Kentucky Equine Research®
World Leaders In Equine Nutrition

Advances in Equine Nutrition

Volume I

Edited by

J.D. Pagan

BLOOD ANALYSIS AND ITS RELATIONSHIP TO FEEDING THE PERFORMANCE HORSE

PER SPÅNGFORS
Euro-VETS AB, Simlångsdalen, Sweden

Introduction

The discussion in this paper concerns the performance horse, and cannot without proper adjustments be extrapolated to the young growing horse or the pregnant mare.

There is a great danger with blood analyses in that you get a figure on a piece of paper. Most people regard that figure as the absolute truth, and that this figure is static until you take the next blood sample. If the person in question also compares that figure with a reference value and finds his horse's value within the expected limits for health, then the clinical picture is of no concern to him, and no matter how the horse looks, he is sound according to the blood test. If the horse starts in a competition and fails, the person raises his eyebrows and wonders why the blood test failed to tell him that the horse was in no condition to compete.

This somewhat pessimistic scenario could be one reason why people are beginning to lose faith in the analyses. This person should have realized that the figure in his hand is a photograph of a dynamic situation, and was only valid for that particular situation. He should also know that the clinical picture of his horse is the truth, no matter what the blood test and normal values say, and he should have consulted a specialist about the interpretation of the analyses, but the absolute truth is never revealed.

Why use clinical chemistry in the feed industry?

The use of body fluids (blood, urine, milk) for the development of new feeds for the performance horse is possible because we now know more about the desirable reactions after feeding. As a complement to classical methods (digestibility trials, field studies) and in conjunction with exercise physiology trials, the benefits for the feed industry to add clinical chemistry to these research models should be:

1) Good estimate of the metabolic responses after feeding, including fuel utilization and hormonal responses.

2) Possible to measure waste product production during rest and exercise.
3) Gives valuable information of the mineral metabolism and turn-over.
4) Can be used to fine tune the balance of nutrients in a complete feed.
5) For cheap and easy follow-up studies after feed composition adjustments.

Basic facts

There is an enormous amount of information hidden in a blood sample and a lot of parameters to choose between. Therefore, it is essential to consider some basic facts before the sample is drawn.

1) The purpose of the test:
   a) Health test
   b) Disease diagnostics
   c) Nutritional control
   d) Performance prognosis

2) What type of blood to take
   e) Venous (blood to the heart)
   f) Arterial (blood from the heart)

3) Parameters to analyze

4) What kind of test-tubes
   g) Serum (no anticoagulant)
   h) Heparin (Sodium or Lithium)
   i) EDTA (Potassium)
   j) Fluoride (for glucose analyses)
   k) Other

5) When to take the sample
   l) Before and/or after feeding
   m) Before and/or after training

6) Method of controlling the results
   n) Lab's reference values
   o) The horse itself (previous tests)

7) Treatment of the sample

o) Immediate analysis
p) Stored in ice water or room temperature
q) Centrifuged or whole blood

Evaluation of feed with blood-tests

Evaluation of feeds by biochemical analyses of blood requires a strategy somewhat different from other sampling techniques. The horse must be its own control and the samples must be taken in narrow intervals. An overnight fast is essential and the first sample is taken before feeding. After feeding, blood is collected with 30 minutes to 1 hour intervals until 4 hours post feeding. The first sample, before feeding, reflects the absorption of nutrients from the hindgut and the metabolic response to hindgut fermentation. The other samples reflect the absorption of nutrients from the small intestines and the metabolic response to that absorptive phase. This is hence a good way to study the hormonal changes after feeding and can therefore be used to interpret how long after feeding the horse is work intolerant. It is also possible to get a rough idea how long the feed takes to pass the small intestines (when certain values return to “before feeding” figures).

Urine and mineral studies

Many studies of minerals including electrolytes use a technique called urine fractional excretion, FE (sometimes called creatinine clearance or fractional clearance). The renal clearance of a mineral is expressed as a percentage of the excretion of creatinine.

