

## The effect of phytoadditives on biochemical indicators and nutrients digestibility in sport horses nutrition

### Vplyv fytoaditív na biochemické ukazovatele a stráviteľnosť živín vo výžive športových koní

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#### Abstract

The aim of the study was to determine the effect of a phytogetic additive on blood serum indicator levels and faecal nutrients digestibility. The experiment was realized in Riding Centre of the Department of Animal Husbandry, Faculty of Agrobiolgy and Food Resources, Slovak University of Agriculture in Nitra. Total 14 warmblood sport horses (geldings) were used (7 horses in control group, 7 horses in experimental group respectively); Slovak warmblood bred, average body weight  $525 \pm 75$  kg and  $6.8 \pm 3$  Years. The control group of horses were fed by crimped barley, meadow hay and mineral feed mixture. Feed rations in experimental group were supplemented with a phytogetic additive containing a blend of essential oils from origanum, anise and citrus, as well as a prebiotic rich in fructooligosaccharides. Blood serum was collected 3 times during the experiment, in the beginning of the experiment, and every 45 days. The experiment lasted 90 days. After the 45 days of phytoadditive supplementation we found a tendency of lower concentrations of serum triglycerides ( $0.07$  vs.  $0.23 \text{ mmol} \cdot \text{l}^{-1}$ ) and total cholesterol ( $2.18$  vs.  $2.27 \text{ mmol} \cdot \text{l}^{-1}$ ) in experimental group of horses ( $P > 0.05$ ). In serum concentrations of glucose, total proteins and urea we find similar values in all of groups. The same tendency we analyzed in activity of AST, ALT, and ALP enzymes ( $P > 0.05$ ). After the 90 days of the phytoadditive supplementation in feed rations of sport horses, we detected a tendency of lower serum triglycerides concentration in experimental group of horses and higher cholesterol, glucose, total protein and urea concentrations ( $P > 0.05$ ). We didn't find a significant effect of a phytoadditive supplementation on blood serum indicator levels. Higher faecal digestibility coefficient of dry matter was found in experimental group of horses (62.98%) in comparison with control group (56.87%). In control group of horses we detected tendency of lower crude protein digestibility (71.36%) in comparison with phytogetic additive group feeding (72.86%). We analyzed a positive effect of phytogetic additive on organic matter digestibility of feed ration. In experimental group of horses we found significantly ( $P < 0.05$ ) higher organic matter faecal digestibility coefficient (73.31%) in comparison with control group (68.32%). We analyzed insignificant ( $P > 0.05$ ) effect of a phytoadditive on blood serum

concentrations during 90 days of experiment. We found positive effect of phytoadditive supplementation on total faecal digestibility of organic nutrients.

