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Effect of dietary grape pomace on fats digestibility in horses

Renata Kolláthová[1], Branislav Gálik[1*], Marko Halo[2], Miroslav Juráček[1], Ondrej Hanušovský[1], Daniel Bíro[1], Michal Rolinec[1], Milan Šimko[1]

[1] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Animal Nutrition, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

[2] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Animal Husbandry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

The present study aimed to analyse dried grape pomace as a possible source of crude fat and polyunsaturated fatty acids in equine nutrition, as well as its effect on apparent digestibility of crude fat and selected fatty acids. Twelve clinically healthy sport horses were used in the feeding trial (Slovak warm blood breed). Animals were divided into three groups; control group (without supplementation) and two experimental groups where diets were enriched by 200 g and 400 g of dried grape pomace, respectively. Digestibility analysis was carried out by total faeces collection method. Crude fat of feeds and faeces, extracted by Soxhlet-Henkel method, was subsequently subjected to fatty acid profile analysis by gas chromatography. Grape pomace contained 96.17 $g.kg^{-1}$ of crude fat with linoleic (70.03% in fat) and oleic (15.86% in fat) as the most abundant fatty acids. An indication ($P>0.05$) of higher digestibility of crude fat and oleic acid in both experimental groups, in comparison with control group, was detected. The digestibility of palmitic, linoleic, α-linolenic and cis-11-eicosenoic acids was not affected by dried grape pomace consumption ($P>0.05$). Based on the results of this experiment, dried grape pomace had no significant effect neither on digestibility of crude fat nor on the selected fatty acids. However, this winery by-product could be used as an alternative source of crude fat in equine diets.

Keywords: crude fat, equine, polyunsaturated fatty acid utilisation, wine by-products

1 Introduction

Dietary fats in mammals play an important role as carriers of fat-soluble vitamins and essential polyunsaturated fatty acids (PUFA) (NRC, 2007). It is well known that PUFA are necessary for the normal course of a wide range of biological functions in animals (Piccione et al., 2014a). The biologically most important essential fatty acids from the omega-3 and omega-6 series are associated with a number of health benefits, in connection with a wide range of physiological and pathological conditions (Piccione et al., 2014b, Ross-Jones et al., 2014). These fatty acids are irreplaceable in the physiological processes, but their endogenous synthesis is limited by the lack of suitable enzymes (Hess et al., 2014). Forage, which constitutes the largest portion of traditional equine diets, is a good source of omega-3, whereas omega-6 fatty acids are very abundant in grains (Kentucky Equine Research, 2016). Higher energy requirements of performance horses are often covered by high-fat commercial concentrates usually containing vegetable oils or dry ingredients containing high levels of fat such as rice bran or flaxseed (Vineyard et al., 2010). In these terms, grape pomace could be used in equine diets as a novel source of fats and omega-6 fatty acids, particularly linoleic acid. In addition, grape pomace is also rich in biologically active substances with antioxidant properties (Georgiev et al., 2014, Lichovnikova et al., 2015) that may contribute to an overall improvement of animal health (Brenes et al., 2016). According to Aslanian et al. (2011) and Hanganu et al. (2012), grape pomace is relatively rich in fat content (5-11%), which is mainly contained in grape seeds (Mironeasa et al., 2016). Therefore, this study aims to evaluate dried grape pomace (DGP) as a possible source of fat for horses and its effects on the apparent digestibility of fat selected fatty acids.

* **Corresponding Author:** Branislav Gálik. Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Animal Nutrition, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia. E-mail: branislav.galik@uniag.sk

2 Material and methods

The experiment took place in cooperation with The Riding Centre of the Department of Animal Husbandry (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra) and lasted 30 days. In the study, 12 individually stabled sport horses were used (Slovak warm blood, six geldings and six mares, average age 9 ± 4 years, average body weight (BW) of 577 ± 34 kg, medium level of exercise). The horses were divided randomly into 3 groups, each comprised of two mares and two geldings: control group (C) and 2 experimental groups (E1 and E2).

