Kentucky Equine Research®
World Leaders In Equine Nutrition

Advances in Equine Nutrition

Volume II

Edited by

J.D. Pagan

A PRACTICAL METHOD FOR RATION EVALUATION AND DIET FORMULATION: AN INTRODUCTION TO SENSITIVITY ANALYSIS

DAVID KRONFELD
Virginia Polytechnic Institute and State University, Blacksburg, VA

Summary

The evaluation of rations and formulation of diets for horses customarily uses mean values for nutritional requirements, compositions of ingredients, and intakes of forages and feeds to yield a single solution, that is, one ration, one diet or one supplement. More realistic is the representation of these variables as ranges (for example, as lower, middle and upper values), and testing the effects of these ranges to yield three or more solutions--*sensitivity analysis*. This method tests the flexibility and robustness of the design of a diet or supplement, and greatly increases the probability of detecting weaknesses in a ration.

Introduction

An orientation of nutritional practice (Figure 1) has been successfully used by the author and associates for many species, including the horse. The focus is the setting of goals for intakes of energy and nutrients. These goals are not specified as single numbers or requirements, but rather as optimal or target ranges, with upper and lower limits as well as middle values (Figure 2). This paper deals with the interactions between nutritional goals and the evaluation of a ration (daily intake) or the design of a diet or supplement. It introduces the use of *sensitivity analysis*, which is borrowed from economics and epidemiology (Anderson, 1974; Martin et al., 1987).

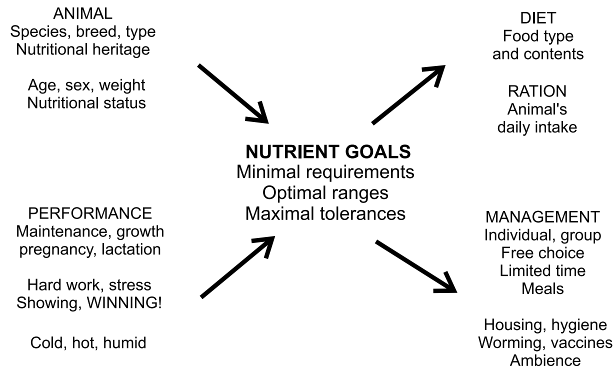

Figure 1. An orientation of nutritional practice focuses on setting nutritional goals for energy and nutrients in keeping with an animal's nature, desired performance, and state of health. The goals are optimal ranges (Figure 2), which are usually specified as lower, middle and upper values.

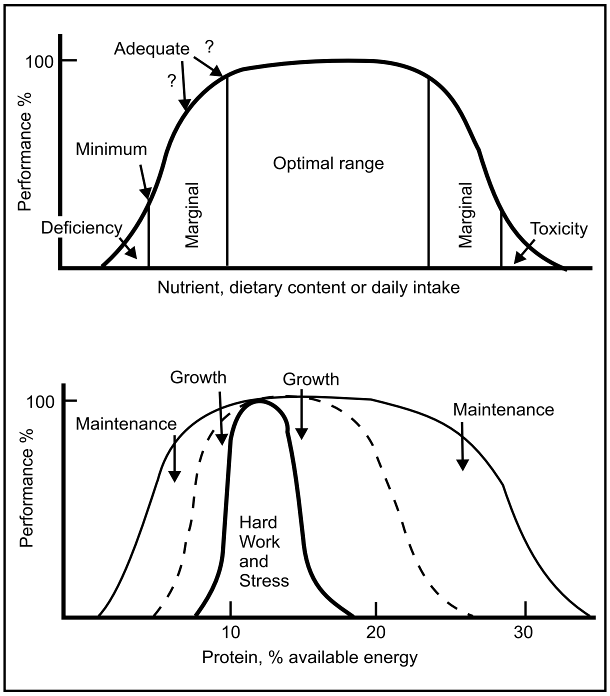

Figure 2. The influence of dietary content or nutrient intake on a specified measure of performance rises to a plateau, the optimal range, then declines. Minimum requirements are usually set to avoid an adverse effect in 50% of the population, and optimal function may be reduced before such an adverse effect becomes evident. For practical purposes, nutritional goals or target zones should be in optimal ranges rather than be restricted to the minimum requirement.

