

Grape pomace in equine nutrition: effect on antioxidant status

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Grape pomace is a bioactive compound rich winery by-product having antioxidant properties. However, its use in equine nutrition in this regard have been unexploited to date. Thus, this study aimed to investigate whether dried grape pomace (DGP) could enhance the antioxidant mechanisms of horses. Redox status was assessed through glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in blood serum, and ferric reducing ability of plasma (FRAP). Twelve horses were assigned to three groups receiving a basal diet (control group) or the basal diet supplemented with 200 g of DGP (experimental group 1), or 400 g of DGP (experimental group 2) for 30 days. Dietary DGP supplementation of horses at level of 200 g positively affected their redox status through increased FRAP ($P < 0.05$). However, no changes in the activity of enzymes GPx and SOD were detected neither at the level of 200 g nor 400 g of DGP. Based on the presented results, further research is required to test other levels of DGP in horse diets and its potential to affect the redox status of these animals.

Keywords: grape pomace, horses, SOD, GPx, FRAP

1 Introduction

Oxidative stress arises from the disproportion in the concentration of antioxidants and free radicals in the body (Hellwig, 2019). Biological systems are protected by endogenous enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (Niedźwiedz et al., 2017). If these are not sufficient to fight free radicals, their deficiency can be compensated by substances from exogenous sources (Santos-Sánchez et al., 2019). Recently, the interest in using natural biological compounds as antioxidants in animal feed is rising (Wang et al., 2019; Hosseini-Vashan et al., 2020; Gungor et al., 2021). These include polyphenols, of which flavonoids, in particular, excel in their remarkable ability to inactivate highly reactive forms of molecules (Lipiński et al., 2017).

Several papers have been published dealing with the antioxidant properties of by-products of wine production rich in polyphenols (Balea et al., 2018; Luchian et al., 2019; Mohamed Ahmed et al., 2020). They prevent and reduce oxidative damage of biological macromolecules caused by free radicals (Makri et al., 2017; Moldovan et al., 2019). Furthermore, they increase the levels of endogenous antioxidants, which leads to enhanced immune system of the animals (Brenes et al., 2016; Oliveira et al., 2017; Kafantaris et al., 2018).

The effect of feeding grape pomace and grape pomace extracts on the antioxidant status was evaluated in poultry (Chamorro et al., 2017; Ebrahimzadeh et al., 2018), pigs (Chedea et al., 2019; Wang et al., 2019) and ewes (Buffa et al., 2020). However, the use of grape pomace in equine nutrition have been unexploited to

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date. In this regard the aim of the present study was to determine the effects of dried grape pomace (DGP) administration in two levels on redox status of sport horses.

2 Material and methods

Twelve horses of Slovak warmblood breed were divided into 3 groups (4 individuals per group). Animals were fed a basal diet (control group), or the basal diet enriched with two different levels of dried grape pomace (*Vitis vinifera*, variety *Pinot gris*) for 30 days. Individually formulated feed rations followed the daily requirement of nutrients (NRC, 2007) and consisted of barley and oat in crimped form (ratio 1 : 1), meadow hay and texturised feedstuff. Table 1 refers to the composition of feed rations in each group and the feeding regime previously published by Kolláthova et al. (2020). The amount of basic nutrients of feeds used in the trial is shown in Table 2.

DGP was further subjected to additional analyzes (Table 3). The determination of antioxidant capacity and total content of polyphenols followed up the methodologies of Sánchez-Moreno et al. (1998) and Singleton, Rossi (1965) previously described in Kolláthová et al. (2019). The evaluation of the content of individual polyphenolic compounds was carried out by liquid chromatography. These analysis were performed in cooperation with the Institute of Food Sciences, Faculty of Biotechnology and Food Sciences and Research Centre of AgroBioTech, Slovak University of Agriculture in Nitra.

At the end of the trial blood samples were collected from *vena jugularis*. The analysis of blood samples

took place in collaboration with the Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra. The activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD) was determined according to the methodologies of Tvrdá et al. (2016) and Kováčik et al. (2019) using the Randox commercial kits (Randox Laboratories, Crumlin, Great Britain) and the semi-automated analyzer Randox RX Monza (Randox Laboratories, Crumlin, UK). The ferric reducing ability of plasma (FRAP) followed the original procedure described by Benzie and Strain (1996).

Statistical analysis was performed using IBM SPSS v. 20.0 software. One-way ANOVA was performed to determine the impact of the feeding group on antioxidant status. The Tukey post-hoc test was conducted to check if differences between feeding groups were statistically significant.