Feed rations, formulated individually depending on the estimated daily requirement of nutrients (NRC, 2007), consisted of crimped barley and oat (at a ratio of 1:1, 0.6 kg per 100 kg of BW), meadow hay (1.5 kg per 100 kg of BW), and texturised feedstuff (0.3 kg per 100 kg of BW). Meadow hay was harvested at the beginning of flowering and dried naturally. The texturised feedstuff was composed of wheat bran, soybean meal, hot-air dried alfalfa, corn, extruded flaxseed, sunflower oil, vitamin and mineral premix. Forage was fed in the morning (50%) and in the evening (50%). The daily amount of concentrates was divided in 3 meals (25% in the morning and at midday, and 50% in the evening). The DGP (*Vitis vinifera* variety Pinot gris) was supplemented in the evening portion of concentrates supplied to E1 and E2 at the doses of 200 g and 400 g/horse/day, respectively. The feed intake was measured individually. No feed refusals or leftovers were detected throughout the experiment.

Representative samples of feeds and faeces were transferred to 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) and processed according to standardised laboratory principles (EC No 152/2009). Feed samples were taken three times during the experiment. The crude fat (CF) content was determined by the Soxhlet-Henkel extraction method using Soxtec apparatus (TECATOR) and weighing the dried petroleum ether extract. The fatty acid composition of analysed samples was carried out by gas chromatography on Agilent 6890A GC machine (Agilent Technologies, USA). The chemical composition and selected fatty acids content in feeds used in the experiment is reported in Table 1.

Table 1 Chemical composition of feeds used in the experiment

	Unit	DGP	Meadow hay	Barley:oat 1:1	Texturised feedstuff
		Mean±Standard Deviation			
Dry matter	$g.kg^{-1}$	927.50±0.14	943.00±0.00	929.55±0.49	937.38±0.07
Organic matter	$g.kg^{-1}$ DM	889.30±0.14	889.25±0.07	897.10±0.85	857.68±0.49
Crude protein		94.20±1.27	57.90±0.99	103.00±1.70	126.95±0.68
Crude fiber		154.35±2.76	322.75±0.92	74.25±0.64	90.23±1.77
NFE		551.55±1.91	496.80±0.42	719.85±0.21	640.50±1.13
Ash		38.20±0.00	53.75±0.07	32.45±0.35	79.45±0.14
Crude fat		96.17±0.59	12.52±0.60	21.74±0.77	35.80±0.71
Palmitic acid	% in fat	7.92±0.03	16.92±0.99	20.60±0.13	13.39±0.42
Oleic acid		15.86±0.01	7.11±0.02	19.85±0.19	24.29±0.60
Linoleic acid		70.03±0.04	15.77±0.84	49.97±0.25	48.18±0.42
α-linolenic acid		0.56±0.00	13.37±1.39	4.60±0.06	6.27±0.26
cis-11-eicosenoic acid		0.19±0.00	0.00±0.00	1.02±0.00	0.66±0.20

DGP - dried grape pomace, NFE - nitrogen free extract

The average intake of fat and selected fatty acids per group is reported in Table 2. The amount of fat in the feed rations was calculated by multiplying the intake of each feed by its fat content. The amount of fatty acids ingested was calculated on the basis of their percentage of the total fat content of each feed.

Table 2 Calculated average intake of fat and fatty acids of the feeding groups (g)

	C group	E1 group	E2 group
Crude fat	210	234.75	252.59
Palmitic acid	35.54	38.34	39.75
Oleic acid	31.86	34.31	37.14
Linoleic acid	70.7	85.67	98.16
α-linolenic acid	19.14	22.06	22.15
cis-11-eicosenoic acid	0.94	0.91	0.94

C group – control group, E1 group – group supplemented with 200 g DGP/horse/day, E2 group – group supplemented with 400 g DGP/horse/day, DGP – dried grape pomace

Concerning the digestibility analysis, the total collection of the daily amount of faeces produced within 24h was carried out individually from each animal during 3 sampling days (3 days per each horse). After weighing and homogenising the daily faeces, average samples were taken from each animal. Apparent digestibility of CF and fatty acids was determined by total faeces collection method (TFC). Digestibility coefficient (D) of nutrients determined from TFC was calculated according to the formula:

$$\% D_{TFC} = (\text{Nutrient Intake} - \text{Faecal Excretion}) / \text{Nutrient Intake} \times 100.$$

Statistical analysis was conducted using IBM SPSS v. 20.0 software. One-way ANOVA was performed to investigate the impact of the feeding group (C, E1, E2) on apparent digestibility of CF and selected fat acids. The Tukey post-hoc test was conducted to check if differences between feeding groups were statistically significant.

