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The effects of dried grape pomace supplementation on biochemical blood serum indicators and digestibility of nutrients in horses

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Abstract: Twelve adult Slovak warmblood sport horses were used to study the effect of dried grape pomace (DGP) on health through blood serum biochemical indicators, and on apparent total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF). The digestibility analysis was carried out by two *in vivo* methods, total faeces collection (TFC) and using lignin as a marker (ADL). Animals were divided into 3 groups: control group (C, without supplementation), experimental group 1 (E1, feed rations + 200 g of DGP) and experimental group 2 (E2, feed rations + 400 g of DGP). In animals, no health problems were detected during the trial. Of the blood serum indicators, only the concentrations of potassium (increase in E2 group compared to C group) and alanine aminotransferase (decrease in E2 group in comparison with E1 and C group) were affected ($P < 0.05$). The ADL method resulted in underestimated digestibility coefficients due to low recovery rates of lignin (less than 90%) in C group and E1 group. According to TFC, in E1 group higher digestibility coefficients were detected for DM, OM and CP ($P > 0.05$) compared to C group. However, in E2 group lower digestibility of all the studied nutrients was found ($P > 0.05$) in comparison with C group and E1 group. These results suggest that DGP could be used in horse diets up to 200 g without negative effect on their health and for a possible digestibility improvement of some nutrients.

Keywords: equine; metabolic tests; nutrition; utilization; wine by-products

Winemaking produces millions of tons of by-products annually, mainly grape pomace and stalks (Makris et al. 2007; Dominguez et al. 2016). These are not currently considered to be profitable and are most commonly used as fertilizer, left on open spaces or burned, which can lead to environmental problems (Rondeau et al. 2013; Bekhit et al. 2016). With respect to animal feed, the nutritional value and the digestibility of these by-products are generally low due to high fibre content. However,

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because of the growing interest in environmentally friendly waste-free technologies and minimising the cost of processing of by-products from the wine industry, innovative procedures are being introduced (Brenes et al. 2016; Galik et al. 2018). Many studies have shown that grape pomace can be used as a substantial source of certain nutrients (e.g. linoleic acid and/or crude protein) and biologically active compounds (polyphenols with antioxidant activity) in livestock nutrition (Viveros et al. 2011; Nistor et al. 2014; Teixeira et al. 2014; Chamorro et al. 2015, Domingues et al. 2016; Kerasioti et al. 2017; Chedea et al. 2018). Grape pomace bioactive substances seem to be a promising feed ration component because of their positive effect on growth performance, total apparent digestibility and potentially better nutrient utilisation from feed rations without negative effects on animal health (Galik et al. 2019). As Somogyvari et al. (2018) and Prochniak et al. (2019) reported, sport horses should be used as an optimal representative model in digestibility trials with horses. Currently, there is only limited information about feeding grape pomace to horses. Therefore, the aim of the present study was to investigate the effects of dietary inclusion of dried grape pomace on biochemical blood serum indicators and digestibility of nutrients in horses.

MATERIAL AND METHODS

Material. Grape (*Vitis vinifera* variety *Pinot gris*) pomace was obtained from the Experimental Farm of the Slovak University of Agriculture located in Kolinany, Oponice farm, Slovakia (48° 28' N, 18° 8' E). The pomace, as a by-product of juice pressing in the wine industry, mainly contained grape skin and seeds, residuals of grape pulp and fragments of grape stalks. To avoid degradation and to increase aerobic stability and shelf life, the grape pomace was pre-dried at 55 ± 5 °C and stored in paper bags until the beginning of the experiment.

Animals and management. The experiment was performed in cooperation with the Riding Centre of the Department of Animal Husbandry (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra) during April–May 2018 and the experimental period lasted 30 days. Twelve clinically healthy, Slovak warmblood sport horses (*Equus caballus*) (six geldings and six mares) were used in the study. Animals were stabled in individual boxes (3 × 4 m, concrete bases + rubber mats, built-in feeder for concentrates and automatic waterer) without bedding. During the experiment horses were in medium-level sport exercise (daily work in afternoons from 14:00 h to 18:00 h, one day per week active rest in the paddock for 3 h). Horses were randomly divided into three groups, two mares and two geldings in each; control group (C group, average weight 580 ± 50 kg) and two experimental groups (E1 group, average weight 600 ± 20 kg and E2 group, average weight 600 ± 30 kg). Average age of the horses in each group was 8 ± 1 years. The experiment was performed in line with the ethical guidelines of the Slovak University of Agriculture in Nitra.