Sensitivity analysis tests the effects of varying parameter values of a model through a defined range and observing the resultant changes in the outcome. A simple example would be the prediction of dry matter intake (DMI):

$$\underset{\text{intake}}{\mathrm{DMI}} = \underset{\text{age}}{a\mathrm{X}} + \underset{\text{weight}}{b\mathrm{Y}} + \underset{\text{activity}}{c\mathrm{Z}}$$

where the measured variables are X, Y and Z; the relating parameters are *a*, *b* and *c*. Sensitivity analysis enables us to cope with variation, including estimating error, in DMI or digestible energy (DE) intake, nutrient content and availability, and DE and nutrient needs of an equine population. It requires that nutritional goals should be represented by a set of optimal ranges rather than a set of single values for DE and each nutrient.

Achieving nutritional goals is simple for those animals fed a complete and balanced diet, for example, dogs and cats, or a total mixed ration, for example, feedlot cattle. Legend has it that the first complete and balanced diet for a domestic animal, Purina Horse Chow, was made by John Danforth in the 1890s to "take the worry out" of feeding horses. This concept has prospered for pet foods more than horse feeds, which commonly are supplements for forages. Most horses are offered a maintenance level of forages (pasture and hay) supplemented with concentrates (mainly grain).

The traditional approach to designing a diet or supplement leads to a single solution, for example, one concentrate formula for each forage analysis, to achieve nutritional goals expressed as requirements (NRC, 1989). In contrast, the procedure summarized here specifies nutritional goals as target ranges and tests the effects of ranges of nutrients in ingredients and proportions of concentrate:forage consumed. It acknowledges and deals with the variations that exist in animals and feedstuffs and, for pasture-fed animals, the huge errors in estimating pasture intake (Figure 3).

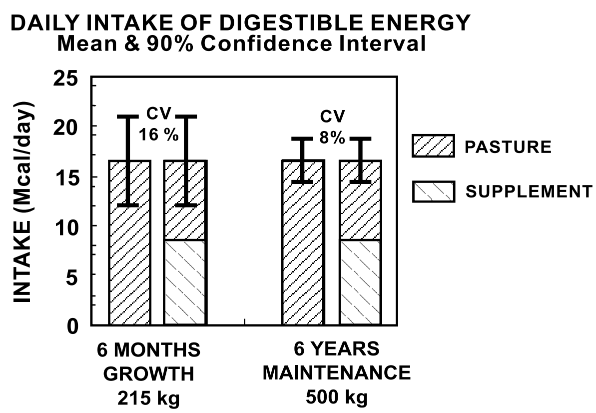

Figure 3. The common proximate analysis of carbohydrates determines the insoluble fibers of plant cell walls as neutral detergent fiber (NDF) and includes the soluble fibers and other non-hydrolyzable, soluble carbohydrates in the nonstructural carbohydrates (NSC), which are calculated by difference: NSC = total carbohydrates - NDF. In view of the digestive physiology and metabolism of hindgut fermenters, such as the horse, hydrolyzable carbohydrates need to be split from fermented carbohydrates, and the latter should be further divided into those fibers that yield lactic acid and those that yield acetic, propionic and butyric acids.

In practice, the diet formulation program is run three or more times instead of just once; this does not require three or more times the work, however, because only one number needs to be changed from one run to the next. By visualizing the effects of ranges, this method generates more general formulas capable of sustaining wider ranges of uses. Engineers would call these more flexible and robust products.

Similarly, for evaluating the ration of a horse, the traditional approach is to assume a single, middle value for pasture intake (Lewis, 1995; Pagan et al., 1996). This approach neglects the huge variation or error in estimating pasture intake, especially when a supplement is fed and all of the variation in daily intake is confined to the pasture intake (Figure 3). The present method addresses the practical importance of this variation or error by running the ration calculations at least three times, using lower and upper limits as well as the middle values for pasture intake.

Nutritional Goals

Nutritional science usually is applied to the determination of minimal nutrient requirements, whereas practical nutrition really needs optimal ranges for specified purposes (Figure 2). The performance traditionally evaluated in farm animal nutrition is energetic efficiency, but other measures of interest would include reproductive efficiency, conformation, tractability, low disease incidence and competitive athletic ability---winning! For horses, optimal ranges of vitamin A have been determined for growth and reproduction (Donoghue et al., 1981; Greiwe-Crandell et al., 1995). Also, parabolic curves have been used to determine the optimal dietary protein for growth in horses (Thiers and Kronfeld, unpublished data) and the optimal dietary fat with respect to muscle glycogen concentration (Kronfeld et al., 1994). An optimal range for dietary calcium for growth has been determined for large dogs (Kronfeld et al., 1994); it may apply equally well to the horse, because the minimum requirements are the same and the upper limit appears to depend on the risk of osteochondrosis in both species. Much remains to be done in equine nutrition to establish nutritional goals in the form of optimal ranges with scientific rigor. Meanwhile tentative optimal ranges or target zones need to be used as nutritional goals; practical experience, as well as science, contributes to craft and technology.

