. The LSe group cows grazed non-Se-fertilized forage. The control group grazed non-Se-fertilized forage and received continuous Se supplementation (CSe) from a free-choice mineral supplement (120 mg/kg Se from sodium selenite). Cows were bled pre and post grazing and then every 4 weeks thereafter for approximately 5 months to assess WB-Se concentration. All cows were immunized with J-5 *Escherichia coli* bacterin at the end of the 6-week supplementation period, and serum was collected for antibody titers 2 and 4 weeks after the third immunization. Covariate adjusted WB-Se concentrations were influenced ($P < 0.0001$) by group, time and their interaction. Cows in the FSe group had higher ($P < 0.0001$) WB-Se concentration (186 ± 5 ng/mL) immediately post-grazing (42 days) compared to LSe (117 ± 5 ng/mL) and CSe cows (130 ± 5 ng/mL). WB-Se concentration in FSe cows remained higher ($P = 0.02$ to $P < 0.0001$) over the next 4 (CSe) and 5 (LSe) months. Higher ($P < 0.05$) WB-Se concentrations were observed in CSe compared to LSe cows over the last 4 months of the study. Treatment group ($P = 0.036$) and time post vaccination ($P < 0.0001$) influenced J-5 *E. coli* antibody titers, with FSe cows having higher titers than LSe cows ($P = 0.01$), although FSe and CSe cows were not different. Short-term exposure of cattle to Se-fertilized forage elevates WB-Se concentrations within several weeks and this exposure is sufficient to maintain adequate concentrations throughout grazing periods when there is limited access to Se supplements. Short term exposure to higher levels of inorganic Se supplementation is not equivalent to ongoing inorganic Se supplementation at lower rates.

Hall JA, Bobe G, Hunter JK, Vorachek WR, Stewart WC, Vanegas JA, Estill CT, Mosher WD, Pirelli GJ. **Effect of feeding selenium-fertilized alfalfa hay on performance of weaned beef calves.** *PLoS ONE* 2013; 8(3):e58188. doi: 10.1371/journal.pone.0058188.

Selenium (Se) is an essential micronutrient in cattle, and Se-deficiency can affect morbidity and mortality. Calves may have greater Se requirements during periods of stress, such as during the transitional period between weaning and movement to a feedlot. Previously, we showed that feeding Se-fertilized forage increases whole-blood (WB) Se concentrations in mature beef cows. Our current objective was to test whether feeding Se-fertilized forage increases WB-Se concentrations and performance in weaned beef calves [41]. Recently weaned beef calves ($n = 60$) were blocked by body weight, randomly assigned to 4 groups, and fed an alfalfa hay based diet for 7 wk, which was harvested from fields fertilized with sodium-selenate at a rate of 0, 22.5, 45.0, or 89.9 g Se/ha. Blood samples were collected weekly and analyzed for WB-Se concentrations. Body weight and health status of calves were monitored during the 7-wk feeding trial. Increasing application rates of Se fertilizer resulted in increased alfalfa hay Se content for that cutting of alfalfa (0.07, 0.95, 1.55, 3.26 mg Se/kg dry matter for Se application rates of 0, 22.5, 45.0, or 89.9 g Se/ha, respectively). Feeding Se-fertilized alfalfa hay during the 7-wk preconditioning period increased WB-Se concentrations ($P = 0.001$) and body weights ($P = 0.002$) depending upon the Se-application rate. Based upon our results we suggest that soil-Se fertilization is a potential management tool to improve Se-status and performance in weaned calves in areas with low soil-Se concentrations.

Hall JA, Bobe G, Vorachek WR, Hujeriletu, Gorman ME, Mosher WD, Pirelli GJ. **Effects of feeding selenium-enriched alfalfa hay on immunity and health of weaned beef calves.** *Biol Trace Elem Res* 2013; 156(1-3):96-110. doi: 10.1007/s12011-013-9843-0.

Previously, we reported that feeding selenium (Se)-enriched forage improves antibody titers in mature beef cows, and whole-blood Se concentrations and growth rates in weaned beef calves [41]. Our current objective was to test whether beef calves fed Se-enriched alfalfa hay during the transition period between weaning and movement to a feedlot also have improved immune responses and slaughter weights [8]. Recently weaned beef calves ($n = 60$) were fed an alfalfa-hay-based diet for 7 weeks, which was harvested from fields fertilized with sodium selenate at 0, 22.5, 45.0, or 89.9 g Se/ha. All calves were immunized with J-5 *Escherichia coli* bacterin. Serum was collected for antibody titers 2 weeks after the third immunization. Whole-blood neutrophils collected at 6 or 7 weeks were

evaluated for total antioxidant potential, bacterial killing activity, and expression of genes associated with selenoproteins and innate immunity. Calves fed the highest versus the lowest level of Se-enriched alfalfa hay had higher antibody titers ($P=0.02$), thioredoxin reductase-2 mRNA levels ($P=0.07$), and a greater neutrophil total antioxidant potential ($P=0.10$), whereas mRNA levels of interleukin-8 receptor ($P=0.02$), L-selectin ($P=0.07$), and thioredoxin reductase-1 ($P=0.07$) were lower. In the feedlot, calves previously fed the highest-Se forage had lower mortality ($P=0.04$) and greater slaughter weights ($P=0.02$). Our results suggest that, in areas with low-forage Se concentrations, feeding beef calves Se-enriched alfalfa hay during the weaning transition period improves vaccination responses and subsequent growth and survival in the feedlot.

Hall JA, Isaiah A, Estill CT, Pirelli GJ, Suchodolski JS. **Weaned beef calves fed selenium-biofortified alfalfa hay have an enriched nasal microbiota compared with healthy controls.** *PLoS ONE* 2017; 12(6):e0179215. doi: 10.1371/journal.pone.0179215.

Selenium (Se) is an essential trace mineral important for immune function and overall health of cattle. The nasopharyngeal microbiota in cattle plays an important role in overall respiratory health, especially when stresses associated with weaning, transport, and adaptation to a feedlot affect the normal respiratory defenses. Recent evidence suggests that cattle diagnosed with bovine respiratory disease complex have significantly less bacterial diversity. The objective of this study was to determine whether feeding weaned beef calves Se-enriched alfalfa (*Medicago sativa*) hay for 9 weeks in a preconditioning program prior to entering the feedlot alters nasal microbiota [42]. Recently weaned beef calves ($n=45$) were blocked by sex and body weight, randomly assigned to 3 treatment groups with 3 pens of 5 calves per treatment group, and fed an alfalfa hay based diet for 9 weeks. Alfalfa hay was harvested from fields fertilized with sodium selenate at a rate of 0, 45.0 or 89.9 g Se/ha. Blood samples were collected biweekly and analyzed for whole-blood Se concentrations. Nasal swabs were collected during week 9 from one or two calves from each pen (total $n=16$). Calculated Se intake from dietary sources was 3.0, 15.6, and 32.2 mg Se/head/day for calves consuming alfalfa hay with Se concentrations of 0.34 to 2.42 and 5.17 mg Se/kg dry matter, respectively. Whole-blood Se concentrations after 8 weeks of feeding Se-fertilized alfalfa hay were dependent upon Se-application rates (0, 45.0, or 89.9 g Se/ha) and were 155, 345, and 504 ng/mL ($P_{\text{Linear}} < 0.0001$). Microbial DNA was extracted from nasal swabs and amplified and sequenced. Alpha rarefaction curves comparing the species richness (observed OTUs) and overall diversity (Chao1, Observed OTU, and Shannon index) between calves fed selenium-biofortified alfalfa hay compared with control calves showed that Se-supplementation tended to be associated with an enriched nasal microbiota. ANOSIM of unweighted UniFrac distances showed that calves fed high Se-biofortified alfalfa hay clustered separately when compared with control calves in the PCoA plot ($R = 0.216$, $P = 0.04$). The bacterial orders *Lactobacillales* and *Flavobacteriales* were increased in healthy control calves compared with *Clostridiales* and *Bacteroidales* being increased in calves fed Se-biofortified alfalfa hay. Although there were strong trends, no significant differences were noted for any of the bacterial taxa. Based upon these findings, we suggest that weaned beef calves fed Se-biofortified hay tend to have an enriched nasal microbiota. Feeding Se-biofortified alfalfa hay to weaned beef calves prior to entering the feedlot is a strategy for increasing nasopharyngeal microbial diversity.

Hall J, Bobe G. Effects of feeding cows Se-yeast or Se-enriched alfalfa hay on baby calves Se status and IgG titers. **11th International Symposium on Selenium in Biology and Medicine and 5th International Conference on Selenium in the Environment and Human Health.** 2017, Stockholm, Sweden.

Introduction: Selenium (Se) is an essential trace mineral important for immune function and overall health of cattle. Two methods of Se-delivery to pregnant cows are organic Se-yeast supplementation and agronomic Se biofortification, whereby the Se content of hay is increased through the use of Se-containing fertilizer amendments. Our objective was to evaluate the effect of these two Se-delivery methods in cows on passive transfer of IgG to calves.

Methods: Se-Yeast Supplementation: During the last 8-wk before calving, dairy cows were fed either 0 ($n=17$) or 105 mg Se-yeast once weekly ($n=20$), in addition to Na-selenite at 0.3 mg Se/kg DM in their ration [43]. The Se-

yeast dosage was calculated to provide 15 mg of Se/d ($5\times$ the maximal FDA-permitted level). After birth, calves were fed pooled colostrum from control or supranutritional Se-yeast supplemented cows. Concentrations of whole-blood (WB)-Se and serum-IgG were measured at birth, 48-h, and 14-d of age.

