GRAPE BY-PRODUCTS

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Prof. Ing. Miloslav Šoch, CSc., dr.h.c.
(University of South Bohemia in České Budějovice, Czech Republic)

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• CONTENT

• ABSTRACT

The agro-food industry generates a large production of by-products. Many by-products have interesting nutritive value and potential to be used in animal nutrition as feed ingredients. In grape processing, about 30% of the production is made of by-products, mainly grape pomace (pulps) and stems. From an environmental point of view, it seems vital to make use of these by-products as sources of nutrients and different bioactive substances in internal nutrition and feeding. The nutritive value of these products depends on different factors, such as the original composition of nutrients and processing technology. Grape pomace is rich in crude protein content, crude fat, crude fibre, as well as ADF and NDF. Regarding mineral composition, grape pomace is rich in Ca, P, Mg, K and Fe content. The quality of fat depends on the fatty acid profile. The content of polyunsaturated fatty acids is up to 70% and the content of α-linolenic acid is high. Grape by-products are also rich in many bioactive compounds, particularly grape pomace. Grape by-products are sources of resveratrol, quercetin, catechin, epicatechin etc.. Published studies reported the positive effect of nutritional supplementation of feed rations with grape pomace on the digestibility of nutrients, mainly crude proteins, and crude fat, and without a negative effect on haematological and biochemical indicators. Dietary grape pomaces intake can positively affect cow colostrum nutrients composition and increase crude protein content; however, it can decrease fat content. In pig nutrition, it is recommended to use grape pomace in fattening. In sow nutrition, there can be a negative effect of some haematological indicators on newborns. A limited ration of grape pomace in horse nutrition can increase the digestibility of nutrients, dry matter, organic matter, crude fibre and fibre fractions. It is important to note that grape pomace also contains some antinutritive factors with a possible negative effect on digestion. However, in geese fattening, grape pomace reduces the body weight at the end of the fattening process and can also increase carcass yield and the content of polyunsaturated fatty acids in the crude fat. Conservation offers various possibilities for drying and silage making of grape pomace. For ensiling, the positive effect of urea as an additive is typical in relation to the protection of the content of crude proteins and fermentation stimulation. However, grape pomaces in particular have the potential to be used as feed ingredients in livestock nutrition and feeding. They are rich in different nutrients, minerals, and bioactive compounds. Additional research is needed to identify the maximum dietary intake and clarify the technological processing prior to the actual use.

Keywords: livestock, by-products, nutrition, performance, quality of the products

• INTRODUCTION

Animal performance is affected by many different factors, both internal and external. One of the major external factors is nutrition and feeding. In the past, the primary role of the feed industry was to produce large amounts of feed for livestock population, such as cattle, sheep, goats, swine, and poultry. One of the important reasons why livestock populations around the world have decreased in the last 20 years is the increase in their productivity. For the same amounts of animal-based products, less livestock population is now needed. Moreover, feed production at present (i.e., forages and concentrated feeds) faces a new challenge: it should be both sustainable and effective.

In contemporary animal nutrition and feeding, great emphasis is placed on economic effectivity and environmental concerns, i.e., it is to be effective both in terms of the production and environmental costs. Nowadays, the focus has shifted to circular economy, climate change, emissions, low-carbon portfolio, and the issues related to the carbon footprint. Indeed, these concerns are also topical in the field of animal production, animal nutrition, and feeding. Circular economy in this field presupposes the use of different by-products generated by the agro-food industry and by-products from the industrial use of agricultural plants. The goal is to use as many by-products as possible, decrease the production of waste and thus eliminate environmental costs. Many by-products from the industry have a good potential to be used as sources of nutrients for animals because they contain various nutrients and different bioactive compounds.

The global grape production is close to 80 million tons per year, more than 10 million tons of which are by-products, like stems and pulps (or pomace). They contain different nutrients like dietary crude fibre, essential unsaturated fatty acids, different minerals, etc. Grape pulps (pomace) have a high concentration of different bioactive compounds such as tannins and flavonoids. Grape by-products have the nutritional potential to be used as sources of different nutrients and active compounds for animal protection, better utilization of feed nutrients, and higher animal performance. The authors of this publication have carried out *in vivo* and *in vitro* experiments with grape by-products with different animal models. The analyzed results in this monograph may be used by all those who wish to use these ingredients in animal (i.e., not only farm) nutrition.