The most widely used nutritional standards for animals are the NRC series for many species. These requirements are performance-oriented for production animals, but they are mean minimums for companion animals, prudently viewed as sufficient to prevent lesions or growth retardation in 50% of animals. Failure to recognize this crucial difference in the NRC series may lead to the improper use of the NRC minimum requirements for dogs, cats and horses in the same way as NRC standards for cattle, swine and chickens (or the recommended dietary allowances, RDAs, for humans). Recognizing that the nutrient requirements for dogs and cats have little practical value, the Association of American Feed Control Officials created nutrient profiles for dogs and cats that were about 1.3- to 2-times corresponding NRC values. The human RDAs are two standard deviations above mean minimum requirements, thereby being sufficient for 98% of the population (Food and Nutrition Board, 1989). When the standard deviation is not known for a nutrient, the CV of 15% for energy is used; thus many RDAs are 1.3-times the mean minimum. Applying the same approach to the horse, a set of equine RDAs would be about 1.2-times the NRC requirements for maintenance and up to 1.5-times the NRC requirement for rapid growth (Kronfeld et al., 1994). Such equine RDAs would be more likely than the NRC requirements to reach the lower regions of the optimal ranges for nutrients (Figure 2), and hence be more useful guides for practical nutrition.

Researchers have recommended that horses should be fed 2- to 5-times the vitamin A requirement specified by the NRC for growth and reproduction (Donoghue et al., 1981; Greiwe-Crandell, 1996). Others have recommended 2- to 3-times the Cu requirement specified by the NRC (Knight et al., 1985). Both vitamin A and Cu interact with several other vitamins and minerals, so elevating these two nutrients but not the other vitamins and minerals is likely to induce imbalances. In practice, our goals for vitamins and minerals are about 1.5 to 3 times the NRC minimums, a range based partly on the literature and partly on our own experiments with vitamin A, phosphorus, calcium, zinc and selenium (Kronfeld et al., 1996). Target ranges are also modified in line with likely availabilities of nutrients in various ingredients.

Goals for dietary protein are historically the most contentious in nutritional science. Applying a quadratic curve to data from three good growth studies in the literature indicates peak weight gain at a crude protein (CP) level of 160 g/kg of DM, with 1.0 SE below the peak at 130 and 190 g/kg, which may be taken to be an optimal range (Thiers and Kronfeld, unpublished). The NRC's minimum requirement for maintenance, 8.0% CP (80 g/kg), seems like a sub-subsistence for wasting tissue in 50% or more horses, especially for old horses (Ralston and Breuer, 1997). Examination of the NRC's Tables 5-2a and 5-2b reveals a range of CP contents for concentrates from 10.8% of DM for light work to 17.3% for rapid growth (NRC, 1989). Most manufacturers make a series of concentrates with CP contents of not less than 12, 14 and 16% as fed (13.3 to 17.8% DM). This series neglects the competitive athlete, which must balance protein benefits against disadvantages. More protein may be needed for hypertrophy and repair, for stress and, especially in the horse, to compensate for nitrogen losses in sweat. On the other hand, the athlete needs less protein to minimize the production of acid, heat and urea. To minimize protein quantity without compromising protein needs, protein quality should be as high as possible in a diet for top athletes. High quality protein is needed also to minimize pasture contamination with nitrogen.

Goals for dietary carbohydrates are currently the most contentious in equine nutrition. The traditional guideline of forage intake equal to 1% body weight (NRC, 1989) needs refinement for various purposes; for example, we use entirely different design objectives for our athlete's diet versus our pasture supplement. The proximate analysis of carbohydrates designed for ruminants is inappropriate for the horse (Figure 4, upper part). For a hindgut fermenter, the two main physiological groups should be hydrolyzed carbohydrates (CHO-H) and fermented carbohydrates (CHO-F), since CHO-H yields mainly glucose, whereas CHO-F yields mainly acetate, propionate and butyrate. Digestive and metabolic processes are much more efficient for CHO-H than for CHO-F. Also, the CHO-F should probably be divided into slowly fermented fibers (CHO-S) and rapidly fermented fibers (CHO-R), because the latter tend to give rise to lactate rather than acetate, thus raising risks of several disorders.

