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Advanced nutritional and stem cells approaches to prevent equine metabolic syndrome

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ABSTRACT

Horses metabolic disorders have become an important problem of modern veterinary medicine. Pathological obesity, insulin resistance and predisposition toward laminitis are associated with Equine Metabolic Syndrome (EMS). Based on pathogenesis of EMS, dietary and cell therapy management may significantly reduce development of that disorder. Special attention has been paid to the diet supplementation with high bioavailability minerals and mesenchymal stem cells (MSC) which increase insulin sensitivity. In nutrition, there is a great interests in natural algae enriched in biosorption process with micro and macroelements. In the case of cellular therapy, metabolic condition of engrafted cells may be crucial for the effectiveness of therapy. Although, recent studies indicated on MSC deterioration in EMS individuals. Here, we described combined nutritional and stem cells therapy for the EMS treatment. Moreover, we specify in details how EMS affects the adipose-derived stem cells (ASC) population. Presented here, combined kind of therapy- an innovative and cutting edge approach of metabolic disorders treatment may become a new gold standard in personalized veterinary medicine.

1. Equine metabolic syndrome (EMS) etiopathology

Over the past decades, obesity has grown into a major global problem not only in human but also in veterinary medicine. Apart from obesity, two other factors i.e. hyperinsulinemia and insulin resistance (IR), play a key role in the development of Equine Metabolic Syndrome (EMS) (Frank, 2009). This term was for the first time introduced by Johnson et al. (2004), who suggested that IR, obesity and laminitis underlie this endocrynologic disorder which affects horses and ponies as well. The term EMS was adopted from human medicine, because of many similarities, in the field of risk factors, although alternative nomenclature for EMS has been proposed: peripheral Cushing's syndrome or prelaminitic metabolic syndrome. However, the term EMS becomes some kind of compromise in veterinary nomenclature and has been well accepted by most of clinicians and what is more becomes the term that is dedicated only for horses.

In the last, approximately ten years, different research groups have proposed a panel of clinical feature's that has to be fully field to diagnose EMS. It includes: increased adiposity in specific locations, IR and predisposition toward laminitis, however increasing evidences indicate on importance of additional components i.e. arterial hypertension, hyperleptinaemia and hypertriglyceridaemia (Frank, 2011; Johnson et al., 2004). Our own research provide a data about molecular changes in liver, adipose tissue and muscle caused by EMS. We have shown that, excessive endoplasmic reticulum stress correlates with impaired mitochondrial dynamics, mitophagy and apoptosis in liver and adipose tissue of EMS horses (Marycz et al., 2018).

Pathological obesity is one of the characteristic feature of clinical picture of EMS horses. Abnormal accumulation of fat deposits are especially localized around the nuchal ligament in the neck (cresty neck), the base of the tail, eyes, and behind the shoulder or in the prepuce or mammary gland region (Frank, 2011). Adipose tissue is an important endocrine and secretory organ, which produce a variety of active molecules, including (i) hormones i.e. leptin, adiponectin, (ii) pro-inflammatory cytokines i.e. interleukin 6, interleukin, tumour necrosis factor alpha and (iii) others pro-inflammatory cytokines i.e. p53

Abbreviations: AGEs, advanced glycosylation end products; ALA, alpha-linolenic acid; CHO-FR, rapidly fermentable groups; CHO-FS, slowly fermentable groups; CHO-H, hydrolyzable groups; DHA, docosahexaenoic acid; DJD, degenerative joint disease; DPA, docosapentaenoic acid; ELISA, enzyme-linked immunosorbent assay; EMS, equine metabolic syndrome; EPA, eicosapentaenoic acid; IECs, intestinal ensheating cells; IPC, insulin producing cells; IR, insulin resistance; KER, Kentucky Equine Research; MSC, mesenchymal stem cells; MVs, membrane-derived vesicles; NO, nitric oxide; NSC, non-structural carbohydrates; PARL, presenillin-associated rhomboid-like; PGC, 1α- peroxisome proliferator–activated receptor gamma coactivator; ROS, reactive oxygen species; SOD, superoxide dismutase

