Applied Trace Element Nutrition in the Bovine Animal

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Foreward

Within the scientific and academic community there have been great accomplishments in understanding the epidemiology and pathogenesis of cattle diseases. Even so, the food animal industry continues to suffer significant monetary losses from disease pathogens and non-infectious disease disorders. As practicing veterinarians, our knowledge is limited only because of our inability to keep pace with the various disciplines within the scientific community. The area of biochemistry and cellular physiology, once thought to be subjects limited to the classroom, may hold the answer to improving the basis of effective and profitable herd health programs.

Many livestock disease situations are a result of multifactorial health damaging situations that revolve around environment, area of origin, genetics, disease exposure, nutrition and agricultural practices. More significant in our industry is the highly aggressive sales approach utilized in animal health advertising that imply production problems are handled and cured through the use of a particular product. These realities are in conflict and create a serious clinical problem for the animal health specialist. Producers expect solutions to be quick and usually revolve around vaccination and treatment programs. Understanding nutrition and the impact it has on overall health is one way in which to remove this conflict and make better use of the biologicals and pharmaceuticals we have in our arsenal.

By incoporating basic knowledge on cellular physiology, assay techniques for the protective oxidative (metallo) enzymes, and the use of computerized spreadsheet data analysis, my practice has developed an exciting and challenging approach to total herd health. It requires discipline, time, and continuing education. Resolving the conflict does not involve finding "new" cures, but rather a re-evaluation of cultural and scientific data already in existence with proper application to the intensified cattle production industry.

Accept the challenge!

R. T. Coffey

Introduction and Review

The fundamentals of trace element nutrition in the bovine and the role this vital area of physiology plays in

overall health and performance has expanded rapidly in recent years. All too often trace element physiology is ignored or regarded as secondary in the pathogenesis of bovine diseases. Nutrition is dealt with in a very basic format and the actual role of the practicing veterinarian in this area is usually minimal. Even with the assistance of a nutritionist it is usually limited to "macro" nutrition which covers protein, TDN, fiber, fat, energy, fat-soluble vitamins, Ca,P,K, and Mg. Trace elements are added in some form of trace element "pack" to theoretically satisfy NRC requirements with very little attention to trace element interactions that may alter those requirements.

Research in private industry, universities, and government has developed basic nutritional guidelines for the various cattle types. (1,2) These guidelines, though fundamentally sound, have their own inherent problems when it comes to application in the field. Cow/calf nutrition in a pasture environment is more difficult to control and relies on palatable vitimin-mineral supplements to complement pasture forage conditions. In contrast, feedlot and daily nutrition have become quite similar to swine nutrition in that there is a tremendous amount of control of total daily intake, ingredient composition and quality of the final ration. The actual interrelationship of trace element nutrition and the physiological health of the animal is still left to chance. The ability of the clinician to troubleshoot this area is dependent upon his or her knowledge and ability to correlate that interrelationship and make the proper adjustments in the nutritional and herd health program.

Some of the inherent problems with bovine nutrition and the involvement of veterinarians and nutritionists in bovine diets include:

- A. Knowledge: nutrition, physiology, applications
- B. Significance of nutrition vs. pathogen
- C. Deficiency(s) acquired over time
- D. Nutritional guidelines: chemical analysis vs. book values
- E. Water quality and quantity
- F. Diagnostic methods: animal tissues, feedstuffs

Knowledge

It is virtually impossible to accumulate the amount of

data available from scientific research and apply it on a daily basis. This does not justify apathy or the inability to utilize the current knowledge. It does present a healthy challenge to those of us involved in veterinary medicine and requires that we utilize computerized informational resources and other experts in livestock production to process this information for the betterment of the client and livestock enterprise. It is mandatory to have basic understanding of nutrition and physiology in order to be involved. Though a concurrent effort between veterinarian and nutritionist is vital, veterinarians must accept their unique role in their ability to correlate the impact of cellular physiology to nutritional parameters.

Significance

It is difficult at times to correlate the significance of nutrition and the role it plays in infectious and non-infectious diseases. The debate on whether or not trace element deficiencies actually cause disease or are precipitated by chronic disease situations continues in full force. (3) It is very apparent that trace element deficient animals are predisposed to physiological insults that lead to a higher incidence of disease and tissue damage, but the question usually arises as to the origin of the original insult. The evidence is leaning toward the fact that biological systems that are compromised due to decreased enzyme activity, decreased amounts, and inhibition of induction of protective enzyme systems that rely on various trace elements are indeed more susceptible to infectious agents as well as cellular damage from uncontrolled oxidative processes that for the most part are considered normal.

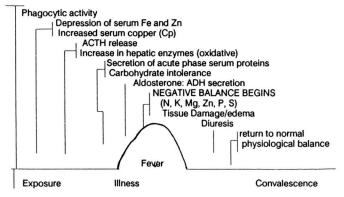
Not just lack of knowledge but the perception that drug and biological usage is mandatory in the "first wave" treatment of disease must be replaced by the perception that alteration in basic physiology is active present, and what can be done to return the animal back to normal to enhance treatment programs (ie. energy and protein metabolism; liver and kidney function). Many times there is little emphasis placed on nutritional parameters. The sequence of physiological events, as shown in Figure # 1, that take place prior to, during, and after the onset of clinical disease must be understood when utilizing nutritional therapy in treatment programs. (4) Anticipation of these same physiological events is also important when nutritional profiles are incorporated into a herd health program.

Acquired Deficiency

Trace element nutritional disorders are usually acquired over an extended period of time. Figure #2 illustrates the multifactorial influence in acquired deficiencies. Though a single antagonist or insufficient supplementation will contribute to a primary deficiency, there is usually more than one situation that must be considered. Whether

FIGURE 1

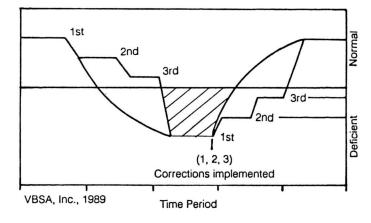
Sequence of Biochemical Changes in Infectious Disease



(From: Nutritional Biochemistry and Metabolism, 1985)

FIGURE 2

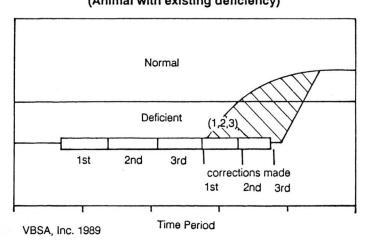
Multifactorial Influence On Trace Element Deficiencies (Acquired deficiency from Inadequate nutrition or antagonist influence)



it starts at parturition, weaning or after improper intake of excessive levels of antagonistic elements or prolonged deficient intake of essential trace elements, it usually takes time. But, after this time period, the consequences are manifested very quickly and in a detrimental fashion.

In some herds there is no starting point indentified. The deficiency syndrome has existed for years and has never been identified as a contributory factor in herd health problems. Accordingly, to establish the fact that a deficiency exists is only the beginning phase of the corrective process. You must identify those contributing factors that precipitated the deficiency. If three factors existed and you identified and corrected two factors, the chance of returning the animal to normal is reduced or at least extended over a greater period of time. Figure #3 illustrates this same scenario except in an animal with an existing deficiency compounded by additional situations that make the deficiency worse. In this instance you would expect the

Multifactorial Influence On Trace Element Deficiencies (Animal with existing deficiency)



animal to be more sensitive to any situation that generates phsyiological distress such as environmental changes, feed change, vaccination, etc.

A good example for Figure #2 would be a group of 450# crossbred calves arriving into the feedlot. Perfectly healthy, they undergo the usual processing, slowly started on feed, and after 30-45 days everything seems to be progressing properly. About 5 months into the feeding period (800-900#) the bovine respiratory disease complex surfaces. The diagnosis of IBR or BRSV with secondary pasteurella is consistent with clinical signs, laboratory data, and post mortem lesions. Response to treatment is only fair, revaccination (bacterin or viral vaccine, killed or MVL) actually makes some of the animals get worse. Secondary lung changes, adenomatosis, emphysema, etc. are found in several animals. The cattle slowly progress through this stress period, gradually get better, and after 2-5% death loss and 30 days lost in gains they go to market - with considerable financial loss to the producer. The nutritional parameters, ration, and serum profile analysis that could definitely influence this endemic disease "break" would go as follows: (Table 1)

Ration Specifications	Serum mineral profile		
1.) NEg > 580 Kcal/lb., NEm > 850 Kcal/lb. 2.) K:Mg > 4:1 3.) Na intake .1% with K > 1%, Mg < .2% 4.) S intake > .3% 5.) Cu meets NRC requirement of 8 - 15 ppm 6.) Fe > 200 ppm	Ca P Mg K Cu Zn	7.8 mg/dl 8.1 mg/dl 1.6 mg/dl 28.0 mg/dl .65 ppm 1.8 ppm	
7.) Mo> 3 ppm 8.) Selenium intake <.2ppm	Fe Se Mo	3.2 ppm .078 ppm .215 ppm	

These eight items could directly contribute to the following physiological changes:

- Liver disease: fatty liver, liver abscesses as a result of high energy intake.
- Copper deficiency: precipitated by the Mo, S, Fe interaction.
- Selenium deficiency: inadequate supplementation,
- Vitamin A deficiency: from increased nitrate reductase activity in the rumen (if nitrates are present). (5)
- Free-radical cellular oxidative stress: from decreased quantity and activity of the primary oxidative enzymes as a result of trace element deficiencies (Cu,Zn-superoxide dismutase, selenium dependent glutathione peroxidase).
- Immune suppression and/or a predisposition to hypersensitivity reactions: alteration of cAMP and cGMP within immunocytes.

To recognize these nutritional problems at the time of the "break" is of little value since the physiological changes have taken place over time, and it will take time to correct those physiological changes. The serum mineral profile analysis doesn't appear to be too striking, but there are several "warning signs" that dictate attention to the total nutrition in the herd. The first sign is the disease break itself. Everything was progressing satisfactorily in this herd and unexpectedly they became ill. Past herd health programs should have prevented, or at least curtailed, the respiratory pathogens from causing a serious problem. The question then arises - is it a primary disease or is it secondary to an underlying nutritional disorder? Understanding that trace elements, by their place in enzyme structure, play a vital role in energy metabolism and immune response, there are several nutritional parameters of concern in this example.