NONSTRUCTURAL CARBOHYDRATE	• ***HYDROLYZABLE*** **Monosaccharides** **Disaccharides** **Some starches** • ***FERMENTED*** **Oligosaccharides** **Resistant starches** **Gums, mucilage, pectins**
NEUTRAL DETERGENT FIBER	**Hemicelluloses**
ACID DETERGENT FIBER	**Celluloses** **Lignocelluloses** **(Lignin)**

- ***HYDROLYZABLE*** **→ glucose**
  Sugars and some starches
- ***FERMENTED RAPIDLY*** **→ lactic acid → acetic acid**
  Resistant starches, oligosaccharides
  Gums, mucilages, pectins
  Hemicelluloses
- ***FERMENTED SLOWLY*** **→ acetic acid**
  Cellulose
  Lignocellulose

Figure 4. The daily intake of DM and DE is highly variable, with coefficients of variation (CV) of about 8% for maintenance and about 16% for rapid growth. For a mean intake of 16.4 Mcal DE, the 90% confidence interval (±1.7 CV) is 14.2 to 18.6 Mcal for the mature horse and 11.9 to 20.9 Mcal for the weanling. Thus, if 20 weanlings are group fed, one will consume nearly twice as much DE as another one. If half the intake is provided as a highly palatable supplement, then all of the variation will be compressed into the pasture intake. Now the mean intake of pasture is 8.2 Mcal, and the range is 6.0 to 10.4 Mcal for the horse and 3.7 to 12.7 Mcal for the weanling. If 20 yearlings are group fed, one will consume 3.4-times as much pasture as another one. The impact of this huge variation in pasture intake should be assessed by sensitivity analysis.

We have enzymatically assayed CHO-H in 130 forages and 30 concentrates and found that CHO-H, hence also CHO-R, can be predicted approximately from nonstructural carbohydrates, NSC (Hoffman and Kronfeld, unpublished data):

CHO-H = 0.3 x NSC in forages
CHO-H = 0.6 x NSC in concentrates
CHO-F = (NSC - CHO-H)

Of course, any overload of CHO-H that escapes hydrolysis in the small intestine will be fermented rapidly in the large bowel. Thus, we are now able to evaluate rations and design diets and supplements with goals specified for physiologically different carbohydrates (Figure 4, lower part).

Most clients of professional nutritionists are seeking help to achieve a high level of performance of various kinds. Each nutritionally competent person or corporation in equine practice has a set of nutritional goals or tentative target ranges, and these goals are usually regarded as proprietary and valuable intellectual properties.

Ration Evaluation

Ration evaluation is needed most often to identify and describe the role of the food and feeding management in poor performance or disease. The traditional approach is to determine amounts of concentrates and hays offered, which is usually fairly precise, then how much remains unconsumed, which is often zero for concentrates but sometimes more difficult to determine for hays (Kronfeld, 1978; Lewis, 1995; Pagan et al., 1996). The cumulative errors are probably less than ±10% for stall-fed horses, but much larger for horses with access to pasture, up to ±20% for horses at maintenance and up to ±40% for young horses (Figure 4). These huge errors are seldom acknowledged by nutritionists and epidemiologists. The procedures described herein, however, are designed to cope with variation and to provide a realistic picture of the lack of precision in ration evaluation.

In practice, one visits the farm or stable and interviews the staff to determine the volumes of hays, concentrates and other supplements that are offered and, if possible, any amounts not consumed. At least two, preferably five, of these volume measures (cans or cups of concentrates, flakes or bales of hay) are weighed. At least two samples of each concentrate and five samples of each hay are taken. Pastures are usually sampled by walking the two diagonals and clipping the grasses and legumes, neglecting the weeds, every 10 paces. For each forage or feed, samples are combined and thoroughly mixed, then duplicate subsamples are submitted for proximate and mineral analyses.