Agronomic Biofortification: During the last 8-wk before calving, beef cows were fed alfalfa hay fertilized with 0 (calculated Se intake: 8.3 mg Se/head/d; $n=15$), 45.0 (27.6 mg Se/head/d; $n=15$), or 89.9g Se/ha (57.5 mg Se/head/d; $n=15$). Concentrations of colostrum-Se and IgG1 were measured at birth, and concentrations of calf WB-Se and serum-IgG1 were measured at birth and 12, 24, 36, and 48-h of age [44].

Chemical Analysis: Concentrations of IgG1 were quantified by ELISA, and Se concentrations by an inductively coupled argon plasma emission spectrophotometry method by commercial laboratories (Michigan State University, East Lansing, MI, for the WB-dairy cow samples and Utah Veterinary Diagnostic Laboratory, Logan, UT, for all other samples).

Results and Discussion: Se-Yeast Supplementation: Calves born to Se-yeast supplemented cows had higher WB-Se concentrations at birth (280 ± 7 vs. 190 ± 9 ng/mL; $P<0.0001$), 48h (323 ± 11 vs. 221 ± 14 ; $P<0.0001$), and d14 (238 ± 7 vs. 184 ± 9 ; $P<0.0001$), and higher IgG absorption efficiency (40 ± 4 vs. $23\pm4\%$ at 48 h; $P=0.004$), resulting in higher serum-IgG concentrations (20.8 ± 1.8 vs. 12.3 ± 2.0 mg/mL at 48 h; $P=0.003$ and 6.8 ± 0.5 vs. 4.8 ± 0.6 mg/mL at 14 d; $P=0.01$) and higher serum-IgG content (58.1 ± 4.5 vs. 31.5 ± 5.0 g at 48 h; $P=0.0004$ and 23.4 ± 1.6 vs. 15.5 ± 1.7 g at 14 d; $P=0.002$), compared with calves born to control cows.

Agronomic Biofortification: Colostral Se concentrations increased with Se-fertilization from 119 ± 51 (0g Se/ha) to 504 ± 51 (45.0g Se/ha), and $1,336\pm51$ ng/mL(89.9g Se/ha), and IgG1 concentrations from 107 ± 5 (0g Se/ha) to 167 ± 34 (45.0g Se/ha), and 198 ± 51 ng/mL(89.9g Se/ha). Calf WB-Se concentrations at birth increased with Se-fertilization from 138 ± 37 (0g Se/ha) to 279 ± 37 (45.0g Se/ha), and 429 ± 37 ng/mL(89.9g Se/ha), but Se-fertilization had no effect on serum IgG1 concentrations during the first 48 h of age. Thus, feeding Se-biofortified alfalfa hay promotes the accumulation of Se and antibodies in colostrum, but a physiologic limitation of small intestinal epithelial cells to absorb additional antibodies may have limited our ability to observe differences in serum antibody concentrations in these calves.

Conclusion: Both Se supplementation strategies for cows during the dry period were effective for maximizing WB-Se and serum-IgG concentrations in calves. The more economical alternative is agronomic biofortification because it involves less labor and costs.

Conclusions

As beef producers enter into niche markets with grass finished beef and restricted use of antibiotics, more knowledge on alternative practices to enhance cattle health and prevent diseases is important. Results of these studies provide evidence for hay producers in Oregon to adopt the practice of Se-fertilization of forages to provide an enhanced quality of hay, which will then be used to benefit performance and health of beef cattle. This is an innovative and economically viable way of supplementing Se to cattle in our Se-deficient state that we hope will be adopted by hay and cattle producers.

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Transition Minerals and Vitamins: Impacts on Cow and Calf Health

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Introduction

Significant economic losses occur in agricultural enterprises as a result of prenatal and natal deaths and neonatal disease processes. Diagnosis of abortion losses still remains below 45% and a significant portion of these "idiopathic" abortions or stillbirths have been hypothesized to be related to nutritional causes. In a path model of risk factors for natal and prenatal deaths in beef herds, nutritional deficiencies and toxins were linked with every primary factor identified in causing these deaths as well as factors leading to inadequate passive transfer and neonatal disease.¹ Hepatic trace mineral concentrations were significantly lower in aborted fetuses compared to "control" slaughterhouse fetal specimens.² However, it has not been determined as to whether this finding suggests a direct role of trace mineral deficiency in abortion or whether it is a consequence of the abortion. Many specific trace mineral deficiencies can result in abortion, stillbirths or weak neonates.³

Assessing trace mineral and vitamin status of the fetus or neonate may provide critical information as to the underlying cause of a given diagnostic dilemma. Nutrient concentration within fetal or neonatal liver specimens or blood can be just as easily determined as is routinely completed for adults. Of concern however, is how one can interpret these concentrations relative to mineral or vitamin status. Can we assume metabolism is the same between fetal, neonatal and adult animals? Is it appropriate to use current criteria for adult animals on fetal and neonatal samples? If the answer to these questions are "no", then we may be mis-diagnosing many perinatal nutritional problems. In this presentation, maternal, fetal and neonatal mineral and vitamin nutrition concepts will be compared and contrasted to gain some understanding of the underlying physiologic processes. Role of these essential nutrients relative to fetal and neonatal health and survival will be discussed with recommendations for assessment and supplementation.

Vitamin and Mineral Metabolic Dynamics

Trace minerals are indirectly or directly associated with a tremendous variety of metabolic processes in animals of all ages. Deficiency diseases affect almost every physiologic and metabolic function and include immune dysfunction (Cu, Zn, Se); developmental abnormalities (Cu, Mn, I); abortion (Cu, I, Se); retained placenta (Cu, Se, I); metabolic disturbances (Co, Fe, Zn, I); and poor growth (Co, Cu, Fe, I, Se, Zn).^{3,4} In addition and more importantly, subclinical disease resulting in reduced productive efficiency (reproduction, growth, lactation) and increased disease susceptibility is a more economically important problem associated with marginal mineral deficiencies. Subclinical disease is often difficult for the producer to identify within the herd without appropriate records evaluation and production bench marking. Presence of trace mineral deficiencies, clinical or subclinical, seem prevalent within the beef and dairy industries. Fat-soluble vitamins (ie, A, D and E) are associated with specific clinical disease manifestations, but much less is known relative to subclinical disease concerns.

Trace minerals and fat-soluble vitamins are not homeostatically regulated, but more controlled through movement between pools. Microminerals can be found in the body as a component to one or more metalloenzymes (biochemical function pool), transported on carrier proteins (transport mineral pool) or stored as a metal complex (storage mineral pool). Fat soluble vitamins are managed in a similar way, though they exert their biological effects as hormones affecting gene expression (A, D) or direct action (A, E). The body makes every effort to maintain a necessary level of activity in the biochemical pool to ensure normal function. The storage pool holds a reserve and is sensitive to nutritional status. If nutrient intake is in excess of requirements, excess intake will be stored until other regulatory processes, reduced absorptive efficiency or increased renal excretion, modify net mineral retention back into balance. Transport pool is dynamic in reflecting changes relative to either deficient or sufficient nutrient state. In a situation of nutritional inadequacy, hepatic storage will be mobilized and used to

maintain biochemical pool activity until absorptive efficiency, reduced excretion or both can be enacted to raise net retention.

Using these concepts of mineral pools within the body, Suttle has described four progressive phases of mineral deficiency disease.^{3,5} Phases of mineral status move from depletion, loss of mineral in storage; deficiency, loss of mineral in transport pool; dysfunction, compromise of the function pool and finally disease (clinical signs associated with critically reduced function of a specific metalloenzyme). For example, copper deficiency reduces tyrosinase activity, which then decreases production of melanin pigment resulting in clinical signs of achromotrichia. Subclinical disease occurs during deficiency and dysfunction phases, often defined as impaired immune function, reduced growth rate or reproductive efficiency or other non-specific declines in productive efficiency. Disease due to deficiency of fat soluble vitamins would follow this same pattern of change in respective biologic pools.

Maternal-Fetal-Neonatal Interrelationships

The developing fetus is totally dependent upon availability of essential nutrients from placental transfer from maternal blood. As a result, fetal nutrient status is reflective of maternal nutrient status. Maternal nutrients available to the fetus would include those from the consumed diet as well as mobilized reserves, if needed. Swenson showed a decline in maternal liver copper concentration during late pregnancy, which would be consistent with maternal transfer of mineral to fetus.⁶ A decline in maternal mineral status with progressing gestation was observed in beef cattle, but not dairy cattle, suggesting differences in supplementation relative to requirements.⁷ Numerous studies have observed a fetal liver concentrating ability for minerals in finding fetal hepatic mineral concentrations to be nearly twice maternal values on a dry weight basis.^{2,7-10}

In contrast to hepatic mineral concentrations, mean maternal serum selenium concentration was twice that of her fetus.¹⁰ However, whole blood and erythrocyte selenium concentrations were not different between fetus and dam. Mean whole blood glutathione peroxidase activity was only slightly greater in the fetus compared to the dam. Whole blood and erythrocyte Se concentrations as well as whole blood glutathione peroxidase activity represent the functional pool of selenium as an antioxidant and these data suggest an approximately equal requirement for both fetus and dam. Observed differences between serum and liver selenium concentrations suggest altered transport and storage pools between dam and fetus. Higher maternal serum selenium concentration provides a substantial concentration gradient necessary for efficient placental selenium transport. Higher fetal liver Se concentrations infers a preferential storage of excess Se by the fetal liver over and above tissue requirements. Given these relationships, fetal liver and serum mineral concentrations must be interpreted differently from adult values.