Feed rations were formulated individually according to daily requirements (NRC 2007) from crimped barley and oats (at a ratio of 1 : 1, 0.6 kg per 100 kg of body weight), meadow hay (1.5 kg per 100 kg of body weight), and supplemental feed mixture in muesli form (0.3 kg per 100 kg of body weight). In the experimental groups, the feed rations were enriched with 200 g (E1 group) and 400 g (E2 group) of dried grape pomace (DGP). Forage was fed twice a day (50% in the morning, 50% in the evening) from a feed table. The daily amount of concentrates was divided into three parts (25% in the morning, 25% at midday, 50% in the evening). DGP in the experimental groups was added to the evening portion of concentrates.

Blood sampling and biochemical analyses. Blood samples were taken from each animal at the beginning and at the end of the experiment by a qualified veterinarian from *vena jugularis* (Danko et al. 2011). Thereafter the samples were transported to the Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra. Subsequently, the samples were centrifuged at 3 000 rpm (1 006 *g*) for 20 min and the blood serum was obtained and stored at –20 °C until analyses were performed. Blood serum concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (Bili), cholesterol (Chol), glucose (GLU), triglycerides (TG), total protein (TP) and urea were measured using DiaSys (Diagnostic Systems GmbH, Germany) commercial kits and a Randox RX Monza semi-automated clinical chemistry analyser (Randox Laboratories,

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UK) (Kovacik et al. 2017). Sodium (Na), potassium (K), and chloride (Cl) ions were analysed using an EasyLite analyser (Medica, USA) provided with an ion-selective electrode (Kolesarova et al. 2008).

Chemical analysis. Nutrient composition of feeds and faeces was analysed in the Laboratory of Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra). Feed samples were taken three times during the experiment in accordance with Commission Regulation EC No. 152/2009. From the sampled portion an adequate amount of incremental samples was taken, then mixed to create an aggregate sample. A reduced sample as a representative part of the aggregate sample was prepared by the process of reduction. Part of the reduced sample was used as a final sample for laboratory analyses. For the purpose of identification of possible risky impacts in feeds, an indicative organoleptic assessment was carried out. After the organoleptic assessment samples were pre-dried at 55 ± 5 °C and milled by a laboratory mill to pass a 1-mm sieve. To evaluate the concentration of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF), standard laboratory methods and procedures were used (AOAC 2000; Commission Regulation EC No. 152/2009). Samples were analysed in duplicates. Nutrient composition of feeds used in the experiment is shown in Table 1.

Digestibility analysis. The faeces were collected during the last 5 days of the experiment. On the 1st, 3rd and 5th day the faeces produced within 24 h were collected from each animal individually in labelled plastic containers. After weighing and homogenising the daily faeces, average samples were taken from each animal which were then transported to the laboratory for processing and analysis of nutrient content and digestibility. Apparent digestibility of nutrients was evaluated by two *in vivo* methods: total faeces collection (TFC) and using acid detergent lignin (ADL) as an internal marker. Digestibility coefficient (D) of nutrients determined from TFC was calculated as follows:

$$D = [(\text{Feed intake} - \text{Faecal output})/\text{Feed intake}] \times 100$$

Digestibility coefficient of nutrients determined using ADL as the marker was calculated as follows:

$$D = 100 - [(\text{Indicator feed} \times \text{Nutrient faeces})/(\text{Indicator faeces} \times \text{Nutrient feed})] \times 100$$

Statistical analysis. The IBM SPSS Version 20.0 statistical package was used. To calculate basic statistical characteristics, determine the significance of differences and compare the results, the analysis of variance, one-way ANOVA, was performed at the level $P < 0.05$.