The subsamples are sent to a laboratory that analyzes forages and feeds. The routine profile is likely to be suitable for ruminants (for dairy cattle in the US, reflecting the importance of the Dairy Herd Improvement Association). Routine profiles usually include dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), nonstructural carbohydrates (NSC, by difference), ether extract (EE or crude fat), ash, Ca, Mg, P, Na and K. Trace element assays are usually available, and Cu, Zn and Se are often requested for the horse, together with Fe, Mn, Mb, I and S, depending partly on expense. The main inadequacies of ruminant nutrient profiles for the horse are the proximate assays for carbohydrates (Figure 4), as discussed above.

The ration and diet can now be calculated as weighted averages (Figure 5). The energy and nutrient content of each dietary component (e.g., g/kg food) are multiplied by the component's daily intake (kg/day/animal) to obtain the daily intakes of energy and nutrients from that component. Then the sums of these component intakes of energy and nutrients comprise the ration. These intake totals for energy and nutrients are divided by the sum of the intake weights to give the overall diet. The example (Figure 5) is for a 215 kg (473 lb) weanling, 6 months old in October. The supplement is a typical, high quality concentrate with an energy density of 3.3 Mcal/kg DM and a guaranteed analysis for CP >15% (170 g/kg of DM). The pasture was browned off in a hot August, so some hay is provided. A single weighted average for energy and all measured nutrients is where the traditional ration evaluation stops.

WEIGHTED AVERAGES

WEANLING, 6 MONTHS, 215 KG

VARIABLE	CONC[a]	HAY	PASTURE	RATION[b]	DIET[c]
DM, kg/d	3	2	1	6 (2.8% W)	
DE, Mcal/kg	3.8	2.0	1.8		2.62
Mcal/kd	9.9	4.0	1.8	15.7	
CP, g/kg	170	100	80		132
g/kd	510	200	80	790	

[a] Concentrate. [b] Ration = Conc + Hay + Pasture. [c] Diet = Ration/5.91.

Figure 5. Rations and diets are calculated as weighted averages. For example, the concentrate intake of 3 kg/d of dry matter (DM) is multiplied by the concentrate's content of digestible energy (DE), 3.3 Mcal/kg DM, to give the intake of 9.9 Mcal/d of DE from concentrate. The same calculation is performed for hay (4 Mcal/d) and for pasture (1.8 Mcal/d), then the three DE intakes are added to give the total intake, 15.7 Mcal/d, which is the energy provided by the ration. This number is divided by the total DM intake, 6 kg, to yield the energy density of the diet, 2.62 Mcal/kg. The procedure is repeated for crude protein (CP). In practice, weighted averages are obtained for energy and all measured nutrients to describe the ration and diet.

Sensitivity analysis may now be applied to the variation in DM intake, hence the error in estimating pasture intake (Figure 6). The DM intake of weanlings has a range of 2% to 3.5% of body weight (NRC, 1989). In this example, the midpoint is taken as 2.8% or 6.0 kg, and the intakes of concentrate and hay are 3 and 2 kg, respectively (from Figure 5), so that the intake of pasture is 1.0 kg. The upper limit is 7.5 kg, and the corresponding pasture intake is 2.5 kg. The lower limit should be 4.3 kg (2% of 215 kg), but this youngster is already consuming 5 kg of DM, so its pasture intake is estimated at -0.7 kg, which we reduce to zero. If we inspect the diets, the one with the lowest pasture content is the best. This is misleading, however, because consuming too little of an admittedly better diet is still underfeeding. In practice, full attention should be given to the ration. Clearly the yearling should consume DM between the middle value and upper limits, 6 and 7.5 kg/day, to obtain the energy and nutrients that it needs--at least 16.1 Mcal DE, 800 g CP, and 32 g Ca (NRC, 1989). This example (Figure 6) supports the need for sensitivity analysis, and emphasizes why the ration should be evaluated, rather than the diet.

SENSITIVITY ANALYSIS

WEANLING, 6 MONTHS, 215 KG

	DM INTAKE, %W		
	2	2.8	3.5
DM, intake, kg/d	5[a]	6.0	7.5
DE, Mcal/day	13.4	15.7	18.4
Mcal/kg DM	2.68	2.62	2.45
CP, gl/day	710	783	912
g/kg DM	124	132	122
Ca, gl/day	38	41	46
g/kg DM	7.6	6.8	6.1

[a] 2% of 215 = 4.3 kg; Conc 3 kg, Hay 2 kg, so pasture intake is *- 0.7 kg!!!*

Figure 6. Sensitivity analysis is applied to the full range of dry matter (DM) intake for a weanling. In this example, solutions are found for the lower limit, 2% of body weight (W), the middle value, 2.8%, and the upper limit, 3.5%. The best diet is found for the lowest intake, but an adequate ration is provided between the middle and upper intakes. The calculation of a negative pasture intake is not uncommon although clearly an indication of lack of precision and accuracy in estimates of intakes. This example emphasizes the need to evaluate the ration rather than the diet of an animal.