A urine sample and a blood sample are taken no more than one hour apart. Both the serum and the urine are analyzed for the studied minerals and creatinine. The fractional excretion ratio is calculated from the equation:

$$\frac{\text{Mineral } \textit{urine}}{\text{Mineral } \textit{serum}} \times \frac{\text{Creatinine } \textit{serum}}{\text{Creatinine } \textit{urine}} \times 100$$

This technique assumes that the urine excretion of creatinine is constant which is not always the case. There are some inborn errors as we shall comment.

Creatinine is a degradation product of creatine, an energy rich compound found in muscles as phosphocreatine. The formation of creatinine in non-meat eating animals is dependent on 1) the rate of synthesis of creatine (from amino acids arginine, glycine and methionine), 2) the formation of creatinine from creatine and 3) the muscle mass (Finco, 1989).

Creatinine is highly soluble in water and is therefore equally distributed in intra and extra cellular tissues. The urine excretion is consequently constant with these important exceptions. A) The formation of creatinine from creatine rises in exercising animals. B) The excretion of creatinine is lowered in kidney diseases (even in sub clinical cases) (Finco, 1989). C) Creatinine excretion can also be affected by diet even if no creatinine is present in the feed (Lindberg and Jacobsson, 1992), and creatinine was found to be significantly lower in urine when NDF (neutral detergent fibre) content in the feed was high due to added sugar beet pulp. The creatinine in plasma was not measured in this study. D) Dehydration probably also affects FE. E) Furthermore, the excretion of the mineral in question is not constant and is sometimes elevated after feeding, for example sodium after grain rich diets, and sometimes delayed, as for phosphorous because of the main absorption site in the hind gut.

When using fractional excretion technique to study mineral losses the horse must not be exercised prior to sampling (24 hours). The horse must be healthy and hydrated and the samples must be taken at a constant interval from feeding, in order to be comparable. The feed itself must be evaluated for the effect of creatinine. If these criteria cannot be filled, total urine collection must be done in a balanced trial.

Calcium sediments in horse urine due its alkaline nature. Repeated measurements are therefore difficult to attain. An inadequate intake of calcium stimulates the secretion of parathyroid hormone (PTH). PTH also decreases the renal reabsorption of phosphate. PTH is often successful in maintaining the serum calcium through mobilization of bone calcium, and increased intestinal uptake of calcium, whereas the serum phosphate tends to drop. Inadequate calcium intake gives us the picture of normal serum calcium, low serum phosphate and increased FE % of phosphate.

Direct methods of assessing calcium nutrition are also possible and several methods are described (Caple, *et al.*, 1982).

Minerals suitable for FE studies or total urine collection trials

Calcium	Magnesium	Silicon
Chloride	Phosphorous	Sodium
Chromium	Potassium	Sulfur
Fluorine	Selenium	Zinc
Lead		

NORMAL FE RANGES

FE %	Hay diet		Grain based diet	
Magnesium	>20		>15	
Phosphate	0-0.2[1]		0-0.5[2]	
Sodium		0.04-0.54[1]		0.02-1[2]
Potassium	35-80[1]		15-65[2]	
Chloride		0.7-2.1[1]	0.04-1.6[2]	

[1)]Harris, *et al*, 1991 [2)]Traver, *et al*, 1976

Statistical revision of the results

The results from a trial, including blood tests, need special procedures when it comes to the statistical handling.

Many parameters from such trials show a non-normal distribution. This makes the figures unsuitable for common parametric techniques, such as ANOVA and regression, for raw data interpretation. A non-parametric method, for example rank sum tests or ANOVA on ranks, must be used. Another way to cope with this problem is to do some form of transformation of the raw data.

As we analyze many parameters from the same individual, the independent variables are all related to one another. This must not be the case in regression procedures. This situation is called multicollinearity. When the independent variables are correlated (as more or less all analyzed parameters are), they all hold some common information. In regression models all independent variables must contribute with unique information (Glantz, *et al*., 1990).

There are two kind of multicollinearity; structural multicollinearity, when the independent variables are a function of each other, and sample-based multicollinearity, which occurs when many parameters are measured on the same individual. There are several ways to measure multicollinearity and to solve the problem. One way to resolve structural multicollinearity is to collect more data under other circumstances to break up the relationship (Glantz, S.A. *et al*., 1990).

