Determination of protein fractions in a sports horse with laryngotracheitis, nonspecifically stimulated with a phytotherapeutic extract (case study)

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Abstract

In a sports horse with signs of laryngotracheitis that hardly achieved the training program, a phytotherapeutic extract with immunomodulating properties was administered. Following the administration of the extract, on the second day, the horse showed a remarkable comeback in the sense that it did the normal training without the presence of fatigue and coughing. The horse was clinically examined, blood samples were collected and the extract was inoculated s.c., blood samples were collected at 24 hours, 7 days and 10 days for haematological, biochemical examinations and also for electrophoresis, in order to determin the protein fractions. Significant changes are noted for fractions β1, β2 and γ. In terms of albumin, they are found initially at physiological values, and 24 hours after the inoculation of the extract, very significant decreased throughout the experiment. The most spectacular variations of the protein fractions are those for gamma globulins: initially they are drastically decreased, and 24 hours after the inoculation of the extract, they increased spectacularly, about 3 times, and remain close to this level (in physiological parameters), throughout the experiment. The albumin / globulin ratio is clearly in favor of globulins.

Key words: *immunomodulation, phytotherapeutic extract, globulins, sport horses, laryngotracheitis.*

Introduction

Over time, the horse has been seen from several points of view: as a tool of battle, fieldwork, transport, then to become closer to the people and to become a symbol of the nobility among animals, winning the laudatory title of "gentleman" animal. However, with the domestication of these animals, the first diseases were found: digestive, locomotory, respiratory diseases. At the moment, respiratory disorders are becoming more frequent. They occur most often due to shelter conditions (microclimate, ammonia level), inadequate food (feed contaminated with mice) or even decreases in immunity in the case of horses subjected to a long effort, which will lead to a low economic yield of the animal. (4,5,8,11) Non-infectious respiratory disease is a common condition that limits performance and affects adult horses of different ages. Inflammatory airway disease is characterized by excessive tracheal mucus, high respiratory tract activity, and poor exercise performance in younger horses. Etiology is unclear, but viral respiratory infection (Equine Herpes Virus 1, 2, 5), allergy (exposure to organic dusts in older horses) and environmental factors may play a role in the pathology of the airway. The severity of clinical signs ranges from exercise intolerance to dyspnea at rest (2,6,7). Clinical manifestations are similar and include pyrexia, nasal discharge, submandibular lympha-denopathy, anorexia and cough. At the present, the choice of treatment for these conditions is chemotherapy, and it is estimated that for 2020 they will not be as effective as now. This creates the premise for the establishment of new therapeutic

protocols, based on plant extracts, to achieve a modulation of immunity with minimal side effects. In this case, immunomodulation in order to increase the immune potential of the body remains the only alternative to infection control. (3,9)

Non-specific immunomodulators are substances that induce a non-antigen-specific improvement in native or acquired defense mechanisms. Immunomodulatory preparations are most commonly used for the treatment of chronic, viral or bacterial infection, with secondary immunosuppression being evidenced. The mechanism of action of these products is macrophage activation and subsequent release of cytokines that improve cell mediated immunity. In equine medicine, immunomodulatory preparations are indicated for the prevention and treatment of chronic respiratory diseases rather than for the treatment of acute infections (1, 10)

Materials and methods

Research and experiences have been carried out within a private horse farm in Bucharest. The farm has 10 horses from the breeds: Andalusian, Lipitan and Arabian. They participate in various local, national and international competitions. All horses have two training sessions a day, morning and evening, consisting of one-hour training,. As food, the horses receive lucerne, hay, oats and barley, according to a well-timed schedule.

Blood samples were taken from four horses for paraclinical examinations, one of them showing clinical signs of laryngotracheitis (horse A, which received the phytotherapeutic extract), and the other three horses were clinically healthy, representing the control sample, as follows: Horse A: LEA - Arabian, female, age 11 years