**Keywords:** additives, aromatic plants, blood serum, digestibility, equine

## Abstrakt

Cieľom experimentu bolo analyzovanie vplyvu fyto génného aditíva na ukazovatele krvného sera a zdanlivú stráviteľnosť živín. Experiment bol realizovaný v spolupráci s Jazdeckým centrom Katedry špeciálnej zootekniky Fakulty agrobiológie a potravinových zdrojov Slovenskej poľnohospodárskej univerzity v Nitre. Do pokusu bolo zaradených celkom 14 teplokrvných športových koní, valachov, ktoré boli rozdelené do 2 skupín (kontrolná skupina a pokusná skupina, v každej 7 koní) plemena slovenský teplokrvák, priemerná živá hmotnosť 525 ±75 kg a vek 6.8 ±3 roky. Krmná dávka koní v kontrolnej skupine pozostávala z miaganého jačmeňa, lúčneho sena a minerálnej krmnej prísady. Krmná dávka koní v pokusnej skupine bola v porovnaní s kontrolnou obohatená o fyto génné aditívum, ktorého účinnými látkami bola zmes esenciálnych olejov z oregano, anízu a citrusových plodov, ako aj prebioticky obohatené fruktooligosacharidy. Odber krvi bol urobený 3 krát počas pokusu, na začiatku a následne každých 45 dní. Experiment trval celkom 90 dní. Po 45 dňoch od suplementácie krmných dávok v pokusnej skupine koní sme zaznamenali tendenciu nižšej sérovej koncentrácie triglyceridov (pokusná skupina 0,07, kontrolná skupina 0,23 mmol\*l<sup>-1</sup>) a cholesterolu (pokusná skupina 2,18, kontrolná skupina 2,27 mmol\*l<sup>-1</sup>). V sérovej koncentrácii glukózy, celkových bielkovín a močoviny sme zistili podobné hodnoty u oboch skupín koní. Podobnú tendenciu sme zistili aj v aktivite AST, ALT a ALP (P>0,05). Po 90 dňoch od suplementácie fyto génnym aditívum v pokusnej skupine koní, sme zaznamenali tendenciu nižšej koncentrácie sérových triglyceridov v pokusnej skupine koní, avšak aj tendenciu vyššej sérovej koncentrácie cholesterolu, glukózy, celkových bielkovín a močoviny (P>0,05). Nezistili sme významný vplyv fyto génného aditíva na sledované krvné parametre. Vyššiu zdanlivú stráviteľnosť sušiny sme zaznamenali v pokusnej skupine koní (62,98 %) v porovnaní s kontrolnou skupinou (56,87 %). V kontrolnej skupine koní sme zistili tendenciu nižšej stráviteľnosti dusíkatých látok (71,36 %) v porovnaní so skupinou koní, ktorých krmné dávky boli obohatené o sledované fyto génné aditívum (72,86 %). Zistili sme pozitívny vplyv fyto génného aditíva na zdanlivú stráviteľnosť organickej hmoty krmnej dávky. V pokusnej skupine koní sme zaznamenali preukazne (P<0,05) vyššiu stráviteľnosť organickej hmoty (73,31 %) v porovnaní s kontrolnou skupinou koní (68,32 %). Zistili sme nepreukazný (P>0,05) vplyv fyto génného aditíva na ukazovatele krvného sera počas 90-dňového experimentu. Zistili sme pozitívny vplyv aditíva na zdanlivú stráviteľnosť organických živín.

**Kľúčové slová:** aditíva, aromatické rastliny, kone, krvné sérum stráviteľnosť

## Detailed abstract in native language

Cieľom experimentu bolo analyzovanie vplyvu fyto génného aditíva na ukazovatele krvného sera a zdanlivú stráviteľnosť živín. Experiment sme realizovali v spolupráci s Jazdeckým strediskom Katedry špeciálnej zootekniky, Fakulty agrobiológie a potravinových zdrojov Slovenskej poľnohospodárskej univerzity v Nitre. Do pokusu