Fat-soluble vitamins A, D and E do not appreciably cross the placenta as evidenced by much lower serum and liver fat-soluble vitamin concentrations in fetal samples compared to adult cattle.^{11,12} Mechanisms for placental transport of fat-soluble vitamins exist, most likely to ensure sufficient amounts to meet fetal metabolic needs. The neonate's primary source of fat-soluble vitamins comes via colostrum ingestion supplied from an adequately supplemented dam. Maintenance of neonatal vitamin status will come from milk consumption.

During the early postnatal period, almost all essential nutrients are adequately provided for by milk consumption. However, a number of critical micronutrients, namely Cu, Fe, Zn and Se, are insufficiently to marginally provided by milk consumption alone, thus requiring additional sources to meet daily needs. Milk will contain some fat-soluble vitamins, but this will depend upon maternal supplementation. Fetal hepatic nutrient reserves play a critical role in maintaining adequate micromineral concentrations to support daily nutrient requirements in the milk-fed postnatal animal. Hepatic mineral reserves are augmented by consumption of colostrum, a highly concentrated source of most essential minerals and fat-soluble vitamins, which is dependent upon maternal nutrient status.

Role in Perinatal Disease

Clinical disease associated with specific trace minerals or fat-soluble vitamins has been described,³ but these situations are not generally prevalent unless serious dietary issues are present. Marginal deficiencies of trace minerals have been clinically implicated in prenatal and postnatal disease issues leading to abortion, stillbirth, weak neonates and impaired immune response, but a definitive cause and effect has not been established through controlled studies.^{1,3,4,13} The role of fat-soluble vitamins is less well defined. Survey studies have associated low hepatic copper, selenium, manganese and zinc concentrations with abortion with and without an identified infectious agent.^{2,14} In a recent survey of stillborn beef and dairy calves, 67% of cases had low vitamin A and one or more trace mineral deficiencies (Van Saun, unpublished data, 2015). This observed role of vitamin A is consistent with other observations of low vitamin A status being associated with weak beef calves.¹⁵

Adequacy of neonatal nutrient reserves might explain differences in time frame and severity of specific nutrient deficiency disease occurrence. If a pregnant dam is severely deficient, mineral transfer to the fetus may be so limiting as to compromise normal functions, resulting in fetal death and abortion. If the deficiency is lessened but still serious, the fetus may die during parturition or soon thereafter. If mineral status is sufficient to maintain fetal development, hepatic reserves may be limited to various degrees. This then may result in clinical deficiency signs in the neonate within a week or two of birth. In other neonates where hepatic mineral reserves were slightly better, one might see clinical signs at one month or later or may not see clinical signs at all, but rather poor growth and performance. At this time, we do not know what mineral storage amount is necessary in the neonatal liver to minimize clinical and subclinical problems. Much more research in this area is needed.

Diagnostic Evaluation

A better database of adequate fetal and neonatal trace mineral and vitamin concentrations is required for proper diagnosis and monitoring. In attempting to determine mineral status, one needs to consider what question is being asked. First if one is interested in determining cause-effect relationship between a mineral deficiency and specific pathologic lesion, then one needs to look at the physiologic or biochemical role of mineral relative to biochemical function. On the other hand, what is most often asked is: What is the nutritional status of the animal? This is entirely different question and reflects the status of a different nutrient pool, the storage pool. Unfortunately collection of serum or whole blood is not the preferred specimen for determining nutritional status. Interpretation of this pool is difficult in many circumstances due to dynamic circumstances of nutrient flux through this pool. As a result of these relationships, collection of a liver biopsy specimen is the preferred sample to determine nutritional status of the animal. Liver samples can be obtained from any portion of the liver. No data has shown any evidence of mineral concentration variation within the liver.¹⁶

Given the described differences between fetal and maternal mineral metabolism, and understanding that neonatal mineral metabolism is a gradual progression from fetal to adult metabolic patterns, it seems obvious that adult-based diagnostic criteria cannot be used for either fetal or neonatal evaluations. Some diagnostic laboratories have recognized these differences and have established age-based criteria. At Michigan State University's Clinical Nutrition laboratory diagnostic criteria have been estimated for fetal, newborn (1-9 days), infant (10-29 days), juvenile (30-300 days), yearling (301-700 days) and adult (>700 days) age categories. This laboratory has a tremendously large database by which these criteria were empirically derived for vitamins A and E and selenium in serum and liver samples. Without age-based criteria, all younger animals and fetuses would be considered deficient in most trace minerals and vitamins. While this approach is a tremendous move forward in diagnostic capabilities, more data is needed to refine these diagnostic criteria. Our ability to make diagnostic interpretations from fetal and neonatal liver mineral concentrations may be improved if evaluations are based on age and hepatic dry matter content. More controlled research is needed to specifically determine adequate hepatic mineral concentrations in bovine fetus and neonate.

Supplementation Approaches

It is absolutely essential that the pregnant animal receive an adequate amount of all minerals and fat-soluble vitamins to support both maternal maintenance and conceptus development throughout the duration of gestation

to minimize deficiency disease problems of either the dam or neonate. House and Bell have suggested NRC mineral requirements were sufficient to support pregnancy,¹⁷ but vitamins A and E were increased with the later NRC publication.¹⁸ In beef cattle it is suggested to increase NRC mineral requirements during pregnancy by 125% to ensure sufficient fetal transfer and maintain maternal status. Dietary supplementation is more physiologic and should be maintained throughout the gestation feeding period. Challenges occur with the rumen in microbial degradation of fat-soluble vitamins (A predominately) and production of interfering agents. Often a recommendation to include between 25 and 30% mineral supplement from chelated or organic forms is made.

Injectable minerals and vitamins have been used to correct diagnosed deficiencies or in the place of dietary supplementation programs. Injectable minerals and vitamins are more biologically available, but increasing serum concentrations may result in a greater percent of the injected dose being excreted. Selenium deficient beef heifers administered a label dose of injectable sodium selenite excreted nearly 25% of the injected dose within 24 hours and selenium status adequacy was not achieved.¹⁹ More recent work has shown some beneficial effects on cow performance with injectable minerals even when the diet fed was within recommendations.^{20,21} A better understanding of how supplemental nutrition can influence immune response is necessary to better define mode and rate of trace mineral and vitamin supplementation to enhance cow and calf health and performance.

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Is Protein the Missing Piece of the Transition Metabolism Puzzle?

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Introduction

For a cow to transition from late pregnancy into lactation successfully, she needs to exquisitely coordinate metabolism in multiple tissues to ultimately provide sufficient glucose to support productive needs.¹ Daily requirements for glucose, amino acids, fatty acids and calcium for an early lactation cow (4 days postpartum; 30 kg milk, 4.7% fat and 4.2% protein) are 2.7, 2.0, 4.5 and 6.8 times greater, respectively, than those needed for pregnancy.² These differences represent changes in nutrient requirements over a period of only 1-to-2 weeks and occur during a period of lowest dry matter intake; highlighting the tremendous metabolic alterations necessary to adequately support lactation. The inability of the cow to metabolically adapt to this transition is the underpinning of most postpartum diseases. The late gestation diet has been shown to play a critical role in modulating a cow's predisposition to periparturient health disorders, though the role of protein remains elusive. The objective of the presentation is to review protein metabolism during transition and its influence on metabolic response relative to cow health, performance and reproduction. Other non-nutritional factors affecting the cow's ability to consume sufficient nutrients also contributes, and may contribute more significantly, to postpartum disease susceptibility. Nuances of how management and environment alter stability of these metabolic changes remains to be highlighted.

Protein Metabolism in Transition

Much emphasis has been placed on energy metabolism and markers of energy balance as underpinning metabolic disturbances of transition and risk for disease. Although elevated concentrations of either NEFA or BHB have been highly associated with disease risk, their presence is not a direct determinant. A population of cows can perform without evidence of disease with elevated concentrations of NEFA suggesting some other factor or protective element. As our understanding of transition metabolism sheds more light on its complicated nature, a more integrated perspective on transition metabolism is needed and central to this is the supply and prioritization of amino acid metabolism as it relates to cow response to diet and management (Table 1). Although the body of published literature does not strongly suggest improved cow performance with higher prepartum dietary protein, there is much interest and anecdotal observations suggesting benefits from feeding diets delivering greater metabolizable protein (MP; >1100 g/day) than models would suggest is necessary to meet the cow's amino acid requirements. This observed response may be due to an underestimation of the MP requirement, providing an essential amino acid or acids, accounting for intake variability within a group allowing for adequate MP intake for cows with lower intake, or some combination of these factors.

