UNIVERSITATEA DE ŞTIINTE AGRICOLE ŞI MEDICINĂ VETERINARĂ CLUJ-NAPOCA
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Heterogeneity of equine metabolic syndrome: between pure expression and comorbidities

(SUMMARY OF Ph.D. THESIS)

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1. Equine metabolic syndrome
1.1. Components of equine metabolic syndrome

Interest in the risk of laminitis associated with obesity in horses has increased in recent years. It is well known that obese ponies and horses are more prone to laminitis. In a consensus statement, the European College of Equine Internal Medicine recommends the use of the term "Equine Metabolic Syndrome" (EMS) for a metabolic dysfunction marked by obesity, insulin resistance and laminitis (DURHAM et al., 2019). In human medicine, metabolic syndrome brings together factors that increase the risk of cardiovascular disease, including obesity, insulin resistance, dyslipidemia, and hypertension. Associated non-alcoholic fatty liver and polycystic ovary syndrome appear. EMS shares some features of human metabolic syndrome, but differs in that laminitis is the complication of primary concern (DARADICS ZSOFIA et al., 2021).

Metabolic syndrome is not a specific disease entity, but rather a clinical syndrome resulting in laminitis (TREIBER et al., 2006; CARTER REBECCA et al., 2009). Excessive and localized adipose tissue, hyperinsulinemia, and insulin resistance are the three main components of this syndrome. Unlike human metabolic syndrome, atherosclerosis and coronary heart disease are not known in horses, and this may be explained by the horse's herbivorous diet or blood lipoprotein composition. The majority of circulating cholesterol is carried in greater proportion bound to these high-density lipoproteins, which are non-atherogenic (WATSON et al., 1993).

EMS is not an independent disease but it is a grouping of several risk factors that lead to complications, the most important being metabolic laminitis. The mandatory condition for the appearance of this syndrome is the dysregulation of insulin functions. The actual term insulin dysregulation indicates the loss of balance between the plasma concentration of insulin, glucose and lipids. Insulin dysregulation can manifest itself in several ways: hyperinsulinemia, excessive and prolonged hyperinsulinemic response to oral or intravenous administration of carbohydrates, followed by prolonged hyperglycemia and peripheral tissue resistance to insulin (FRANK and TADROS, 2014).

Obesity is expressed by the excessive deposition of adipose tissue in certain areas, and it damages the individual's health. The accumulation of adipose tissue can be generalized or regional (TREIBER et al., 2006; CARTER REBECCA et al., 2009), and can be accompanied by a predisposition to gain weight and resistance to weight loss (ARGO CAROLINE et al., 2012). However, there are exceptions, some individuals having body mass within normal limits (BAILEY et al., 2008).

Other common features can be hypertension, tachycardia, myocardial hypertrophy (BAILEY et al., 2008; HELICZER NATALIE et al., 2017) and abnormal values of plasma adipokines: hypoadiponectinemia and hyperleptinemia (CARTER REBECCA et al., 2009; MENZIES- GOW et al., 2017).

Until now, the metabolic syndrome in the equine species is under continuous research. However, future studies are needed to fully understand the pathophysiological components and mechanism of the syndrome.

1.2. The physiopathological mechanism of the metabolic syndrome – Hyperinsulinemia

Hyperinsulinemia plays a central role in the pathophysiology of the disease, acting both hormonally and metabolically on the individual (DE LAAT et al., 2016). Normally hyperinsulinemia is a response to insulin resistance, but in the case of MES hyperinsulinemia is a phenomenon that cannot be explained by insulin resistance alone, as it can even occur independently of it (JACOB et al., 2018). The acute insulin response to glucose justifies the presence of compensatory insulin resistance. This response is assessed by the results of intravenous glucose tolerance tests, and the insulin response is represented by the total insulin secretion of the pancreas. The value of the insulin response is more pronounced in horses and ponies with low insulin sensitivity (TREIBER et al., 2005).

Oral administration of glucose stimulates a much greater insulin response than intravenous administration. This fact is explained by the participation of the gastrointestinal tract in the release of factors that stimulate pancreatic insulin secretion (DÜHLMEIER et al., 2001). In horses, three hormonal factors are known that contribute to insulin secretion and are called incretins: GIP (gastric inhibitory polypeptide), GLP-1 (glucagon-like peptide-1), GLP-2 (glucagon-like peptide-2). Active GLP stimulates insulin secretion, and its role in insulin dysregulation remains unknown (DE LAAT et al., 2016). Also, the role of GLP-2 in this context is currently not understood, as it does not influence insulin secretion, but increases the bioavailability of glucose (DE LAAT et al., 2018).