bolo zaradených 14 teplokrvných športových koní (valachov), ktoré boli rozdelené do 2 skupín, kontrolnej a pokusnej. Kone zaradené do experimentu (plemeno slovenský teplokrvnik) mali priemernú živú hmotnosť  $525 \pm 75$  kg a vek  $6,8 \pm 3$  rokov, počas pokusu boli kone v strednej športovej záťaži. Kone boli počas experimentu individuálne ustajnené v boxoch s prístupom k vode *ad libitum*. V kontrolnej skupine koní bola počas experimentu skrmovaná krmná dávka pozostávajúca z miaganého jačmeňa, lúčneho sena a minerálnej krmnej prísady. Krmná dávka v pokusnej skupine koní bola v zložení z rovnakých krmných surovín, avšak bola obohatená o fytogénne aditívum, ktorého účinnými látkami bola zmes esenciálnych olejov z oregána, anízu a citrusových plodov, ako aj prebioticky obohatené fruktooligosacharidy. Dávka sledovaného aditíva bola zostavená individuálne pre každé zviera v množstve 1 g na 1 kg prijatého jadrového krmiva. Krmná dávka bola pre jednotlivé kone zostavená individuálne na základe živej hmotnosti a záťaže. Frekvencia kŕmenia u koní v pokuse bola 2 krát denne (50 % ráno, 50 % večer). Počas experimentu bola u sledovaných koní 3 krát odobratá krv, na začiatku experimentu, na 45. resp. 90. deň pokusu. Po 45 dňoch experimentu sme u koní v pokusnej skupine, ktorej bola skrmovaná obohatená krmná dávka, zaznamenali tendenciu nižšej sérovej koncentrácie triglyceridov ( $0,07 \text{ mmol} \cdot \text{l}^{-1}$ ) v porovnaní s kontrolnou skupinou športových koní ( $0,23 \text{ mmol} \cdot \text{l}^{-1}$ ). Podobný efekt sledovaného fytogénneho aditíva sme zistili aj v sérovom obsahu cholesterolu. V pokusnej skupine koní sme zaznamenali nižšiu priemernú koncentráciu cholesterolu v krvnom sere ( $2,18 \text{ mmol} \cdot \text{l}^{-1}$ ) v porovnaní s kontrolnou skupinou ( $2,27 \text{ mmol} \cdot \text{l}^{-1}$ ). Rozdiely boli v oboch prípadoch štatisticky nepreukazné ( $P > 0,05$ ). V sérovej koncentrácii glukózy, celkových bielkovín a močoviny sme zistili podobné hodnoty u oboch skupín koní. Rovnakú tendenciu sme zaznamenali aj v enzymatickej aktivite AST, ALT a ALP ( $P > 0,05$ ). Po 90 dňoch od začiatku experimentu sme u koní kŕmnych kŕmnu dávkou obohatenou o fytogénne aditívum zistili tendenciu nižšej sérovej koncentrácie triglyceridov a vyššiu koncentráciu cholesterolu, glukózy, celkových bielkovín a močoviny ( $P > 0,05$ ). Nezistili sme preukazný vplyv analyzovaného fytogénneho aditíva na krvné sérové ukazovatele športových koní. Počas experimentu, v jeho poslednej fáze, sme realizovali bilančný kŕmny pokus s cieľom zistenia vplyvu testovaného aditíva na zdanlivú *in vivo* stráviteľnosť sušiny, dusíkatých látok a organickej hmoty kŕmnej dávky. Bola použitá klasická priama metóda. Bilančný pokus trval po adaptačnom období 5 dní, počas ktorých boli individuálne a kvantitatívne zachytávané výkaly, ktoré boli následne analyzované na obsah vylúčených živín. Priemerne vyšší koeficient stráviteľnosti sušiny sme zistili v pokusnej skupine koní, ktorých kŕmna dávka bola aditovaná o sledované fytogénne aditívum (účinné látky zmes esenciálnych olejov z oregána, anízu a citrusových plodov, ako aj prebioticky obohatené fruktooligosacharidy) a to 62,98 % v porovnaní s kontrolnou skupinou koní (56,87 %). V kontrolnej skupine koní sme zaznamenali tendenciu nižšej stráviteľnosti dusíkatých látok (priemerný koeficient stráviteľnosti 71,36 %) v porovnaní s pokusnou skupinou koní (72,86 %). Zistili sme pozitívny vplyv fytogénneho aditíva na zdanlivú *in vivo* stráviteľnosť organickej hmoty. V pokusnej skupine koní sme zistili preukazne vyšší priemerný koeficient stráviteľnosti organickej hmoty (73,31 %) v porovnaní s kontrolnou skupinou (68,32 %). Zistili sme nepreukazný vplyv ( $P > 0,05$ ) vplyv fytogénneho aditíva na ukazovatele krvného sera športových koní počas 90-dňového experimentu. Zistili sme pozitívny vplyv testovaného aditíva na zdanlivú *in vivo* stráviteľnosť sušiny, dusíkatých látok a organickej hmoty kŕmnej dávky.