Authors

1• FEED ADDITIVES IN ANIMAL NUTRITION

In the past, antibiotics were frequently used as growth promoters, especially in non-ruminant nutrition. It was a preventive measure to protect animals against different pathogens and certain diseases. Antibiotic growth promoters were also effective in stimulating growth, improving the conversion ratio, increasing daily weight gain and productivity, and in other aspects. However, since the time antibiotics were first used in animal nutrition in aquaculture more than 70 years ago, it has been causing antibiotic resistance in animals, as well as in humans. For more than 50 years, researchers and scientists around the world have been looking for alternatives, i.e., feed additives like probiotics, prebiotics, acidifiers, enzymes, combined additives, and plant-originating additives. Since 2006, antibiotics have no longer been considered feed additives (Gálik, 2012). Feed additives are substances, microorganisms, or preparations that differ from feeds (or premixtures) added into feed or drinking water, and are to perform a single or multiple functions, such as better quality of feed, animal product, better animal nutritional needs, better animal production, or welfare. Feed additives must have a positive effect on gastrointestinal flora and feed digestibility. Additionally, feed additives can also have coccidiostatic and/or histomonostatic effects. There are different functional categories of feed additives: i) **technological additives** (like conservants, antioxidants, emulgators, etc.), ii) **sensory additives** (mainly colours), iii) **nutritional additives** (vitamins, minerals, amino acids, etc.), iiii) **zootechnical additives** (for the gastrointestinal tract, for improved nutrients digestibility improve). The use of feed additives in animal nutrition is regulated by EC Regulation No. 1831/2003.

In modern animal nutrition, zootechnical additives have been used in routine practice. The oldest additives are ***probiotics***. They have been tested for more than 60 years now, mainly in poultry nutrition, as an alternative concept to antibiotics. Probiotics are live microorganisms with a positive effect on the host animal. They can reduce the activity of undesirable microorganisms with different modes of action (volatile fatty acid production, intestinal pH reduction, antimicrobial production, and possible immunostimulation). However, the results have sometimes been disputable (Leibetseder, 2004). If probiotics intake is adequate, positive effects on animal production include better conversion ratio, higher daily weight gain, better animal health status; typical is also the elimination of undesirable microorganisms in the gastrointestinal tract (Vila et al., 2010). Regarding the mode of action, ***prebiotics*** represent a completely different type of additives. This additive is indigestible for the host animal; originally, prebiotics are saccharides such as FOS (fructooligosaccharides), MOS (mannanoligosaccharides) and similar compounds, i.e., they are sources of energy and nutrients for the naturally existing probiotics in the animal body. The principle of the prebiotic mode supports the activity of lactic acid bacteria in the gastrointestinal tract and their lumen colonization. Prebiotics represent an alternative to probiotics also from an economical point of view (Gálik, 2012). A relatively new group of feed additives are known as ***phytogenics***, or phytogenerics. These additives are obtained from different aromatic plants and/or herbs with active compounds with beneficial effects. They can improve feed digestibility, feed conversion, and growth rate stimulation. Moreover, they are physiological, environmentally friendly and have insecticide effects (Li et al., 2007, Kroismayr et al., 2007, Windish et al., 2008, Ying et al., 2007). In the last two decades, we have observed some interest in the use of phytogenics in animal nutrition. Different studies have shown that these additives also contain different compounds and active units with beneficial effects on the animal body. Some compounds can protect the animal against different metabolic problems; some phytogenics are rich in the content of some enzymes helping in digestion. Phytogenic feed additives can positively affect animal performance, such as growth intensity, feed conversion ratio, laying intensity, and the quality of meat and/or table eggs. In poultry feeding, phytogenic additives can reduce cholesterol content in meat, as well as in table eggs. In pigs fattening, phytogenics can improve the quality of fatty acid profile in the meat. In horse nutrition, phytogoenics increase the digestibility of nutrients. However, the amount of daily intake is very important. Phytogenics also often contain some anti-nutritive factors, such as phenols, tannins, etc., which can reduce nutrients transfer and cause some metabolic problems. In the years to come, we will quite probably see further interest in using these additives in animal nutrition. Their major benefit is that they are physiological and natural. (Gálik et al., 2015).