Table 3 Antioxidant capacity and polyphenolic content of dried grape pomace

Antioxidant activity (mg TEAC g ⁻¹)	9.06 ± 0.13
Total polyphenols (mg GAE g ⁻¹)	42.69 ± 1.66
Gallic acid (mg kg ⁻¹ of DM)	101.23 ± 0.18
Catechin (mg kg ⁻¹ of DM)	2,280.39 ± 12.14
Epicatechin (mg kg ⁻¹ of DM)	1162.84 ± 59.64
Rutin (mg kg ⁻¹ of DM)	51.37 ± 1.42
Resveratrol (mg kg ⁻¹ of DM)	20.25 ± 9.28

TEAC – trolox equivalent, GAE – gallic acid equivalent, DM – dry matter

Table 1 Ingredients composition of the basal and experimental diets of horses

	Control group	Experimental group 1	Experimental group 2
Meadow hay (kg 100 kg ⁻¹ of body weight)	1.5	1.5	1.5
Barley : oat (1 : 1) (kg 100 kg ⁻¹ of body weight)	0.6	0.6	0.6
Texturized feedstuff (kg 100 kg ⁻¹ of body weight)	0.3	0.3	0.3
Dried grape pomace (kg)	0	0.2	0.4

Meadow hay fed 2 times per day. Concentrates fed 3 times per day. Dried grape pomace fed ones per day with the evening portion of concentrates

Table 2 Nutrient levels of the feeds used in the trial

	Dried grape pomace	Meadow hay	Barley : oat 1 : 1	Texturised feedstuff
Dry matter (%)	92.8	94.3	92.3	93.7
Organic matter (% of dry matter)	88.9	88.9	89.7	85.8
Crude protein (% of dry matter)	9.42	5.8	10.3	12.7
Crude fat (% of dry matter)	9.6	1.3	2.2	3.6
Crude fiber (% of dry matter)	15.4	32.3	7.4	9.2
Ash (% of dry matter)	3.8	5.4	3.2	7.9

3 Results and discussion

An increased FRAP was observed in groups E1 and E2, with significantly higher ($P < 0.05$) values being found in group E1 in comparison with the control group (Table 4). Higher blood serum antioxidant activity through increased FRAP was also detected by Buffa et al. (2020) in dairy ewes supplemented with grape marc in an amount of 100 g per day. This fact is probably related to the higher content of polyphenols, especially catechins, in the feed rations of horses in experimental groups. The antioxidant ability of these compounds has been confirmed by several authors (Kalli et al., 2018; Olejar et al., 2019; Yammine et al., 2020). The activity of SOD and GPx was not affected by DGP consumption. Similar results for SOD were reported by Kerasioti et al. (2017) in sheep fed grape pomace silage.

To the date, little information is available about feeding winery by-products to horses. However, studies with other animal species can provide a good overview of how feeding GP effects their antioxidant status, although found results may be inconsistent. The inconsistency may be particularly due to differences in the digestive tract of ruminants and non-ruminants based on fermentation in the rumen (Ishida et al., 2015).

Yang et al. (2017) found an elevated activity of SOD in broilers blood serum when administering proanthocyanidin extract from grapes. GP enhanced the total antioxidant status (TAS) of broilers through raised serum GPx and SOD levels (Ebrahimzadeh et al., 2018, Hosseini-Vashan et al., 2020, Gungor et al., 2021). The dietary inclusion of GP increased the TAS of plasma and serum in chickens (Brenes et al., 2008; Chamorro et al., 2017; Makri et al., 2017). Supplementation with polyphenols from red GP increased the antioxidant mechanisms of growing lambs (Kafantaris et al., 2017). Wang et al. (2019) detected better redox status in multiparous sows during late gestation and lactation after dietary grape seed polyphenols administration (increased activity of SOD and GPx). The GPx and SOD activities reflect the efficiency of the first-line antioxidant defense system of cells and tissues against the damaging effects of free-radicals.

Hence, increased level of these enzymes in blood or tissues is desirable (Surai, 2016). Hao et al. (2015) and Fan et al. (2015) detected significantly increased antioxidant capacity of serum in weaned pigs and sows after grape seed procyanidins and catechins supplementation. An elevated antioxidant activity was found in the liver, kidney and spleen (Chedea et al., 2019), as well as an enhancement in the overall redox status (Kafantaris et al., 2018) of pigs after GP supplementation. According Alía et al. (2013) the increased TAS in is a result of intestinal absorption of polyphenolic compounds and their reaching the target organs and plasma. However, Goñi et al. (2007), Brenes et al. (2008), and Pascariu et al. (2017) reported no change in TAS in the serum of broilers fed GP. Ishida et al. (2015) found no effect of supplementary GP of TAS in wethers. When a low level of antioxidants is offered, their impact on the TAS, in comparison with endogenous antioxidative defenses, is ommissible (Gladine et al., 2007). In this regard the improvement of TAS may be limited by amount of GP in feed rations.

4 Conclusions

Based on results of the current study, dried grape pomace supplementation can potentially improve the antioxidant status of horses, but more experiments need to be carried out. The mechanisms behind the effect of biologically active substances from grape pomace on the redox status of horses needs to be clarified. The proper level of dietary grape pomace in equine diets also needs to be determined.

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Table 4 Effect of different levels of dried grape pomace on antioxidant mechanisms of horses

	Control group	Experimental group 1	Experimental group 2
	mean \pm standard deviation		
FRAP ($\mu\text{M Fe}^{2+}$)	258.0 \pm 39.6 ^a	329.0 \pm 32.5 ^b	276.2 \pm 70.0 ^{ab}
SOD (U g ⁻¹ TP)	46.6 \pm 11.2	42.5 \pm 1.5	45.6 \pm 6.6
GPx (U g ⁻¹ TP)	20.5 \pm 10.3	18.9 \pm 6.5	16.0 \pm 1.8

FRAP – ferric reducing ability of plasma; SOD – superoxide dismutase; GPx – glutathione peroxidase; TP – total protein

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