- Though copper intake meets NRC guidelines, the sulfur, molybdenum, and iron content in this ration forces the copper requirement to be closer to 50 ppm. Serum analysis shows copper levels to be adequate at .65 ppm but since molybdenum is .lppm, some of this copper may not be bioactive (ceruloplasmin copper) and probably ionic. (6)
- 2. The elevated Mo is consistent with ration intake and excess Mo in the ration makes these animals less tolerant to any nitrate present in the rumen.
- Serum magnesium is deficient. Evaluation of the magnesium metabolic pathways shows that dietary K and Na are not correct. One would also suspect inhibition of the active transport system involving Na⁺, K⁺ ATPase required for magnesium uptake.
- 4. Serum Ca and P is inverted and the dietary P intake does not correlate to these elevated levels.

Hyperphosphatemia can be due to kidney failure, or excess molybdenum which will alter phosphorus metabolism. (6,7) Also, completion of the cellular terminal respiratory enzyme sequences yielding ATP requires cytochrome c oxidase, a copper enzyme. Therefore in copper deficiency, incomplete phosphorylation can lead to a buildup of inorganic serum phosphorus.

- 5. Control of free radical accumulation within oxygen consuming cells requires superoxide dismutase, a copper-zinc containing enzyme, glutathione peroxidase, a selenoenzyme, and catalase, a heme containing enzyme. Deficiencies of these elements would compromise the level and activity of these enzymes.
- 6. An anamnestic IgG immunoglobin response and/or an IgA immunoglobin response in the upper respiratory tract dramatically increases oxygen consumption by immunocytes increasing the demand for the protective oxidative enzymes. (8)
- 7. Alteration of the cyclic AMP/GMP ration within lymphocytes will alter the type of immune response initiated, if any.
- 8. Insufficient GSH-Px activity due to selenium deficiency can lead to excessive leukotriene production and exaggeration of lipid peroxidation within the lung tissue. This leads to pulmonary edema and pathological changes associated with immune-initiated type responses.(9)

There are numerous other physological processes that are taking place, but this brief scenario should give you some insight into the necessity of adequate trace element nutrition and the importance of being able to evaluate these situations prior to disease. Indeed, nutritional consultation is an integral part of any herd health program.

If this same scenario were applied to Figure #3, the feeder cattle that "appeared" perfectly normal upon arrival but already trace element deficient (copper, selenium, magnesium), one would expect the endemic disease pattern to start earlier in the feeding period.

Therefore, when confronted with a disease situation or production disorder in which a trace element problem exists, it is that much more difficult to diagnose, interpret, and correct. Also herd health vaccination programs may not be as effective in these situations. (Figure #4).

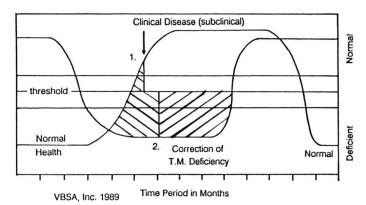
Even after corrective measures are taken, the disease pattern may persist for several weeks due to the time necesary for cellular systems to return to normal. This is especially significant in feedlot cattle when time factors are the difference between economic success and failure.

Nutritional Guidelines

Nutritional recommendations and records utilized to

FIGURE 4

Trace Element Deficiencies vs. Incidence of Disease



keep track of nutritional data are usually formulated from "programmed" information. Very seldom is actual analytical data for feedstuffs amd water utilized. What is in the book may not be what is actually present in the product. There is wide variation in trace element and heavy metal content of feedstuffs based on area of production and production technique used. Certain forages are extremely detrimental to trace element physiology in ruminants based on forage type, stage of growth, soil pH, and heavy metal content. Micronutrient interactions and availability factors must be considered.

An example of the feedlot nutritional guidelines recommended is shown in Figure 5. Though this ration meets all accepted standards for those nutrients shown, there are a number of parameters that must be considered when

FIGURE 5

Sug	gested Nutrients to	or Receiving Stre	ssed Cattle
	(450 - 600	lbs. body weight)	
Guideline	s are taken from NRC, Texas Io, Texas and Sh	Agricultural Experiment S hiloh-LVS, Newton, Iowa	Station (TAES), Amaril-
RIENT	SUGGESTED		0.00.000

NUTRIENT	SUGGESTED	TAES	SHILOH-LVS
	RANGE	Receiving Ration	Receiving Ration
Dry Matter %	70 - 85	88	70 - 85
Crude Protein %	12.5 - 14.5	13.9	12.5
NEm Mcal/cwt	70 - 75	72	70
NEg Mcal/cwt	37 - 40	39	36 - 40*
Ca %	.68	.7	.71
Р%	.46	.5	.40
К%	1.2 - 1.4	1.3	1.2 - 1.4
Mg %	.23	.3	.3235
Na %	.23	.3	.31
Cu ppm	10 - 15	15.7	54 ~
Zn ppm	50 - 75	81.7	159 ~
Mn ppm	20 - 30	26.3	99 ~
Fe ppm	100 - 200	210.7	< 200 ~
Co ppm	.12	.15	.3 ~
Se ppm	.12	.15	.3 ~
Vit A IU/Ib		3000	7500
Vit E IU/Ib		50 - 100	300 - 500
Vit D IU/Ib			1200
Mo ppm			<2
Silicon ppm			< 100
Aluminum ppm			< 200
Water analysis must	be taken into considerati	on	

Dependent on cattle type and feedstuffs

Elemental ratios, physiologic state of the animal, type of nutrient and area of origin must be considered

suggesting nutritional guidelines whether it be the receiving diet, grower, or finisher diet. Type of cattle, breed, area of origin, herd health program, feedstuffs available, and feedstuff assay must all be considered. This is a "textbook" ration. Actual feedstuff assay may show a completely different nutrient profile and require different levels of supplementation for important micronutrients based on these assays.

Water

Water quality and quantity have a dramatic influence on livestock performance and health. Water analysis is an essential piece of data in any herd health program. Of greatest significance are nitrates, sulfates, iron, salts, and heavy metals. Table #2 on the following page lists some of the more important analytical guidelines and the tolerable levels.

TABLE 2

Water Quality Guidelines (partial listings)

Chromium	< .05 PPM	Cobalt	< 1.0 PPM	Copper	< 1.0 PPM
Flouride	< 1.2 PPM	Hardness	< 2000 PPM	Iron	< .4 PPM
Lead	< .05 PPM	Magnesium	< 1000 PPM	Mn	< .05 PPM
Mercury	< .003 PPM	Molybdenum	< .06 PPM	N	< 100 PPM
pН	5.5 - 8.3	Phosphate	< .7 PPM	к	< 20 PPM
Selenium	< .01 PPM	Sodium	< 800 PPM	Zinc	< 5.0 PPM
Sulphates	< 500 PPM				

Source: 1) Mineral Levels in Animal Health, Sherpa International, 1988 2) Nutrients and Toxic Substances in Water for Livestock and Poultry, National Academy of Sciences, 1974

All too often we assume that water intake is adequate just because it is offered. There are several tables and formulas that can be used to estimate water requirements such as the following formula for feedlot cattle: (10)

Gals./day = -4.939 + (0.104 x Temp F) + (0.2923 x DMI lbs)-(2.5971 x Precip. inch.) - (1.1739 x % dietary salt)

It is amazing how often water requirement is underestimated and the impact water analysis will have on trace element metabolism.

Example:

A 800# steer consuming 10 gallons of water a day with a water analysis of 200 ppm sulfates and 1.3 ppm iron. This will add 8 grams of sulfur (an additional .1% to the ration) and 53 mg of iron which is 20% of the NRC requirement. Iron dissolved in water is very available. Both the S and Fe in the water will increase the copper requirement by 20-30 ppm depending on ration type, cattle type, and other antogonistic factors present in the feed such as the amount of molybdenum. Water is discussed in more detail in the applications part of this presentation.

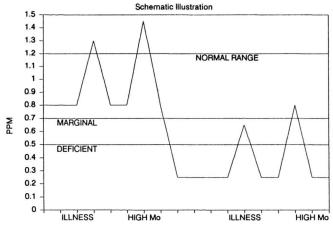
Diagnostic Methods

Current diagnostic methods for blood, serum, plasma,

urine, liver and kidney provide important information but all too often the diagnostician or practitioner will hang his hat on one piece of data and fail to analyze the whole problem. Interpretation of data is more important than the actual numbers generated. A classic example of this is serum copper as a parameter for copper status in the animal. Figure #6 is a schematic that illustrates some of the problem of interpretation. During illness, serum copper will usually rise as ceruloplasmin activity increases (and serum iron should decrease); with high molybdenum intake, especially from fresh forages (> 4ppm) serum copper levels will rise once again. (6)

FIGURE 6

SERUM COPPER AS A DIAGNOSTIC PARAMETER



As can be seen in Figure 6, depending on where and when you take the serum sample, the results have better than a 75% chance of being inaccurate and may even indicate a normal serum level. The increase in serum copper from illness is biologically active copper; the increase from excess Molybdenum is not biologically available. This presents one more piece of data that has to be interpreted properly and if you have no idea of the Mo intake and serum Mo levels in the animal you cannot make a proper evaluation. When analyzing feedstuffs, absolute values must be correlated to bioavailability and antagonstic parameters of one element to another. Per the above example on Mo and its influence on copper in serum, many clinicians and nutritionists do not recognize Mo levels in feedstuffs as significant unless present in very high levels. 3ppm are not uncommon, and dependent on the Values source, can be very detrimental to cattle. Of all species, cattle are the most susceptible to Mo toxicosis, especially subclinical forms with feed intake in the 4-6ppm range. Any time molybdenum exceeds 5 ppm in the total ration there is need for concern as regards copper metabolism, xanthine oxidase activity in vivo, and nitrite formation in the rumen. Cattle on low Mo intake are more tolerant to nitrates in feedstuffs and water; high Mo intake may actually be conducive to nitrate formation with increased activity of nitrate reductase (a Mo containing enzyme) within the rumen microflora.

There has been significant advances in predicting the availability of specific trace elements based on antagonistic factors of one element to another. A good example of this is the ruminant copper availability equations developed by Suttle (Figure 7).(6,11) When used in combination with optimum elemental ratio's it is possible to estimate the needed copper requirement in rumanints for a specific diet. There are no mathematical formulas that can be specific enough to address the everchanging biological systems, but it does give a better idea as to the needs of the animal.