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2 • INDUSTRIAL BY-PRODUCTS AS INGREDIENTS IN ANIMAL NUTRITION

In the past, there used to be significant interest in industrial by-products with nutritional potential. This was at a time when the population of livestock was much larger than today. With the decline in livestock population, interest and search for by-products of industrial agricultural plants also gradually decreased. However, this interest in using by-products with nutritional potential in animal nutrition has now been revived (Gálik et al., 2015, 2018). Many by-products are rich in some nutrients, such as proteins, amino acids, fatty acids, or some minerals and vitamins. Agriculture and food industry now produce large amounts of residues, by-products, almost 30 % of worldwide agricultural production (Ajila et al., 2012). As ingredients, by-products come from cereal processing (e.g., meals, germs, and others) and from the brewery and fat industry (pulps, expellers, meals, and others). Modern animal nutrition includes the use of ingredients such as DDGS and by-products from grape processing. This mixture of ingredients is used in animal nutrition for two reasons: they are rich in the content of some organic nutrients, as well as specific compounds, such as vitamins, enzymes, and polyphenols. Moreover, their use in animal nutrition is environmentally friendly.

For some time now, we have been hearing calls to reduce waste production in Europe and in the global context, i.e., the key challenge is the concept of a circular economy. Clearly, there seems to be a large potential for using these products as a source of nutrients for animals. Indeed, with the use of agricultural by-products of in animal nutrition, there will be lower production of biological waste. In addition, many agro-food by-products are rich in many enzymes with beneficial effects on digestion. This means the retention of many nutrients in the body is higher and excretion lower. This is a positive moment in relation to the excretion of some critical analytes, such as proteins, phosphorus, and nitrogen.

Different studies have analyzed the nutritional content of grape by-products. The main idea is that grapes are rich in various minerals; however, they are also rich in many bioactive compounds with antioxidant, protective, and other effects on animal organisms. Compounds such as resveratrol, quercetin, catechin, and others are a very interesting issue in modern livestock nutrition and feeding. Moreover, the crude fat composition of grapes, mainly in seeds, is of high quality. There is also an increased occurrence of essential polyunsaturated fatty acids. However, grape pomace also contains antinutritive compounds with depressive effects on animal organisms. This means the key issue at stake is the amount of daily intake. Additionally, modern feed technologies can help reduce the negative effect of grape pomace, e.g., nanotechnologies.