RESULTS AND DISCUSSION

No problems with feed intake were observed either in the control or in the DGP supplementation groups during the experiment. The inclusion of DGP had no effect on the feed intake of horses. These findings are similar to those in other animal studies where DGP or grape seed extract was fed (Davies et al. 2008; Ishida et al. 2015; Aditya et al. 2018).

At the beginning of the trial, blood samples were taken from all animals to monitor possible health problems (Table 2, Start of the trial). Parameters were in the reference intervals (Doubek et al. 2010). The effect of different doses of DGP on biochemical indicators of horse serum is shown in Table 2 (End of the trial). Although an increase or a decrease of some parameters was recorded, these were all in the reference range. Of the macrominerals, higher K concentration was detected

Table 1. Nutrient composition of feeds used in the experiment (values are means ± SD)

	DGP	Meadow hay	Barley : oat, 1 : 1	Müsli
DM (%)	92.8 ± 0.1	94.3 ± 0.0	93.0 ± 0.5	93.7 ± 0.1
OM (% DM)	88.9 ± 0.1	88.9 ± 0.1	89.7 ± 0.8	85.8 ± 0.5
CP (% DM)	9.4 ± 1.3	5.8 ± 1.0	10.3 ± 1.7	12.7 ± 0.7
ADF (% DM)	25.0 ± 0.9	35.0 ± 0.7	8.0 ± 1.6	11.1 ± 0.4
NDF (% DM	28.4 ± 1.3	55.9 ± 0.1	18.8 ± 1.2	21.8 ± 2.8

DM = dry matter; OM = organic matter; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; DGP = dried grape pomace

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Table 2. Effect of dried grape pomace on selected blood serum parameters (values are means ± SD)

	Unit	C group	E1 group	E2 group
Start of the experiment				
Total protein	g/L	73.4 ± 17.1	70.1 ± 3.9	77.3 ± 8.8
Glucose	mmol/L	3.8 ± 0.4	3.6 ± 0.2	3.4 ± 0.1
Triglycerides	mmol/L	0.4 ± 0.5	0.3 ± 0.1	0.2 ± 0.1
Cholesterol	mmol/L	2.0 ± 1.1	2.1 ± 0.4	2.7 ± 1.3
Urea	mmol/L	3.3 ± 0.4	3.3 ± 0.9	3.1 ± 1.9
Bilirubin	μmol/L	31.5 ± 9.7	32.1 ± 6.8	35.0 ± 7.9
AST	μkat/L	4.7 ± 0.9	4.2 ± 0.6	3.6 ± 0.3
ALT	μkat/L	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
ALP	μkat/L	1.7 ± 0.4	1.8 ± 0.9	1.6 ± 0.2
Calcium	mmol/L	3.6 ± 0.4	3.8 ± 0.7	3.6 ± 0.0
Phosphorus	mmol/L	0.3 ± 0.3	0.7 ± 0.1	0.4 ± 0.2
Magnesium	mmol/L	1.01 ± 0.25	1.02 ± 0.13	0.83 ± 0.04
Sodium	mmol/L	135.5 ± 3.0	137.8 ± 5.4	138.6 ± 2.4
Potassium	mmol/L	3.6 ± 0.5	3.7 ± 0.1	3.4 ± 0.2
Chloride	mmol/L	102.4 ± 0.9	102.8 ± 2.1	104.3 ± 2.0
End of the experiment				
Total protein	g/L	65.5 ± 7.7^{ab}	70.0 ± 1.9^{a}	66.6 ± 1.5^{b}
Glucose	mmol/L	3.5 ± 0.1	3.4 ± 0.5	3.4 ± 0.3
Triglycerides	mmol/L	0.5 ± 0.5	0.3 ± 0.1	0.3 ± 0.1
Cholesterol	mmol/L	1.5 ± 0.7	1.6 ± 0.4	1.6 ± 0.5
Urea	mmol/L	2.0 ± 0.4	2.9 ± 0.6	2.5 ± 0.6
Bilirubin	μmol/L	25.4 ± 9.0	34.0 ± 9.2	34.1 ± 14.2
AST	μkat/L	4.4 ± 0.5	4.3 ± 0.5	3.9 ± 0.4
ALT	μkat/L	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}
ALP	μkat/L	1.3 ± 0.2	1.4 ± 0.5	1.3 ± 0.5
Calcium	mmol/L	2.8 ± 0.3	2.6 ± 0.2	2.7 ± 0.1
Phosphorus	mmol/L	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.3
Magnesium	mmol/L	1.2 ± 0.5	1.2 ± 0.3	1.3 ± 0.2
Sodium	mmol/L	134.8 ± 2.2	134.8 ± 3.4	133.9 ± 1.0
Potassium	mmol/L	3.9 ± 0.3^{a}	4.2 ± 0.5^{ab}	4.4 ± 0.2^{b}
Chloride	mmol/L	102.4 ± 1.8	104.7 ± 1.9	103.4 ± 1.4

AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

^{a,b}values followed by different superscripts differ within a row at $P < 0.05$

in E2 group compared to C group ($P < 0.05$). An increasing tendency ($P > 0.05$) was also observed for Mg and Cl. The serum concentration of Ca in experimental groups was lower ($P > 0.05$), while P and Na concentrations were not practically affected by the DGP consumption. Most of the parameters of enzymatic, energy and protein profile of the animals was not significantly ($P > 0.05$) affected by the addition of DGP to their feed rations, except for ALT where the dose of 400 g of DGP significantly ($P < 0.05$) lowered the activity of this enzyme compared to C group. A tendency ($P < 0.1$) of higher BILI, CHOL, TP and UREA concentrations was analysed as the effect of DGP consumption. In contrast, in experimental groups a decreasing tendency ($P < 0.1$) of AST, GLU and TAG was found.

There are several methods for determining the apparent digestibility of feeds and feed rations in horses (Strakova and Suchy 2013). Total collection

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of faeces is considered to be the most accurate of them (Bergero et al. 2009). Digestibility coefficients obtained by TFC (Table 3) in E1 group compared to C group (feed rations enriched with 200 g of DGP) indicate a tendency ($P < 0.1$) of higher digestibility of DM, OM and CP ($P > 0.05$). The reason for these results could be explained by the presence of polyphenolic compounds in grape pomace, which at the optimal level could improve nutrient digestibility (Makkar 2003) by modifying the gut morphology and intestinal microflora (Viveros et al. 2011). However, in E2 group (feed rations and 400 g of DGP) the digestibility coefficients, compared to C group and E1 group, were lower ($P > 0.05$). This undesirable digestibility decrease could be related to a higher amount of polyphenols in feed rations, especially tannins, which are known to form indigestible tannin complexes with nutrients (Baumgartel et al. 2007; Nistor et al. 2014). The tendency of higher digestibility of some nutrients shows that DGP in the amount of 200 g could be used as a feed ingredient for horses. According to Galik et al. (2019), from a nutritional point of view the effect of grape pomace is mainly affected by the amount of daily intake. Therefore, further studies are needed to determine the optimum feeding amount of DGP and the mechanism underlying its action in nutrient digestibility for adult horses as an animal model.

Table 3. Effect of dried grape pomace on apparent digestibility of nutrients (%) determined by total faecal collection and acid detergent lignin methods (values are means ± SD)

	Method	C group	E1 group	E2 group
DM	TFC	71.4 ± 10.0	72.7 ± 4.4[a]	67.4 ± 8.9
	ADL	67.4 ± 2.5	63.8 ± 0.9[b]	66.6 ± 12.5
OM	TFC	72.7 ± 9.2	73.8 ± 4.6[a]	69.0 ± 7.9
	ADL	68.8 ± 2.4	65.2 ± 0.8[b]	68.0 ± 12.6
CP	TFC	72.7 ± 8.6	73.4 ± 5.4	71.6 ± 7.0
	ADL	68.6 ± 3.6	64.7 ± 3.9	70.4 ± 12.3
ADF	TFC	56.9 ± 14.3	56.2 ± 7.5[a]	43.3 ± 14.3
	ADL	50.6 ± 5.0	41.9 ± 1.3[b]	41.8 ± 21.0
NDF	TFC	61.7 ± 12.7	61.6 ± 7[a]	53.7 ± 11.8
	ADL	56.1 ± 4.7	49.1 ± 1.0[b]	52.2 ± 18.8