Most studies evaluating prepartum protein nutrition essentially looked at milk yield or composition as metrics for a measured response.³ Most observations and research would suggest the primary benefit of prepartum protein feeding comes from disease prevention and improved reproductive performance.^{4,5} Curtis first reported higher prepartum protein diets decreased incidence of ketosis.⁶ Cows fed a higher protein prepartum diet, independent of energy content, had lower serum NEFA concentration, NEFA to cholesterol ratio, and fatty liver score.⁷ Van Saun also reported lower clinical ketosis prevalence for mature Holstein cows fed 1300 g MP/day compared to cows fed 1100 g/d.⁸ In this study all cows maintained a higher body condition score (mean 3.87 BCS at calving), thus were more predisposed to ketosis problems. Using 3-methylhistidine (3-MH) as a marker of skeletal muscle degradation, van der Drift and colleagues showed muscle mobilization occurring prepartum through 4 weeks postpartum for dry cows fed a diet composed of grass silage and corn silage containing approximately 12.6% crude protein.⁹ Cows having higher 3-MH concentrations generally had lower BHB concentrations, suggesting a protective effect. Cows with extreme hyperketonemia had excessive muscle and fat mobilization. Amount of muscle mobilization was highly variable among cows in this study, though supplementing methionine may

mitigate body protein mobilization.¹⁰

Table 1. Comparison of fetal and maternal protein sources and metabolic adaptations during transition and association with deficiency disease risks.^{a,b}

Protein	Fetal	Maternal
Supply	Placental active transport of amino acids from maternal blood supply (70-80% of maternal circulating amino acids)	Microbial and dietary bypass proteins provide the metabolic source of amino acids to support body functions; mobilization of labile proteins (blood proteins, skeletal muscle) can make up for dietary deficiencies
Metabolism	Amino acids deposited into fetal tissues and placenta to support growth, oxidized for energy in fetus, amino acid exchange between fetus and placenta	Amino acids used to support body protein turnover and new protein synthesis; Up-regulation of many metabolic regulatory enzymes in support of gluconeogenesis, lipid metabolism, milk protein synthesis; Amino acids serve as intermediates in TCA cycle facilitating CHO and lipid metabolism; Amino acids may be redirected toward acute phase protein synthesis in face of inflammatory response
Disease Risks	Reduced birth weight, Inability to thermoregulate, weak calf syndrome, FPT?	Subclinical and clinical ketosis; Varying degrees of hepatic lipidosis; Reduced blood transport proteins for minerals and vitamins; Impaired immune response with increased infectious disease susceptibility, RFM, Udder edema?

^aAbbreviations: FPT = failure of passive transfer; RFM = retained fetal membranes

^bAdapted from Van Saun R, Sniffen CJ. Vet Clinics NA: Food Anim Pract 2014; 30:689-719.

Feeding additional rumen undegraded protein (RUP) prepartum showed improved insulin status in mature dairy cows relative to BCS and BCS score change.¹¹ Additionally cows fed a balanced protein diet postpartum compared to a high rumen degraded protein (RDP) diet had higher insulin sensitivity across body condition scores.¹² Insulin not only is an important regulator of glucose homeostasis, but also influences reproductive performance. Cows consuming more MP prepartum (>1300 g/d) had improved reproductive performance⁸ and ovulation time was not influenced by negative energy balance nadir.¹³ In contrast cows consuming lower prepartum MP intake (1100 g/d) followed by a postpartum diet high in RDP had their first ovulation time highly correlated with negative energy balance nadir.⁸

Using production data from 55,000 lactations it was found milk protein and milk fat-to-protein ratio in early lactation were associated with reproductive performance.^{14,15} Cows with low milk protein on first or second test day had lower first service and overall conception risks. Mobilized protein from skeletal muscle and involuting uterine tissue provide a primary source of amino acids to the mammary gland to support milk protein synthesis.¹⁶ Lower milk protein content may reflect inadequate dietary MP supply and repartitioning of amino acids to support the immune response or gluconeogenesis.

Blood albumin concentration reflects dietary amino acid supply and metabolic responses repartitioning available amino acids. Increasing dietary protein in early lactation increased albumin concentration.¹⁷ Albumin is synthesized in the liver and is considered a negative acute phase protein meaning its rate of synthesis is decreased during an acute phase response to inflammatory cytokines.¹⁸ Albumin concentration pre- and postpartum was associated with greater risk for postpartum disease.¹⁹ Blood albumin concentration of 3.5 g/dL or greater was found in primarily healthy cows compared to lower concentrations being predominately associated with cows having one or more disease events.¹⁹ Cows experiencing endometritis postpartum had lower prepartum albumin

concentration.²⁰ Lower prepartum albumin concentrations were observed in pasture-fed cows consuming a high (31.8%) nonfiber carbohydrate (NFC) compared to low (13.2%) NFC diet.²¹ Lower albumin concentration may reflect inadequate dietary MP supply, liver dysfunction, an active inflammatory response, or some combination and may provide a marker of transition cow health status.²²

Role of Inflammation in Metabolic Regulation

A growing body of research is recognizing an association between the activated inflammatory response mediated by proinflammatory cytokines interleukin (IL)-1, IL-6, and Tumor Necrosis Factor (TNF)- α and altered metabolism leading to greater disease risk, poor production, and impaired reproduction.^{18,23-28} Proinflammatory cytokines can be released from adipose tissue during mobilization as well as from any stress response.²⁴ Hepatic activation by these cytokines initiates the acute phase protein response resulting in up-regulated synthesis of positive acute phase proteins (+APP; i.e., ceruloplasmin, haptoglobin, serum amyloid-A, C-reactive protein, complement components) as well as enzymes and other physiologic mediators. Both IL-1 and TNF- α have profound metabolic effects promoting an increased basal metabolic rate (BMR) to produce fever in concert with reducing appetite. Reduced appetite in the transition cow is a recognized lynchpin to metabolic disease susceptibility. Mobilized skeletal muscle provides amino acids to support gluconeogenesis in maintaining the higher BMR. This response is in an effort to promote the immune response in responding to some pathogen or stressor, but is quite costly nutritionally to the animal.²⁹

Mobilization of skeletal muscle will further exacerbate negative protein balance in early lactation and may account for the predilection for more than one disease process once one has been established.³⁰ In addition to mobilization of skeletal muscle, constitutive proteins synthesized by the liver such as albumin, retinol binding protein, apoproteins, and transferrin (e.g., negative acute phase proteins, -APP) are not synthesized most likely to further provide amino acids to support the acute phase protein response. Reduction of these constitutive proteins may adversely affect mineral and vitamin metabolism through the loss of transport proteins. Additionally, loss of apoproteins would reduce the liver's ability to synthesize very low density lipoproteins (VLDL) and potentially increase fatty infiltration in the face of elevated NEFA concentrations. An activated immune response is necessary during transition to deal with uterine clearance and protection from potential mastitis pathogens, but excessive stimulation of this response through environmental, social, or dietary factors will predispose to poor transition cow performance.

Gestational Protein Requirement

Modeling gestational protein requirements is much more complicated as evidenced by model variation depicted in recent NRC publications. A proportion of the differences among these models is due to assumed efficiency of converting MP (i.e., absorbed amino acids) to net protein (i.e., retained within the fetus). Models prior to 1995 used an efficiency of 50%,³¹ whereas Bell summarized data suggesting this efficiency was much lower at 33%.² This difference in efficiency increases pregnancy MP requirement by 150%. Other challenges in predicting gestational protein requirements result from the dynamic metabolic functions of amino acids in supporting placental and uterine growth as well as the significant role amino acids play in fetal energy metabolism; none of which contribute to fetal protein retention that is the measured end point.² Another consideration is whether or not experimental diets were properly formulated to meet or exceed cow requirements to maintain a stable labile "reserve" protein pool in the cow. This is an underlying assumption of NRC models; maternal skeletal muscle is not used in support of pregnancy. McNeil and colleagues showed lamb birth weights not be different from ewes fed energy adequate diets with either 12% or 15% crude protein (CP) diets.³² Body compositional analysis; however, showed ewes fed the 12% CP diet had significant skeletal muscle protein loss accounting for the indifference in birth weights. Ewes fed the 15% CP diet had significant skeletal muscle accretion suggesting these ewes may be better positioned metabolically to adapt to negative energy balance and mobilize amino acids to support lactation. Cows in the Bell et al., study consumed 10-12 kg dry matter of a total mixed ration (TMR) containing 13% and 14% (after 250 days gestation) CP diets.³³ No measure of maternal protein status was determined in this study. The CNCPS system now recognizes the importance of mammary growth and protein reserves; however, it does not recognize the importance to labile protein reserves relative to immune function as

well as the need in the early postpartum period when cows can mobilize 800 to 1000 g/day. This puts greater emphasis on the maintenance of labile protein reserves in the last 60 to 80 days of gestation. This is a period in late lactation and during the dry period when lower energy rations are being fed, reducing microbial protein output and MP balance can easily become negative.

ENSURING ADEQUATE PROTEIN INTAKE

One of the primary challenges of dry cow group management is formulating the diet for an appropriate intake level. Even if one provides a balanced diet for a defined average intake for a given feeding group, statistics tell us 50% of the animals in the group consume less than the average intake. French presented summarized prepartum intake data from Phillips et al.¹⁰ for multiparous Holstein cows.³⁴ In this analysis the average DM intake was 12.3 ± 2.5 kg/d for the last 21 days precalving with 15% of the cows consuming less than 10 kg/d (1 standard deviation below group average) and being in a state of negative nutrient balance. A recommendation from this analysis was to formulate the close-up dry diet to 1300 g or 1400 g MP as a safety factor to ensure adequate 83% or 95%, respectively, of the cows consume a desired 1,080 g MP from the diet.

In another multiparous cow dataset, 21 day prepartum intake was 13.5 ± 2.6 kg/d.⁸ In this study prepartum diets differed in MP content (1100 vs. 1350 g/d) but DMI was not different across treatments. The cows consuming the higher MP diet had less metabolic disease and improved reproductive performance compared to the lower MP diet. These results would seemingly support the concept promoted by French, though a higher MP requirement is not out of consideration in explaining such responses. Clearly, large variation (higher standard deviation) of DMI within a group will result in more cows, and especially heifers in mixed groups, having lower intake and potentially experiencing negative protein balance.