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(Minamino et al., 2009; Steppan and Lazar, 2002). These factors are involved in the development of metabolic diseases and closely related with the accompanying systemic inflammation. In our studies we investigated the production and distribution of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) in subcutaneous adipose tissue and their correlation with serum concentrations in Welsh ponies with equine metabolic syndrome. Based on clinical examination findings, the animals were divided into two groups: ponies affected with EMS (n = 8) and obese ponies (n = 8). The adipose tissue was examined using immunohistochemical analysis while concentrations IL-6 and TNF-α were measured using enzyme-linked immunosorbent assays (ELISAs). Additionally, histological characterization of the adipose tissue was performed. The results obtained showed that IL-6 expression in adipose tissue biopsies derived from animals with EMS was enhanced while TNF-α levels of both groups were comparable. Compared to the obese ponies, EMS animals also had significantly elevated levels of serum IL-6 and TNF-α. Histological analysis revealed macrophage infiltration and fibrosis in adipose tissue preparations from the EMS group. These data suggest that IL-6 may play a key role in the course of EMS in Welsh ponies. Our findings also demonstrated that analysis of pro-inflammatory cytokines levels in serum may serve as an additional tool for diagnosing EMS (Basinska et al., 2015).

Moreover, Waller et al. (2011) showed, that insulin resistance is closely associated with elevated expression of Toll-like receptor 4 (TLR-4) and suppressor of cytokine signaling 3(SOCS-3) in skeletal muscle (SM) and visceral tissue, without a significant change in subcutaneous (SC) site. In addition, positive correlation between TLR-4 content and SOCS-3, as well as a significant negative correlation between SOCS-3 content and glucose transporter 4 (GLUT-4) trafficking was confirmed. Results indicated that IR develops following systemic inflammation in horses and suggested that adipose tissue may contribute to this inflammatory response. Methods to regulate insulin sensitivity may improve clinical outcome in critically ill patients. However, further research that will correlate pro-inflammatory cytokines expression in abnormal accumulated fat deposits and their impact on developing systemic and/or local inflammation are required.

The EMS is also associated with the development of laminitis- metabolic disease causes serious pathological changes in equine hoof and it is also manifested by lack of the attachment between the distal phalanx and the inner hoof wall (French and Pollitt, 2004; Johnson et al., 2010). Juan et al. (2004) showed that, endothelin-1 plays a crucial role in pathophysiology of laminitis via vasoconstriction. Moreover, it seems that, excessive expression of endothelin-1 can be induced by hyperinsulinaemia. It was reported that disorders of insulin action are associated with an increased risk for development of laminitis (Freestone et al., 1992). de Laat et al. (2010) confirmed that results as they observed that the high concentration of insulin in the blood (approximately 1000–1100 μiu/ml) can induce laminitis in horses.

Furthermore, multiple studies indicated that excessive obesity leads to oxidative stress, due to excessive activity of reactive oxygen species (ROS) (De Marchi et al., 2013). Furukawa et al. (2004) reported, that ROS are increased in the adipose tissue of obese mice, which is associated with reduced expression of antioxidant enzymes i.e. superoxide dismutase (SOD). ROS can induce DNA damage and subsequent expression of p53, leading to cellular senescence and increased risk of metabolic diseases. It was shown, that deficiency of riboflavin enhances not only ROS production but also activates pro-inflammatory macrophages response in adipose tissue (Mazur-Bialy and Pocheć, 2017). In consequence, pro-inflammatory activity of adipocytes, which secrete robust amount of leptin, leads to IR and chronic inflammation (Mazur-Bialy and Pocheć, 2016). Thus supplementation of EMS horses' diet in vitamin B seems to be fully justified.