FIGURE 7

Copper Amilability (Acu%) Coefficient

Pasture Grass $A_{Cu}\% = 5.72 - 1.297S - 2.785 (1nMo) + .227 (Mo x S)$

Hay $A_{Cu}\% = 8.9 - .70 \text{ lnMo} - 2.61 \text{ lnS}$ Silage $A_{Cu}\% = 10.6 - 6.65 \text{ lnS}$ (12)

(Results are expressed as a percentage)

Limits of the equation: .4% sulfur; 6 ppm Mo

Suttle

Another example of the bioavailability and antagonistic factors that must be considered when evaluating cattle diets is that of the sodium, potassium, and magnesium relationship.(12) Though not considered trace elements *per se*, they are very important in overall electrolyte and trace element physiology. Magnesium is an essential element for many enzyme systems, antioxidants, and bone formation. In ruminants, its absorption is an active transport process within the reticulo-rumen and to a limited extent in the omasum. This active transport process is controlled by the enzyme Na⁺,K⁺-ATPase. When there is elevated dietary potassium intake (>2%) it will diminish magnesium availability. This in part is due to suppression of the active transport process due to excess potassium intake.

If the rumen Na:K molar ratio is 4:1 there will be decreased magnesium absorption.(13) This ratio is altered by both the dietary K and Na content as well as the Na content of saliva. Since a great deal of the rumen sodium content comes from saliva and not directly from the diet, the prolonged effect of excess potassium intake is to replace salivary Na by K. Potassium can replace up to 50% of the sodium in saliva if there is prolonged excess potassium intake. The rumen Na:K ratio is maintained at a normal level as long as the dietary K:Na ratio is approximately 5:1.

The Na⁺, K^+ ATPase enzyme can also be irreversibly inhibited by an accumulation of the superoxide free radical.(14) Therefore, with deficiencies in copper, zinc, or manganese, the superoxide dismutase enzyme activity and quantity is diminshed, allowing for free radical accumulation in various tissues. Indirectly, copper deficiency may have a definite impact on magnesium metabolism. Similarly, selenium deficiency will lead to decreased activity and quantity of selenium dependent glutathione peroxidase. As this enzyme decreases in activity or quantity the potential for hydrogen peroxide buildup increases in tissues. Hydrogen peroxide will inhibit Cu, Zn-superoxide dismutase activity.(15) This would allow superoxide accumulation, and as in copper deficiency, may inhibit the Na⁺,K⁺AT-Pase enzyme. Therefore, it is reasonable to assume that Cu and Se deficiencies will also impact Mg metabolism.

To complicate the situation, as subclinical magnesium deficiency develops there is a potential problem in glutathione formation, a necessary substrate for glutathione peroxidase activity, as well as formation of other Mg-dependent enzymes. If this were to occur, accumulation of the oxidized form of glutathione (GSSG) may oxidize glutathione peroxidase to an inactive state. Hence, even with adequate selenium supplementation in the diet it does not necessarily mean there will be adequate glutathione peroxidase activity at the cellular level to elevate a buildup of hydrogen peroxide.

This whole scenario demonstrates a complex interrelationship for just two elements but adequately explains why subclinical magnesium deficiency (< 1.8 mg/dl) is present in many clinical situations and how copper and selenium deficiencies, though not directly related to magnesium metabolism, will impact the biological availability and activity of magnesium.

In practice, this is one reason for utilizing the oxidative enzyme assays as an aid in evaluating copper, zinc, selenium, and iron status.(16) This, along with the extensive analytical work on feedstuffs and water, conventional diagnostic parameters, and detailed history allows for better insight into the trace element status in cattle.

Trace Element Problems

It is essential that the clinician develop a uniform approach when evaluating nutritional relationships. It should already be clear that there are many factors involved and simultaneous evaluation is impossible without this structured approach. Six basic steps are used to analyze nutritional influences on overall cattle health and performance. These are:

A. Anamnesis

- B. Environment
- C. Tissue Collection and Analysis
- D. Feedstuff Analysis
- E. Interpretation
- F. Applications

Anamnesis

The first and most important aspect of any clinical or

subclinical medical problem is a complete anamnesis (clinical history). Experience has shown that this is the most neglected aspect of practice. It is time consuming and for the most part, many do not understand the significance of this kind of data or how to put it into a meaningful format. Since trace element deficiencies are usually acquired over an extended period, it is essential to have as much background data as possible on the cattle involved.

Feedlots have found over the years that there is some consistency to cattle origin as it influences the overall performance and health. Many times it does not pertain to just breed type. We would like to think that it does pertain to an animal that arrives in the feedlot which is physiologically sound and able to adequately respond to the stresses that it is forced to overcome. A portion of this favorable response is due to the sound nutritional status of the cow-/calf herd and the recognition by the cow/calf producers that their type of cattle have specific nutritional demands and the producer takes the appropriate steps needed to insure proper nutrition. Also, it is this type of producer that utilizes a well conceived herd health program to aid in overall cattle performance. Having a good picture of the origin of the cattle will assist in the interpretation of any nutritional problems that develop.

There are two key questions that should always be asked and substantiated in any clinical history:

■ What is the response to vaccinations?

■ What is the response to treatment?

Repeated antigenic stimulation will promote oxidative reactions within immunocytes. If the reactions are uncontrolled at the cellular level there can be serious damage done to these cells with an overflow of free radical damage to target organs such as the lung or intestine. Antigen recognition, lymphocyte proliferation, inflammatory responses, etc, all involve biochemical reactions that directly or indirectly rely on enzymatic control from enzymes that require specific trace elements to function. More often than not, when nutritional clinical or subclinical deficiencies are involved there will usually be a generalized poor response to vaccination programs (Figure 8). Also, even with timely treatment regimes the tissue damage is so great that control of secondary bacterial infections seems to be of little value.

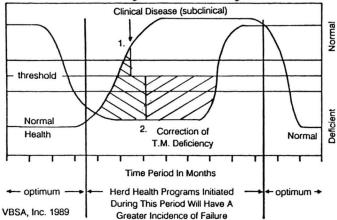
Micronutrient deficiencies are usually related to immuno-suppression, that is, a lack of a normal immune response. It also should be related to "lack of control" of an immune response. An example of this is arachidonic acid metabolism that leads to prostaglandin, thromboxane, and leukotriene synthesis.(9) Acceleration of leukotriene production will create an allergic type of reaction in pulmonary tissues. One reason for this is decreased seleniumdependent glutathione peroxidase activity within the cell.

The clinician needs to apply his or her knowledge to the physiological clinical course of disease processes (Figure 1) and observe whether animals are capable of respon-

FIGURE 8

Trace Element Deficiencies vs. Incidence of Disease





ding in a normal and timely fashion. As stated before, once nutritional problems are identified, it is tough problem to deal with, but the sooner it is realized and corrected the overall herd health program will proceed with greater benefit to the animal.

Predominant breed, sex, and age all significantly influence the trace element requirements over time. Exotic breeds, such as Simmental and Limousin have a 20-30% greater copper requirement than crossbreed calves or standard English breeds such as Angus and Hereford. Likewise, rapidly growing calves (3lbs/day ADG) will have a greater trace element demand and be more susceptible to trace element antagonists than those calves in a backgrounding program (1.0-1.5 lbs/day AGD). The genetically superior cattle of all breeds, if allowed to perform at maximum capacity, will have a considerably greater trace element requirement when raised under a wide variety of nutritional and environmental programs.

Environment

Understanding of the environment, past and present, is similar to the anamnesis. Be aware of the environmental factors that increase or decrease nutrient requirements. Minimize stress especially in regards to housing and feeding practices. Physiologic response to stress and its detrimental effect on performance and immune response is very well documented.(17,18)

Cow/calf herds that run on fescue, quackgrass, or pastures high in molybdenum will develop subclinical copper deficiency.(19) High sulfate intake and excess iron will also compound the problem. Total iron intake >250 ppm, regardless of bioavailability, will interfere with copper availability.(20) This level is not too difficult to surpass when "red" minerals are fed and >.4ppm iron in the water. Stocker calves pastured on irrigated soils or similar pasture conditions can be exposed to the same situations. Grazing on certain southern warm-climate grasses can cause cobalt deficiency.(21)

It is a well known fact that up to 75% of all beef and dairy cattle in the United States are exposed to crop and forage conditions that are selenium deficient. (22) In the United Kingdom it is estimated that 60% of all cattle are copper deficient due to the forage conditions. (23) It is not too far fetched to make that same assumption here in the United States. Part of the difficulty is the reluctance of researchers and academic institutions to accept that fact, let alone take the time to evaluate the nutritonal trace element status of the animal. Supplying NRC requirements does not necessarily mean adequate diets.

It is important to realize that trace element deficient cattle do not necessarily look bad to the eye, but when stressed from their environment, nutrition, processing, hauling, etc., they are unable to handle this stress and eventually succumb to disease situations. Recognizing the problem at this point is usually too late.

Tissue Collection and Analysis:

Know how to collect the proper tissue in the proper container when you are doing the diagnostic workup. Improper tissue collection gives results that are meaningless. Avoid contamination of tissue samples.

Recommendations:

Blood and serum analysis:

Utilize the Vacutainer collection technique to minimize any outside contamination to the sample. There are specific vacuum tubes for serum and whole blood trace element analysis. The recommended tubes are:

- •Vacutainer[®] #6526, 7 ml, No additive, Royal blue top.
- •Vacutainer[®] #6527, 7 ml, Sodium heparin, Royal blue top.

Using the standard red stopper serum tube or the "bangs" bleeding tubes are not adequate for serum trace element analysis. You should not use disposable syringes; contact to rubber and plastic will alter zinc levels. Any hemolysis at all will give erroneous results for serum iron and copper.

Table 3A illustrates a standard mineral profile analysis; become familiar with the normal bovine values. Table 3B points out some of the significant serum elemental relationships that should be regarded as significant in the interpretational process.