Summary

Protein nutrition of the dry cow has been misunderstood and is still a controversial area of investigation. The controlled studies in this area have many times been confounded by the method of balancing to meet the protein requirement of the pregnant cow. The NRC recommendations for protein supply were based on research that unfortunately was limited and the experimental rations were formulated incorrectly providing wrong conclusions. Further the recommendations did not recognize the importance of the mammary requirement and the protein reserves. Field observations would suggest that there is a need to exceed the NRC 2001 recommendations for protein and meet and not exceed the ME requirements. Couple this with variation in DMI within a group of cows being fed a balanced ration, dictates that there be an adequate concentration of MP in the rations being fed during this time in order to ensure that all cows will be able to maintain the protein reserves that were replenished in mid-lactation. Additionally recent work has suggested that protein quality may be important as well. This would suggest that it is important to pay attention to the source of MP as well as the amount of MP. All cows experience a period of negative protein balance in early lactation that seems somewhat independent of prepartum protein feeding. However, if dietary protein is sufficiently deficient prepartum tissue protein mobilization may occur and the reservoir of labile protein to be utilized in early lactation may be compromised resulting in greater risk for impaired health, productive efficiency, and reproductive performance.

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Small Ruminant Nutritional Case Studies

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Nutritional problems in small ruminants are challenging as often sheep and goats are considered “equivalent” from a nutrient requirement perspective; however, there are a number of important differences as well as similarities in disease issues. This presentation will demonstrate fundamental principles of nutritional diagnostics in field cases where Cu nutrition was either inadequate or excessive leading to clinical signs.

Case 1. Meat goat herd experiencing reproductive losses

This was a meat goat herd of 75 does located in western Pennsylvania. The herd maintains approximately a 165% kidding crop. The herd is primarily managed as a grazing herd and hay cuttings are harvested off some pastures to provide forage during the non-grazing winter period. Pastures and hay are predominately a cool season grass with only a small amount of clover. A free choice mineral product is available for use and late pregnant does are provided an on-farm grain mix comprised of soybean meal, corn grain, molasses and a vitamin/mineral premix.

Presenting Problem: Herd was experiencing poor fertility during breeding season. During the winter 8 does were lost for various reasons including pregnancy toxemia. A total of 29 kids were lost due to late term abortion or stillbirths. Owner admitted the does looked “rough” this year and samples had been submitted to evaluate herd parasite status.

Diagnostic Process: Three stillborn kids were submitted for necropsy. Necropsy results showed no infectious agents responsible for kid deaths, all liver mineral samples indicated low copper (Cu) status (10.5-16.6 µg/g wet weight; Reference: 25 – 100 µg/g wet weight). No other abnormalities were found to indicate cause of death. Forage samples were collected and submitted for nutrient analysis (Table 1).

Table 1. Hay sample nutrient content results (all values other than moisture on dry matter basis).

Parameter	Units	Grass Hay #1 1 st cutting 2013	Grass Hay #2 2 nd cutting 2012	Kalmbach Goat Mineral (label values)
Moisture	%	12.4	10.7	
Dry Matter	%	87.6	89.3	99
Crude Protein	%DM	10.4	14.9	
Adjusted Protein	%DM	9.9	14.6	
Soluble Protein	%CP	27.6	17.3	
Rumen Degradable Protein	%CP	63.8	58.6	
TDN	%DM	58.6	59.1	
Acid Detergent Fiber	%DM	39.3	38.4	
Neutral Detergent Fiber	%DM	65.0	59.8	
Ash	%DM	6.67	9.39	99.5
NFC	%DM	17.1	14.5	
Calcium	%DM	0.39	0.85	15.5-18.5

Parameter	Units	Grass Hay #1 1 st cutting 2013	Grass Hay #2 2 nd cutting 2012	Kalmbach Goat Mineral (label values)
Phosphorus	%DM	0.28	0.28	8.0
Magnesium	%DM	0.11	0.29	1.5
Potassium	%DM	2.35	2.95	1.0
Salt	%DM			18.5-22.0
Sodium	%DM	0.005	0.008	
Iron	ppm	56	98	
Manganese	ppm	33	79	
Zinc	ppm	26	51	7500
Copper	ppm	6	9	1450-1850
Molybdenum	ppm	2.25	3.09	
Selenium	ppm			26

Key to the herd's current situation, both hay samples contain lower Cu content and higher molybdenum (Mo) content, which will result in lower Cu availability from these forages. Molybdenum and sulfur in the diet or water are metabolized by the bacteria in the rumen to generate compounds termed thiomolybdates. Thiomolybdates are well known for binding (chelating) Cu and making it unavailable to the animal. To address this potential interference between Mo and Cu we would like to have the dietary Cu:Mo ratio range between 6-to-8:1 in sheep and 6-to-10:1 in goats and cattle. Ratios below 4:1 are consistent with inducing a dietary copper deficiency problem. Both hay samples have low Cu:Mo ratios of 2.7:1 and 2.9:1, respectively. This would suggest that consumption of the mineral supplement is essential to balancing the consumed total diet in meeting the daily Cu requirements of the goats.

Dietary evaluation indicated total Cu intake was below requirement for pregnant does (9.35 mg/d vs. 26.8 mg/d) as well as dietary Cu availability being compromised. Consumption of at least 0.5 oz of the mineral could improve Cu intake to requirement needs. As the owner reviewed historical information on mineral feeding and herd performance there was a strong relationship between improved reproduction and less kid losses when more finances were spent on mineral supply.

Nutritional Recommendations: The mineral product being used can adequately supply sufficient Cu to the diet to meet the daily needs of the does, but the mineral needs to be continuously consumed at sufficient quantity. A minimum of 0.5 oz/head/day would be suggested, though this may be increased to 0.75 oz/head/day for pregnant does to ensure sufficient transfer to the fetuses. It was suggested to incorporate the mineral into the grain supplement and provide a minimum amount to all the animals. Allowing free choice intake leads to highly variable intake with some animals overconsuming (not a significant issue) and others underconsuming.

Case Outcome: Owner immediately starting adding mineral to the diet of the does and called a few weeks later to state some concern that he has not seen his does in estrus. The bucks had been with the does the entire time. The following spring he experienced an overwhelming kidding period where nearly all does kidded within a 1 week period indicating all does were bred when he was not seeing estrus activity. Kid losses were negligible that kidding season. The herd regularly incorporates mineral into the grain feeding practices and offers it free choice while grazing.

Case 2: Commercial sheep and goat herd with stillborn and weak lambs and kids

This case involved a 250 ewe flock of Finn, Dorset, Suffolk and crossbred sheep with a 200% lambing crop as well as 60 Boer does with 185% kidding crop. Farm is located in west-central Ohio and is managed intensively in

providing a formulated total mixed ration (TMR) throughout the year.

Presenting Problem: The flock/herd had experienced problems with animal losses and Cu related diseases (swayback, enzootic ataxia) approximately 6 years previously. Unable to correct the underlying problem resulting in ongoing losses of lambs and kids. Approximately 17-25% stillbirth or weak neonates year to year. High perinatal death loss within 3-4 days of age.

Diagnostic Process: Flock owner had reached out to a number of sheep nutritionists to help identify and resolve the problem. A number of dietary changes were made but with little improvement in the overall situation. A number of retained livers from dead lambs and kids were submitted for mineral analysis (Table 2).

Table 2. Hepatic copper and molybdenum concentrations in population of kids, lambs and ewes from affected flock.

Liver Minerals	Kids, n=9	Lambs, n=4	Ewes, n=3
Cu, $\mu\text{g/g DW}$	28.6 ± 22.2	36.3 ± 16.7	18 ± 13.1
Mo, $\mu\text{g/g DW}$	3.6 ± 1.1	1.9 ± 0.6	3.3 ± 1.6
Cu Reference	75-300 $\mu\text{g/g DW}$	60-300 $\mu\text{g/g DW}$	60-300 $\mu\text{g/g DW}$
Mo Reference	1.5-3.0 $\mu\text{g/g DW}$		

In recognizing the issues of copper and molybdenum in the liver samples, forage samples were submitted. Table 3 summarizes forage testing results for copper and molybdenum over the past 5 years on the farm. Water tests from the farm indicated high sulfur (>500 ppm) and iron (>1 ppm). All forages are grown in the local area.

Table 3. Forage copper and molybdenum concentrations in farm forages over the past 5 years.

Forage Crop	Year	Cu, ppm DM	Mo, ppm DM
Clover hay	2011	5.1	2.52
Corn silage	2011	14.8	0.51
Clover hay	2011	9.8	5.04
Corn silage	2012	6.6	5.48
Oatlage	2012	7.4	5.31
Corn silage	2013	7.0	5.33
Oat forage	2013	7.0	5.77
Clover silage	2014	14.8	1.5
Clover hay	2015	12.5	8.43
Corn fodder	2015	6.5	2.97
Corn silage	2015	3.6	7.15
Corn silage	2015	4.3	6.87
Oat baleage	2015	8.4	2.47

Nutritional Recommendations: The primary assessment was inadequate dietary Cu availability based on forage testing and liver mineral concentrations. A custom premix used in the TMR formulation was modified to increase

Cu content to account for dietary Mo to achieve a total dietary Cu:Mo ratio of 6-8:1. Due to the excessive Mo in the forages the total dietary Cu content was an amazing 43 ppm DM, extremely above any recommendations for feeding sheep.

Case Outcome: New diets with high Cu were provided to early and late gestating rations. The following lambing and kidding season saw tremendous improvement in lamb and kid survival and ewe health. Diets were reformulated with the same approach the ensuing years following forage nutrient analysis to account for Cu and Mo. The third lambing season since adding the higher Cu diets starting with a horrific lamb loss of 40 out of 80 lambs born. A number of lambs or liver samples were submitted for mineral analysis (n=13) and necropsy (n=4). Again liver Cu concentrations were low in all samples that had not been treated with injectable mineral supplements. A new alfalfa hay had been purchased and fed in the gestation diets and subsequent nutrient analysis showed a Cu and Mo content of 15.4 and 22.1 ppm DM, respectively. The forage was removed from the diet and the TMR reformulated as previously to properly balance Cu and Mo. Lamb losses were resolved following the change for the latter lambing ewes.