3 Results and discussion

The DGP used in the experiment (Table 1) was characterized by higher CF content than that reported by Azevêdo et al. (2012) and Gülcü et al. (2019). The predominance of linoleic acid is in line with that reported in literature (Hussein and Abdrabba, 2015, Ribeiro et al., 2015). In comparison with Guerra-Rivas et al. (2016) lower amounts of all the fatty acids were detected for grape pomaces. On the other hand, Gülcü et al. (2019) measured higher content of the same fatty acids, except for linoleic acid. Russo et al. (2017) studied the fatty acid profile of six grape pomaces with very similar results to those reported in the present study.

In equine nutrition, the relation between hematologic and clotting parameters and dietary PUFA was investigated by Piccione et al. (2014b) and Ross-Jones et al. (2014). The positive effect of PUFA supplementation on athletic horses' oxidative metabolism was confirmed by Hinchcliff et al. (2013). This can be explained by the increased metabolisation and oxidation of fatty acids in muscles, which in turn leads to high glycogen content and its better utilization within the tissue during performance (Burke, 2009). Moreover, in terms of joint health, the long chain derivates of these fatty acids, known as eicosanoids, play an important role during inflammatory events (Hess et al., 2014).
The effect of DGP on apparent digestibility of CF and selected fatty acids is reported in Table 3. An indication ($P>0.05$) for an increased digestibility of CF in both experimental groups, in comparison with C group, was detected. The same result ($P>0.05$) was also observed for oleic acid digestibility. The supplementation with DGP did not affect the utilisation of palmitic and linoleic acids. The *α*-linolenic and cis-11-eicosenoic acids were not recovered in faeces, likely due to the low concentration observed in the feeds. Therefore, they can be considered as completely digested in the C group, as well as in both experimental groups. According to Gálik et al. (2019) the effect of grape pomace is mainly affected by the daily intake. However, in this experiment, the level of DGP inclusion in horse diets had no effect on apparent digestibility of the studied nutrients. Currently, only few references in the literature regarding the use of grape by-products in horse diets are available and information from other animal studies is limited. Davies et al. (2009) recorded a positive effect on fermentation in hindgut of Thoroughbred race horses when 150 mg of grape seed extract/kg BW was fed. Kolláthová et al. (2020) reported an indication of better dry matter, organic matter and crude protein digestibility in sport horses fed 200 g of DGP; however, 400 g of DGP in horse diets affected the utilisation of nutrients negatively. An improved utilisation of some nutrients was detected also in studies with dairy steers fed grape pomace powder at 2% of dry matter intake (Foiklang et al., 2016), and broilers fed

1.5% red grape pomace powder (Lichovnikova et al., 2015). Viveros et al. (2011) suggested that these results could be due to polyphenolic compounds in grape pomace and their effect on gut morphology and intestinal microflora. The mechanism underlying the action of DGP in nutrients digestibility is still not completely clear. Therefore, further studies are needed to determine the optimum feeding concentration of DGP and its effect on nutrient digestibility in horses.

Table 3 Apparent digestibility of crude fat and selected fatty acids related to dried grape pomace administration (%)

	C group	E1 group	E2 group	Feeding effect
	Mean±Standard Deviation			P-value
Crude fat	65.83±10.56	68.11±6.63	68.24±7.90	0.90
Palmitic acid	77.71±7.35	77.48±5.07	77.54±7.09	0.94
Oleic acid	89.40±6.17	92.45±1.80	90.34±4.27	0.76
Linoleic acid	96.13±1.41	96.69±1.20	96.00±1.11	0.48
α-linolenic acid	100.00±0.00	100.00±0.00	100.00±0.00	-
cis-11-eicosenoic acid	100.00±0.00	100.00±0.00	100.00±0.00	-

C group – control group, E1 group – group supplemented with 200 g DGP/horse/day, E2 group - group supplemented with 400 g DGP/horse/day, DGP – dried grape pomace

4 Conclusions

Due to its fat content and quality, grape pomace could be used as an alternative source of fatty acids in horse diets. Feeding grape pomace to horses had no significant impact either on digestibility of CF or selected fatty acids in both levels of inclusion. However, a suggestion of improved CF and oleic acid utilisation was detected. In the future additional research is required to determine the optimal dose of grape pomace in horse feed rations and confirm the indicated trends.

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