Liver, Kidney, Brain:

The most common tissue utilized is the liver. All tissues should be perfused in sterile isotonic saline and free of outside contamination. Adequate samples should be packaged in leak-proof bags, refrigerated or iced and shipped immediately to the diagnostic laboratory. When assaying the liver you should take sections of the various

TABLE 3A

Standard Mineral Profile Analysis

Element	Units	Normal Range
Ca	mg/dl	9.0 - 12.0
P	mg/dl	4.5 - 7.0
К	mg/dl	15.0 - 23.0
Mg	mg/dl	2.0 - 3.0
Cu	ppm	.7 - 1.5
Zn	ppm	1.5 - 1.8
Fe	ppm	.6 - 1.7
Mn	ppm	.0107
Se	ppm	.0730
Мо	ppm	.0205

Other elements should be assayed depending on need

TABLE 3B

Serum Elemental Relationships

(Abnormal conditions)

Ca. Inversion: Serum Ca < P Ca 8 mg/dl, P 12 mg/d Elevated serum potassium: > 22 mg/dl
Serum magnesium < 2.0 mg/dl
Cu > .7 ppm or > .6 ppm with elevated serum Mo (> .0505 ppm)
Cu >.7 ppm with Fe >1.5 ppm
Cu >1 ppm with Fe >2.0 ppm in clinical disease
Se <.070 ppm with >.2 ppm in diet
Se <.070 ppm with Cu <.7 ppm
Manganese - normal or deficient
Zinc > 2.0 ppm; contamination or dietary excess
Molybdenum > .05 ppm; > 1.0 ppm highly significant

Serum profiles must be correlated to dietary intake and current health conditions

lobes and request that a consolidated assay be done since there is a difference in trace element concentration in the different lobes (copper).

Besides fresh tissue assay, formalized tissue sections should also be submitted for detailed histopathology on the tissues. Get in the habit of relating some detailed history to the pathologist and specifically request cellular descriptions, such as:

- lymphocytic proliferation
- hemosiderin in cells
- neutrophil aggregates
- basement membrane degeneration (EM)
- perivascular cuffing
- encephalomalacia, myelitis
- bone and cartilage demineralization

Review the significance of these histopathological descriptions. All too often the diagnostic laboratory is left in the dark on the true clinical picture which detracts from the value of a good pathologist and diagnostician.

Specialty Laboratory Assays Utilized in Clinical Research:

Enzyme Assays

Iron Assays

- RBC Catalase
- Iron profile (serum, UIBC, TIBC, % sat.)

• BleomycinAssay

- RBC selenium dependent glutathione peroxidase
- RBC Cu,Zn-superoxide dismutase

The red blood cell oxidative enzyme assays have given a better insight into bioactive copper, zinc, selenium and iron.

Feed, Forage, Water Sample Collection and Analysis:

The first thing to realize in any type of feedstuff analysis is that you are utilizing very specific numbers from the assay to generate conclusions for a very non-specific condition. Therefore, view your data as an "average" intake with the understanding that there will be variation to this data. What is important are observations such as:

- iron intake is always >250 ppm
- molybdenum intake is >3ppm while on pasture
- molybdenum intake is >5ppm in bunk rations
- the Ca:P ratio is > 2:1
- selenium values are always < .1ppm
- the Posassium:Sodium ratio is always >6:1 even with salt intake

It is these types of observations that set the trend for the herd or feedlot that you are investigating. More of these interrelationships will be discussed in the applications section of this presentation.

Table 4 lists the standard assays for feedstuffs and water that are commonly used in evaluating elemental intake.

TABLE 4

FEEDSTUFF ANALYSIS - STANDARD ASSAY

Moisture %	Silicate ppm	Zinc ppm
Nitrogen %	Sulfur %	Copper ppm
Crude Protein%	Sodium %	Cobalt ppm
ADF %	Chloride%	% Dry Matter
Calcium %	Molybdenum ppm	TDN %
Phosphorus %	Iron ppm	NEg Kcal/#
Potassium %	Aluminum ppm	NEm Kcal/#
Magnesium %	Manganese ppm	NEI Kcal/#

Optional (recommended): Selenium, iodine, cadmium, nitrate. Any suspected heavy metal toxicities should be assayed separately (Pb, Arsenic, Hg, Fluoride, etc.)

WATER ANALYSIS - STANDARD ASSAY

Nitrates, Sulfates, pH, Ca, Mg, Na, Cl, TDS, Fe - other metals as warranted from diagnostic and observed data.

When analyzing pasture grasses it is vital to walk part of the different grazing areas similar to the grazing pattern of the herd. Obtain grass samples realistic of the true grazing pattern. Make sure there is adequate forage for dry matter analysis. One pound of pasture grass may dry down to 3-4 ounces in the ovens! If there are several pastures grazed at various times, then analyze the pastures during the actual grazing period. Rapidly growing forages in early season will usually have a markedly different analysis from late or dry season forages. Develop an understanding for the value of forage analysis and relate to the dietary changes that the cattle are exposed to.

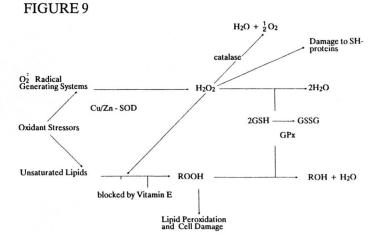
Utilize a forage sampler when collecting hay samples and drill a significant percentage (5-10%) of the various hays that are going to be fed over a 90 day period of time. This "average" sample is satisfactory as long as there is not a wide variation of forage type.

Complete feeds or total mixed rations (TMR) samples need to be collected in such a way there is a representative well mixed sample for analysis. This also brings to mind the importance of the feed mill or feed truck operator and the important job they have in delivering not only balanced, but well mixed rations to the livestock.

Water samples should be respective of what the cattle are consuming. Compare water analysis from the fresh source as well as water that is standing in large tanks. Leaching of iron, zinc, lead, or copper in large old water systems may precipitate a problem. This reinforces the argument for fresh, clean drinking water. Make sure that the cattle know how and where to drink from the water source. (Refer to Table 1 for water quality guidelines).

Specialty Laboratory Diagnostics

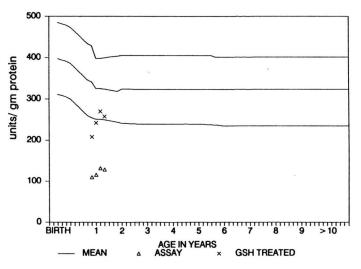
In our laboratory, heparinized whole blood, collected in the proper trace element vacutainers, is utilized to assay for the primary red blood cell oxidative enzymes that are reflective of trace element status. These include catalase (heme, Fe), Selenium dependent glutathione peroxidase (GSH-Px), and Cu,Zn-superoxide dismutase. Figure 9 is a schematic representation of the role these enzymes play in protecting the cell from free-radical damage.



Utilizing this type of enzyme assay gives a better insight into the bioavailability and bioactivity of the trace element involved. There are many other enzymes that could be assayed but on a clinical basis, these three are comparatively easy to assay, the tissue is readily available and the cost is reasonable. By measuring the activity of the enzyme it also allows for the evaluation of the cellular oxidative state. There are some excellent reviews on the significance of oxygen radical toxicity and the role these free radicals play in overall tissue damage.(16) I would highly recommend further study in this area to understand the relationship of trace element physiology to overall health and performance.

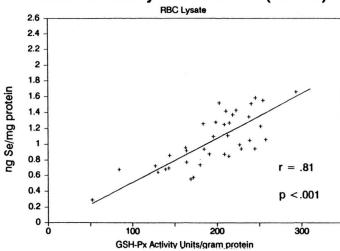
Figures 10 through 14 summarize the data utilized with the oxidative enzyme assays. To go into more detail than a brief description goes beyond the scope of this presentation, however, these illustrations will help the practitioner to visualize some of these relationships.

Figure 10: Bovine RBC glutathione peroxidase activity versus age; the feedlot animal will consistently show a higher normal value since this activity is age dependent.



BOVINE RBC GSH-Px ACTIVITY vs AGE

Our assay shows a positive correlation to selenium content and GSH-Px activity in the bovine RBC from two assay techniques. RBC lysate that is assayed direct: (Figure 11A) and RBC Lysate that is incubated in 10_m M GSH (Figure 11B).





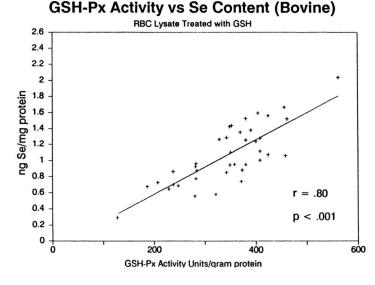
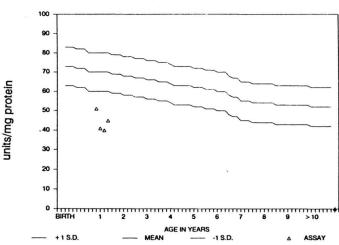
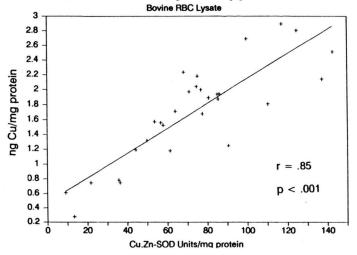


Figure 12: Bovine RBC Cu,Zn-superoxide dismutase activity versus age; again, like GSH-Px activity there is age variation with this enzyme. Some of this observation maybe due to the relative age of the RBC's assayed.

BOVINE RBC Cu, Zn-SOD ACTIVITY vs AGE



Cu,Zn-SOD Activity vs Copper Content



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Figure 13 and 14: Both of these regression plots show a positive correlation of RBC copper content and Cu,Zn-SOD activity either by measuring SOD activity in the lysate or by precipitating the proteins and measuring just the cytosol Cu,Zn-SOD activity. Either way, it gives a biological parameter with which to assay copper availability.

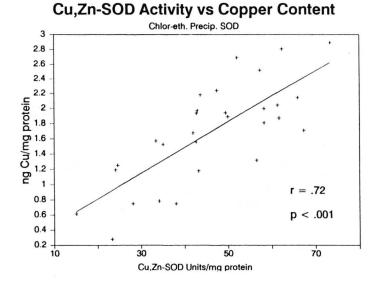


Table 5 is a summary of this data. These oxidative enzyme assays provide a valuable insight into the overall picture of trace element metobolism and interpretation of the nutritional data generated. There are pro and con arguments as to the significance of the oxidative enzymes within the red blood cell and whether or not they give a true profile of the trace elements that are contained in each enzyme. The RBC is peculiar in its demand for protection to oxidative stress. This would make it an ideal tissue for these assays. Catalase is one of the most common enzymes present in most cells that utilize oxygen. Very seldom is there decreased RBC catalase activity. Its significance lies in the fact that it has a relationship to iron metabolism and the selenium status of the animal. It is known that selenium deficiency will increase monoheme oxygenase activity in the liver of laboratory animals which in turn promotes heme catabolism.(24) Heme, an iron protein, is the primary component of the catalase enzyme. Therefore, if catalase activity is decreased we would suspect a problem in iron and selenium metabolism, usually iron excess and selenium deficiency.

