Lactofermented sugar beet pulp as an insulin sensitizing factor in horses with metabolic syndrome EMS

Abstract

The equine metabolic syndrome (EMS) is an endocrine disease whose pathogenesis is related to insulin resistance (IR), obesity, abnormal fatty tissue deposition at specific spots, chronic or past laminitis, and local and / or systemic inflammation. Currently, more and more interest is found in lactofermented foods that contain beneficial bacteria, referred to as natural probiotics. It has been shown that some LAB strains of the genus *Lactobacillus spp.* conduce to a number of pro-health activities, including antidiabetic properties by increasing the sensitivity of tissues to insulin.

The aim of the research in the first stage was to determine the effect of sugar beet pulp fermentation without the addition of probiotic microorganisms and with the participation of heterogeneous strains probiotic bacteria of *Lactobacillus rhamnosus* and *Pediococcus acidilactici* on the chemical composition, pH values, as well as the total content of polyphenols along with antioxidant activity by DPPH, FRAP and ABTS methods. Moreover, the content of short-chain fatty acids (SCFA) and simple carbohydrates with the isolation of sucrose in the fermentation materials was determined. Dried molassed sugar beet pulp in the form of pellets, fermented according to the scheme: sugar beet pulp without the addition of probiotic microorganisms (CTRL), lactofermented sugar beet pulp with the addition of *Lactobacillus rhamnosus* (LR) and lactofremented sugar beet pulp with the addition of *Pediococcus acidilactici* (PA). The fermentation process lasted six days, on each day a sample of the fermented material was collected for further analysis.

The chemical composition and SCFA analysis was carried out in fresh and dried material. The total content of polyphenols, antioxidant activity and the content of simple sugars were detected in the dried material.

The study showed a positive effect of the use of probiotic bacteria strains on the physico-chemical properties of the biomass from beet pulp compared to spontaneously fermented sugar beet pulp. Particularly, the *Lactobacillus rhamnosu* strain is noteworth. Fermentation carried out with its participation was characterized by the best profile, both in terms of nutrients, short-chain fatty acids, the total content of polyphenols and antioxidant properties and the reduction

of simple sugars. Due to the obtained results, the *Lactobacillus rhamnosus* strain was selected for molecular testing.

In addition, the aim of the study was to evaluate the effect of fermented sugar beet pulp extracts without the addition of microorganisms (CTRL) and lactofermented beet pulp extracts with the addition of the *Lactobacillus rhamnosus* bacterial strain in in vitro tests on progenitor cells (ASC) of adipose tissue collected from horses. Molecular and cytobiological studies included determination of migratory activity, viability and proliferation rate, apoptosis, a marker of cell destruction, oxidative stress and the dynamics of ASC mitochondria. In addition, the expression of pro-inflammatory and anti-inflammatory genes and genes related to insulin resistance as well as miRNA expression were determined. Immunofluorescence staining was also performed for the marker Ki-67, the pro-inflammatory cytokine IL1β and the caspase 3. The morphology of ASC cells was visualized by fluorescent staining.

In the course of in vitro studies it was shown that the extracts taken from the fermentation of beet pulp with the addition of *Lactobacillus rhamnosus* increase the rate of migration, proliferation, and show a higher clonogenic potential and a shorter doubling time of the population. Culturing ASC cells with aplication of *Lactobacillus rhamnosus* extracts reduced inflammation and the expression of genes related to apoptosis. Additionally, these compounds had a positive effect on the dynamics of mitochondria. The use of extracts with the addition of *Lactobacillus rhamnosus* reduced reactive oxygen species (ROS) compared to control group and improved the expression of SOD2, which is responsible for protecting cells against mitochondrial apoptosis.

To summarize, extracts of lactofermented sugar beet pulp with the addition of the probiotic strain *Lactobacillus rhamnosus* improved the nutritional value of the fermented biomass. In addition, studies on ASC cells confirmed that lactofermented beet pulp can be a therapeutic tool in counteracting the occurrence of the metabolic syndrome in horses.

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