Horse 1: ALFRED - Andalusian, male, age 4 years

Horse 2: NERO - Lipiţan, male castrated, age 12 years

Horse 3: MARCO - Andalusian, female, age 15

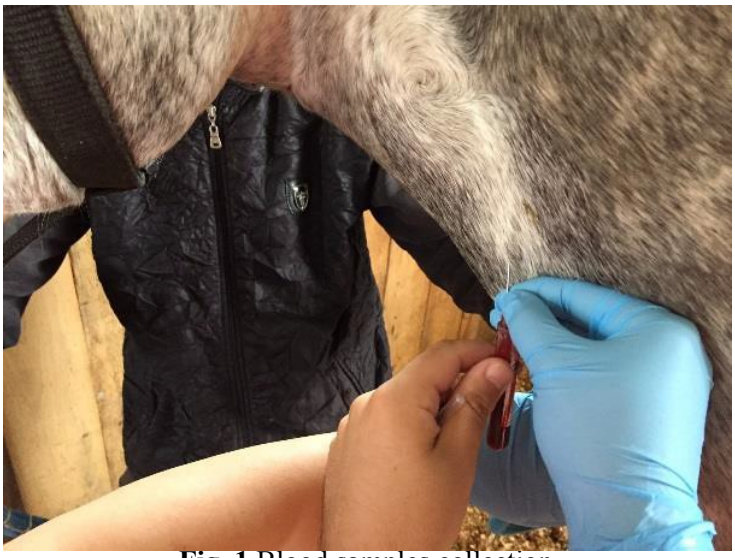

Fig. 1 Blood samples collection

The hematological examination was performed with the "Abacus Junior Vet 5" and the biochemical examination of the blood was performed with the "Arkray" analyzer. For electrophoresis it was used the EP SA200 electrophoresis system, all within the discipline of

"Medical Pathology and Clinical Lectures by Species" of the Faculty of Veterinary Medicine Bucharest. The phytotherapeutic product used has immunomodulatory action (Patent Application No. 467 / 20.04.2016) and has been used by subcutaneous administration in the horse A in the prescapular area.

Results and discussions

At the clinical examo, the horse A showed repeated and spontaneous coughing, triggered by exertion, at the exit of the pad, in contact with cold air, exhaustion in effort, resulting in a shortening of the training period, normothermia and absent nasal discharge. There were rallies in the thoracic auscultation, and the visceral tingling of the cheeks. The coughing challenge was easily accomplished by palpation of the laryngotracheal area, which demonstrates the sensitivity of the mucosa.

The experimental protocol focused on determinations (hematology, biochemistry, electrophoresis) at baseline (I) for the 4 horses, and for the horse showing the symptoms of a respiratory disease (laryngotracheitis), blood was harvested at 24 hours and 5 days and also at 10 days after administration of the phytotherapeutic product and the results are presented in Table 1.

Tabel 1. The Results of the Haematological Exam

Parameter	U/M	Physiolog. Values	Healthy Horses (I)			CLINIC CASE		
			1	2	3	(I)	24h	5days
WBC	$10^{-9}/mm^3$	5,4-14,3	7,96	8,39	7,87	8.75	9.5	8.4
LYM	$10^{-9}/mm^3$	1,5-7,7	2,94	2,05	2,26	**36.1**	**19.3**	**32**
MON	$10^{-9}/mm^3$	0-1,5	0,36	0,47	0,04	0.6	**4.7**	**4.7**
NEU	$10^{-9}/mm^3$	2,3-9.5	4,36	5,52	5,47	**58.2**	**75**	**65**
EOS	$10^{-9}/mm^3$	0,1-1	0,27	0,32	0,09	**4.7**	0.7	1
BAS	$10^{-9}/mm^3$	0-0,5	0,02	0,03	0,01	0.5	0.3	**0.7**
RBC	$10^{-12}/mm^3$	6,8-12,9	8,93	6,95	8,95	8.88	7.80	8.20
Hgb	g/dl	11-19	16,1	13,2	16,6	14.8	10.8	13.0
Ht	%	32-53	39,14	34,54	39,93	35.27	37.1	35.3
MCV	fl	37-59	44	50	45	40	47.6	42.3
MCH	pg	12,3-19,7	18,1	18,9	18,5	16.6	13.8	15.6
MCHC	g/dl	31-39	41,2	38,1	41,5	41.9	**29.1**	36.8
PLT	$10^{-9}/mm^3$	100-400	98	106	99	106	175	205

At 24 hours after inoculation of the extract, circumscribed spherical or ellipsoidal swelling was noticed, with increased of consistency, warm and sensitive, adherent to subcutaneous connective tissue at the site of inoculation, showed a slight decrease in appetite and exercise fatigue and body temperature increased by 1-2^{o}C. After 2-3 days, the overall condition of horse A has improved, appetite returned to normal, and the cough disappeared.

The results of the biochemical examination were not influenced, therefore Uric Acid (UA) was found below the physiological limits in all horses, with no clinical significance. Uric acid results from protein burning, being the final product of degradation of free purines: adenine, hypoxanthine, guanine. In our case, the horse's values have no pathological significance, these uricemic values being reported after uricosuric medication. Twenty-four hours after dosing there

was a significant increase in LDH levels, total protein and alkaline phosphatase, and the other parameters remained within physiological limits.