As we often use a rather small number of horses in nutritional research, it is extremely important to check the raw data for influential data points which are outliers. There are several ways to identify and quantify influential data points (Glantz, S.A. *et al*., 1990).

In nutritional research we deal with a multi-variate situation. To conduct classical trials with a small number of experiments and using t-test and ANOVA for interpretation, is not always the best way, but definitely the cheapest. A better, but far more expensive method, is to design the trial for multi-variate evaluation, such as principal component analysis (Mardia, K.V. et al, 1989).

Discriminant analysis is also a good way to see that your trial was correct, in that afterwards you can let the computer suggest which feed the horses probably have eaten, according to the blood results. If the computer does the right classification as in reality, your trial was successful. You can also see which blood-parameters have the strongest influence on the classification (Mardia, K.V. *et al.*, 1989).

Pitfalls and errors

In all analytical work there are inherent errors. Sampling technique, storage after sampling and laboratory errors are the most prominent errors affecting the result of a blood test.

One type of laboratory error is suspected if we make ten analyses on the same sample, and we get ten different values. We measure the variation in the analytical results as coefficient of correlation (CV=Standard deviation/mean *100). As the horse itself has a very low variation in most parameters, an inferior analytical machine is responsible for most of the variation in the test results. From such a machine it is difficult to see the variation contributed by the feed. CV under 2% is a must for most parameters when dealing with horse-blood. Just as important as the precision, is the accuracy of the measured parameters. This means that the values from the machine must have a clinical significance. Not many machines and analytical methods can give these low variations and acceptable accuracy. The laboratory must spend a lot of effort to accomplish this, with methods validated for horses.

Robots for pipetting and highly automated routines are essential for a good result. For hematology, automated systems for measuring, cell counting and white cell differential counts are necessary. This equipment costs of course a lot of money, but unfortunately it is necessary when dealing with blood tests from horses.

Blood tests to evaluate nutritional status in individuals

Interpretation of blood tests is an art. It takes knowledge, intuition and fantasy to do a good job. You have to see patterns in the figures and understand the dynamics between the different parameters. To be an experienced interpreter, the feed-back from the veterinarians, trainers and horse owners is essential. First when you compare the real situation with the test results in discussion with the horse handler, you can develop your skill in reading test results.

From the discussion above it is clear that in order to use blood samples to evaluate nutritional status, it all depends on how you do it. If you have a reliable laboratory and know which parameters to analyze, and when to take the samples, it is possible to make a good guess on the nutritional status for some nutrients (see list above).

As a general rule, it is much easier to see when something is wrong, than to see when the situation is optimal. In other words, it is easy to see when you can expect poor performance, but almost impossible to see when the horse is in perfect shape. Or, it is easy to see a copper deficiency, but impossible to see when copper for this horse is optimal.

We use blood tests to scan all the individuals in a stable, and we can get a rough picture of the health status, performance status and nutritional status by doing this. We encourage the trainers/horse owners to take the samples at least once a month. One must use the test results as a complement to what you see and what you feel about the horse when in training. We find a good clinical relevance in the test results, but we also find imbalances among the parameters, and are often able to correct these imbalances before clinical symptoms occur.

What is normal?

Normal range is a statistical way to help understanding non-normal situations. But as it is just a statistical value, many exceptions exists. Furthermore, we can have many different normal ranges, one for the whole population (widest range), one for clinical situations (most used range), and one for performance evaluations (narrowest range). Every laboratory must have their own set of normal values, as analysis procedures, analytical temperatures, hardware and sample handling differ among laboratories. To simply use literature values is meaningless.

The best normal values come from the horse itself. Previous blood tests from the same horse can be used if the samples are sent to the same laboratory and have been taken on similar occasions as the previous ones. It is hence a good practice to standardize your sampling procedures and make notes of sampling conditions for future use.

Interpretations

Most of the parameters analyzed from blood give an unspecific picture of the biochemical processes. Consequently, we have to analyze many unspecific parameters in order to get a more specific illustration of the situation. By doing this we see patterns in the figures, and do not necessarily have to have values outside the normal ranges to spot pathologic situations. This in turn makes each blood test more difficult to explain.