In general, a diet rich in easily digestible carbohydrates reduces insulin sensitivity and also reduces adiponectin levels. A high-fiber, high-fat diet does not induce the same changes (BAMFORD et al., 2016; TREIBER et al., 2005).

Hyperinsulinemia can also result from decreased hepatic clearance. The liver removes 70% of insulin through portal vein circulation in healthy individuals. In those with metabolic syndrome, a reduced clearance compared to normal is evident (TÓTH et al., 2010). In some horses with metabolic syndrome, an increase in liver enzymes (GGT, AST) and an accumulation of lipids in hepatocytes can be seen. The impact of obesity on liver functions is reflected by the reduction of hepatic insulin clearance capacity, generating insulin resistance (SAMUEL et al., 2010).

1.3. The development of the primary complication – laminitis

Hyperinsulinemia is the key element in the experimental induction of laminitis. In one study, laminitis was induced in a group of horses by administering insulin and glucose within 48 hours (ASPLIN KATIE, 2007; DE LAAT et al., 2010). The threshold level of hyperglycemia (mean 11 mmol/L) and hyperinsulinemia (mean 208 μIU/mL) induce lesions characteristic of laminitis (DE LAAT et al., 2012). For the diagnosis of laminitis, Obel's scoring system (Obel Grading System) is the most accepted and widely used by clinicians and researchers. Depending on the severity, points are given from 1 to 4 (OBEL and PHIL, 1948).

The lesions of metabolic laminitis initially appear at the level of the secondary lamellae of the epidermis that are part of the gear structure of the hoof horn. These histologically examined secondary lamellae are more elongated and thinner than normal, have a more pronounced angulation on the long axis of the primary lamellae, and their tips are tapered (PATTERSON-KANE JANET et al., 2018). In lamellar epithelial cells, this stretch is observed even after 6 continuous hours of hyperinsulinemia and indicates cytoskeletal disruption (PATTERSON-KANE JANET et al., 2018). These changes can induce cell apoptosis and accelerated proliferation. This explains the deformation of the hoof horn with the presence of macroscopically visible "laminitic rings" (DE LAAT et al., 2013; KARKOSKI et al., 2014, 2015).

The most accepted theory behind the onset of laminitis is represented by a change in intracellular insulin signaling, resulting in endothelial dysfunction. In healthy individuals, stimulation of insulin receptors initiates the sending of intracellular messages through the metabolic pathway, which produces vasodilation through the action of nitric oxide. In insulin-resistant horses this pathway is blocked and the MAP-kinase pathway, a protein that leads to endothelin-1-mediated vasoconstriction, is favored. The effect of MAP-kinase translates into the constriction of digital vessels and lamellar arteries (VENUGOPAL et al., 2011; WOOLDRIDGE et al., 2014; MORGAN RUTH et al., 2016).

The insulin-like growth factor-1 (IGF-1) receptor is found on both lamellar epithelial cells and the endothelium, whereas the insulin receptor is found only on endothelial cells (BURNS et al., 2013). This IGF-1 gained ground because of its similarity to the insulin receptor, and high concentrations of insulin produce its activation and result in intracellular signaling through the MAP-kinase pathway (DE LAAT et al., 2013), leading to vessel vasoconstriction of the acropodium. The increase in intracellular signaling by the action of IGF-1 has been experimentally reproduced by the administration of exogenous carbohydrates and insulin.

In another study, BURNS et al. (2015) highlight changes in COX-2 expression. It is assumed that this enzyme, responsible for the transformation of arachidonic acid into prostaglandin E2 (following the release of arachidonic acid through the lysis of membrane phospholipids by phospholipases C and D and then A), can thus control the local inflammatory reaction through a paracrine effect, i.e. it can stimulate cell division, but also the transition between cell types, especially the transition from the epithelial cell type to the mesenchymal type. Although this transition would have a positive impact on the local recovery and reconstruction of the destroyed structures, from a biomechanical point of view, the area of the horse's hoof is an area subject to significant stressors and such a cellular transition could further weaken the area, having adverse effects. Given these, although metabolic syndrome-specific laminitis differs fundamentally from endotoxic laminitis, it may benefit from treatment with specific COX-2 inhibitors, which are already available for the treatment of other pathologies in multiple species.