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3 • NUTRITIONAL COMPOSITION OF GRAPE BY-PRODUCTS

3.1 Basic nutritional characteristics of grape pomace

The nutritive value of wine by-products depends on the technical processing of wine production. There may also be differences caused by grape origin (Baumgärtel et al., 2007). The nutritive value of grape pomace is defined by a higher concentration of DM, CP, EE, CF, ADF, NDF and L compared to the stem of grapes (Hanušovský et al., 2020). The DM and OM content of grape pomace is highly variable. For example, in the studies of Baumgärtel et al., 2007, González-Centeno, 2010 and Azevêdo et al., 2012, the DM content of 273 to 408 $g.kg^{-1}$ (in fresh matter) and the OM content of 933 to 943 $g.kg^{-1}$ were determined. Similar results were reported by Hanušovský et al. (2020), with variable dry matter content in grape pomace from 327.00 to 393.70 $g.kg^{-1}$ and the OM from 929.21 to 956.71 $g.kg^{-1}$. Furthermore, high variability can also be found in the content of dried grape pomace. In the research of Hanušovský et al. (2019a), the DM content in dried grape pomace was observed in the interval of 927.40 to 943.20 $g.kg^{-1}$. On the contrary, the content of CP in grape pomace ranged from 77 to 175 $g.kg^{-1}$ (Peiretti et al., 2017; Russo et al., 2017). Similarly, Hanušovský et al. (2020) determined the CP content in fresh grape pomace from 73.10 to 131.09 $g.kg^{-1}$ and in dried grape pomace from 97.39 to 103.75 $g.kg^{-1}$ (Hanušovský et al., 2019a). In the research of Azevêdo et al., Zalikarenab et al. (2007) and Molina et al. (2008), the EE concentration 74 $g.kg^{-1}$, respectively from 50 to 71 $g.kg^{-1}$ was reported. However, the EE content can be more variable. For example, Hanušovský et al. (2020) reported the EE content in grape pomace from 30.59 to 100.87 $g.kg^{-1}$ with lower content in white grape by-product varieties. In dried grape pomace, on the other hand, the EE concentration interval was narrower (84.19 – 100.58 $g.kg^{-1}$) (Hanušovský et al. 2019a). Furthermore, in the research carried out by Hanušovský et al. (2020), the CF content in grape pomace was found between 120.07 to 246 $g.kg^{-1}$, with a higher content in the red varieties. However, Baumgärtel et al. (2007) reported a higher EE content of up to 312 $g.kg^{-1}$. In the experiments of Zalikarenab et al. (2007) and Molina et al. (2008), the ash content was found between 62 to 79 $g.kg^{-1}$. According to Hanušovský et al. (2020), the ash content can be lower (from 43.29 to 70.79 $g.kg^{-1}$. That was also confirmed by Hanušovský et al. (2019a), where the ash content ranged from 36.83 to 41.19 $g.kg^{-1}$. The starch concentration in grape pomace was presented in minimum quantities of 0 to 41.87 $g.kg^{-1}$ (Hanušovský et al., 2020). A similar level of starch was reported by Corbin et al. (2015) from 27 to 40 $g.kg^{-1}$. A very high variety, especially in the NFE content was presented by Hanušovský et al. (2019a) and Hanušovský et al. (2020) in fresh and dried grape pomace from 487.35 to 730.75 $g.kg^{-1}$. On average, lower but very variable concentrations of NFE were also determined by Corbin et al. (2015) to be between 314 and 539 $g.kg^{-1}$. Obviously, higher NFE has been determined in the white grape pomace varieties (Hanušovský, et al. 2020). Regarding TS concentrations, in the research of Corbin et al. (2015) the content of TS ranged from 148 to 430 $g.kg^{-1}$, however, the SG content in the red grape pomace varieties has been found to be lower in comparison with the white grape pomace varieties. It should be noted that the variability is very wide, indeed (4.73 to 542 $g.kg^{-1}$), (Hanušovský et al., 2020).

Table 1 The basic nutritional composition of grape pomace

Nutrients g.kg^{-1}	Hanušovský et al., 2019a		Hanušovský et al., 2020	
	Mean ± SD			
	Min	Max	Min	Max
DM	934.78 ± 8.40	942.25 ± 1.00	327.00 ± 2.79	393.70 ± 0.10
CP	98.70 ± 1.02	103.75 ± 3.03	73.10 ± 0.67	131.09 ± 0.91
EE	84.19 ± 0.63	100.58 ± 5.20	30.59 ± 0.17	100.87 ± 0.94
CF	180.85 ± 1.67	183.98 ± 1.04	120.07 ± 2.93	246.20 ± 1.14
Ash	39.06 ± 2.45	39.72 ± 0.36	43.29 ± 0.24	70.79 ± 0.33
NFE	575.76 ± 21.88	593.42 ± 2.69	487.35 ± 1.96	730.75 ± 4.81
OM	960.28 ± 0.36	960.94 ± 2.45	929.21 ± 0.33	956.71 ± 0.24
Starch	ND	ND	ND	41.87 ± 0.80
TS	ND	ND	4.73 ± 1.22	542.02 ± 3.39