The calculation of diets and rations by means of weighted averages is simple but tedious, time consuming and subject to errors that are hard to find and usually repeated. For these reasons, ration evaluations were usually limited to energy, protein, calcium and phosphorus before the advent of the personal computer. The mathematical drudgery of ration evaluation has been eliminated by the availability of inexpensive, user-friendly computer programs. Commonly available spreadsheets, such as Lotus and Excel, readily perform weighted averages.

Specifically designed dietetic programs calculate the total diet and the ration or daily intakes of energy and nutrients, then compare these sets of values with nutritional goals, such as the nutrient requirements of horses (NRC, 1989). In addition, some programs will calculate diets and rations from combinations of feed ingredients, either from specified amounts or on a least-cost basis if the cost of each ingredient is entered into the program.

The relative merits of rival programs are arguable both objectively and subjectively. I became familiar with *Animal Nutritionist* (Version 2.5, 1987, N-Squared Computing, Silverton, OR) a decade ago and continue to use it. This software can be used to evaluate a ration, calculate a diet from specified amounts of ingredients (stored in an adjustable data bank), or calculate a least cost diet from a stipulated set of ingredients. Much simpler to use and inexpensive is *Spartan* (Cooperative Extension, Michigan State University, East Lansing, MI). A recent program for ration evaluation is *Microsteed* ™ (Kentucky Equine Research Inc., Versailles, KY).

This software has several advantages: it is designed solely for the horse; it is simple to use; it is the subject of detailed explanatory articles (Pagan et al., 1996); it is likely to be updated and expanded; and, most of all, it offers an option of using the minimum requirements of the NRC (1989) or Kentucky Equine Research's own set of nutritional allowances.

Diet or Supplement Formulation

A maintenance level of nutrition is usually provided by forage-pasture or pasture conserved as hay, preferably at peak energy and nutrient contents. For above maintenance purposes, such as growth, reproduction and hard work, forage intakes are usually supplemented with concentrates, which have higher contents of energy and essential nutrients. One might expect, perhaps, that a single forage supplement could be designed to meet all of these purposes by appropriate feeding management, that is, by varying the concentrate:forage ratio. This ratio may be varied from 0:100 at maintenance to 70:30 for rapid growth, as recommended by the NRC, changing the energy density from 2.0 to 2.9 Mcal/kg.

Pasture varies in nutritional quality through the seasons, so presents wide ranges of contents of most nutrients. In north-central Virginia, we found 90% confidence intervals for 130 samples as for the first 33 samples, so believe that these ranges are reasonably well defined for this region (Hoffman et al., 1996; Wilson et al., 1997). Thus, we had the information needed for the statistical exercise of designing a robust, flexible pasture supplement capable of reaching target zones for all nutrients of interest when used to provide 25 to 50% of the ration. The concentrate would be used to increase energy and nutrient densities above the maintenance level, that is, for growth, reproduction and hard work.

Copper is given as an example of the use of sensitivity analysis in designing a pasture supplement (Figure 7), because it was often marginal or deficient in our survey, and because of its potential importance in developmental orthopedic disease. The minimum requirement is 10 mg/kg DM (NRC, 1989). Our target zone is set by lower, middle and upper values of 15, 20 and 30 mg/DM, respectively. Virginia forages have a mean of 8 mg/kg and a 90% confidence interval of 6-12 mg/kg. We start with the middle values, 9 mg/kg Cu in forage, 20 mg Cu target and 33% concentrate (concentrate:forage, 1:2). A Pearson square is used to calculate that the concentrate should contain 42 mg/kg of Cu (Figure 7). Next we practice sensitivity analysis, using the 6-12 mg/kg Cu range in the forages and the range of 25-50% concentrate (concentrate:forage, 1:3 to 1:1). The resulting range of Cu in the mixtures is 15-27 mg/kg, right on our target of 15-30 mg/kg. If the initial estimate of concentrate Cu had given a value outside the target range, further iterations would be explored to find a best fit.