**Klíčové slová:** aditíva, aromatické rastliny, kone, krvné sérum stráviteľnosť

## Introduction

In the last 20 Years, there is in the World an increase of horses nutrition and feeding interest. This point is affect mainly by their growth of popularity. In modern horses nutrition are very actual feed additives and detection of additives on metabolism and nutrients utilization (Gálik et al., 2011; Lacková, Šťastný, 2004). In recent Years, there were published some papers with possible using of phytogenics in horses nutrition, and the effect of phytogenics on horses performance Williams and Lamprecht (2008) published that plant extract are useful for horses mainly for their immune-protective effects. Some studies shows significantly effect for digestion, and by this point significantly effect on nutrients utilization and lower feeding costs (Fors, 2009). Xie, et al. (1999) analysed the effect of additive (*Glycyrrhiza glabra*) which were fed in 57 horses. These authors found higher digestibility of organic nutrients in experimental horses group in comparison with control group (without additive supplementation). Epp, et al. (2005) analysed the effect of phytogenic additive on biochemical blood serum parameters. They found significantly lower content of serum cholesterol and triglycerides. Pearson, et al. (2007) observed effect of additive with active components from origanum, fennel and anise, with any significant effect on blood serum of sport horses. Metabolic profile tests are used in horses management as indicator of nutrition level (Gálik, et al., 2009, Halo, et al., 2009a; Lacková, et al., 2000). Some of energy metabolism parameters can be affected by nutrition (Halo, et al., 2009b).

## Material and Methods

The experiment was realized in cooperation with Riding centre (Department of Animal Husbandry, Faculty of Agrobiological and Food Resources, Slovak University of Agriculture, Nitra) in 2009 and 2010. In the trial were used total 14 adult and clinically healthy sport horses (geldings, Slovak warm-blood bred, average body weight  $525 \pm 75$  kg, average age  $6.8 \pm 3$  years). Horses were during the experiment in medium exercise (daily work from 4 to 6 hours in basic and light level of sport exercise). Horses were divided into two groups; control group (C) and experimental group (E). Feed rations were formulated individually according daily requirement (NRC, 2007) from crimped barley, meadow hay and mineral feed mixture. The feeding time was the same for all of horses (50% in the morning, 50% in the evening respectively). The average nutritive value of feed ration was: digestible energy  $107.7 \text{ MJ} \cdot \text{kg}^{-1}$  of dry matter, crude protein  $1256 \text{ g} \cdot \text{kg}^{-1}$  of dry matter. Nutrients composition of feeds used in the experiment is shown in Table 1.

Table 1. Nutrient composition of feed ration feeds in the experiment

Tabuľka 1. Obsah živín v kŕmnej dávke počas experimentu

| Feed            | DM                 | CP                               | CF    | NFE   | ADF   | NDF   | DE                        |
|-----------------|--------------------|----------------------------------|-------|-------|-------|-------|---------------------------|
|                 | g*kg <sup>-1</sup> | g*kg <sup>-1</sup> of dry matter |       |       |       |       | MJ*kg <sup>-1</sup> of DM |
| <b>CB</b>       | 869.0              | 109.8                            | 42.6  | 798.1 | 53.8  | 189.5 | 14.57                     |
| <b>MH</b>       | 850.5              | 107.9                            | 271.0 | 525.9 | 310.2 | 527.6 | 10.08                     |
| <b>MFM</b>      | 869.3              | 181.1                            | 70.2  | 635.5 | 90.7  | 200.8 | 13.40                     |
| <b>Additive</b> | 872.0              | 74.3                             | 221,4 | 623.3 | N.D.  | N.D.  | 10,56                     |

CB: crimped barley, MH: meadow hay, MFM: mineral feed mixture, DM: dry matter, CP: crude protein, CF: crude fibre, NFE: nitrogen free extract, ADF: acid detergent fibre, NDF: neutral detergent fibre, N.D.: not detected, DE: digestible energy (determined by Zeyner and Kienzle, 2002).