Another interesting side note to this situation was an unexpected group of ewes lambing due to some rams not be removed. This ewe group was fed the maintenance diet and never exposed to the late gestation diets since it was not recognized they were pregnant. There were a number of issues with lamb viability in this group. The maintenance diet did address the Cu availability issue, but was not formulated to account for lower intake capacity and higher energy and protein needs. It was a testament to what a difference proper nutrition during critical periods makes for animal performance and health.

Case 3. Sheep flock experiencing high lamb losses

In this situation there were two adjacent farms owned by a father and his daughter and son-in-law. The farms were located in western Pennsylvania and the family has been in the sheep industry for multiple generations and active in 4-H showing of sheep. Both flocks are predominately grazed on cool season grass pastures on the respective farms and hay is harvested from these pastures and other fields to provide forage during the winter non-grazing period. A custom grain mix is provided at 1 lb/head/day for late gestating ewes. Trace mineral salt is provided free choice.

Presenting Problem: On the daughter's farm they experienced high stillbirth and neonatal lamb losses (>25%). Twenty four of 25 2-year old ewes died and no lambs from these ewes survived. The affected ewes were described as reducing feed intake and segregating from the flock, looking thin to cachexic and then found dead within 1-3 days from first signs. There were no lamb or ewe losses on the father's farm.

Diagnostic Process: Stillborn lambs were submitted for necropsy with a similar finding of no infectious agents or other obvious cause for their demise. Liver mineral concentrations showed low Cu, but no other issues. Two-year old ewes also had no significant findings accounting for their demise. Liver mineral concentrations of these ewes showed normal Cu, but very elevated Mo (6.68 µg/g DW; Reference 1.5 – 3.0 µg/g DW). The farms were visited to review management and feeding programs and evaluate the ewes. Forage samples were collected on each farm for nutrient analysis (Table 4). Water samples were submitted from both farms and from various sources within each farm. A sample of a new limestone product used to lime all fields on the daughter's, but not the father's, farm was collected. This product is a byproduct of the steel polishing industry from Pittsburgh and sold at a low cost compared to traditional agricultural limestone products.

Table 4. Comparison of forage copper and molybdenum content for the two farms.

Forage	Cu (ppm)	Mo (ppm)
Daughter's Farm		
Baleage	13	7.81
1 st Cut Hay	8.0	3.46
1 st Cut Round Bale	7.0	5.66
2 nd Cut Round Bale	11.0	7.15
Grain mix	6.0	1.9
Father's Farm		
1 st Cut Hay	12.0	1.45
2 nd Cut Hay	11.0	2.13
Grain mix	7.0	1.62

Nutritional Recommendations: The forage test results indicated a significant difference in Mo content between farms. No significant issues were identified in water samples from either farm. The daughter's farm had a new gas well drilled 2 years ago. Results of the limestone product analysis showed a high Mo content (21 ppm), which was confirmed by further DEP analysis on the farm as requested by the Pennsylvania Department of Agriculture once they were aware of the animal loss issues. A group of affected ewes were moved from the daughter's to father's farm and fed only forages from the father's farm. The remaining ewes were provided a 2 g CuO pellet for the remainder of the grazing season as it was decided a high Cu free choice mineral would not be a workable solution. Copper content of the premix incorporated into the grain mix was increased for the daughter's farm.

Case Outcome: There were no further animal losses for the remainder of the grazing season and all ewes seemingly were bred for early February lambing. Serum Cu concentrations were determined in 3 groups of 18 ewes to assess Cu status 6 months after dietary changes (Table 5). Lambing season went well with no lamb losses and good viability. The last 2-year old ewe and 2 other ewes died after all lambs were weaned. Liver mineral concentration showed a higher Cu (352 µg/g DW) and high Mo (6.8 µg/g DW) in this ewe. Ewes on the daughter's farm were given another 2 g CuO bolus for the grazing season. Forage testing is being completed to monitor the Mo status. How long it will take for the Mo to be removed from the soil and forages remains uncertain. Ongoing Cu status monitoring is shown in Figure 1.

Table 5. Comparison of ewe serum Cu concentrations.

Group	Serum Cu (µg/mL)	
	Mean	Range
Home farm	1.09 ± 0.20 ^a	0.82 – 1.46
Transferred	0.87 ± 0.13 ^b	0.63 – 1.26
Boluses	0.60 ± 0.28 ^c	0.26 – 1.01
Blood collected in December from 18 ewes for each group		
25% of "Bolus" ewes had serum Cu < 0.3 µg/mL		

Take Away Points to the Case Studies

These case studies all suggest a new clinical presentation of copper deficiency in sheep and goats characterized by stillbirths and weak, unthrifty neonates. This has not been a recognized consequence of copper status documented in any nutrition text. Other trace minerals, especially selenium, zinc and manganese, may also be involved in such clinical issues. Recent and past research has suggested lower hepatic trace mineral status in aborted and stillborn

fetuses, but no direct cause and effect relationship has been documented. These cases also underscore the critical need for timely forage analysis to recognize potential nutritional issues. Many small ruminant clients do not utilize this diagnostic resource, but it is critical to identifying potential nutritional risks. Mineral analysis of forages must be completed using wet chemistry and not near infrared spectroscopy (NIR) methods. One should always include molybdenum analysis with copper and possibly sulfur to ascertain potential for dietary copper availability.

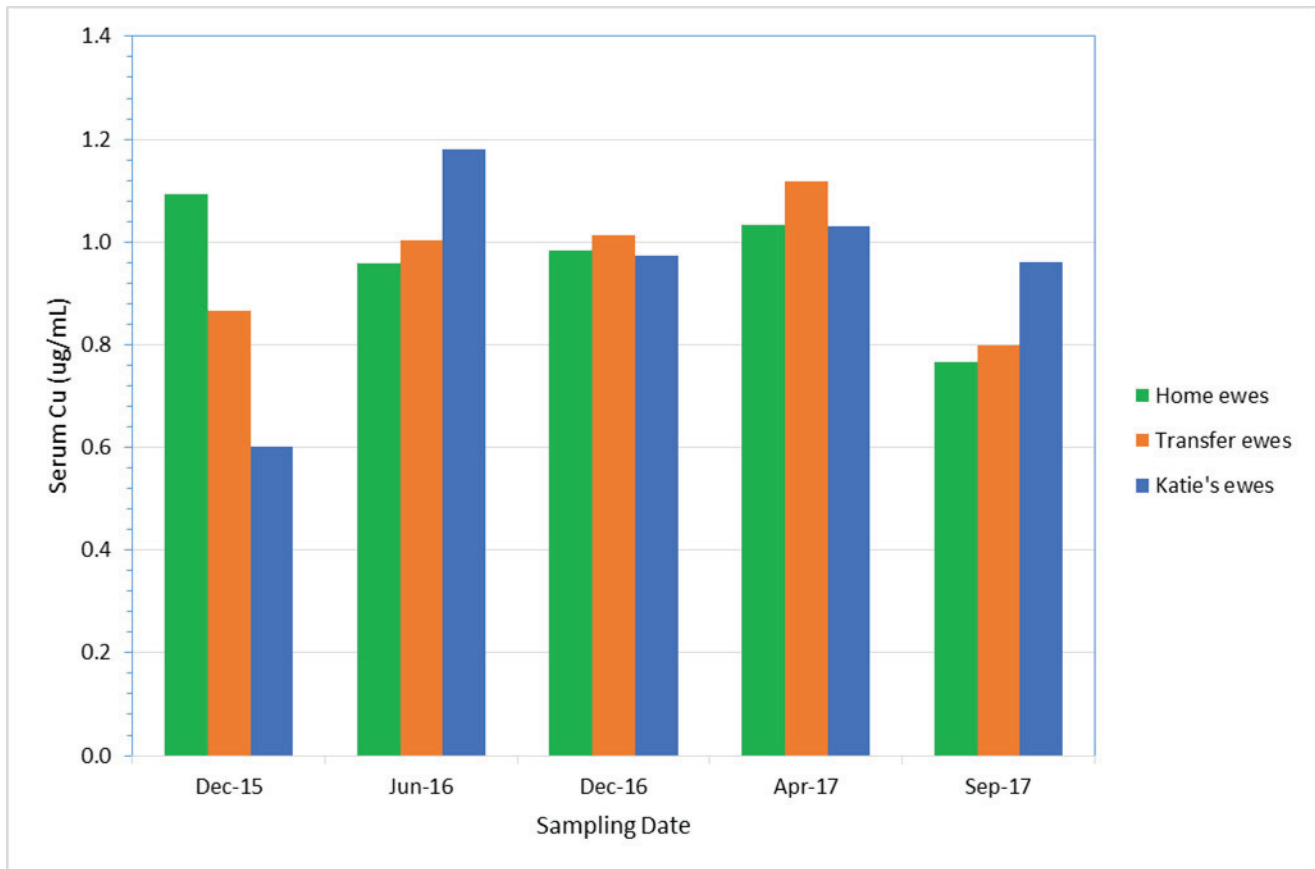


Figure 1. Comparison of serum Cu concentrations over time for three groups of ewes with different exposure to high forage molybdenum. Katie's ewes group was administered 2 g CuO boluses at three time points.