Abbreviations: **Min** – minimal average, **Max** – maximal average, **DM** – dry matter, **CP** – crude proteins, **EE** – ether extracts, **CF** – crude fibre, **NFE** – nitrogen free extracts, **OM** – organic matter, **TS** – total sugars, **ND** – not detected

3.2 Composition of the grape pomace fibre complex

Russo et al. (2017) reported the NDF content in grape pomace to be between 215 to 614 g.kg^{-1} and Hanušovský et al. (2019a) from 306.47 to 459.67 g.kg^{-1}. A very wide interval was also published in Hanušovský et al. (2020), namely 221.64 to 529.55 g.kg^{-1}, with a higher content in the red grape pomace varieties. The concentration of ADF ranged from 173 to 561 g.kg^{-1} (Russo et al., 2017). Subsequently, Baumgärtel et al. (2007) reported the ADF concentration from 202 to 267 g.kg^{-1}. However, higher ADF content was found by Hanušovský et al. (2019a), from 269.06 to 380.87 g.kg^{-1}. Additionally, white grape pomace varieties tend to have a lower ADF content compared to red grape pomace (Hanušovský et al, 2020). The lignin content of grape pomace was found in the interval from 133.85 to 285.34 g.kg^{-1} (Hanušovský et al., 2020). A similar interval (216.93 to 236.90 g.kg^{-1}) of the lignin concentration was reported by Hanušovský et al.

(2019a). The content of C in the research by Zalikarenab et al. (2007) was determined to be from 80 to 90 $g.kg^{-1}$ and Molina et al. (2008), (110 $g \cdot kg^{-1}$). However, the C content may be higher, and Hanušovský et al. (2020) found the C content in grape pomace from 77.00 to 155.39 $g.kg^{-1}$; the red variety showed a higher content. Zalikarenab et al. (2007) determined the H content from 31 to 54 $g.kg^{-1}$ and Molina et al. (2008) 89 $g.kg^{-1}$. Similar content was also reported by Hanušovský et al. (2019a) from 37.41 to 78.80 $g.kg^{-1}$. However, the content of hemicellulose tends to be higher in red grape pomace (Hanušovský et al, 2020).

Table 2 The fibre complex composition of grape pomace

Nutrients $g.kg^{-1}$	Hanušovský et al., 2019a		Hanušovský et al., 2020	
	Mean ± SD			
	Min	Max	Min	Max
ADF	269.06 ± 0.95	380.87 ± 4.03	210.85 ± 2.53	440.73 ± 2.04
NDF	306.47 ± 1.40	459.67 ± 0.39	221.64 ± 0.84	529.55 ± 1.64
L	216.93 ± 4.37	238.06 ± 1.64	133.85 ± 2.18	285.34 ± 0.17
C	31.00 ± 0.69	163.95 ± 2.37	77.00 ± 4.71	155.39 ± 1.93
H	37.41 ± 0.45	78.80 ± 3.76	6.85 ± 1.99	88.82 ± 1.89

Abbreviations: **Min** – minimal average, **Max** – maximal average, **ADF** – acid detergent fibre, **NDF** – neutral detergent fibre, **L** – lignin, **C** – cellulose, **H** – hemicellulose