Many nutritional imbalances affect the hormonal system. And as metabolic active hormones always have more than one target organ, and there are many correlations between parameters, you can be certain to find more than one parameter diverging from normal. This is why you can see a pathologic pattern if you analyze many parameters.

Many metabolic active hormones are possible to analyze even in mailed blood samples, such as insulin, cortisol, T4 and T3, while others, growth hormone (GH), insulin-like growth factor-1 (IGF-1), glucagon, parathyroid hormone (PTH) and calcitonin, are for research or hospitalized horses only. To measure hormone levels requires special attention and is best used to confirm a suspected disturbance discovered by the ordinary blood test.

It is of little use to analyze hormones without making a stimulation or inhibition test, because hormone levels show a natural variation during the day due to feeding, exercise or circadian rhythm. Thus, insulin, T4, T3, and cortisol can be stimulated by glucose tolerance tests and T4 and T3 alone by giving thyroid releasing hormone (TRH). Tolerance tests often give valuable information of an individual's response-status, and can give information on how this particular horse should be fed.

Table 1. SOME COMMONLY ANALYZED PARAMETERS IN HORSE BLOOD AND THE POSSIBLE USE OF THE DIFFERENT PARAMETERS FOR THE FEED INDUSTRY AND FOR EVALUATION OF NUTRITIONAL STATUS.

Group	*Used for feed Parameter*	*Evaluation*	*Comments*
1) Hormones	insulin	Yes	Metabolic evaluations
	thyroxine (T4)	Yes	and to follow responses
	triiodothyronine (T3)	Yes	from feed and exercise
	cortisol		
2) Fuels	glucose	Yes	Responds on feeding
	triglycerides	Yes	and exercise
	fatty acids	Yes	
	cholesterol	Yes	
	glycerol		
	amino acids	Yes	
	pyruvate		
3) Waste products	urea	Yes	Responds on feeding
	lactate	Yes	and exercise
	ß-OH butyrate	Yes	
	bilirubin		
	ammonia	Yes[1]	
	creatinine	Yes[2]	
	urate		
4) Enzymes	AST[3]	Yes	High values can reflect cell
	CK[4]	Yes	damage or increased production.
	ALP[5]		In general, high starch diet, high
	GT[6]	Yes	enzymes.
5) Minerals	calcium	Yes[1]	Apart from copper the
	magnesium	Yes	interpretations of the
	phosphorus	Yes	test results are very
	copper	Yes	difficult except when low
	zinc	Yes[1]	values are found which has
	iron	Yes[1]	a high diagnostic value.
	selenium	Yes	
6) Electrolytes	sodium	Yes[1]	Along with some of the
	potassium	Yes[1]	minerals, urine is the best fluid
	chloride	Yes[1]	for analysis
7) Vitamins	B_{12}	Yes	Good indicators of feed quality.
	folate		Retinol requires special
	retinol	Yes	procedures for evaluation[7]
	tocopherol	Yes	

Table 1. (CONT'D)

8) Serum proteins losses	total protein albumin globulins fibrinogen		Reflects indirectly nutritional status (but also excessive via intestine). Albumin is a big protein reservoir
9) Acid/base balance	O_2 CO_2 HCO_3-	 Yes Yes	Can reflect acid/base status after feeding. O_2 ,CO_2 is independent, HCO_3-, pH pH dependent of CO_2[8]
10) Haematology	red/white cells hematocrit haemoglobin MCV[9] differential count		Requires sophisticated equipment for nutritional studies. Haemoglobin concentration is more affected by horones and protein intake than dietary iron.

1) Requires special care and/or sampling techniques for evaluation
2) In mineral studies in conjunction with analysis of urine
3) Aspartate aminotransferase (GOT) [4)5)]
4) Creatine kinase
5) Alkaline phosphatase
6) Glutamyltransferase
7) Loerch, *et al.*, 1979
8) Stewart, 1981
9) Mean Corpuscular Volume (Mean red cell volume)

Table 2. SOME BLOOD PARAMETERS AND THEIR RESPONSE TO FEEDING.

