Haematological Changes Observed in Andalusian Horses with Laminitis

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ABSTRACT. Clinical blood chemistry was studied on 13 Andalusian horses with laminitis. Ten healthy horses were used as controls. Blood samples were taken within the first 24 hr after the onset of the disease in Obel grade 3 before any type of treatment was given. No significant differences were observed in the number of red blood cells and white blood cells between Andalusian horses affected by laminitis and healthy animals. Significant decrease in the number of neutrophils and increased number of lymphocytes were noted. A significant increase in the enzymatic activity of CPK, LDH and AST in these animals strongly suggested the presence of muscular and hepatic disorders. The K ion increased, meeting with a slight acidosis. There was also an increase of glucose and total proteins. This fact may be a result of an excessive intake of carbohydrates (which could cause hyperglycaemia) and a diminution of the plasma volume, as a consequence of fluid movement as well as the sweating process (which could cause protein haemoconcentration).—KEY WORDS: Andalusian horse, haematological change, laminitis.

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Laminitis is a metabolic disease of a great complexity resulting in a diminution of blood perfusion in the capillary of the foot [3, 5, 8, 10, 19].

Due to their characteristics of beauty, docility, rusticity, etc., Andalusian horses have been introduced on the world market as an alternative for driving horses. However, they have a certain predisposition to suffer from laminitis [6]. There is a risk of founder due to their constitution, unsystematic handling, unsuitable feeding and possibly a hereditary trait [18].

Laminitis or founder has been demonstrated as being a highly complex metabolic disease associated with disorders of the cardiovascular system [7], the endocrine system [11], the renal and gastrointestinal systems, and causing both acid-basic [5] and blood coagulation [12, 17] imbalance.

Since laminitis is a local manifestation of a more generalized metabolic disorder and due to the scarcity of data available, this study was focused on the blood chemical analysis in Andalusian horses with laminitis.

A total of 23 Andalusian horses were studied, 13 affected by laminitis (with no signs of any other disease) and 10 were perfectly healthy (control group). The characteristics of age and sex are shown in Table 1. The laminitis-affected horses belonged to various stock farms in the Province of Córdoba. However, the control animals belonged to the same stock farm.

For a period of 3 months, the animals with laminitis have received a diet made up of 85% barley and 15% bran in amounts ranging from 4 to 4.5 kg. The control group, during the same period of time followed a diet with similar amounts to those received by the animals with laminitis, composed of oats (55%), barley (25%), soya flour (10%), bran (10%), together with mineral and vitamin supplements. Both groups of individuals consumed hay *ad libitum*.

Samples were taken where the animals were stabled during the period from May to July. Samples were taken in the acute phase of laminitis during the first 24 hr of the onset of clinical symptoms and before receiving any

treatment. All of them were clinically found to have Obel grade 3 lameness [8, 17]. Blood, 10 ml per animal was withdrawn from the jugular vein. Two blood smears were also made.

In blood, erythrocyte and leucocyte counts, packed cell volume (PCV) and haemoglobin (Hb) concentration were studied. In plasma, total bilirubin, creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total plasma proteins (TPP), lactate dehydrogenase (LDH), potassium, glucose and creatinine were analysed. The remaining samples were refrigerated (2–8°C) until analysis.

For the determination of the number of erythrocytes and leucocytes, a particle counter was used (Ibercell HF-24). PCV was determined by the microhaematocrit method. The blood pH was ascertained by means of a pH meter. The total plasma protein was observed by an inversion refractometer, and K⁺ was measured with an ions analyzer (644, Ciba-Corning). The determination of total bilirubin, CPK, AST, ALT, haemoglobin, LDH, glucose and creatinine was made by spectrophotometry.

Basic statistics were calculated for each group and parameter (laminitis, healthy individuals, males, females, young animals and adult animals). Simple variance analyses were also made with the aim of obtaining the possible significance between the different groups, by the Snedecor-F, for each parameter analyzed.