3.3 Mineral composition of grape pomace

Corbin et al. (2015) found the Ca content between 2170 and 3867 $mg.kg^{-1}$. Chikwanha et al. (2018) determined similar Ca concentrations, from 2290 to 3730 $mg.kg^{-1}$. However, Šimko et al. (2019) reported that the Ca content in grape pomace was between 4457.50 to 5517.50 $mg.kg^{-1}$. Subsequently, Botelho et al. (2018) observed almost the same Ca content, up to 4295 $mg.kg^{-1}$. On the other hand, Hanušovský et al. (2019b) found a lower Ca content in grape pomace, from 2225.58 to 3286 $mg.kg^{-1}$. The content of P in the research by Corbin et al. (2015) was found between 2367 and 2733 $mg.kg^{-1}$. Hanušovský et al. (2019b) reported a wider interval of P in grape pomace, from 2259.71 to 3160.03 $mg.kg^{-1}$. A similar maximal P content was determined in Šimko et al. (2019), from 3180.00 to 3210 $mg.kg^{-1}$. However, the concentration of P in grape pomace can also be up to 3420 $mg.kg^{-1}$ (Chikwanha et al., 2018). In contrast, Bennemann et al. (2016) reported the minimal level of P in grape pomace from 330.5 to 497.6 $mg.kg^{-1}$. Chiwkanha et al. (2018) reported Mg concentrations of 950 to 1370 $mg.kg^{-1}$. But Bennemann et al. (2016) found a wider interval, from 587.3 to 1547 $mg.kg^{-1}$, compared to the study mentioned. Within this interval was also the content of Mg (1200.00 $mg.kg^{-1}$) found by Šimko et al. (2019). In contrast, Corbin et al. (2015) determined a lower concentration of Mg compared to 710 to 987 $mg.kg^{-1}$. Similarly, Hanušovský et al. (2019b) reported a lower minimal Mg content in grape pomace from 687.40 to 1176.35 $mg.kg^{-1}$. Subsequently, Chikwanha et al. (2018) determined the Na content was between 328 and 1210 $mg.kg^{-1}$. In contrast, Corbin et al. (2015) observed the Na content only from 58 to 61 $mg.kg^{-1}$. Hanušovský et al. (2019b) reported a very high variability in Na concentration, namely 361.45 to 1555.59 mg kg^{-1}. On the other hand, Šimko et al. (2019) determined the Na content from 260.00 to 462.50 $mg.kg^{-1}$. The content of K in grape pomace is very variable. Thus, Chikwanha et al (2018) observed K concentrations from 15000 to 25300 $mg.kg^{-1}$. Then, Corbin et al. (2015) determined the content of K up to 27333 $mg.kg^{-1}$. Šimko et al. (2019) reported a similar K content, 12892.50 to 15055.00 $mg.kg^{-1}$. Botelho et al. (2018) observed a narrow interval in the K contents in grape pomace from 59.6 to 258.2 $mg.kg^{-1}$. In contrast, the K content determined by Hanušovský et al. (2019b) was very wide, from 23825.40 to 58549.82 $mg.kg^{-1}$. A microelement profile was also described by only a few authors. A wide interval in Cu content from 92.5 to 2964.2 $mg.kg^{-1}$ was observed during the last years (Bennemann et al., 2016; Botelho et al., 2018). The lower Cu content in the grape pomace determined Kolláthová et al. (2019) from 16.68 to 60.53 $mg.kg^{-1}$. In addition, Šimko et al. (2019) reported an even lower Cu content, from 13.24 to 14.17 mg.kg^{-1}. In the study by Corbin et al. (2015), Fe concentrations of 60 to 85 mg.kg^{-1} were found. However, different research observed a wide Fe content of 587.3 to 2865.0 mg.kg^{-} observed (Bennemann et al., 2016). The lower Fe content was described by Kolláthová et al. (2019), between 42.37 to 152.86 mg.kg^{-1}. Within this interval was also the Fe content in grape pomace reported by Šimko et al. (2019), between 68.22 to 96.82 mg.kg^{-1}. Then Botelho et al. (2018) determined the Mn concentrations from 90.2 to 366.4 mg.kg^{-1}. In the research of Kolláthová et al. (2019) was the Mn content from 7.11 to 17.81 mg.kg^{-1}. A similar content of Mn was determined by Šimko et al. (2019), from 11.44 to 12.51 mg.kg-1. Finally, a very wide interval was reported by Bennemann et al. (2016) and Botelho et al. (2018) from 0.8 to 335.9 mg.kg^{-1}. On the contrary, Kolláthová et al. (2019) observed a Zn content to be between 18.48 to 41.32 mg.kg^{-1}. Similarly, Šimko et al. (2019) determined the Zn concentration in grape pomace from 16.42 to 28.99 mg.kg^{-1}.