Selenium dependent glutathione peroxidase (GSH-Px) has been recognized in domestic animals as a reliable parameter of selenium status in the animal. Only recently has it been suggested that bovine Cu,Zn-superoxide dismutase may be a better indicator of copper status than serum or liver elemental values.

Besides the oxidative enzyme assays and serum mineral profile analysis, evaluation of the iron status of the animal is very relevant. This is an area that is basically very elementary but yet provides some excellent insight to the overall problem in the animal. Iron supplementation in livestock is usually overdone when compared to actual requirement. Failure to realize the longterm impact of high iron exposure can lead to excess iron accumulation in tissues (hemosiderin). Bacterial proliferation in vivo is highly iron dependent and the ability to overcome infectious diseases depends on the host response to withhold iron from these infectious agents. This is one reason why serum iron levels drop dramatically in infectious diseases. Simultaneously serum copper levels (as ceruloplasmin) will increase. If this normal response is not observed further investigation is warranted. Serum iron, total iron binding capacity and % serum transferrin saturation along with hemoglobin and MCHC (Mean Cell Hemoglobin Concentration) determinations provide some basic clinical pathology for evaluating iron status.(Table 6)

SUMMARY DATA

OXIDATIVE ENZYME ASSAY

Catalase	Se-GSH-Px	Cu, Zn-SOD				
(Un	its of activity d	ependent on age)				
k/Gm	Units/Gm	Units/mg				
80 - 140	250 - 400	45 - 60				
Se-GSH-P	Se-GSH-Px activity vs RBC selenium content:					
	r = .82	p <.001				
Cu, Zn-SOD activity vs RBC copper content:						
	r = .78	p <.001				

Normal Parameters: Bovine

Hemoglobin	9-15 Gm/dl
MCHC	30-36
Serum Iron	57 - 162 ug/dl
TIBC	120-240 ug/dl
UIBC	63 - 168 ug/dl
% Transferin Satur	ation - 45%
Catalase	80-140 k/Gm RBC protein

Excess iron in feedstuffs and water and low manganese intake (Fe:Mn > 2.5:1), along with blood and serum data, are all indicators of potential iron overload. During conditions that impose oxidative stress such as hydrogen peroxide buildup in selenium deficiency due to decreased activity of GSH-Px, the primary iron storage protein ferritin is converted to the more insoluble hemosiderin. This unavailable source of iron may be part of the reason why animals develop iron overload since the feedback mechanisms for iron uptake continue to transport more iron into the system.(25)

The use of the Bleomycin assay or Electron Spin Resonance (ESR) technique for the detection of biologically reactive iron in body fluids are other assays to be considered.(26,27) These are very sophisticated assays and very difficult and expensive to perform. ESR techniques are usually confined to research institutions (ESR CORE Laboratory, Radiation Research, University of Iowa, Dr. Garry Beuttner). I have done the bleomycin assay on several serum samples over the past two years with positive results indicating biologically reactive iron. Part of the difficulty in utilizing serum is the tremendous binding capacity of serum transferrin for free iron. Transferrin must be approaching the saturation point (>50%) in order to suspect free radical iron as an initiator of lipid peroxidation and cellular damage. As assay techniques are improved, this valuable observation will be very helpful in evaluating the iron status of the animal and the role that reactive iron plays in initiating early cell damage.

Interpretation & Applications

Applications

Due to the tremendous amount of data that is generated when utilizing the Uniform Methods as I have described, it is easier to utilize some form of spreadsheet applications for interpretation. There are several good spreadsheet software packages available such as Lotus 123 (Registered trademark of Lotus Development Corporation).

Veterinary Biological Systems Analysis, Inc. (VBSA, Inc.) has developed the Nutritional Data Analysis^w bovine version spreadsheet to assist the user in processing this information into a workable format. As with any software its value is only as good as the knowledge of the person inputting and interpreting the data. Sample printouts of nutritional profiles are included in Appendixes A and B but the use of the NDA program was limited to the actual presentation and all examples are not shown here. Appendix A deals with Feedlot Ration. Appendix B deals with cow/calf pasture ration.

Conclusion

In this high technology world, the days of the local

country practition er are gone. Demands that are placed upon us to be versed in many areas of livestock specialization are indeed overwhelming. To be a primary participant in food animal production, not only is our knowledge of animal disease important, but we must also be versed in nutrition, biochemistry, and applied physiology. Accept the challenge!

Acknowledgements

I would like to thank the following people for their help in developing my laboratory and taking the time from their academic responsibilities to share their knowledge and expertise: Professors Larry Oberley and Garry Beuttner, University of Iowa, Radiation Research Laboratory, College of Medicine, Iowa City, Iowa; Drs. Tom Carson and Gary Osweiler, Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa. Dr. Frank Ahrens, Department of Veterinary Physiology, College of Veterinary Medicine, Iowa State University, Ames Iowa; to Ms. Marty Froah for her assistance in the laboratory and dedication to my clinical research effort, and to Ms. Ruth Bourgeois, Design Graphics, Newton, Iowa for her assistance in the preparation of this manuscript.

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Appendix A

This is a sample printout from the Nutritional Data Analysis software (VBSA., Inc.) that assists in organizing nutritional data. The actual ration being fed in this example is basically correct. Further investigation into the ration analysis and review of the "Estimated Copper Requirement", and "Warning Statements" will show that there are several problem areas in micronutrient intake that may lead to deficiency situations during the feeding period.

Data input includes client, species and breed, body weight, and ingredients in the ration. Approximate daily intake and dry matter content must be known.

	SHILL			EWTON, IA	SERVICES	,	
			515-792-				
Client Number: Client:	EXAMPLE	FEEI	DLOT RAT	LON			
	xx 1.00	(In year	s)			Sex:	
	Select 1	Liters =	= 37.74	Gal.=	as a % of = 9.97 edient Lis		8.50 (output) TOTAL
							AMOUNT FED
Feed label	CORN ,	HAY	SILAGE	GLUTEN	BALANCER		
Lbs. as fed							
% Dry Matter	89.00	85.60	56.00	43.00	94.00	0.00	63.66

			Select	Output Da	ata %DM	Intake=	= 3.03%
			********			*******	
Kgs. as fed	5.00	1.36	9.09	4.55	0.23	0.00	20.23
Lbs.DM fed							
Kqs. DM fed	4 45	1 17	5 09	1 95	0 21	0 00	12.88

The actual laboratory assay (on feed table values) is entered for feedstuffs and water. The spreadsheet calculates the final total intake.

Feed I.D.	CORN	***** 10 HAY	O% DRY SILAGE	gredient MATTER BA GLUTEN	BALANCER	*	(output) % or PPM IN RATION
<pre>% Protein % Calcium % Phosphorus</pre>	8.90 0.03 0.26	16.00 1.60 0.40	7.00 0.23 0.34	20.00 0.36	0.00 18.00 0.00	0.00 0.00 0.00	10.33 0.61 0.39
<pre>% Potassium % Magnesium % Sulfur % Sodium</pre>	0.31 0.22 0.18 0.04	1.60 0.35 0.34 0.24	1.20 0.24 0.23 0.30	0.36	2.87 1.35 0.12 10.00	0.00	0.87 0.28 0.24
% Solum % Chloride % Nitrogen	0.04 0.13 1.42	0.24 0.67 2.56	0.30	0.25	12.00 0.00	0.00 0.00 0.00	0.50 0.49 1.65
PPM Zinc PPM Manganese PPM Copper PPM Iron	14.00 45.00 2.00 68.00	56.00 67.00 18.00 340.00	34.00 56.00 22.00 500.00	26.00 12.00	1200.00 879.00 220.00 4657.00	0.00 0.00 0.00	54.20 62.30 16.49 404.87
PPM Aluminum PPM Silicon PPM Cobalt	45.00 23.00 0.05	321.00 78.00 0.50	234.00 78.00 0.60	487.00 90.00 0.10	3467.00 187.00 4.00	0.00 0.00 0.00	268.61 62.62 0.38
PPM Selenium PPM Molybdenum PPM Iodine	0.05	3.50	0.10 3.00 0.05	2.00	4.00 5.00 2.00	0.00 0.00 0.00	0.14 2.93 0.09
				Intake C			(output) Kcal/lb
NEg Kcal/lb NEm Kcal/lb NEl Kcal/lb	640.00 910.00 890.00	634.00	438.00 768.00 765.00	895.00		0.00 0.00 0.00	508.63 811.46 797.00
		Wate	er Analy	sis Data	****		
	ELEMENT			ELEMENT	(input) ppm	(output) Gm/day	
(Nitrogen as (Sulfur as)			0.081 2.264 0.000 0.000 0.055	4 Cl D Mg D Ca	81.00 58.00 12.00 34.00	3.056 2.189 0.453 1.283	
	Mo Mn	0.00	0.000				

Output data includes the gram or milligram intake of each element from each ingredient as well as total intake.

					D.M.BASI		
	IOCAI G				ater if en		ike/bay
Feed I.D.	CORN	HAY	SILAGE	GLUTEN	BALANCER	cerea,	TOTAL
Gm. Protein	396.05	186.76	356.36	390.91	0.00	0.00	1330.09
Gm.Calcium	1.34	18.68	11.71	7.04	38.45	0.00	78.49
Gm. Phosphorus	11.57	4.67	17.31	16.03	0.00	0.00	49.58
Gm.Potassium	13.80	18.68	61.09	12.51	6.13	0.00	112.20
Gm.Magnesium	9.79	4.09	12.22	7.04	2.88	0.00	36.47
Gm.Sulfur	8.01	3.97	11.71	4.50	0.26	0.00	30.70
Gm.Sodium	1.78	2.80	15.27	20.52	21.36	0.00	64.80
Gm.Chloride	5.79	7.82	16.29		25.64	0.00	62.61
Gm.Nitrogen	63.37	29.88	57.02	62.55	0.00	0.00	212.89
			*******				*********
mg Zinc	62.30	65.37	173.09			0.00	697.85
mg Manganese	200.25	78.21	285.09			0.00	802.15
mg Copper	8.90	21.01	112.00			0.00	212.37
mg Iron	302.60	396.87				0.00	5213.25
mg Aluminum	200.25	374.69		951.86	740.68	0.00	3458.76
mg Silicon	102.35	91.05	397.09		39.95	0.00	806.35
mg Cobalt	0.22	0.58	3.05			0.00	4.91
mg Selenium	0.04	0.11	0.51			0.00	1.81
mg Molybdenum		4.09	15.27		1.07	0.00	37.69
mg Iodine	0.22	0.06	0.25	0.14	0.43	0.00	1.10

	RIENT RAT				
	Optimum Ratio	Actual		Optimum Ratio	Actual
Ca:Phos	1.5:1	1.58	Cu:Mo	8.0:1	5.64
Na:Cl	0.8:1	1.03	K:Mg	3.5:1	3.08
Zn:Cu	4.0:1	3.29	K:Na	6.0:1	1.73
Fe:Mn	2.5:1	6.50	N:S	10:1	6.93

The nutrient ratios from this ration are calculated.