The mean values obtained for each of the blood parameters analyzed are shown in Table 2. In animals with

Table 1. Characteristic of sex, age and healthy condition in the Andalusian horses studied

	SEX		AGE	
	MALE	FEMALE	≤ 3 YEARS	> 3 YEARS
HEALTHY	5	5	2	8
WITH LAMINITIS	9	4	5	8

Table 2. Haematological values ($\times \pm$ SD) found in Andalusian horses with laminitis and in healthy ones (N=n° of analyses made; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Determination	Healthy		Laminitis	
	$\times \pm$ SD	N	$\times \pm$ SD	N
Erythrocytes (mill./mm ³)	6.73 $\pm$ 1.13	10	8.75 $\pm$ 2.91	9
PCV (%)	39.30 $\pm$ 7.82	10	39.37 $\pm$ 7.17	12
Haemoglobin (gr/dl)	13.20 $\pm$ 1.56	10	14.06 $\pm$ 3.32	12
Leucocytes (thous./mm ³)	5.33 $\pm$ 1.22	10	6.54 $\pm$ 2.89	9
Neutrophils (%)	58.93 $\pm$ 6.30	9	42.46 $\pm$ 10.44***	9
Eosinophils (%)	1.53 $\pm$ 2.32	9	1.88 $\pm$ 2.62	9
Basophils (%)	0.13 $\pm$ 0.40	9	0.16 $\pm$ 0.35	9
Lymphocytes (%)	37.24 $\pm$ 7.00	9	50.32 $\pm$ 12.09*	9
Monocytes (%)	3.32 $\pm$ 2.48	9	4.13 $\pm$ 2.24	9
Neutr./Lymfoc. (Ratio)	1.65 $\pm$ 0.44	9	0.93 $\pm$ 0.43**	9

laminitis, the mean value of RBC and the mean haemoglobin concentration were slightly higher than those found in healthy animals, but the PCV value was practically the same.

The number of white cells was higher in horses with laminitis (Table 2). With regard to the differential count of leucocytes ($p < 0.05$) a significant decrease was found ($p < 0.001$) in the percentage of neutrophils in animals with

laminitis at the expense of a rise in the percentage of lymphocytes ($p < 0.05$). The other white cell types remained statistically invariable. The variation in the neutrophil and lymphocyte numbers conditioned a reversal in the neutrophil/lymphocyte quotient which was over 1 in healthy animals and under 1 in animals with laminitis ($p < 0.01$).

The CPK, LDH and AST enzymes displayed a substantial and significant increase ($p < 0.05$; 0.01; 0.01, respectively) in horses with laminitis in relation to the healthy ones (Fig. 1). Similarly, the animals affected by the disease studied exhibited higher plasma concentrations of glucose and potassium, although these differences did not become statistically significant (Fig. 2).

Finally, in Fig. 3 mean values were observed for the plasma concentrations of bilirubin, creatinine, TPP and pH values. Although the data shown by Andalusian horses with laminitis were higher in all cases, they were not significant differences.

It should be noted that no significant differences existed in practically any of the parameters analyzed when taking into consideration the sex and age of the animal.

In Andalusian horses with laminitis, an increase in the number of red cells was observed. This rise in RBC may be

Fig. 1. Mean values found for enzymatic activity of CPK, LDH, AST, ALT in Andalusian horses affected by laminitis and healthy ones (* $p < 0.05$, ** $p < 0.01$).

Fig. 2. Mean values for plasmatic levels of glucose and potassium found in Andalusian horses affected by laminitis and healthy ones (** $p < 0.01$).

Fig. 3. Mean values found for plasmatic levels of bilirubin, creatinin, total proteins and blood pH in Andalusian horses affected by laminitis and healthy ones.

due both to the inflammatory response, which releases inflammatory mediators and the pain which stimulates the release of catecholamines from the adrenal glands. Other causes may be the sweating process, which also occurs in horses with laminitis and the appearance of compartmental fluid shifts in the microvasculature. Thus, Moore *et al.* [15] described a reduction of the plasma volume.