THESIS STRUCTURE

The work entitled "Heterogeneity of equine metabolic syndrome, between pure expression and comorbidities" comprises 192 pages and is written according to the norms in force, being structured in two parts.

The first part, namely the bibliographic part, consists of a bibliographic study with 24 subchapters and comprises 28 pages. In this part of the thesis, I have synthesized the current general framework of knowledge of the components, physiopathology, clinical aspects, consequences, respectively diagnostic and treatment methods in equine metabolic syndrome.

In the second part, extended over 142 pages, structured in 7 chapters and 8 studies, I detailed the personal research carried out in the period 2019-2021 on the North-West area of Transylvania. The first chapter presents the aim and objectives of the work, and the second the materials and methods used. The rest of the chapters are divided into sub-chapters that present the purpose and objectives, respectively the results obtained with the related discussions on their novelty compared to other studies carried out up to the present moment and the partial conclusions brought after the completion of each individual study. The research results were illustrated by a number of 139 figures and summarized in 66 tables.

The present work ends with the cited bibliography, totaling 175 titles.

RESEARCH RESULTS

In the second part of this paper, we studied the clinical, epidemiological, molecular and pathological aspects of equine metabolic syndrome, during the years 2019-2021, in the North-West part of Transylvania. Our aim was to evaluate some nutritional, clinical and humoral aspects in the EMS diagnosed in the Semigreu Romanian breed from the North-West area of Transylvania, respectively to demonstrate the fact that the secondary conditions of the metabolic syndrome can lead to the appearance of various complications, the most important in horses being laminitis.

The comparison of the values of the studied variables between various populations was carried out, depending on the scale on which the variables were measured, the sample volumes and the number of populations compared, through the tests: Shapiro-Wilk, $\chi 2$, Kruskal-Wallis, Mann-Whitney, ANOVA, the t for independent samples or the t test for paired samples.

Chapter 5 presents the following studies:

Study I., entitled "Determination of macronutrients in the ratio of horses with metabolic syndrome" had as its objective the correlation of clinical and humoral aspects with nutritional ones and the possible involvement of fructans in the occurrence of laminitis or other comorbidities. In order to achieve this objective, the following analyzes were carried out: the determination of the floral composition of the feed samples corresponding to each individual or group of individuals from each geographical area, following the main types of plants that predispose to obesity, respectively the determination of carbohydrates, lipids and fructans from the ration of horses and evaluating the effects of these macroelements on body condition.

Following the completion of this study, the following emerges:

1. The deposition of localized adipose tissue varies depending on the type of diet, especially at the level of the cervical ridge.

2. The concentrates have a much higher content of fructans and fats than the other types of fodder analyzed.

3. The degree of adiposity of the cervical ridge increases with the content of carbohydrates and lipids in the ratio.

4. Horses that showed advanced chronic laminitis had a low level of carbohydrates in the feed and were fed mainly natural mixed hay.

5. The hyperglycemic diet leads to an increase in insulinemia and a decrease in leptinemia.

6. No correlations were found to support the involvement of fructans in the development of laminitis, at least in this breed of horses, and further studies are needed.

Study II.,, entitled "Qualitative, quantitative and histomorphometric evaluation of adipose tissue", aimed to evaluate the association of hormonal markers of obesity (leptin and adiponectin) and insulin resistance with morphometric and ultrasonographic measurements of adipose tissue in the neck and body in semi-heavy horses, respectively histological and immunohistochemical evaluation of adipose tissue.

Fig. 1 Evaluation of body condition score according to Henneke (1983). A 5-year-old female with a Body Condition Score of 8 (Original)

Fig. 2 Evaluation of the neck crest by inspection and palpation of the area in an 8-year-old individual. (Original)

This study was structured in four distinct parts:

1. The first part was focused on the clinical assessment of the degree of obesity in horses by determining the Body Condition Score and the Neck Crest Score (figures 1 and 2);

2. The second part focused on the quantitative assessment of localized adiposity by measuring the cervical circumference and ultrasound measurement of the adipose tissue layer in the seven predilected areas in horses where hyperinsulinemia and hyperleptinemia were identified.

3. The third part, or the morphometric study of adipose tissue in hyperinsulinemic and normoinsulinemic horses, aimed to establish a correlation between adipocyte sizes and the state of obesity in the two distinct areas, namely the ridge area and the perirenal area.

4. The fourth part focused on the morphological and physiological evaluation of the adipose tissue in overweight and normal-weight horses through the histological and immunohistochemical examination of the adipose tissue in the two areas of interest.