Feed rations of horses in the experimental group were supplemented with a commercial phytogetic additive (vehiculum wheat flour, active compounds: a blend of essential oils from origanum, anise and citrus fruits, as well as a prebiotic rich fructo oligosaccharides) in dosage 1g per 1kg of concentrated feed. The experiment lasted 90 days. During last two weeks of the experiment digestibility trial was realized. Adaptation period lasted 7 days (period for fix stabling), during next 7 days were faeces individually collected. Nutrient composition of the feed and faeces was analysed in the laboratory of quality and nutritive value of feeds (Department of Animal Nutrition, Faculty of Agrobiolgy and Food Resources, Slovak University of Agriculture in Nitra) by standard laboratory methods<sup>[13]</sup> for the content of nitrogen free extract, crude fibre, acid detergent fibre and neutral detergent fibre. Coefficients of nutrient digestibility were calculated by the formula:

$$\% D = (\text{Intake} - \text{Faecal Excretion}) / \text{Intake} \times 100$$

To calculate basic statistic characteristics, determine significance of differences and compare the results the analysis of variance, one-way ANOVA and t-test were performed using a P level less than 0.05. The SAS statistical package was used (SAS Inc., New York City, U.S.A.).

## Results and discussion

Metabolic profile tests are very good indicators of nutrition level in horses nutrition (Gálik, et al., 2009, Halo, et al., 2009a). In the experiment, there was analysing the effect of phytogetic additive on selected blood serum parameters. First blood collection was realized in the beginning of experiment (results show Table 2). Second blood collection was obtained after 45 days of experiment. We didn't find significantly differences in concentration of glucose, total proteins and liver enzymes activity after additive supplementation. We found a tendency lower concentration of triglycerides and total cholesterol in blood serum of experimental horses group with a phytogetic additive supplementation. After the qualitative changes of feed ration was found significantly differences of sport horses serum triglycerides by Gálik, et al. (2011). The 3rd blood collection was realized at the last day of experiment. We didn't find



significantly differences between control (without additive) and experimental (with additive) horses group. After the phytoadditive supplementation in feed rations we didn't detect marked differences in AST, ALT and ALP activity. We found similar results in comparison with Halo, et al. (2009) or Gul, et al. (2007). Monitoring of serum parameters in horses nutrition is often use for diseases prognosis and diagnostic of nutritive status (Mason, Kwok, 1977, Gupta, Varshney, 1993). However, Gul, et al. (2007) reported, that haematological and biochemical parameters have only territorial significance, and they aren't useful for different conditions of animal husbandry.

Table 2. The effect of phytoadditive on blood serum indicators during the experiment (mean ±S.D.)

Tabuľka 2. Vplyv fytogénneho aditíva na ukazovatele krvného séra počas experiment (mean ±S.D.)

|         | TRG                             | CHOL       | GLU        | TP                           | AST                             | ALT        | ALP        |
|---------|---------------------------------|------------|------------|------------------------------|---------------------------------|------------|------------|
|         | mmol <sup>l</sup> <sup>-1</sup> |            |            | g <sup>l</sup> <sup>-1</sup> | μkat <sup>l</sup> <sup>-1</sup> |            |            |
|         | 1 <sup>st</sup> collection      |            |            |                              |                                 |            |            |
| Group C | 0.24 ±0.04                      | 2.25 ±0.27 | 6.03 ±0.55 | 60.11 ±1.48                  | 3.72 ±0.61                      | 0.12 ±0.02 | 4.50 ±0.49 |
| Group E | 0.19 ±0.03                      | 2.46 ±0.17 | 5.94 ±0.48 | 62.24 ±4.12                  | 3.76 ±0.39                      | 0.13 ±0.01 | 3.69 ±0.43 |
|         | 2 <sup>nd</sup> collection      |            |            |                              |                                 |            |            |
| Group C | 0.23 ±0.03                      | 2.27 ±0.38 | 5.73 ±0.12 | 68.93 ±6.23                  | 3.81 ±0.20                      | 0.12 ±0.01 | 3.27 ±0.53 |
| Group E | 0.07 ±0.01                      | 2.18 ±0.24 | 5,90 ±0.21 | 68.48 ±1.50                  | 3.93 ±0.20                      | 0.13 ±0.02 | 3.14 ±0.46 |
|         | 3 <sup>rd</sup> collection      |            |            |                              |                                 |            |            |
| Group C | 0.31 ±0.03                      | 2.18 ±0.29 | 5.33 ±0.15 | 60.55 ±3.62                  | 3.49 ±0.39                      | 0.13 ±0.10 | 4.07 ±0.51 |
| Group E | 0.30 ±0.04                      | 2.34 ±0.36 | 5.94 ±0.42 | 62.79 ±4.18                  | 3.76 ±0.20                      | 0.16 ±0.01 | 3.52 ±0.61 |