The regression formulas in Figure 7 as well as nutrient ratios are used to calculate the estimated copper requirement.

ESTIMATED Cu AVAILABILIT	
Ref: Suttle et al (see)	NDA manual)
IMPORTANT: To use this portion of the	Nutritional Data Analysis program
you must have entered the f	ollowing in "Select Input Data
For Each Ingredient Listed"	
Ing.#1 = PASTURE	* VALIDITY RANGE
Ing. #2 = HAY	* Sulfur: .16%4%
Ing. #3 = SILAGE	* Mo: .6ppm - 6ppm
Input only those ingredients that are	used. PASTURE refers to almost
any type of pasture grazing by ruminan alfalfa/grass mixtures of stored hay:	ts; HAY refers to grass or

There are different linear regression (slope,y-intercept) formulas for each type of forage. If you do not use this exact (upper case) form of entry then the program will not work properly and the data will be invalid. Any other input in these columns will be ignored by this portion of the program. *(Max.=y-intercept)

-	Max. & A	Avail.	mg Cu Present	
		1 MIN THE NO. 222 TO 223 TO 224	********	********
Ing.#1-PASTURE	5.30	0.00	0.00	0.000
Ing.#2- HAY	8.03	4.70	21.01	0.987
Ing.#3-SILAGE	7.47	4.57	112.00	5.120

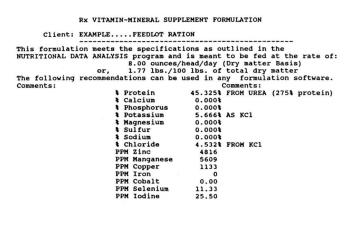
There is a breed variation in copper requirement.

The software user can now formulate the proper supplement needed to balance the ration.

Client:		******	CAL SUPPLI	******	DRMULATION	
	How many (Normall	ounces/ y 3-8 of		mineral	om this rat al is to be ;	
Feed + water NUTRIENT I.D.	total in this ration		Computer to add to PPM	with	to add (INPUT)	<pre>% or PPM in 1 ton of Custom Rx Supplement</pre>
						KX Supprement
<pre>% Protein</pre>	10.33	******	VARIABLE	11.130	0.80	45.3248%
& Calcium	0.61	1.5%	-0.032	0.610		0.0000%
Phosphorus	0.39	.75%	0.015	0.385	0.00	0.0000%
1 Potassium	0.87	2.0%	0.529	0.971	0.10	5.6656%
Magnesium	0.28			0.283	0.00	0.0000%
\$ Sulfur	0.24	. 3%	-0.101	0.238	0.00	0.0000%
Sodium	0.50	.8%	-0.103	0.503	0.00	0.0000%
& Chloride	0.49	1.0%	0.143		0.08	4.5325%
						ROR #4
ppm Zinc	54.20					4815.8
ppm Manganese		500.00		161.3		5608.9
ppm Copper	16.49	100.00		36.5	20.00	1133.1
ppm Iron	404.87	500.00				0.0
ppm Cobalt	0.38	5.00		0.381		0.0
ppm Selenium	0.14	2.00				11.3
ppm Iodine	0.09	20.00	0.915	0.535	0.45	25.5
	intake t	hat would	ld be con	sidered	a level of safe on lo 50% of the	

Vitamin A supplementation (IU)/head/day (125,000 Max.):=	50,000
Vitamin D supplementation (IU)/head/day (20,000 Max) :=	8,500
Vitamin E supplementation (IU)/head/day (500-1200): =	300
Thiamine supplementation in mg/head/day: (Range=75-500 mg)=	125
Rumensin in mg/hd/day: =	0
or, Bovatec in mg/hd/day: =	0

The final supplement formula is used to blend the proper vitamin-mineral supplement.



100,000
17,000
600
250.0
0.0
0.0

With addition	of this	Rx Formul	lation t	o the fo	llowing	feedstuf	fs:
	CORN	HAY S	SILAGE	GLUTEN	BALANCER	2	
(lbs. as fed)) 11.00	3.00	20.00	10.00	0.50	0.00	
the nutrient	ratios wi	ll be at	the fol	lowing 1	evels:		
Element	Prior	Current	Optimum	Element	Prior	Current	Optimum
Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
Ca:P	1.58	1.58	1.50	Cu:Mo	5.64	12.47	8.00
Na:Cl	1.03	0.89	0.80	K:Mg	3.08	3.43	3.50
Zn:Cu	3.29	3.81	4.00	K:Na	1.73	1.93	6.00
Fe:Mn	6.50	2.51	2.50	N:S	6.93	7.47	10.00

It is recommended to use yeast cultures, fermentation solubles, midds, probiotics, and/or soyoil, and flavorings as needed for palatability and added nutritional benefit in the RX Vitamin-Mineral Supplement.

Elemental ratios are also displayed graphically as prior, current and optimum levels.

FIGURE A1

NUTRIENT INTAKE RATIOS

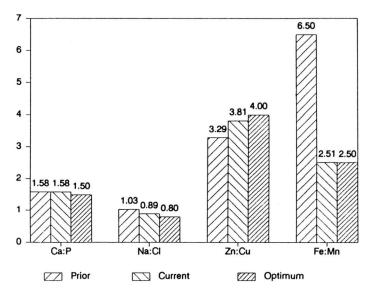
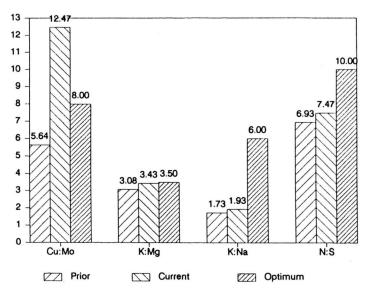


FIGURE A2

NUTRIENT INTAKE RATIOS



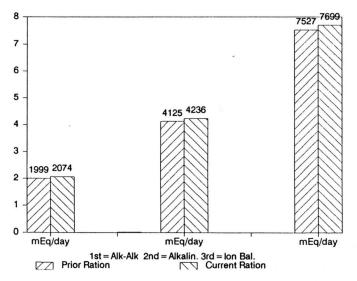
The caution-anion balance is very important in drylot dairy cows and gestating beef cows.

Client:	EXAMPLI	2F	EEDLOT RAT	ION		
Ingredients:	CORN	HAY	SILAGE	GLUTEN	BALANCER	
	Alkali	-			Ion	
	Alkali	nity	Alkalin	ity	Balance	
	mEq/day	1	mEq/day		mEq/day	Comment
				-	20 10 10 10 10 10 10 10	*********
Prior Ration	199	9	4125		7527	Acceptable
Current Ration	1 207	4	4236		7699	Acceptable
	Alkali	-Alkali	nity = (Na	+K) - (S+C	:1)	
	Alkali	nity =	(Ca+Mg+Na+	K) - (S+C1	+PO4)	
	Ion Ba	lance =	(Ca+.333F	04+Na+K)	-(S+C1) [USD	A/ARS]

All equations are based on milli-equivalents (mEq) of each ion ingested on a daily basis. Ion Balance >8000 mEq/day is conducive to faulty Ca, P, Mg metabolism predisposing to metabolic disease in ruminants (pre-calving, dairy cow). The "Current Ration" figures reflects the use of the Rx Vitamin- Mineral Supplement formulated from this program.

FIGURE A3

NUTRIENT CATION-ANION INTAKE



The real power of the Nutritional Data Analysis[™] software is in the automatic warning statements generated. Over 150 conditions can be recognized as either deficiencies, toxicities, or improper relationships. Newer versions of NDA[™] also include complete "help screens" on each element. Handling data in this manner offers the clinician a powerful tool in evaluating the nutritional status of the herd.

WARNING STATEMENTS - Original Ration	
The information and formulation data in this printout is valid only while the feedstuffs mentioned are being fed. It is highly recommended that your nutritional program be re-evaluated on a quarterly basis. Unless specified, all figures/calculations are based on 100% dry matter The following conditions exist within the original ration that constitute a potential deficiency, excess, or toxicity situation:	
The K:Mg ratio is <3.2:1, evaluate K, Mg levels. The K:Mg ratio is <3.2:1, evaluate K, Mg levels. The N:Na ratio is >1:1 in this ration, evaluate K, Na levels. The N:Cl ratio is >1:1 in this ration, evaluate Na, Cl levels. The Zn:Cu ratio is <3.8:1 in this ration, evaluate Zn, Cu levels. This diet is below the Zn requirement based on Ca intake. The Fe:Mn ratio is >2.8:1 in this ration, evaluate Zn, Mn levels. The Fe:Mn ratio is >2.8:1 in this ration, evaluate G, Mn levels. Cu intake from this ration is below the estimated requirement. Fe intake is >250 ppm in this ration, this is a low level.	

Se intake is <.25 ppm in this ration, this may be too low. Se intake is < .15 ppm in this ration, this is deficient. Iodine intake is <.5 ppm in this ration, this may be deficient.