After carrying out this study we concluded the following:

1. The accumulation of adipose tissue differs according to the geographical areas studied, physical activity and hormonal status.

2. Horses with confirmed metabolic syndrome were hyperinsulinemic and both cervical and ultrasound measurements correlated positively with serum adipokines.

3. Through the histomorphometric analysis of the adipocytes, it was found that the adipocytes in the metabolic group have a significantly increased diameter compared to the healthy group, where a decrease in diameter was found, respectively an increased number of them.

4. In the histopathological evaluation, the most important pathological change was represented by adipocyte hypertrophy in both anatomical areas studied

5. An increased immunoexpression of chemerin was observed in adipose tissue in the SME group, but its involvement in the metabolic syndrome requires studies involving a larger number of animals.

Study III., entitled "Determination of the hematological and biochemical profile in overweight and obese horses", aimed to correlate humoral markers of glucose homeostasis (insulin and glycated hemoglobin), adipokines (leptin, adiponectin, omentin and chemerin), serum lipids (total cholesterol and non-esterified fatty acids) and kynurenines with the morphological features of the horses studied.

To achieve this objective, the following determinations were necessary:

1. Determination of insulin, leptin and adiponectin from serum samples taken from horses suspected of metabolic syndrome during the intra-vital stage;

2. Determination of the plasma levels of insulin, glycosylated hemoglobin, omentin, chemerin, respectively non-esterified fatty acids and total cholesterol in samples from overweight and obese horses in the *post-mortem* stage;

3. Determination of kynurenine from plasma samples from the *post-mortem* stage.

After carrying out this study we concluded the following:

1. In individuals diagnosed with EMS, hyperinsulinemia is followed by hyperleptinemia.

2. Foals have higher leptin and adiponectin levels than adults.

3. Serum insulin correlates statistically significantly with upper cervical measurement in the entire studied group.

4. Adiponectin correlates positively with the presence of the male sex.

5. Insulin and leptin had random increases and decreases between groups, but adiponectin in most correlations remained low.

6. Free fatty acids as a dependent variable correlate positively with both weight and age, Body Condition Score and Cresty Neck Score.

7. Total cholesterol correlates significantly with weight, being a valuable marker in the diagnosis of EMS.

8. Omentin correlates significantly with the Cresty Neck Score, the cervical area being predisposed to the accumulation of adipose tissue in individuals with EMS.

9. Free fatty acids correlate positively with both weight and Height Score, being a useful predictor of the degree of obesity in individuals with metabolic syndrome.

10. Body scores correlate positively with weight within group 4.

11. Groups 1 and 4 resulting from the cluster analysis are contrasting groups, regarding the values of free fatty acids, omentin, chemerin, respectively of the body scores.

12. Horses with metabolic syndrome show higher levels of kynurenine and glycosylated hemoglobin compared to control individuals.

13. In non-SME individuals, when glycosylated hemoglobin increases, the Cresty Neck Score values, respectively cholesterol, decrease.

14. Kynurenine increases symmetrically with omentin and chemerin, so it can be considered a useful marker of EMS.

Study IV., entitled "The mineral composition of hair in horses with metabolic syndrome" had as its objective the evaluation of the elemental mineral composition of hair collected from horses of the Semigreu breed affected by equine metabolic syndrome with the aim of introducing this type of analysis as a valuable and non-invasive auxiliary tool in the diagnosis of this pathology.

To achieve this goal, the spectrometric analysis method preceded by the hair digestion protocol was used.

After carrying out this study we concluded the following:

1. Within the group of healthy horses, compared to individuals with metabolic syndrome, significantly increased levels of minerals were detected.

2. Hair analysis regarding the content of micro and macro elements is a valuable diagnostic tool and subsequently later it could have an important role in the prevention and treatment of obesity associated with EMS.

3. Although during this study the batches to be researched were not very numerous, the obtained result seems to be promising in terms of simplifying the EMS diagnostic protocol. This method is non-invasive and very fast.

4. We recommend supplementing the diet of horses with metabolic syndrome with minerals such as Zn, Mg, Ca and Cr.

5. Within the EMS group, as K values increase, so do cholesterol values, so K can be considered an important metabolic marker. At the same time, the concentration of K in the hair increases with age.

6. Within the full sample, Cu increases symmetrically with chemerin, omentin, and K and Na increases with cholesterol. Pb and Zn increase symmetrically with glycosylated hemoglobin.