Update on Camelid Nutrition

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The most recent National Research Council (NRC) report on nutritional requirements of small ruminants was published in 2007. For the first time nutritional requirement recommendations for llamas and alpacas were included. Much of the information presented to validate llama and alpaca requirements were based on a single paper that modeled requirements for various physiologic states based on extrapolations from sheep and goat models (Van Saun, 2006). Due to the lack of published feeding trials, no models were suggested for predicting mineral requirements of llamas and alpacas. As with any NRC publication, newly published information becomes available providing opportunities to improve upon the initial recommendations. This presentation will summarize more recent publications addressing nutrient requirements and other pertinent information in addressing nutritional management of llamas and alpacas.

Feed Intake Quandary

Feed intake is the cornerstone of nutrition and ensuring adequate nutrient intake in support of various physiologic states. Characterizing feed intake in llamas and alpacas has been a challenge. Early information suggested that maintenance feed intake was lower compared with other species based on observations documenting a longer retention time for forage particles in compartment-1 (C-1). The prolonged retention time provides for greater fiber digestibility by the fermentative microflora in C-1. This is an adaptive mechanism facilitating animal survival in the more hostile environment with only sparse, low quality forage available.

The NRC (2007) shows expected dry matter intake (DMI) for llamas and alpacas to range from 1.0 to 1.5% of body weight (BW). Summarized data from South America suggested higher intake rates of 2.0% and 1.8% of BW for alpacas and llamas, respectively (San Martin and Bryant, 1989). Data from Chile suggested slightly lower DMI expectations for llamas (1.5% BW) and alpacas (1.7% BW) (Lopez and Raggi, 1992). If models to predict amounts of nutrients required are correct, then these differences in expected DMI will result in variable expectations for dietary nutrient density in providing these nutrients. This may explain differences in dietary energy and protein concentrations between North American and South American feeding guidelines.

To better address these differences studies directly measuring daily DMI in llamas and alpacas are needed. Unfortunately, estimating DMI for animals managed on pasture is extremely difficult. Eight published studies were identified that had sufficient individual intake data and adequately characterized feed composition to evaluate expected DMI and dietary factors that might control intake (Carmean et al., 1992; Lopez et al., 1998; Sponheimer et al., 2003; Robinson et al., 2005, 2006; Davies et al., 2007ab; Liu et al., 2009). Across these studies with 25 different forage comparisons, averaged DMI was $1.5 \pm 0.4\%$ BW for both llamas and alpacas.

Intake potential in llamas and alpacas was suggested to be related to dietary crude protein content with depressed intake resulting from low protein diets. In ruminant animals, microbial fermentation of fiber is the rate limiting step of intake and dietary NDF content is highly associated with feed intake regulation. From these seven studies, relationships between intake and dietary NDF and crude protein content were investigated. Unfortunately, no clear predictive relationships among DMI and protein or NDF intake were identified. In ruminants, NDF intake is maximized at approximately 1.2% BW. In these data, NDF intake as a percent of BW was lower ($0.87 \pm 0.26\%$ BW) compared with other ruminant animals. This observation would be consistent with fiber retention within C-1 and greater degree of NDF digestibility. Using these data we might set desired NDF intake to range between 0.9 and 1.0 % BW as a guideline for predicting DMI potential or identifying amount of needed dietary fiber.

Energy Requirements for Lactation

Two studies have described maintenance energy requirements for llamas (Schneider et al., 1974; Carmean et al., 1992), though these studies had somewhat divergent determinations. However, both studies had similar determinations of fasting energy requirement. Other studies have estimated maintenance energy requirement for alpacas with similarity to the averaged value ($72.85 \text{ Mcal/BW}_{\text{kg}}^{0.75}$) defined by NRC (2007). Other South American data are consistent with this energy requirement for alpacas (Flores et al., 1989). Two studies from New Zealand either suggested an energy requirement similar to NRC (Newman and Paterson, 1994) or a much higher energy requirement (Russel and Redden, 1997). Neither of these studies was designed to estimate maintenance energy requirements.

New studies have better characterized milk production in llamas over a lactation and changes in milk composition through the lactation (Pacheco and Soza, 2004; Vargas et al., 2004; Riek and Gerken, 2005). Milk composition was not found to differ in alpacas (Parraquez et al., 2003). Based on these new numbers it is recommended to increase energy requirements for milk production from 946 kcal ME/kg milk to 1296 kcal ME/kg milk. Using the data from Riek and Gerken (2005), lactation curves were modeled to help better predict milk output and total energy requirements in support of lactation.

New Perspectives on Protein Nutrition

There is only a single study that determined maintenance crude protein requirement ($3.5 \text{ g CP/BW}_{\text{kg}}^{0.75}$) of llamas. Some recent studies from the BYU group have used individual feeding trials to assess protein requirements (Sponheimer et al., 2003; Robinson et al., 2005, 2006; Davies et al., 2007ab). A more recent study has also provided some data for evaluating protein requirement in alpacas (Liu et al., 2009). Although one study (Davies et al., 2007a) suggested a much higher ($5.2 \text{ g CP/BW}_{\text{kg}}^{0.75}$) for llamas, regression analysis of retained nitrogen onto intake nitrogen per unit of metabolic BW is consistent with the previous protein requirement value. There was a suggestion that protein requirements may differ between high and low altitude, but there is not sufficient data to support this hypothesis.

There are large differences in recommended dietary protein content necessary to support llamas and alpacas in differing physiologic states. The recommendations in the NRC (2007) report suggest 9% crude protein in dietary dry matter for maintenance. This is in contrast to recommendations in South America that range from 6.5 to 8.8% CP in maintenance diets. Both systems are using the same requirement model, but the difference comes from the differing feed intake expectations.

A protein feeding study was undertaken in Australia, but insufficient information was provided to use this study to assess protein requirement. Of interest in this particular study are the objectives of determining the amount of undegradable protein (UDP) in the diet relative to fiber production and reproductive performance (Blache et al., 2011). Supplementation of UDP numerically increased fiber yield, but diameter was greater in supplemented groups compared with the unsupplemented group. There also was not documented improvement in fiber quality with specific supplementation of the amino acid methionine. Similarly, no beneficial effects of UDP supplementation were found on improvement of male fertility and reproductive development. Clearly more research needs to be undertaken to better clarify dietary protein fractions in llamas and alpacas.

Mineral Requirement Modeling

The primary missing piece of describing nutrient requirements for camelids is the lack of published feeding studies defining requirements for minerals and vitamins. Initially models extrapolated from mineral requirements for beef cattle, sheep and goats in converting a requirement to an amount per unit body weight and adjusting for differences in intake (Van Saun, 2006). These models seemingly depicted current feeding practices, but had not been truly validated through controlled feeding trials. As a result the NRC (2007) did not use any suggested model and instead suggested using the models predicting mineral requirements for sheep. Unfortunately, the models generated by the NRC committee for sheep mineral requirements are based on a factorial approach rather than a dietary concentration. To use these models directly, one would have to assume the bioavailability of

mineral sources was similar across species and the utilization of mineral in support of various bodily functions was similar in need and utilization efficiency.

Comparisons were made between the new NRC (2007) requirement models for sheep and goats in generating an appropriate model for llamas and alpacas. A summary of the adjusted recommendations for the microminerals are provided in Table 1. These suggested requirements are within typical feeding practices of llamas and alpacas in North America. These should be considered lower end of requirements and under certain circumstances may be adjusted upward to ensure the desired animal response. Inhibitory mineral interactions are not accounted for in these recommendations, so again dietary mineral content will need to be adjusted accordingly.

Table 1. Suggested dietary concentrations for the essential microminerals in llamas and alpacas for various physiologic states.

Nutrient	Averaged Requirement ¹	Extrapolated Requirement		
		Intake, mg/day ²	Diet, ppm ³	Group ⁴
Cobalt	1.76 µg/kg BW	0.11–0.28	0.12–0.15	M, G, P, L
Copper	0.12 mg/kg BW	7.2–19.2	8–12	M, G
	0.15–0.18 mg/kg BW	9–27.2	9–12	P, L
Iodine	8.8 µg/kg BW	0.5–1.4	0.55–0.65	M, G, P
	15.7 µg/kg BW	0.9–2.5	0.65–0.75	L
Iron	0.6 mg/kg BW	36–96	35–40	M, G, P, L
Manganese	0.33 mg/kg	19.8–52.8	22–25	M, G, L
	0.52 mg/kg	31.2–83.2	28–30	P
Selenium	6.5–6.8 µg/kg BW	0.4–1.07	0.42–0.45	M, G
	7–7.5 µg/kg BW	0.44–1.2	0.46–0.5	P, L
Zinc	0.56 mg/kg BW	33.6–89.6	45	M, G
	0.8–1.3 mg/kg BW	60–160	55–60	P, L

¹Extrapolated from nutrient requirements for beef cattle (National Research Council: *Nutrient requirements of beef cattle*, ed 7, Washington, DC, 1996, National Academy Press), sheep and goats (National Research Council: *Nutrient requirements of small ruminants: sheep, goats, cervids and New World camelids*, Washington, DC, 2007, National Academic Press).

²Estimated daily requirement based on a range of adult body weights from 60 to 160 kg.

³Dietary concentration (mg/kg) on dry matter (DM) basis. Nutrient density calculations based on an assumed range of DM intake between 1.25 and 1.5% of body weight.

⁴Physiologic states of maintenance (M), growth (G), lactation (L), and pregnancy (P) for which the requirement is defined.