Parameter	*Postprandial reaction*	*Optimal reaction for performance*	*Comments*
Glucose and insulin	elevated[1]	higher peaks-longer work intolerance	The higher starch content of the feed higher peaks of both.
Cortisol	NA[1,2]	is a stress factor if mean level is high	Cortisol increases the breakdown of glycogen and body fat. Exercised induced release.
Thyroxine (T4)	sparse elevation	normal levels enhances performance	Is an antagonist to insulin but is sometimes lowered (hypothyroidism) in the post absorptive phase (adverse reaction to the feed).
Triiodothyronine (T3)	elevated	as T4, is an insulin antagonist	The conversion of T4 to T3 is stimulated by insulin-higher peaks than T4. T3 enhances performance.
Fatty acids	lowered	high levels - glycogen sparing effect	Elevated in the hindgut-absorptive state. Excellent fuel for all horses. Utilization of FA as a fuel is proportional to plasma concentration in man[3].
Cholesterol	NA[2]	important carrier of vitamins	The mean level is strongly to the fat content of the feed up to a certain limit[4].
Urea	sparse elevation	high levels - negative	High levels increase water consumption and urine formation Uses bicarbonate when synthesized. Reflects the protein quantity and quality of feed. (Protein/energy balance).
Ammonia	lowered	high levels in fatigue	Strong inverse Correlation to ATP levels in muscles of exercised horses[5].
AST	NA[2]	low levels good for performance	Mean levels higher for starch rich diets than in fiber rich diets.

Table 2. (CONT'D)

CK (CPK)	variable	as for AST	Mean levels higher for starch rich diets than in fat rich diets.
GT	NA[2]	low levels good for performance	High grain diets increase synthesis due to higher stress hormone levels and gives high mean levels in serum (feed stress indicator). Fibre/fat rich diets give lower values. Sometimes very high levels when long term feeding of high fat diets.
Magnesium	elevated	low levels negative for performance	In magnesium deficient horses serum levels are normal due to decreased urinary output. Muscle and urine levels are better indicators of Mg-status.
Phosphate	often lowered	narrow range for optimum performance[7]	Low levels not only by low absorption but also by high urinary output. Inorganic phosphorous is the lowest level of energy, ATP the highest.
Copper	NA[2]	as for phosphorous	Mean plasma levels is a good indicator of feed levels on a long term basis. Liver storage normalizes low intake for several months. Strong inverse correlation to plasma iron (and zinc of healthy horses). Copper deficiency increases MCV in exercising horses and lowers white cell count in nonexercising horses.
Iron	NA[2]	as for phosphorous	Iron deficiency rarely seen. Often high levels in copper deficient horses.
Sodium	sometimes elevated	as for phosphorous	High grain diets give higher peaks. Glucose sodium coupled uptake as in pigs not demonstrated in horses but likely.

Table 2. (CONTD)

Potassium	Often lowered	as for phosphorous	High feed levels in conjunction with high sugar levels (molasses) can give a mild transient hyper-kalemia. Low dietary cation/ anion balance give low concentration in plasma.
Chloride	varies	influencing acid/base status	Abundant negatively charged ion in serum. Increased urinary excretion in hay diets.
Vitamin B_{12}	NA[2]	high levels good for performance	Reflects cobalt status and hindgut microbe activity. Synthesized by hindgut microbes. High levels are seen in well balanced diets with high fibre quality and adequate cobalt intake.
Tocopherol	elevated	high levels good performance	Plasma values follow feed content. Levels must be correlated to plasma lipids[8].
pH	varies		Often postprandial acidosis in high grain diets. Fibre and/or fat rich diets usually don't affect pH levels.
Haemoglobin	NA[2]	low and high levels negative	Is a poor indicator of iron status. Affected by hormones (T3) and protein intake. Low plasma copper influence HGB.
MCV	NA[2]	as for phosphorous	MCV can, on a long term basis, be affected by nutrients, such as copper.

[1)]Stull, *et al*., 1988
[2)]NA-Not Affected, means that the concentration in blood is not markedly raised or lowered up to 4 hours after feeding but the mean levels are influenced by feeding.
[3)]Jansson, *et al.*, 1982.
[4)]Hambleton, *et al*., 1980
[5)]Harris, *et al*., 1991
[6)]Snow, *et al*., 1987
[7)]Denny, *et al*., 1987
[8)]Horwitt, *et al*., 1972

Summary

Blood analysis is a good complement to conventional methods for the development of new feeds or for characterizing existing feeds. It is necessary for the laboratory to use validated methods with high precision and accuracy. Several parameters must be analyzed to get a more specific picture of the dynamic processes. Sampling technique and sample handling is a crucial factor, as well as the statistical revision.