Table 3 The macromineral profile of grape pomace

Minerals mg.kg^{-1}	Šimko et al., 2019		Hanušovský et al., 2019b	
	Mean ± SD			
	Min	Max	Min	Max
Ca	4457.50 ± 102.43	5517.50 ± 322.63	2225.58 ± 73.73	5366.94 ± 109.82
P	3180.00 ± 326.80	3210.00 ± 69.28	2259.71 ± 65.74	3797.84 ± 120.76
Mg	1200.00 ± 58.31	1205.00 ± 54.47	687.40 ± 6.35	1173.35 ± 19.01
Na	260.00 ± 8.16	462.50 ± 181.91	341.30 ± 19.36	1555.59 ± 0.25
K	12892.50 ± 85.00	15055.00 ± 365.29	23825.40 ± 1287.83	58549.82 ± 729.13

Abbreviations: **Min** – minimal average, **Max** – maximal average, **Ca** – calcium, **P** – phosphorus, **Mg** – magnesium, **Na** – natrium, **K** – potassium

Table 4 The micromineral profile of grape pomace

Minerals mg.kg^{-1}	Šimko et al., 2019		Kolláthová et al., 2019	
	Mean ± SD			
	Min	Max	Min	Max
Cu	13.24 ± 1.74	14.17 ± 0.93	16.68 ± 0.71	60.53 ± 0.72
Fe	68.22 ± 2.55	96.82 ± 1.51	42.37 ± 2.88	152.86 ± 2.82
Mn	11.44 ± 0.10	12.51 ± 1.46	7.11 ± 0.22	17.81 ± 0.63
Zn	16.42 ± 2.11	28.99 ± 3.62	18.92 ± 0.42	41.32 ± 0.65

Abbreviations: **Min** – minimal average, **Max** – maximal average, **Cu** – copper, **Fe** – iron, **Mn** – manganese, **Zn** – zinc

3.4 Basic nutritional characteristics of the grape stem

The nutrient composition of grape stem has not been examined properly in the scientific literature so far. Hanušovský et al. (2020) reported a concentration of DM of 223.30 to 326.67 g.kg^{-1}. Similar DM content was found by Basalan et al. (2011), (314 g.kg^{-1}). After that, Spigno et al. (2013) determined the CP concentration in grape stalks from 37 to 97 g.kg^{-1}. Hanušovský et al. (2020) found a narrower interval in the CP content (42.79 – 85.60 g.kg^{-1}). Subsequently, Basalan et al. (2011) determined the EE content on average to be 12.2 g.kg^{-1}, which was in the interval reported by Spigno et al. (2013) from 3 to 21 g.kg^{-1}. Also, Hanušovský et al. (2020) claimed a similar EE content in grape stem, from 5.37 to 14.30 g.kg^{-1}. Peiretti et al. (2017) and Basalan et al. (2011) determined the ash content to be between 53.6 and 76.4 g.kg^{-1}. Hanušovský et al. (2020) reported a similar, only slightly higher ash concentration, namely from 67.84 to 84.50 g.kg^{-1}.

Table 5 The basic nutritional composition of the grape stem

Nutrients g.kg^{-1}	Hanušovský et al., 2020	
	Mean ± SD	
	Min	Max
DM	223.30 ± 0.06	326.67 ± 57.71
CP	42.79 ± 1.93	85.60 ± 1.63
EE	5.37 ± 0.34	14.30 ± 0.28
CF	188.54 ± 2.74	160.40 ± 0.43
Ash	67.84 ± 1.56	84.50 ± 2.07
NFE	656.01 ± 0.72	700.26 ± 0.84
OM	915.50 ± 2.07	932.16 ± 1.56
Starch	ND	53.32 ± 1.14
TS	179.60 ± 1.27	437.57 ± 2.98

Abbreviations: **Min** – minimal average, **Max** – maximal average, **DM** – dry matter, **CP** – crude proteins, **EE** – ether extracts, **CF** – crude fibre, **NFE** – nitrogen free extracts, **OM** – organic matter, **TS** – total sugars, **ND** – not detected