WARNING STATEMENTS - Current Ration (With Supplement)
The information and formulation data in this printout is valid only while the feedstuffs mentioned are being fed. It is highly recommended that your nutritional program be re-evaluated on a quarterly basis. Unless specified, all figures/calculations are based on 100t dry matter. The following conditions exist within the current ration, after supplemental recommendations, that constitute a potential deficiency, excess, or toxicity situation:
The K:Na ratio is <5.8:1 in this ration, evaluate K, Na levels. The N:S ratio is <9:1, evaluate protein, N, and S intake. The Cu:Mo ratio is >10:1 in this ration, evaluate Cu, Mo levels. Fe intake is >250 ppm in this ration, this may interfere with Cu. Si intake is < 16m/day in this ration, this is a low level. Se intake is < 30 ppm in this ration, this is a low level.

Appendix B

This is a sample printout from the Nutritional Data Analysis software (VBSA, Inc.) that assists in organizing nutritional data. This example is for 1400# cows on pasture with access to a "pasture" mineral blend commonly referred to as a "12-12" mineral. The basic nutritional intake in this animal is satisfactory but there are several problem areas that may lead to micronutrient deficiencies during grazing.

	SHILO	H - LIVE	STOCK VET	TERINARY	SERVICES	3	
		RR 1 BOX	362 NEW	TON, IA	50208		
			515-792-0				
Client Number:	C	8					
		001	VONTE DI		ACTUDE		
cifenc.		CO					
Breed:						in lbs:	1400.00
Age:	1.00	(In year	s)			Sex:	COW
-		Dai	ly water	intake a	sa to	E B.W.:	11.00
		Liters =	71.97	Gal.=	19.01		(output)
	Select 1						TOTAL
							AMOUNT FED
	angi ja	1.9.72	119.75			119170	
Feed label	PASTURE			1	INERAL		
Lbs. as fed	78.00	0.00	0.00	0.00	0.35	0.00	78.35
% Dry Matter	32.00	0.00	0.00	0.00	94.00	0.00	32.28

		-	Select O	utput Dat	a %DM	Intake=	1.82%
	*******					********	
Kgs. as fed	35.45	0.00					35.61

Lbs.DM fed							
Kgs. DM fed	11.35	0.00	0.00	0.00	0.15	0.00	11.50

The actual laboratory assay (on feed table values) are entered for feedstuffs and water. The spreadsheet calculates the final total intake.

		Input - F			SIS *****		(output) % or PPM
Feed I.D.	PASTURE				MINERAL		IN RATION
% Protein	11.00				0.00	0.00	10.86
& Calcium	0.76				12.00	0.00	0.93
Phosphorus	0.18				12.00	0.00	0.33
& Potassium	2.10				2.87	0.00	2.11
% Magnesium	0.22				1.35	0.00	0.24
% Sulfur	0.21				0.12	0.00	0.25
& Sodium	0.04				10.00	0.00	0.22
% Chloride	0.63				12.00	0.00	0.81
% Nitrogen	1.76	0.00	0.00	0.00	0.00	0.00	1.74
PPM Zinc			********				
	34.00				1200.00	0.00	49.17
PPM Manganese	45.00				879.00	0.00	55.85
PPM Copper	11.00				220.00	0.00	13.72
PPM Iron	278.00				5678.00	0.00	357.02
PPM Aluminum	112.00				3467.00	0.00	155.65
PPM Silicon	23.00				187.00	0.00	25.13
PPM Cobalt	0.05				4.00	0.00	0.10
PPM Selenium	0.01				3.00	0.00	0.05
PPM Molybdenum	2.60				2.00	0.00	2.59
PPM Iodine	0.05				2.00	0.00	0.08

		Input - Energy Intake Ca			(output) Kcal/lb
NEg Kcal/lb	287.00		0.00	0.00	283.27
NEm Kcal/lb	564.00		0.00	0.00	556.66
NEl Kcal/lb	523.00		0.00	0.00	516.20

		Wate	er Analys	sis Data	****	
	ELEMENT	(input) ppm	(output) Gm/day	ELEMENT	(input) ppm	(output) Gm/day
(Nitrogen as	Nitrate	12.00	0.155	Na	81.00	5.829
(Sulfur as)		180.00	4.317	Cl	58.00	4.174
	Zn	0.00	0.000	Mg	12.00	0.864
	Cu	0.00	0.000	Ca	34.00	2.447
	Fe	1.40	0.101			
	Mo	0.00	0.000			
	Mn	0.00	0.000			

Output data includes the gram or milligram intake of each element from each ingredient as well as total intake.

	Total Gm	(or mg)					ake/Day
		(Includes	intake	from wa	ter if en	tered)	
Feed I.D.	PASTURE				MINERAL		TOTAL

Gm.Protein	1248.00	0.00	0.00	0.00	0.00	0.00	1248.00
Gm.Calcium	86.23	0.00	0.00	0.00	17.95	0.00	106.62
Gm. Phosphorus	20.42	0.00	0.00	0.00	17.95	0.00	38.37
Gm. Potassium	238.25	0.00	0.00	0.00	4.29	0.00	242.55
Gm.Magnesium	24.96	0.00	0.00	0.00	2.02	0.00	27.84
Gm.Sulfur	23.83	0.00	0.00	0.00	0.18	0.00	28.32
Gm.Sodium	4.54	0.00	0.00	0.00	14.95	0.00	25.32
Gm.Chloride	71.48	0.00	0.00	0.00	17.95	0.00	93.60
Gm.Nitrogen	199.68	0.00	0.00	0.00	0.00	0.00	199.83
	********		*******	*******	********		
mg Zinc	385.75	0.00	0.00	0.00	179.45	0.00	565.20
mg Manganese	510.55	0.00	0.00	0.00	131.45	0.00	642.00
mg Copper	124.80	0.00	0.00	0.00	32.90	0.00	157.70
mg Iron	3154.04	0.00	0.00	0.00	849.12	0.00	4103.90
mg Aluminum	1270.69	0.00	0.00	0.00	518.47	0.00	1789.17
mg Silicon	260.95	0.00	0.00	0.00	27.96	0.00	288.91
mg Cobalt	0.57	0.00	0.00	0.00	0.60	0.00	1.17
mg Selenium	0.11	0.00	0.00	0.00	0.45	0.00	0.56
mg Molybdenum	29.50	0.00	0.00	0.00	0.30	0.00	29.80
mg Iodine	0.57	0.00	0.00	0.00	0.30	0.00	0.87

NUTRIENT	RATIO'S	OF	TOTAL	DAILY	INTAKE
*******	*******	***	******	******	*****

	Optimum Ratio	Actual	Element Ratio	Optimum Ratio	Actual

Ca: Phos	1.5:1	2.78	Cu:Mo	8.0:1	5.29
Na:Cl	0.8:1	0.27	K:Mg	3.5:1	8.71
Zn:Cu	4.0:1	3.58	K:Na	6.0:1	9.58
Fe:Mn	2.5:1	6.39	N:S	10:1	7.06

The nutrient rations from this ration are calculated.

The regression formulas in Figure 7 as well as nutrient ratios are used to calculate the estimated copper requirement.

	DOMTHAMDO	CU AVAILABILI	TH DODLARD	
		CU AVAILABILI		
		tle et al (see		
			Nutritional Data	
			following in "Sel	ect input Data
		redient Listed		
	Ing.#1 =		* VALIDITY	
	Ing.#2 =		* Sulfur:	
		SILAGE	* Mo: .6p	
			used. PASTURE re	
			nts; HAY refers t	
alfalfa/grass	mixtures	of stored hay;	SILAGE refers to	corn silage.
There are diff	erent lin	ear regression	(slope, y-interce	pt) formulas for
			e this exact (upp	
			properly and the	
			umns will be igno	red by this
portion of the		intercept)		
		ctual &		
			mg Cu	
			Durant	
	Avail.	Avail.	Présent	
	Avail.	Avail.		
Ing.#1-PASTURE	Avail.	Avail.	124.80	
Ing.#1-PASTURE	Avail.	Avail.	124.80	1.634 0.000
Ing.#1-PASTURE	Avail.	Avail.	124.80	1.634 0.000
Ing.#1-PASTURE	Avail.	Avail.	124.80	1.634 0.000
Ing.#1-PASTURE	Avail. 5.30 8.03 7.47	Avail. 1.31 0.00 0.00	124.80	1.634 0.000
Ing.#1-PASTURE Ing.#2- HAY Ing.#3-SILAGE	Avail. 5.30 8.03 7.47	Avail. 1.31 0.00 0.00 EQUIRED DIETAR	124.80 0.00 0.00	1.634 0.000 0.000
Ing.#1-PASTURE Ing.#2- HAY Ing.#3-SILAGE Based on the N	Avail. 5.30 8.03 7.47 RUTRITIONA	Avail. 1.31 0.00 0.00 EQUIRED DIETAR	124.80 0.00 V COPPER INTAKE	1.634 0.000 0.000 intake computed
Ing.#1-PASTURE Ing.#2- HAY Ing.#3-SILAGE Based on the N	Avail. 5.30 8.03 7.47 RUTRITIONA gram, the	Avail. 1.31 0.00 0.00 EQUIRED DIETAR L DATA ANALYSI estimated copp	124.80 0.00 0.00 Y COPPER INTAKE S input and daily wer requirement is	1.634 0.000 0.000 intake computed
Ing.#1-PASTURE Ing.#2- HAY Ing.#3-SILAGE Based on the N	Avail. 5.30 8.03 7.47 RUTRITIONA gram, the 1.) Total	Avail. 1.31 0.00 0.00 EQUIRED DIETAR L DATA ANALYSI estimated copp mg of copper	124.80 0.00 V COPPER INTAKE	1.634 0.000 0.000 intake computed determined by:

2.) Ratio's, mineral interactions and comp with Mo, Zn, S, Fe, Mn.
 3.) Breed variations.
 * 4.) Linear regression availability factor.

Estimated Cu requirement = 69.60 PPM (Min.) all breeds. Total dietary Cu present = 13.72 PPM from all sources.

Breed Dietary Cu(ppm) Requirement (est.) SHO HER

ORTHORN	(SS)	LIMOUSIN	(LM)	
REFORD	(HH)	GALLOWAY	(GA)	
AROLAIS	(CH)	SIMMENTAL	(SM)	

There is a breed variation in copper requirement.

Fee

The software user can now formulate the proper supplement needed to balance the ration.