In the same time determinations of the protein fractions for horse A were also performed by electrophoresis. This is a method of analysis and separation based on the migration of solid particles dispersed in a liquid under the action of an electric field. Determinations were made at baseline (I) for all 4 horses taken into consideration, respectively at 24 hours, then at 5 and 10 days after inoculation of the extract. for the horse presenting the symptoms of laryngotracheitis, the results are shown in Table 2.

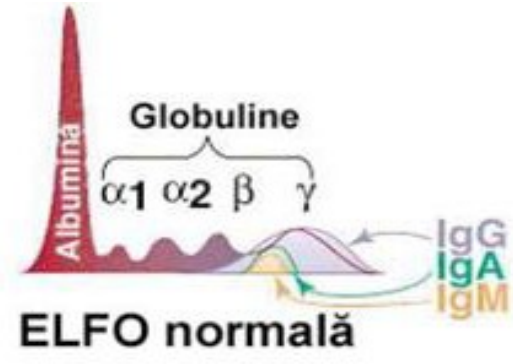

Fig. 2 Normal electrophoresis

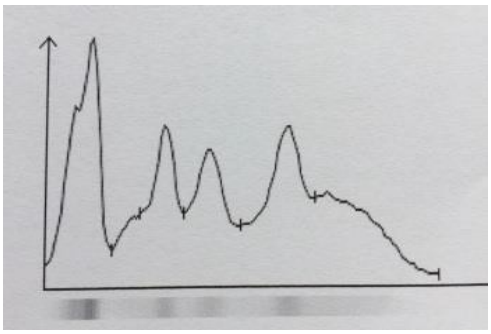

Fig. 3 Horse A Electrophoresis at 5 days postinoculation of the extract

Table 2. The Results of Determination of Protein Fractions

Fraction	U/M	Physiolog. Values	1	2	3	A
			(I)	(I)	(I)	(I)
Total Protein	g/dl	5,1-7,2	5,90	6,40	6,30	5.1
Albumin	g/dl	0,30-0,38	**0,22**	**0,21**	**0,20**	**3,20**
α_1	g/dl	0,19-9,31	**0,15**	0,25	0,25	0.42
α_2	g/dl	0,53-0,87	**0,49**	0,54	0,57	0.63
β_1	g/dl	0,28-0,73	**0,87**	**0,88**	**0,90**	0.61
β_2	gd/l	0,22-0,60	**0,18**	0,41	0,30	0.41
γ	g/dl	0,58-1,27	**1,97**	**2,19**	**2,20**	**0.29**
Alb/Glob Ratio	/	0,95-1,65	**0,50**	**0,49**	**0,61**	**0.66**

According to the data from table 2, Albumin are at a low level at all horses, except the horse A. The Albumin/Globulin ratio is also low. Hypoalbuminemia might be caused by deficiency of intake: nutrition, food deficiency, vomiting, diarrhea, digestive disorders, intestinal absorption

disorders, insufficient synthesis, hepatic failure, loss of albumin (glomerulonephritis, nephrosis), ascites, bleedings, shock. In our case, it can only be a matter of defective problems.

Globulins (α1, α2, β1, β2 and γ) are recorded and modified as follows:
Fraction β1 is slightly increased in all clinically healthy horses without clinical significance. Fraction β2 is slightly low in horse 1, probably due to young age and within normal limits at the other horses; the ß-globulin fraction contains transferin, hemopexin, complement factors and beta and pre-beta lipoproteins (LDL and VLDL).

Fraction γ is significantly increased in horses from the control group. This shows an increased reactivity of the body and we can see it as a cosequence of the physical effort and not as a pathological problem (there are no clinical and laboratory data to prove it). The ɣ-globulin fraction contains lots of immunoglobulin (IgG, A, D, E and M).