Group: control group without additives, Group E: group with a phytoactive additive supplementation, TRG: triglycerides, CHOL: cholesterol, GLU: glucose, TP: total protein, AST: aspartat transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase.

In recent years, there is much attention oriented in digestibility trials in horses nutrition (Blažková, et al., 2009). We found a positive effect of phytoactive additive on some nutrients digestibility of sport horses. In control group of horses we found significantly ( $P < 0.05$ ) lower dry matter faecal digestibility in comparison with experimental group. Higher dry matter digestibility indicates higher satiate (Chachulowa, et al., 1991) Similar results in dry matter digestibility were published by Lattimer, et al. (2007). Crude proteins have in animal nutrition mainly structural importance. Their utilization from feed rations in horses nutrition affect by their biological quality. Average digestibility of crude proteins on horse feeds is form 60 to 75%. In crude protein faecal digestibility we didn't find significantly effect of additive with active compounds of essential oils from origanum, anise and citrus fruits, as well as a prebiotic rich of fructooligosaccharides. However, we found a positive tendency ( $P > 0.05$ ), in experimental group of horses we found in average higher crude protein faecal digestibility (72.86 %) in comparison with control group of horses without additive supplementation (71.36 %). The significant ( $P < 0.05$ ) positive effect after a phytoactive additive supplementation we analysed in organic matter digestibility. After total faeces collection during digestibility trial we found higher coefficient digestibility

of organic matter in experimental group of horses (73.31 %) than in control group (68.32 %) which feed rations we composted only from forage and concentrated feeds, without any additives. Probably, this result was affected by origanum, however origanum might help digestion by increasing bile flow and fighting against some undesirable intestinal microorganisms (Steiner, 2009). We found similar organic matter digestibility coefficients in comparison Miraglia, et al. (1999), Tagaki, et al. (2003) and Lindberg and Ragnarsson (2010).

Table 3. The effect of phytoadditive on some nutrients faecal digestibility (mean  $\pm$ S.D.)

Tabuľka 3. Vplyv fyto génného aditíva na zdanlivú stráviteľnosť niektorých živín (mean $\pm$ S.D.)

|                | DMD                           | CPD              | OMD                           |
|----------------|-------------------------------|------------------|-------------------------------|
|                | %                             |                  |                               |
| <b>Group C</b> | 56.87 <sup>a</sup> $\pm$ 4.35 | 71.36 $\pm$ 4.49 | 68.32 <sup>a</sup> $\pm$ 3.19 |
| <b>Group E</b> | 62.98 <sup>b</sup> $\pm$ 4.64 | 72.86 $\pm$ 4.96 | 73.31 <sup>b</sup> $\pm$ 2.26 |

Group: control group without additives, Group E: group with a phytogetic additive supplementation, DMD: dry matter digestibility, CPD: crude protein digestibility, OMD: organic matter digestibility. Values with different superscript in the column are significant at P less than 0.05.

## Conclusions

The aim of the experiment was to find the effect of phytogetic additive on blood serum indicators and some nutrients digestibility of sport horses. In experiment, we used additive rich in origanum, anise and citrus fruits essential oils as well as prebiotics. We didn't find significantly effect of additive on observed blood serum indicators. We found positive effect of additive on nutrients digestibility. Significantly higher faecal digestibility was analysed in crude protein and organic matter.

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