

INVESTIGATING WEIGHT LOSS – CLINICOPATHOLOGICAL SCREENING TESTS

Although clinical pathology ideally makes use of a very few selected tests, in weight loss cases with no clinical leads on which to base narrow and specific test selection one may have to cast a speculative net a little wider. In these circumstances one must always be aware that some results will be outside the normal range even in completely normal horses and marginally abnormal results (especially if not supported by other clinical or clinicopathological data) should be repeated with possible confounding variables in mind. The following tests often provide a useful initial insight into differential diagnosis and should all be considered as a first clinicopathological step in all unexplained “examination negative” weight loss cases.

1. HAEMATOLOGY

Anaemia is commonly seen in weight loss cases. If it is marked or chronic a bone marrow aspirate and biopsy should be considered to investigate the type and therefore potential cause of the anaemia. Mild non-regenerative anaemia is a very common, non-specific finding in chronic weight loss cases and is often unhelpful in the determining the diagnosis. A regenerative anaemia is more helpful diagnostically and is suggestive of a chronic source of blood loss or immune-mediated haemolysis.

Neutrophilia can be a feature of septic and non-septic conditions such as infectious challenge (viral, bacterial or parasitic), IBD, neoplasia, autoimmune diseases and Cushing’s disease. A band neutrophilia (left shift) is more suggestive of septic than non-septic conditions.

Neutropaenia is common in acute sepsis (especially when loss into effusions occurs – e.g. peritonitis) but uncommon in chronic inflammatory diseases.

Eosinophilia is a general indicator of inflammation in addition to being an indicator of allergic or infiltrative eosinophilic diseases. Parasitism and multisystemic eosinophilic epitheliotropic disease (MEED) are potential causes of weight loss which may be associated with peripheral eosinophilia.

2. BIOCHEMISTRY

Proteins

Total serum protein has a circadian rhythm and may vary by as much as 10-15g/L over the day (high evening, low noon) usually associated with hydration status and intestinal secretory activity. Albumin is one of the first parameters to check in weight loss cases. Marked hypoalbuminaemia (<20g/L) strongly indicates protein losing enteropathy although occasionally enteropathy cases will maintain a normal plasma albumin. Mild to moderate hypoalbuminaemia (20-29g/L) may result from hepatopathy, malnutrition, chronic blood loss), chronic inflammation or (rarely) protein-losing nephropathy. Increased globulin levels are common in weight loss cases and may indicate hepatopathy, parasitism, chronic inflammation or neoplasia. High, normal or low globulins may occur in protein losing enteropathy cases.

Acute Phase Proteins

Fibrinogen and SAA are sensitive indicators of inflammation or infection. Highest levels tend to suggest bacterial infectious processes with milder increases associated with viral disease and non-septic tissue inflammation (e.g. neoplasia).

AST (aspartate aminotransferase)

AST arises from many tissue sources but elevated plasma levels are usually of hepatic and/or muscular origin (cross-check with CK, GGT, and GLDH). It has a long half-life and can remain elevated for 1-2 weeks after resolution of the inciting cause.

GT (gamma glutamyltransferase)

GGT is a very sensitive indicator of hepatopathy but increased levels are sometimes misleading and not associated with severe liver disease. The pancreas contains high concentrations of GGT but pancreatic disease is rare in horses. Damaged renal tubules may also release GGT but this appears in urine rather than blood. Anecdotally, enteropathies and colics may often have raised GGT in the absence of liver disease – perhaps due to the close anatomic and vascular association between the gut and the liver. GGT may remain elevated for a long time after hepatic insult is resolving (possibly due to biliary hyperplasia). Colon displacements, particularly right dorsal displacements frequently have high circulating GGT concentrations.

ALP (alkaline phosphatase)

ALP arises from many sources but high levels in adult horses are usually from hepatopathy or enteropathy cases. ALP is also released from monocytes and may non-specifically reflect inflammation. Young, growing horses normally have high levels derived from bone sources.

Glutamate dehydrogenase (GLDH)

GLDH is liver specific (hepatocellular) with a short half-life (12 hours) and therefore persistently increased levels suggest an ongoing hepatic insult. It is very sensitive and primary intestinal insults sometimes cause increases in GLDH (possibly from increased endotoxin reaching the liver?) and even dramatically increased concentrations do not have prognostic relevance in cases of liver disease.

Bilirubin

Total bilirubin is usually increased in hepatic failure and also in other conditions such as haemolysis and anorexia. Very high levels ($>300 \mu\text{mol/L}$) often indicate biliary obstruction or haemolysis whereas increases of lesser magnitude could indicate hepatocellular disease (30-150) or anorexia (typically up to 100-150). In these equivocal cases direct bilirubin is more useful for indicating hepatopathy. Direct bilirubin should be no more than 10-20% (usually $<5\%$) of total bilirubin, however if hepatobiliary disease and cholestasis are present then direct bilirubin may account for a greater percentage of total bilirubin.

Creatine Kinase (CK)

CK is useful to check for the site of tissue damage if AST or LDH are increased (i.e. determining muscle vs liver). Modest increases in CK are also seen in cases of polysaccharide storage myopathy or motor neuron disease which may present as little more than weight loss. Muscle catabolism itself can also lead to mild increases in CK (e.g. 400-800 iu/L) as can increased recumbency or IM injections.

Creatinine and urea

If renal disease is severe enough to be causing weight loss urea and creatinine are likely to be increased. Mild increases in urea may be due to wasting, dehydration (worth checking urine specific gravity) or high protein diets. Urea may be low in hepatic failure. Urea is often a few mmol/L higher in morning than the evening.