Table 3. Determination of protein fractions in the horse with laryngotracheitis

Fraction	U/M	Physiolog. Values	Horse A			
			Initial	24 h	5 days	10 days
Total Protein	g/dl	5,1-7,2	**5.1**	**5.1**	**5.7**	**5.6**
Albumin	g/dl	3,0-3,8	**3,20**	**1.60**	**1.86**	**1.88**
α1	g/dl	0,19-0,31	**0.42**	0.25	0.24	0.29
α2	g/dl	0,53-0,87	0.63	0.49	0.54	0.58
β1	g/dl	0,28-0,73	0.61	**0.83**	**0.90**	**0.99**
β2	g/dl	0,22-0,60	0.41	**0.81**	**0.95**	**0.78**
γ	g/dl	0,58-1,27	**0.29**	**1.14**	**1.21**	**1.09**
Alb/Glob Ratio	/	0,95-1,65	**0.66**	**0.46**	**0.49**	**0.50**

The albumin/globulin ratio is almost constant below 1, indicating a constant disorder throughout the experiment, although the clinical condition has become very good after receiving the phytotherapeutic preparation. Albumins show a decrease, whereas globulins grow in the bloodstream and are mobilized for emergency situations.

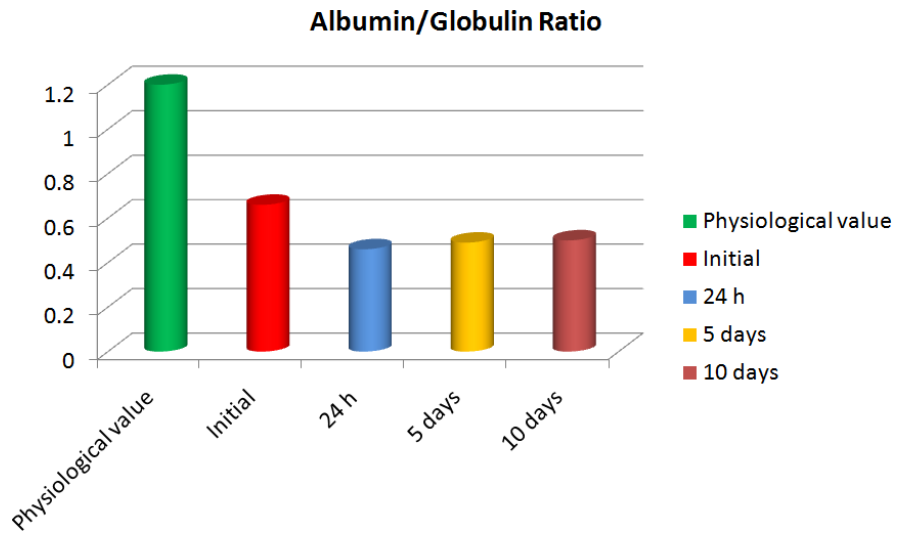

Fig. 4 A/G Ratio Variation Chart

The electrophoresis profile reflects the chronic inflammation demonstrated by the increased gamma globulin band. Fraction α1 shows growth over physiological values before the phytotherapeutic administration and starting to 24 hours, until ten days, the α1 was normal. The α1 fraction is recorded in all inflammatory reactions as the acute phase reaction. The α1 surface reflects the α1 anti-hypsal serum so that its reduction occurs in the α1 deficiency of the antitrypsin deficiency associated with a certain lung disease, probably at the onset of emphysema.

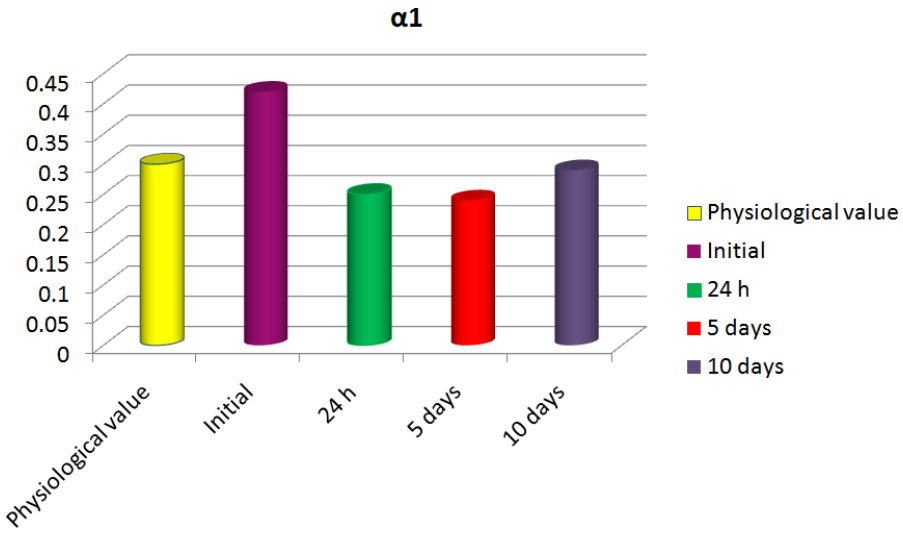

Fig. 5 α_1 Variation Chart

Fractions β1 and β2 remain elevated throughout the experiment. Fractions β include the C3 and C4 complement fractions, which are being elevated too throughout the experiment period.