For the IR diagnosis, several methods exist including (i) fasting glucose/insulin measurement or (ii) dynamic evaluation of glucose and insulin responses. Both of them are usually insufficient and impractical in diagnostics process and may threat animal's life. Thus, available diagnostic methods may only confirm or exclude the syndrome occurrence. Although, metabolic diseases- including EMS are characterized by still limited treatment strategies and it seems that biologically active ingredients could reduce the risk of EMS development in horses. Moreover, searching for an bioactive ingredients, that might reduce expression of pro-inflammatory cytokines in fat tissue, and what is more reduce systemic inflammation seems to be fully reasonable.

More and more frequent, EMS is highly connected with improper feeding strategy and individual predispositions. Some breeds especially ponies and/or cold-bloods are more prone to develop EMS particularly under improper diet management (Geor and Frank, 2009). The proper feeding strategy, becomes an important factor in the course of EMS as it is believed, that low non-structural carbohydrates (NSC) diet reduces glycemic and insulinemic responses to meals and reduce digestible energy helping to control the body weight ("Influence of Functional Nutrients on Insulin Resistance in Horses with Equine Metabolic Syndrome," 2018). In general, NSC consists of simple sugars that can be digested by enzymes produced by horse, for example glucose, fructose, lactose, sucrose and starch. On the other hand, structural carbohydrates (SC) must be fermented by bacteria. That group includes mainly cellulose and hemicellulose. NCS overload can contribute to serious complications as laminitis, IR and colic. High levels of circulating glucose from digested NCS, stimulates pancreas to secrete robust amount of insulin leading in consequence to the development of IR and/or laminitis. Thus reducing NSC levels in horse' diet helps to maintain energy balance. For that reason, algal biomass seems to be promising food adjective for EMS individuals as they are low in NCS and rich in SC. Algae are rich source of protein, polyunsaturated fatty acids, polysaccharides, exogenous amino acids, dyes, fiber and minerals and vitamins (including riboflavin, especially important in metabolic syndrome development) (see Table 1). Moreover, biological active compounds as polyphenols, diphlorethohydroxycarmalol and phlorotannins are present in algal extracts. Our own research have indicated that horses fed with a diet based on *Spirulina platensis* supplementation lost weight and improved insulin sensitivity (Nawrocka et al., 2017) and that *Spirulina platensis* filtrates can considered as an agent with anticancer properties (Michalak et al., 2017; Śmieszek et al., 2017). Furthermore, it was showed that impairment of ASC isolated from EMS individuals (Marycz et al., 2016a, 2016b, 2016c) can be partially reversed by *Cladophora glomerata* enriched by biosorption process in Cr (III) extract (Marycz et al., 2017). Because of those facts, we would like to propose to use algae enriched with elements in the biosorption process as a source of nutrients in feeding of EMS horses.

2. Nutritional management of EMS

As EMS belongs to the family of metabolic disorders, it can be, at least, to some extent, managed by the application of well-balanced diet. The EMS horses feeding strategies are oriented on reaching two main goals. The first one is to induce body weight loss, by energy amount reduction, while second aims to reduce insulinemic response to meals, mostly by lowering the NSC content in diet. In general, EMS is developing in horses that were overfed with both: energy and NSC. In turn, eliminating grass hay from diet seems to be most important factor in case of preventing risk of pasture-associated laminitis (Geor and Harris, 2009; PJ and JD, 2008). Recent study indicated on significant variable between hay samples what additional complicate creation well balance diet. That is why, most of researcher recommends forage diet for obese and EMS horses, with proper mineral and vitamins qualified concentration. It was suggested, that forage NSC concentration in EMS horses diet should be reduce to 10–12% of dry matter or even less. It might significantly reduce the risk of hyperinsulinaemia as well as laminitis. However, it should be pointed out, that feeding obese EMS horses versus EMS not obese individuals comprise two individual nutritional strategies (Geor and Harris, 2009).

The impact of carbohydrate in EMS diet and laminitis prevention