Study V.,, entitled "Evaluation of the genetic factor involved in the etiopathogenesis of the metabolic syndrome: expression of the FAM 174A gene", aimed to determine the genome-wide positive association between a risk allele for equine metabolic syndrome and the phenotype showing obesity and insulin resistance.

To achieve this goal, the PCR amplification method targeting the FAM174A gene region was used.

After carrying out this study we concluded the following:

1. A significantly higher number of horses with metabolic syndrome were found to have the 11-G nucleotide pair.

2. In the non-EMS group more than half of the horses have the 8-G, 9-G or 10-G allele of the FAM 147A gene.

3. Within the metabolic syndrome group, 13 of the 15 horses have a very high cholesterol or insulin z-score, this result correlating with the presence of the 11-G nucleotide pair.

4. Kynurenine values were positively correlated with the presence of the 8-G allele.

5. Horses with the 10-G allele have the lowest values for Mn in the hair, and horses with the 8-G allele of the FAM 147A gene have the highest values.

Study VI., entitled "Reproductive disorders associated with equine metabolic syndrome", aimed to determine the correlations between mares' fertility and insulin resistance, hypoadiponectinemia, hyperinsulinemia and hyperleptinemia.

To achieve this objective, serum levels of insulin, leptin and adiponectin were determined in all mares included in the study.

After carrying out this study we concluded the following:

1. Pregnant mares had lower insulin levels than other horses, respectively pregnant mares had lower insulin levels than other mares.

2. For adiponectin, significant differences are only between males and females.

3. No statistically significant difference was recorded for leptin.

Study VII., , entitled Endocrinopathic laminitis, the major complication of equine metabolic syndrome: clinical, histological and immunohistochemical evaluation" aimed at the clinical, histological and immunohistochemical evaluation of endocrinopathic (chronic) laminitis and the correlation with morphological aspects and metabolic markers.

To achieve this objective, the following methods were used:

1. Clinical and radiological evaluation of endocrinopathic (chronic) laminitis in affected individuals.

2. Evaluation of histological and immunohistochemical changes of the hoof affected by endocrinopathic (endocrinopathic) laminitis.

After carrying out this study we concluded the following:

1. The different degrees of lameness were confirmed by radiological examination, by the presence of local inflammation, the loss of gearing between the podophilus and the cheraphillus lamellae, respectively the rotation of the 3rd phalanx.

2. The histological examination confirmed the changes characteristic of endocrinopathic (chronic) laminitis in the soft tissue and bone of the 3rd phalanx, namely severe necrosis, inflammatory infiltrate and aspects of bone remodeling.

Study VIII., entitled "The histomorphological study of the liver and kidney in equine metabolic syndrome" had as its objective the histomorphological investigation of liver and kidney changes caused by weight gain in horses affected by metabolic syndrome.

In order to achieve this objective, the qualitative morphological and histopathological evaluation of the samples collected from the liver and kidneys was carried out, following the appearance of certain lesions specific to obesity in the equine metabolic syndrome.

After carrying out this study we concluded the following:

1. Following the histopathological examination of the liver, the presence of pathological changes specific to the metabolic syndrome was noted, therefore we can state that steatosis caused by weight gain plays an important role in the development of comorbidities associated with obesity in horses.

2. Renal lipidosis in horses, in the context of metabolic syndrome, is another important comorbidity, still little studied in this species.

In chapter 6 I described the general conclusions and recommendations. The identification of certain correlations between free fatty acids, new adipokines and morphological traits both in humans and in horses affected by metabolic syndrome, supports the idea that the evaluated blood biochemical constituents can be considered relevant markers in the diagnosis of this new condition. Moreover, the determination of these markers could constitute a new screening method in specialized laboratories with the aim of preventing the occurrence of comorbidities of this disease.

The results of the study are amplified by the increased specificity of DNA sequencing, ELISA determinations, spectrometry, spectrofluorimetry, respectively immunohistochemistry, methods used in this work, to highlight the contribution of hormones (insulin, leptin and adiponectin), adipokines (chemerin and omentin) and kynurenine in equine metabolic syndrome.

The presence of chemerin both in plasma and in adipose tissue could be considered a new biomarker for metabolic syndrome, but requires further studies. Another accurate diagnostic method was to investigate the functions of a gene that predisposes to equine metabolic syndrome through its role in cholesterol homeostasis. Another useful diagnostic marker investigated in metabolic individuals was the determination of hair mineral composition, which proved to be a new non-invasive method that can be successfully used in field conditions.