DM = dry matter; OM = organic matter; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; TFC = total feacal collection; ADL = acid detergent lignin
[a,b]difference between TFC and ADL methods at $P < 0.05$

Indigestible markers have found a widespread use in animal nutrition studies due to less time-consuming and laborious experiments compared to conventional direct digestibility methods (Sales 2012). Digestibility coefficients obtained by ADL method (Table 3) were lower in comparison with those found by TFC. Moreover, low recovery rates of lignin were detected in C group (89.13%), as well as in E1 group (75.44%). Using lignin as an internal marker in digestibility trials with horses has received criticism from the scientific community. Many authors reported the failure of this method because of low recovery rates of lignin in faeces and consequent great underestimation of digestibility coefficients (Chachulowa et al. 1994; Miraglia et al. 1999; Araujo et al. 2000; Oliveira et al. 2003; Goachet et al. 2009; Siqueira et al. 2009; Arbabi et al. 2016). Digestion of a fraction of lignin by microbial activity, source of plant lignin and content of lignin in the diet might be the factors responsible for incomplete faecal recoveries (Sales 2012). Assuming the possibility of partial digestibility of lignin, Oliveira et al. (2012) suggested using only the indigestible fraction of lignin as a marker in digestibility studies. Peiretti et al. (2003) and Bergero et al. (2005) found out that a higher level of ADL in feed rations can lead to more precise determination when studying digestibility of nutrients by using lignin as a marker in horses. This is consistent with results detected for E2 group, where the recovery rate of lignin in faeces was the highest (108.07%) and the differences in digestibility between the ADL and TFC methods were less pronounced. It is likely that the higher amount of DGP increased the ADL content of the horse feed rations in E2 group and thus increased the reliability of the use of lignin as an internal marker.

Currently, only few references in the literature regarding the use of grape by-products in horse diets are available (Davies et al. 2008) and information from other animal studies is limited, while found results are inconsistent. Digestibility improvement of CP, ADF and NDF due to the grape pomace powder inclusion was reported by Foiklang et al. (2016) in dairy steers. Previously Foiklang et al. (2015) found that supplementation of grape pomace powder at 2% of feed increased *in vitro* true digestibility. According to Bahrami et al. (2010) digestibility of DM, OM, CP and NDF was improved by increasing DGP content with

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the highest values observed for the inclusion of 10% DGP in diet for fattening male lambs. On the contrary, Ozduven et al. (2005), Baumgartel et al. (2007), Zalikarenab et al. (2007), and Ishida et al. (2015) reported the exactly opposite results for sheep. Decreases in digestibility of DM, ADF and NDF were also observed by Vinyard and Chibisa (2019) as an effect of feeding grape pomace in finishing cattle. Aditya et al. (2018) found that the apparent total tract digestibility of nutrients in broilers was not affected by the inclusion of grape pomace to their diets, but Lichovnikova et al. (2015) stated that grape pomace increased the apparent ileal digestibility of several amino acids in broilers.

CONCLUSION

Based on the results of this study, supplementation of DGP up to 400 g did not affect the feed intake of horses. Neither health nor metabolic problems were observed during the trial. According to TFC, the addition of 200 g of DGP to feed rations for horses showed a positive trend of nutrient digestibility, while 400 g of DGP affected their digestibility negatively. However, to confirm the indicated positive trend of digestibility, further experiments with additional levels of DGP in horses are needed. The digestibility coefficients obtained by ADL in C group and E1 group came out as unreliable due to low lignin recovery rates. This method appears to be more efficient in the case of a higher amount of indicator in feed rations. In this regard, other methods of determining nutrient digestibility should be applied. Overall, it can be concluded that the use of DGP as a feed component in horse nutrition is promising, but further research is required.

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