These observations about the number of red cells were similar to those reported by Garner [6] and Moore *et al.* [14]. On the other hand, PCV value of the affected group (Obel grade 3 lameness) was the same as the control group. The cause of this rise in RBC, with no parallel increase of the PVC or Hb may be due to the deformation undergone by the red cells caused by acidosis which was revealed by a significantly lower blood pH. This acidosis may be regarded as being the agent which triggers a reduction in the mean corpuscular volume (MCV) so that any increase in the RBC would not be accurately reflected in the PCV value.

The increase in the white blood cell count was not significant. The same results were observed in the Moore *et al.* [14] experiment, although the values of these authors were considerably higher. In relation to the different types of white cells, Garner *et al.* [6] showed an increase in the number of neutrophils during the clinical period of this disease. Likewise, Green *et al.* [8] observed the development of a stress leucogram characterized by a fall in the number of lymphocytes, eosinophils and platelets, together with an increase in the cortisol concentration. The results of the present paper, however, do not coincide with the laboratory experiments of the aforementioned authors. A lower number of neutrophils and, therefore, a higher number of lymphocytes were found in horses with laminitis in comparison to healthy horses. These results may be due to the disease being at a stage of full recovery. For this reason, the neutrophil/lymphocyte quotient, which in healthy horses is always over 1, in Andalusian horses with laminitis was reversed and appeared as under 1.

Increased enzymatic activity of CPK, LDH and AST in Andalusian horses with laminitis may indicate the presence of muscular lesions due to the greater effort that horses with this disease are obliged to make in order to remain stationary. Significant increases in the concentrations of these enzymes have been described by other authors [1, 2, 4]. Similarly, this change in the LDH and AST concentrations might reveal the presence of hepatic problems possibly induced by the endotoxins released after the death of the intestinal bacteria [8]. However, in this study, the ALT concentration did not display any significant differences between the control group and the one with laminitis. Andalusian horses with laminitis exhibited a higher TPP concentration than healthy ones, in spite of hepatic damage, as shown by the concentration of certain enzymes. This may be because the hepatic lesion was not serious enough to cause a decrease in protein production, although the AST concentration was 5 times higher than that of the healthy horses. This rise in the TPP levels may also be related to the diminution of the plasma volume

described in horses affected by this disease although it has not been possible to verify this change in the present study.

In Obel grade 3 laminitis, there is a diminution in plasma concentrations of sodium, potassium, chloride, as corroborated by the results of Garner [6] and Moore *et al.* [14]. In the Andalusian horses studied, however, substantial rises (from 4.67 to 7.32 mmol/l) in potassium. This fact might be regarded as being indicative of muscular damage with the subsequent regression of that ion from inside the muscular fibres. However, it has not been possible to verify this possible explanation owing to the fact that no significant correlations were found between the enzymes LDH, CPK, AST and the potassium concentration.

A second possible cause of the rise experienced by the plasma concentration of potassium might be a loss of plasma fluid. Although not determined exactly, this was upheld by the movement of fluid to the intestine, especially in those cases of laminitis developed as a result of an excessive consumption of carbohydrates.

In this paper, the glucose concentrations in plasma were higher in horses with laminitis although these differences did not become significant and the results agreed with those shown by Moore *et al.* [15].

The normal intestinal flora is determined by dietary content. Intestinal overload with carbohydrate disrupts intestinal bacteria balance. Lactic acid, produced by bacteria flourishing is an end-product of carbohydrate metabolism, which is overproduced, rendering the normally neutral intraintestinal environment acidic. Intraluminal endotoxins (released upon the death of resident Gram-negative bacteria) act in synchrony with lactic acid to damage the intestinal mucosa, allowing endotoxins and lactate to gain access to the portal circulation [9]. The passage of the lactate to the system circulation may be the cause of the reduction of pH found in this study for horses with laminitis. Moreover, the loss of pH may be a result of the lactacidosis in the muscle fibres due to a local hypoxia or stress [13].

No significant differences among sex and age of the animal indicates that neither of these factors has an influence on the clinical aspects of the disease.

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