Client:	EXAMPLE	c	OW/CALF R	ATION -	P	AST	JRE				
			r intake/h /hd/day o:								1.82%
	(Normal]	y 3-8 o	z/day on 1	nineral		15	to be	rec	1/=		4.00
	8-32 oz/	day on	Supplement	=)							
d + wate	r total	*Safe	Computer	Total	*	or	PPM	*	or	PPM	in

reed + waler	LOCAL	*Sale	computer	TOCAL	S OF PPM	S OF PPM IN
NUTRIENT I.D.	in this	Upper	to add	with	to add	1 ton of Custom
	ration	Limit	% or PPM	RxSup	(INPUT)	Rx Supplement
<pre>% Protein</pre>	10.86	******	VARIABLE	10.857	0.00	0.0000%
% Calcium	0.93	1.5%	-0.427	0.928	0.00	0.0000%
% Phosphorus	0.33	.75%	0.066	0.394	0.06	6.0694%
% Potassium	2.11	2.0%	0.000	2.110	0.00	0.0000%
% Magnesium	0.24	.5%	0.098	0.342	0.10	10.1156%
% Sulfur	0.25	.3%	-0.102	0.246	0.00	0.0000%
% Sodium	0.22	.8%	0.307	0.340	0.12	12.1387%
& Chloride	0.81	1.0%	-0.214	0.954	0.14	14.1618%
ppm Zinc	49.17	250.00	174.4	232.2	183.00	18511.5
ppm Manganese	55.85	500.00	87.0	143.9	88.00	8901.7
ppm Copper	13.72	100.00	55.9	63.7	50.00	5057.8
ppm Iron	357.02	500.00	-237.0	357.0	0.00	0.0
ppm Cobalt	0.10	5.00	0.199	0.401	0.30	30.3
ppm Selenium	0.05	2.00	0.351	0.299	0.25	25.3
ppm Iodine	0.08	20.00	0.925	0.455	0.38	38.4

*Safe upper limit refers to the a level of nutrient intake that would be considered safe on long term consumption. Generally this is 50% of the LD50 level.

Vitamin A supplementation (IU)/head/day (125,000 Max.):= Vitamin D supplementation (IU)/head/day (20,000 Max):= Vitamin E supplementation (IU)/head/day (500-1200): = Thiamine supplementation in mg/head/day(Range=75-500 mg)= Rumensin in mg/hd/day: = or, Bovatec in mg/hd/day: = 85,000 11,500 650 60 0 0

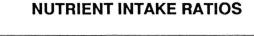
The final supplement formula is used to blend the proper vitamin-mineral supplement.

F	X VITAMI	N-MINERA	L SUPPL	EMENT FOR	RMULATIO	N	
Client: E	XAMPLE .	cow	/CALF R	ATION - 1	PASTURE		
his formulatio	n meets	the spec	ificati	ons as ou	utlined	in the	
UTRITIONAL DAT							e rate of:
		4.00 0	ounces/h	ead/day	(Dry mat	ter Basis	s)
	or,					ry matte	
the following n	ecommend	ations of	can be u				software.
comments:					Comments	::	
		Protein		0.000%			
		Calcium		0.000%			
		Phospho		6.069%			
		Potass		0.000%			
		Magnes		10.116%			
		Sulfur Sodium		0.000%			
		Chlorie		12.139%			
		PM Zinc		18512			
			anese	8902			
		PM Copp			COPPER (XIDE & C	HEIATE
		PM Iron			NONE ADD		ILLAID
		PM Coba		30.35			
	P	PM Sele		25.29			
	P	PM Iodi	ne	38.44			
	v	itamin A	A IU/#	340,000			
	v	itamin	D IU/#	46,000			
			E IU/#				
	т	hiamine	mg/#	240.0			
	R	umensin	mg/#				
	В	ovatec 1	ng/#	0.0			
with addition of	of this P	Y Formu	lation t	o the fo	llowing	feedstuf	fer
	PASTURE	A TOLMU	Lucion c		MINERAL	recuscur	13.
(1bs. as fed)	78.00	0.00	0.00	0.00	0.35	0.00	
the nutrient ra							
Element				Element	Prior	Current	Optimum
Ratio		Ratio		Ratio	Ratio		Ratio
Ca:P	2.78	2.36	1.50	Cu:Mo	5.29	24.58	8.00
Na;Cl	0.27	0.36	0.80	K:Mg	8.71	6.17	3.50
Zn:Cu	3.58	3.64	4.00	K:Na	9.58	6.20	6.00

It is recommended to use yeast cultures, fermentation solubles, midds, probiotics, and/or soyoil, and flavorings as needed for palatability and added nutritional benefit in the Rx Vitamin-Mineral Supplement.

Elemental ratios are also displayed graphically as prior, current and optimum levels.

FIGURE B1



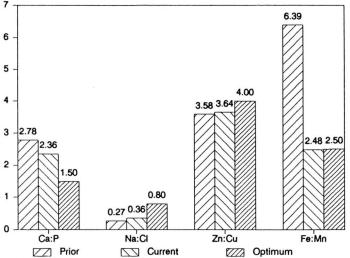
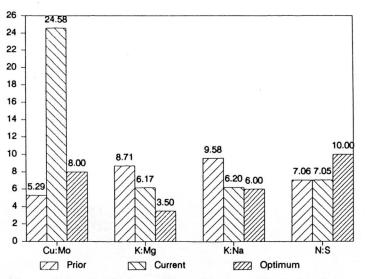


FIGURE B2

NUTRIENT INTAKE RATIOS



The cation-anion balance is very important in drylot dairy cows and gestating beef cows.

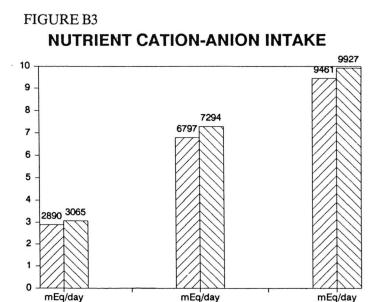
CATION - ANION BALANCE

Client:	EXAMPLE	COW/CALF RATION	- PASTURE	
Ingredients:	PASTURE		MINERAL	
	Alkali-		Ion	
	Alkalinity	Alkalinity	Balance	
	mEq/day	mEq/day	mEq/day	Comment
Prior Ration	2890	6797	9461	WARNING
Current Ratio	n 3065	7294	9927	WARNING

Alkalinity = (Ca+Mg+Na+K)-(S+C1+PO4) Ion Balance = (Ca+.333PO4+Na+K)-(S+C1) [USDA/ARS]

All equations are based on milli-equivalents (mEq) of each ion ingested

All equations are pased on mini equivalence (may, or control indicated on a daily basis. Ion Balance >8000 mEq/day is conducive to faulty Ca, P, Mg metabolism predisposing to metabolic disease in ruminants (pre-calving, dairy cow). The "Current Ration" figures reflects the use of the Rx Vitamin- Mineral Supplement formulated from this program.



mEq/day mEq/day 1st = Alk-Alk 2nd = Alkalin. 3rd = Ion Bal. Ration Current Ration Prior Ration

The real power of the Nutritional Data Analysis software is in the automatic warning statements generated. Over 150 conditions can be recognized as either deficiencies, toxicities, or improper relationships. Newer versions of NDA[™] also include complete "help screens" on each element. Handling data in this manner offers the clinician a powerful tool in evaluating the nutritional status of the herd.

nerd.
WARNING STATEMENTS - Original Ration
Client: EXAMPLE COW/CALF RATION - PASTURE
The information and formulation data in this printout is valid only while the feedstuffs mentioned are being fed. It is highly recommended that your nutritional program be re-evaluated on a quarterly basis. Unless specified, all figures/calculations are based on 100% dry matter. The following conditions exist within the original ration that constitute a potential deficiency, excess, or toxicity situation:
The Ca:P ratio is > 2:1, evaluate Ca, P levels. The K:Mg ratio is >3.7:1, evaluate K, Mg levels. The K:Mg ratio is >6.5:1 in this ration, evaluate K, Na levels. The Na:Cl ratio is <6:1, evaluate protein, N, and S intake. The S: ratio is <3:1, evaluate protein, N, and S intake. The Zn:Cu ratio is <3:1 in this ration, evaluate Zn, Cu levels. Zn intake is <50 ppm in this ration, this is deficient. This diet is below the Zn requirement based on Ca intake. The Cu:Mo ratio is >2.8:1 in this ration, evaluate Fe, Mn levels. The Fe:Mn ratio is >2.8:1 in this ration, evaluate Cu, Mo levels. Cu intake from this ration is below the estimated requirement. Fe intake is <50 ppm in this ration, this may interfere with Cu. Si intake is < 10m/day in this ration, this is deficient. Co intake is <30 ppm in this ration, this is deficient. Se intake is <.55 ppm in this ration, this is deficient. Se intake is <.55 ppm in this ration, this may be too low. Se intake is <.55 ppm in this ration, this may be deficient. Indine intake is <.55 ppm in this ration, this may be deficient. Indine intake is <.55 ppm in this ration, this may be deficient. Indine intake is <.55 ppm in this ration, this may be deficient. Indine intake is <.55 ppm in this ration, this may be deficient. Indine intake is <.55 ppm in this ration may be deficient. Indine intake is <.55 ppm in this ration may be deficient. Indine intake is <.55 ppm in this ration may be deficient. Indine (cation intake) is >8000 mEq; this is an alkaline diet.
WARNING STATEMENTS - Current Ration (With Supplement)

Client: EXAMPLE COW/CALF RATION - PASTURE The information and formulation data in this printout is valid only while the feedstuffs mentioned are being fed. It is highly recommended that your nutritional program be re-evaluated on a quarterly basis. Unless specified, all figures/calculations are based on 100% dry matter. The following conditions exist within the current ration, after supplemental recommendations, that constitute a potential deficiency, excess, or toxicity situation:

The Ca:P ratio is > 2:1, evaluate Ca, P levels. The K:Mg ratio is >3.7:1, evaluate K, Mg levels. The Na:Cl ratio is <.6:1, evaluate Na, Cl levels. The N:S ratio is <.6:1, evaluate protein, N, and S intake. The Zn:Cu ratio is <3.8:1 in this ration, evaluate Zn, Cu levels. The Cu:Mo ratio is >10:1 in this ration, evaluate Cu, Mo levels. Cu intake from this ration is below the estimated requirement. Fe intake is >250 ppm in this ration, this may interfere with Cu. Si intake is <.5 ppm in this ration, this may be deficient. Iodine intake is <.5 ppm in this ration, this may be deficient. Energy intake (NEM) from this ration may be deficient; check intake Ion balance (cation intake) is >8000 mEq; this is an alkaline diet.