Blood analysis is also an excellent tool for determining nutritional status in an individual horse. But the exceptions are many. Knowledge of the dynamic processes involved and a critical eye is a must. Normal values should be used with great care and previous samples from the same horse to compare with is the preferable method.

References

Caple, I.W., Doake, P.A. and Ellis, P.G., 1982; Assessment of the Calcium and Phosphorous Nutrition in Horses by Analysis of Urine. Australian Veterinary Journal, vol. 58:125-135.

Denny, J.E.F.M., 1987; Thoroughbred Blood Serum Inorganic Phosphate Concentrations in Relation to Feeding Regime and Racing Performance. Journal of the South African Veterinary Association, June: 85-87.

Finco, D.R.; 1989, Kidney Function, In Clinical Biochemistry of Domestic Animals, Ed. Jiro J. Kaneko, 4th ed., s 527-537.

Glantz, S.A., Slinker, B.K., 1990. Primer of Applied Regression and Analysis of Variance. New York:McGraw-Hill.

Hambleton, P.L., Slade, L.M., Hamar, D.W., Kienholz, E.W. and Lewis, L.D., 1980; Dietary Fat and Exercise Conditioning Effect on Metabolic Parameters in the Horse., J. Anim. Sci. 51, 1330-1339.

Harris, P.A. and Snow, D.A.; 1991, Role of Electrolyte Imbalances in the Pathophysiology of the Equine Rhabdomyolysis Syndrome; In Equine Exercise Physiology 3:435-442.

Harris, R.C., Marlin, D.J., Snow, D.H. and Harkness, R.A., 1991; Muscle ATP Loss and Lactate Accumulation at Different Work Intensities in the Exercising Thoroughbred Horse. Eur. J. Appl. Physiol. 62:235-244.

Horwitt, M.K., Harvey, C.C., Dahm Jr. C.H., et al., 1972; Relationship Between Tocopherol and Serum Lipid Levels for Determination of Nutritional Adequacy. Ann. NY Acad. Sci., 203:223-236.

Jansson, E. and Kaijser, L. 1982; Effect of Diet on the Utilization of Blood-borne and Intramuscular Substrates during Exercise in Man. Acta Physiol. Scand. 115:19.

Lindberg, J.E. and Jacobsson, K.G., 1992; Effects of Barley and Sugarbeet Pulp on Digestibility, Purine Excretion and Blood Parameters in Horses. Proc. Europäische Konferenz ber die Ernährung des Pferde, 116-118.

Loerch, J.D., Underwood, B.A. and Lewis, K.C., 1979; Response of Plasma Levels of Vitamin A to a Dose of Vitamin A as an indicator of Hepatic Vitamin A Reserves in Rats., J. Nutr. 109:778.

Mardia, K.V., Kent, J.T. and Bibby, J.M., 1989. Multivariate Analysis. Academic Press, Inc. San Diego, CA 92101.

Snow, D.H., Gash, S.P. and Rice, D., 1987; Field Observations on Selenium Status, Whole Blood Glutathione Peroxidase and Plasma Gamma-Glutamyl Transferase Activities in Thoroughbred Racehorses. Equine Exercise Physiology 2, 494-505.

Stewart, P.A.; 1981. How to Understand Acid-Base. Elsevier, North Holland, Inc. New York, N.Y.

Stull, C.L. and Rodiek, A.V., 1988; Responses of Blood Glucose, Insulin and Cortisol Concentrations to Common Equine Diets. J. Nutr. 118:206-213.

Traver, D.S., Coffman, J.R., Moore, J.N., Salem, C.A., Gamerr, H.E., Johnsson, J.H., and Tritchler, L.G.; 1976, Urine Clearance Ratios as a Diagnostic Aid in Equine Metabolic Disease. Proc. Am. Ass. Equine Practors. 22, 177-183.