Following the results of this study we **concluded** the following:

1. The deposition of localized adipose tissue varies depending on the type of diet, especially at the level of the cervical ridge, an area characteristic of equine metabolic syndrome.

2. Concentrates have a higher content of fructans and fats compared to other types of feed.

3. Horses diagnosed with metabolic syndrome were hyperinsulinemic and both cervical and ultrasound measurements correlated positively with serum adipokines.

4. An increased immunoexpression of chemerin was observed in adipose tissue in the SME group, more specifically in the area of the cervical ridge, but its involvement in the metabolic syndrome requires studies involving a larger number of horses.

5. Free fatty acids, as a dependent variable, correlate positively with both weight and age, Body Condition Score and Cresty Neck Score.

6. Omentin correlates significantly with the Cresty Neck Score, respectively increases symmetrically with kynurenine and chemerin, these being considered useful markers for metabolic syndrome in humans, but now also in horses.

7. Within the whole group of horses, copper increases simultaneously with chemerin and omentin, and potassium and sodium symmetrically with glycosylated hemoglobin.

8. It was found that a significantly higher number of horses with metabolic syndrome presented the allele of the FAM174A gene with 11-G, and in the non-metabolic group alleles with 8-G, 9-G or 10-G predominated.

9. Within the metabolic syndrome group, horses with high z-score for insulin and cholesterol showed the FAM174A gene with 11-G.

10. Pregnant mares had lower insulin levels than the other horses studied.

11. For adiponectin, significant differences are only between males and females.

12. Corroboration of the clinical and radiological examination, confirmed the characteristic changes of metabolic laminitis.

13. The different degrees of lameness were confirmed by radiological examination, by the presence of local inflammation, the loss of gearing between the podophilus and the cheraphillus lamellae, respectively the rotation of the 3rd phalanx.

14. The histological examination confirmed the changes characteristic of endocrinopathic (chronic) laminitis in the soft tissue and bone of the III phalanx, namely severe necrosis, inflammatory infiltrate and aspects of bone remodeling.

15. Renal lipidosis, in the context of metabolic syndrome in horses, is another important comorbidity, but still little studied in this species.

16. Although during this study the number of evaluated subjects was not very large, nevertheless the results obtained are promising regarding the simplification of the diagnostic protocol in EMS.

Following the analysis of the results of this extensive study on equine metabolic syndrome, we **recommend** the following:

1. Since concentrates are hyperglycemic and hyperlipidic feeds, we recommend avoiding their excess in the ratio of horses diagnosed with metabolic syndrome as well as healthy ones.

2. Soaking the fibrous feed in water before consumption is an effective method in reducing the content of water-soluble carbohydrates.

3. Moderate physical exercise introduced into the program of horses with metabolic syndrome of at least 30 minutes, 5-6 days a week with the aim of using lipids in energy production. Exceptions to this program are individuals affected by laminitis.

4. If space permits, we recommend placing the feeding stations as far apart as possible to encourage the movement of the horses.

5. Body Condition Score and Cresty Neck Score are useful methods in the diagnosis of obesity in horses.

6. For obese and laminitic horses, it is mandatory to include a diagnostic protocol regarding blood determinations of glycosylated hemoglobin, insulin, leptin and adiponectin.

7. A new non-invasive paraclinical method of diagnosis would be the determination of minerals in hairs, easily performed in the field.

8. We recommend supplementing the diet of horses with metabolic syndrome with minerals such as zinc, magnesium, calcium and chromium.

9. In case of endocrinopathic (chronic) laminitis or in the case of breed improvement, a useful diagnostic tool would be to evaluate the expression of the FAM174A gene.

10. The evaluation of the genetic factor involved in the etiopathogenesis of equine metabolic syndrome is a fast and very accurate tool in the diagnosis of this metabolopathy.

11. Overweight mares and stallions are prone to fertility problems, precisely for this reason we recommend a hormonal screening before using them for breeding.

12. In equine endocrine diseases, not only SME has an important role in reproductive status, but also Equine Cushing's Syndrome (PPID), so we recommend hormonal tests all the more.

13. For any lameness observed clinically, it is recommended to evaluate both the body condition and the limb, respectively radiological examination.

14. The diet and physical activity of clinically and paraclinically confirmed horses with laminitis will be reassessed.

15. To avoid as much as possible liver and kidney comorbidities, we recommend performing biochemical analyzes in overweight and obese horses, with emphasis on liver and kidney markers.

16. The most effective way to avoid complications of equine metabolic syndrome is to educate owners about nutrition and exercise.

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