3. FAECAL ANALYSIS**Parasite eggs/Larvae**

An adult parasite burden is greatly overestimated as a cause of weight loss (especially as owners will invariably have dewormed a thin horse) but larval cyathostomiasis is a common cause of acute (and sometimes chronic) weight loss usually but not necessarily with diarrhoea. Overreliance on fenbendazole could lead to a significant parasite problem in horses which are reportedly 'well wormed'.

Sand

Chronic weight loss may result from an abrasive enteropathy due to voluntary or involuntary sand consumption. Sand can easily be detected by sedimentation in a suspended faecal sample but the quantity that is regarded as normal in a horse grazing sandy pasture is debatable. Colonic sand accumulation may be identified radiographically or ultrasonographically.

Occult blood

In general, positive faecal occult blood indicates colonic bleeding rather than gastric or small intestinal - bleeding e.g. colitis, NSAID toxicity, neoplasia or merely prior rectal examination. High numbers of leucocytes in stained smears may be significant. There is a hand-held test available for faecal occult blood that it is claimed can identify the presence of gastric and colonic ulcers. However, a recent study in which horses were subsequently gastroscoped demonstrated that it was of no value.

Faecal Bacterial Culture

Is rarely helpful in chronic weight loss cases.

Clostridial toxin immunoassay (*C.difficile* Tox A/B, *C.perfringens* enterotoxin)

In the absence of diarrhoea, faecal samples positive for clostridial toxins have been associated with necrotic intestinal lesions.



1. FURTHER SIMPLE BLOOD TESTS

Bile acids

Bile acids (BA) are the only test of liver function that are commercially available and also have prognostic relevance. After excretion in bile, BAs are reabsorbed into the circulation via the ileum and should then be removed from the portal circulation by the liver for recycling. The test is very specific for liver failure but can sometimes be increased to 10 or 20µmol/L with anorexia without liver disease. Normal levels are higher in foals.

Serum protein electrophoresis

This is an exceedingly overinterpreted test that rarely, if ever, provides reliable evidence of cause of disease. Its only established value is in the detection of monoclonal globulin spikes caused by plasma cell myelomas. In all other conditions such as infection, parasitism neoplasia, hepatopathy etc... results have a very poor sensitivity and specificity.

Acid-base balance and electrolytes

Hypercalcaemia may arise in some cases of neoplasia or chronic renal failure. Hypophosphataemia may occur in the latter cases too. An increased serum chloride along with metabolic acidosis is suggestive of renal tubular acidosis which may present as weight loss and lethargy without marked azotaemia.

2. TESTING FOR EQUINE CUSHING'S DISEASE

Cushing's disease is an uncommon cause of weight loss in isolation but may be a contributing factor. (See later chapter on the diagnosis of Cushing's disease).

3. ORAL GLUCOSE ABSORPTION TEST (OGAT)

The OGAT is a valuable test for the detection of small intestinal malabsorption syndromes and is generally performed if a protein losing enteropathy is suspected. This test has no relevance to large intestinal disease. (see chapter on Intestinal Disease).

4. PERITONEAL FLUID ANALYSIS

Peritoneal fluid analysis may be useful in the investigation of intra-abdominal disease, particularly septic peritonitis or neoplasia.

5. ULTRASONOGRAPHIC EXAMINATION

Ultrasonography may provide evidence of peritoneal effusion, intestinal thickening, diffuse pathology of parenchymatous organs or the presence of neoplasms or abscesses.

6. GASTROSCOPIC EXAMINATION

Gastroscopy may provide evidence of equine gastric ulcer syndrome or (rarely) gastric neoplasia. Also provides a means of examining and biopsying the duodenum.

7. URINALYSIS

Urinalysis is worthwhile in cases of polydipsia/polyuria, dysuria/stranguria/pollakiuria, unexplained hypoproteinaemia or cases with significant increases in serum urea and/or creatinine.

8. TISSUE BIOPSIES

Rectal or intestinal biopsies are generally required to provide a definitive diagnosis if disease has been localised to the intestinal tract.

9. LAWSONIA SEROLOGY AND PCR

Lawsonia intracellularis is an intracellular organism that typically causes a marked protein losing enteropathy in post-weaning foals of 3 to 13 months of age although it has been seen rarely in older horses. The disease is characterised by thickening of the intestinal epithelium due to enterocyte proliferation and results in weight loss and diarrhoea. Abdominal ultrasound may reveal small intestinal wall thickening (normal measurement 2-3mm). As the organism is intracellular it cannot be cultured via conventional techniques, however PCR can be used to identify *Lawsonia* DNA in faeces. Excretion of *Lawsonia* may also be short-lived after initiation of antimicrobial therapy and PCR may not be reliable once treatment has commenced. Serology may be a more sensitive technique than PCR but merely indicates exposure rather than active infection. Seroconversion can occur within days of clinical signs developing and may persist for up to 6 months. Identification of intracellular bacteria within crypt epithelial cells would be the gold standard for diagnosis but necessitates collection of biopsies either at surgery or by duodenoscopy.

DIFFERENTIAL DIAGNOSES FOR WEIGHT LOSS

Inadequate ingestion of nutrients:

- Insufficient feed
- Poor quality feed
- Social factors within a herd
- Appetite suppression
- Abnormal prehension or mastication – dental disease, masseter myodegeneration, cerebral disease
- Dysphagia – guttural pouch or pharyngeal disease
- Oesophageal disease
- Gastric ulceration

Abnormal digestion/malabsorption:

- IBD
- Neoplasia
- Parasitism
- *Lawsonia intracellularis* infection
- Hepatic disease

Increased demand for nutrients:

- Increased physical activity, pregnancy, lactation
- Low environmental temperature
- Neoplasia
- Sepsis
- Pain
- Cardiac failure
- PPID
- Renal disease

Neuromuscular wasting disorders

- Neurogenic atrophy
- Equine motor neuron disease
- Immune-mediated myositis