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Review of Vitamin D Metabolism and Diagnostics in Camelids

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Introduction

Abnormal bone growth is a commonly diagnosed problem in young growing animals of all domestic species and is usually related to an array of nutritional deficiencies. A rickets syndrome in juvenile llamas and alpacas characterized by a shifting leg lameness and enlargement of the joints, most noticeably the carpus, has been described.^{1,2} Affected crias have variably shown a slowed growth rate, reluctance to move, and kyphosis. Radiographic evidence of physal ectasia and irregular growth plates were consistent with a diagnosis of rickets. A common clinical pathologic finding of low serum phosphorus concentration (< 3 mg/dl) led to the suggestion of inadequate phosphorus intake being the underlying etiology.

We showed that affected crias were vitamin D deficient and therapeutic administration of vitamin D would correct serum phosphorus concentration and reverse bony changes with clinical recovery.³ Further research characterized seasonal changes in calcium, phosphorus and vitamin D relative to disease risks,⁴ parenteral injections for prevention and treatment (Van Saun, published data),⁵ and dietary supplementation requirements (Van Saun, unpublished data). Unfortunately, vitamin D is one of the more toxic essential nutrients. Given the usual adage that if a little is good, a lot is better, the potential for vitamin D intoxication is of concern and needs further study. This presentation will review available data on vitamin D intoxication based on research data and clinical cases in llamas and alpacas.

Vitamin D Metabolism in Camelids

Metabolism of calcium (Ca) and phosphorus (P) is intertwined with vitamin D. Specific actions of the active form of vitamin D (1,25 dihydroxycholecalciferol) are mediated by the presence or absence of the counter-regulatory hormones parathormone (PTH) and calcitonin (CT). There is a dynamic balance between dietary ingestion and absorption of Ca and P from the intestines, resorption or deposition in the bone, recycling of P via the saliva and elimination of Ca and P via the urine, feces and milk. Vitamin D is of central importance in Ca and P metabolism having a direct effect on the rate of intestinal absorption, bone deposition and urinary loss. Of particular importance is the role of vitamin D in stimulating intestinal absorption and decreasing urinary losses of Ca and P. Without sufficient vitamin D activity, intestinal absorption efficiency of dietary P is greatly diminished. Renal excretion of P is a minor regulatory pathway in ruminants compared to the recycling of P in the digestive tract through saliva.⁶ Reduced intestinal absorption of P resulting from vitamin D inadequacy would potentially result in greater losses of endogenous P from salivary recycling, thus, inducing a hypophosphatemic condition. The question is whether or not camelids respond similarly to other species in the face of vitamin D toxicity with varying severity of hypercalcemia, or is serum phosphorus concentration more indicative of toxicity? In all our studies supplementing vitamin D either parenterally or orally, serum Ca was minimally influenced by vitamin D status whereas serum P concentration was highly associated with vitamin D status.

Vitamin D Toxicity

I am not aware of any published study characterizing physiologic response to high doses of vitamin D in llamas and alpacas, though we have data from an unpublished study. There is one published case study⁷ and information from various clinicians regarding suspected or confirmed cases of vitamin D intoxication of llamas and alpacas.

Following our studies on the pathogenesis of vitamin D deficiency disease and its treatment, we initiated a study to assess the degree of toxicity vitamin D has in the llama and alpaca. Initially 12 llamas and alpacas were assigned to 1 of 4 treatment groups with varying levels of a single vitamin D intramuscular injection (0, 8000, 16000 and 32000 IU/kg BW). These values were based on toxicity studies in other species with 32,000 IU/kg BW

resulting in acute death in dairy cattle.⁸ Serum vitamin D concentrations showed a dose-dependent response; however, no clinical evidence of acute vitamin D toxicity was appreciated. Following these results, a single animal was treated with a higher dosage (64,000 IU/kg BW) and again no acute toxicity was observed on clinical or postmortem evaluation. In reviewing these results, serum vitamin D and P concentrations were in the toxic range for most other species, but serum Ca remained within normal limits. The high serum P values are of concern since they may result in a precipitation of Ca and P crystals in blood, urine and body tissues over time. A third trial using 9 llamas at 3 vitamin D treatments was initiated using an emulsified form of vitamin D, as was used in the previous supplementation studies. Again dose-dependent vitamin D responses were observed, but no clinical evidence of acute toxicity. Clinical chemistry panels were completed on all animals and no evidence of renal dysfunction or other abnormalities were evident. Of the animals euthanized, there was evidence of metastatic mineralization of blood vessels in those receiving the higher doses (>8000 IU/kg BW). Long-term toxicity problems were not addressed in this study and need to be of concern given the observed changes in serum P concentrations.

In the published case study, crias were intoxicated with oral vitamin D at doses of 3750 IU/kg/day and 12,987 IU/kg/day for 7 and 5 days, respectively.⁷ These crias presented with both hyperphosphatemia and hypercalcemia and had azotemia with nephrocalcinosis identified on necropsy. In comparing the two different studies, response to vitamin D intoxication may depend upon dosing method, duration and age of the animal.

Diagnostic Evaluation

Concentration of 25-hydroxycholecalciferol (25VitD) in serum is the standard for assessing nutritive status relative to vitamin D. The challenge here is having appropriate reference ranges for interpreting the values (Table 1).⁹ The two vitamin D intoxicated crias of the case study had serum 25VitD exceeding 600 nmol/L.⁷

Table 1. Age-Based Criteria for Serum Vitamin D (25-Hydroxycholecalciferol) Concentrations in Llamas and Alpacas

Age Category	Months of Age	Units	Mean	Stand. Dev.	Median	Expected Range
Cria	1–6	nmol/L	136.4	116	76.0	26–344
		ng/mL	54.6	46.4	30.4	10.4–137.6
Weanling (Tuis)	7–12	nmol/L	85.2	58.3	73.0	28–316
		ng/mL	34.1	23.3	29.2	11.2–126.4
Yearling	13–24	nmol/L	112.7	80.4	81.0	40–359
		ng/mL	45.1	32.2	32.4	16–143.6
Adult	>24	nmol/L	119.2	91	91.0	30–414
		ng/mL	47.7	36.4	36.4	12–165.6

ng/mL, nanogram per milliliter; nmol/L, nanomole per liter.

Note: Concentrations below 25 nmol/L (10 ng/mL) or above 500 nmol/L (200 ng/mL) are consistent with deficiency and toxicity disease risks, respectively.

Serum vitamin D concentrations are expensive and often not available in a timely fashion. Animals may be exposed or at risk for vitamin D intoxication, yet may have normal renal function. Elevations in serum Ca or P concentrations may not be sufficient to adequately diagnose risk from vitamin D intoxication. With the uncertainty in using either hypercalcemia or hyperphosphatemia as a diagnostic indicator, another possibility is using the product of Ca and P concentrations as a measure of risk for metastatic calcification. The calcium x

phosphorus (CaP) concentration product (mg^2/dl^2) has been used in human medicine as an indicator for risk of renal mineralization. A threshold of $55 \text{ mg}^2/\text{dl}^2$ has been used, but its applicability to camelids needs to be validated.

Using multiple collections of serum Ca and P concentrations ($n=1189$) from survey and vitamin D administration studies, CaP product values were evaluated. Overall CaP product was highly associated ($r^2=0.37$; $P<.0001$) with vitamin D status. Age group was not significant, but there was a significant interaction between CaP product and Age group ($P=.0002$). Healthy, non-vitamin D treated adult camelids had a CaP of $48.1 \pm 3.1 \text{ mg}^2/\text{dl}^2$. Age influences this parameter with yearlings ($75.9 \pm 5.1 \text{ mg}^2/\text{dl}^2$) and crias ($95.3 \pm 3.2 \text{ mg}^2/\text{dl}^2$) having higher values. Association between CaP product and vitamin D was stronger for higher vitamin D concentration than for low vitamin D, though these preliminary data would suggest CaP product could be a proxy for diagnosing vitamin D deficiency. This parameter was used to evaluate potential vitamin D intoxication in an alpaca herd exposed to a high dietary vitamin D (191,000 IU/lb) supplement over a period of months. The percent of adult samples having a CaP greater than or equal to $60 \text{ mg}^2/\text{dl}^2$ was significantly higher in samples from vitamin D injected camelids (43.8%) and the samples collected from the vitamin D supplement exposed camelids (31.9%) compared to camelids not supplemented with vitamin D (16.2%). The two vitamin D intoxicated crias from the case study presented with CaP products of 194.6 and $155 \text{ mg}^2/\text{dl}^2$; values much higher than non-exposed crias.⁷

Clinical Presentation and Treatment

Vitamin D intoxication results in non-specific clinical signs of anorexia, weight loss, lethargy, depression and renal dysfunction. Additionally cardiac arrhythmias or lameness may be present. One challenge here is that many of these signs are often associated with problems of vitamin D deficiency and often a first response in young camelids is to administer parenteral vitamin D. Additional clinical signs associated with cardiac, renal or respiratory function may be present depending upon the degree of soft tissue mineralization.

There is no therapeutic correction for vitamin D intoxication. One should remove the source of vitamin D and provide supportive care to ensure hydration and renal function. Dietary content of calcium and phosphorus should be minimized to reduce available mineral for absorption. Intensity of supportive care will depend upon the severity of renal dysfunction.

Summary and Conclusions

Llamas and alpacas seemingly are seemingly tolerant of acute vitamin D toxicity. However evidence is present that higher doses of vitamin D may result in altered P metabolism with the possibility of Ca and P precipitation in urine and tissues. Vitamin D supplementation should be approached carefully and under the guidance of a veterinarian. Increasing the suggested treatment dosage of vitamin D is not recommended as there is no evidence of improved effect and suggestions of deleterious effects to animal health are evident.

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