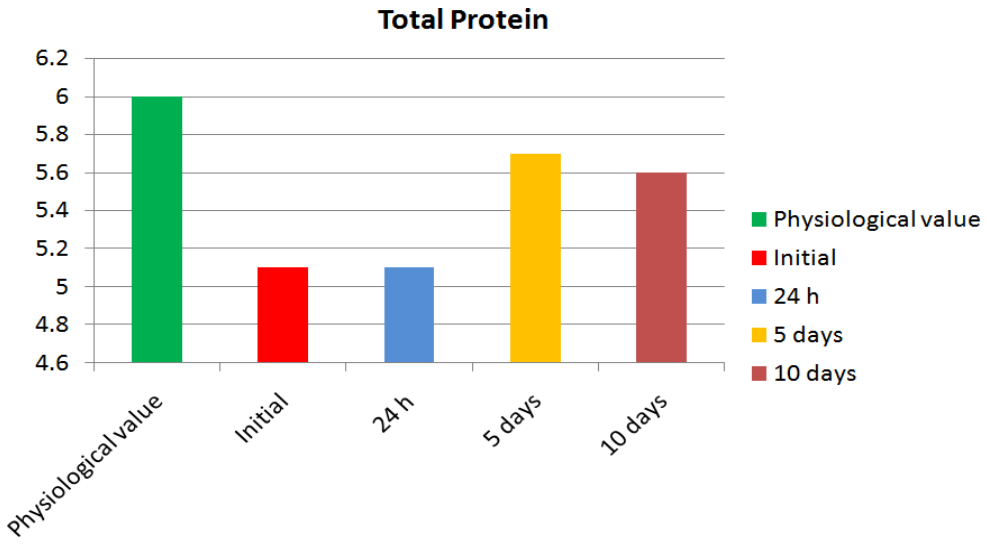

Fig. 6 Total Protein Variation Chart

The gamma area contains immunoglobulin (IgG, IgA, IgM, IgD and IgE). Increases in polyclonal gammaglobulins indicate a chronic immunological process.

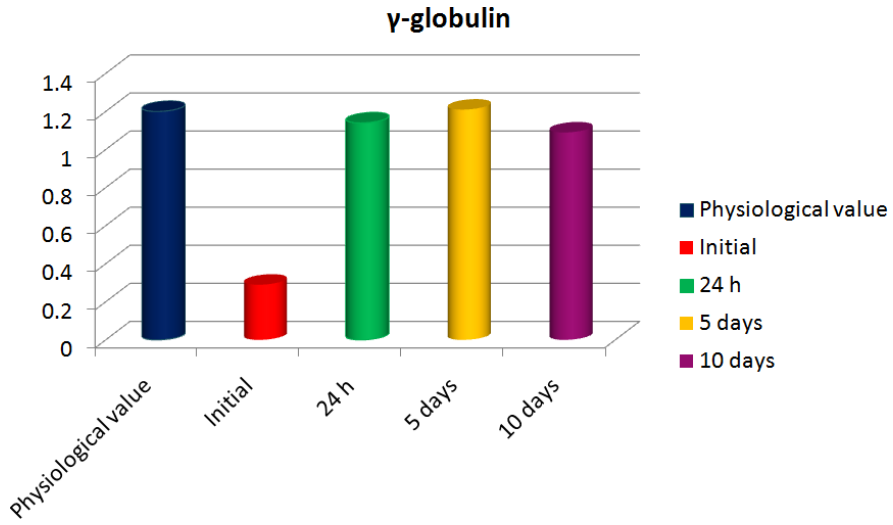

Fig. 7 γ-Globulin Variation Chart

Conclusions

1. At baseline, total proteins are at the lower limit compared to physiological values, and 24 hours after inoculation of the preparation, they reach normal values and remain throughout the study.
2. At the opposite end, the Albumin / Globulin ratio is decreased throughout the experiment, due to increased globulinemia and decreased albuminemia.
3. The fraction α1, if at the beginning of the experiment was very increased, after the inoculation of the phytotherapeutic extract, its values returned to normal until the end of the experiment.
4. Fraction γ had the most spectacular evolution, initially it was very low, than it returned to normal, from 24 hours after inoculation of the preparation up to 10 days postinoculation.

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