Similar calculations were performed for iron, manganese, zinc, molybdenum, selenium, iodine, calcium, phosphorus, magnesium, potassium, and sodium. We could determine mineral specifications for a single pasture supplement for all grass forages and all grass:legume mixtures that contained up to about 35% legumes. Another formula would be needed for alfalfa hay.

SENSITIVITY ANALYSIS in DIET FORMULATION

GIVEN: **VIRGINIA FORAGES, COPPER 6 - 12 mg/kg DM**
TARGET: MIDDLE 20, LIMITS 15 AND 30 mg/kg
CONCENTRATE FORAGE 25:75 to 50:50.
TASK: **SELECT CONCENTRATE COPPER.**

1. Calculate for middle values, Cu 9 mg/kg, C:F 33:67.

9 ↘		↗ **22**	**67%**
	20		
42 ↗		↘ **11**	**33%**

2. Do sensitivity analysis for ranges.

Cu	**CONCENTRATE:FORAGE**		
mg/kg	**25:75**	**33:67**	**50:50**
6	**15**	**18**	**24**
9	**17.2**	**20**	**25.5**
12	**19.5**	**22**	**27**

Figure 7. Sensitivity analysis is applied to determine the copper (Cu) content of a pasture supplement. First, middle values for the nutritional goal (20 mg/kg), forage Cu content (9 mg/kg), and concentrate:forage ratio (33:67) are used in a Pearson square to calculate a first estimate of Cu content (42 mg/kg) in the concentrate. Second, sensitivity analysis is applied to the lower, middle and upper values for forage Cu content and concentrate:forage ratio. In this case, there was no need to attempt further iterations of the concentrate's Cu content.

Conclusion

The availability of computer software for ration evaluation and diet formulation enables the practical use of sensitivity analysis to explore the effects of reasonable ranges of pasture intakes, ingredient composition and nutritional goals. Balancing rations and diets by means of ranges with specified lower, middle and upper values, instead of single, middle numbers, gives a truer picture of the real world. It improves the chances of detecting weakness in rations, and it enables the design of more flexible and robust diets and pasture supplements for horses.

References

Anderson JR. 1974. Simulation methodology and application in agricultural economics. Rev Markt Agric Econ 42: 3-25.

Donoghue S, Kronfeld DS et al. 1981. Vitamin A nutrition of the equine: growth, serum biochemistry and hematology. J Nutr 111: 365-374.

Greiwe-Crandell KM, Kronfeld DS et al. 1995. Seasonal variation in vitamin A status of grazing horses is assessed better by the relative dose response test than by serum retinol concentration. J Nutr 125: 2711-2716.

Hoffman RM, Kronfeld DS et al. 1996. Dietary starch and sugar versus fat and fiber: growth and development of foals. Pferdeheilkunde 12: 312-316.

Knight DA, Gabel AA et al. 1985. Correlation of dietary mineral to incidence and severity of metabolic bone disease in Ohio and Kentucky. Proc Am Ass Equine Practnr 31: 445-461.

Kronfeld DS. 1978. Feeding on horse breeding farms. Proc Am Ass Equine Practnr 24: 461-464.

Kronfeld DS, Cooper WL et al. 1996. Supplementation of pasture for growth. Pferdeheilkunde 12: 317-319.

Kronfeld DS, Ferrante PL, Grandjean D. 1994. Optimal nutrition for athletic performance, with emphasis on fat adaptation in dogs and horses. J Nutr 124: 2745S-2753S.

Martin SW, Meek AH, Willeberg P. 1987. Veterinary Epidemiology: Principles and Methods. Iowa State University Press, Ames.

National Research Council. 1989. Nutrient Requirements of Horses, ed. 5. National Academy Press, Washington, DC.

Pagan JD, Jackson S, Duren S. 1996. Computing horse nutrition: How to properly conduct an equine nutrition evaluation using Microsteed™ equine ration evaluation software. World Equine Vet Rev 1(2): 10B1-7.

Ralston SL, Breuer LH. 1996. Field evaluation of a feed formulated for geriatric horses. J Equine Vet Sci 16: 334-338.

Wilson JA, Kronfeld DS et al. 1997. Seasonal variation in nutrient composition of northern Virginia forages. Proc Equine Nutr Physiol Soc