3.5 Composition of the grape stem fibre complex

Basalan et al. (2011) determined the content of ADF and NDF in grape stem to be between 324 g.kg^{-1} and 433 g.kg^{-1}. This finding was comparable to Hanušovský et al. (2020), with ADF concentrations ranging from 270.67 to 387.37 g.kg^{-1} and NDF concentrations of 287.47 to 445 g.kg^{-1}. However, the stem of the white grape variety had a lower content of ADF compared to the stem of the red grape variety. Furthermore, Spigno et al. (2013) reported a L content of 173 to 263 g.kg^{-1}. Lower L content was determined by Hanušovský et al. (2020) from 117.41 to 237.76 g.k^{-1}. Furthermore, Spigno et al. (2013) determined the C concentrations in the grape stem to be between 171 and 241 g.kg^{-1}. A narrower interval in C content was published by Hanušovský et al. (2020) from 149.61 to 171.76 g.kg^{-1}. Finally, Spigno et al. (2013) reported the H content of 43 to 64 g.kg^{-1}, which was similar to the research of Hanušovský et al. (2020), namely between16.80 and 61.31 g.kg^{-1}.

Table 6 Composition of the fibre complex of the grape stem

Nutrients g.kg^{-1}	Hanušovský et al., 2020	
	Mean ± SD	
	Min	Max
ADF	270.67 ± 2.10	387.37 ± 1.01
NDF	287.47 ± 2.65	445.30 ± 2.74
L	117.41 ± 1.32	237.76 ± 1.03
C	149.61 ± 1.70	171.76 ± 2.17
H	16.80 ± 4.75	61.31 ± 0.34

Abbreviations: **Min** – minimal average, **Max** – maximal average, **ADF** – acid detergent fibre, **NDF** – neutral detergent fibre, **L** – lignin, **C** – cellulose, **H** – hemicellulose

3.6 Grape stem mineral composition

Research focused on the mineral composition of the grape stem has been rare in recent years, but several studies have described the concentration of minerals in the grape stem as well as their pattern. In the ash of the grape stem, one can detect more than 20 minerals with the major content of K, followed by Ca, Mg and Na (Prozil et al., 2012). For example, Arrobas et al. (2014) found the K content in the grape stem at 28 600 mg.kg^{-1}. Similar concentrations of K were also found by Spigno et al. (2013), from 25 800 to 31 700 mg.kg^{-1}. However, the higher K content was determined by Hanušovský et al. (2019), from 29431.54 to 39539.82 mg.kg^{-1}. Subsequently, Arrobas et al. (2014) observed a Ca concentration of 5900 mg.kg^{-1}. This content was within the interval reported by Hanušovský et al. (2019), i.e., from 4397.57 to 8173.72 mg.kg^{-1}. A similar interval was published by Spigno et al. (2013), in which the Ca content in the grape stem was 4 800 to 9 400 mg.kg^{-1}. Subsequently, Arrobas et al. (2014) determined the P concentration of 1 800 mg.kg^{-1}. A higher P content in the grape stem was reported by Hanušovský et al. (2019), from 2112.23 to 3226.61 mg.kg^{-1}. A wide range of Mg content was determined by Spigno et al. (2013) from 800 to 3 400 mg.kg^{-1}, which corresponds to Hanušovský et. al. (2019), where the Mg content was found between 926.34 and 2093.59 mg.kg^{-1}. The Mg concentration reported by Arrobas et al. (2014) was also found in this interval, namely 1 400 mg.kg^{-1}.

Table 7 The mineral profile of the grape stem

Nutrients mg.kg^{-1}	Hanušovský et al., 2019; Kolláthová et al., 2019	
	Mean ± SD	
	Min	Max
Ca	4397.57 ± 24.49	8173.72 ± 77.33
P	2112.23 ± 67.15	3226.61 ± 0.53
Mg	926.34 ± 0.44	2093.59 ± 22.64
Na	227.91 ± 10.43	2631.28 ± 26.24
K	29431.54 ± 1047.97	39539.82 ± 1147.76
Cu	12.90 ± 2.22	44.12 ± 0.79
Fe	42.86 ± 1.27	229.21 ± 2.16
Mn	21.02 ± 0.22	47.72 ± 0.80
Zn	17.50 ± 0.33	43.55 ± 1.11

Abbreviations: **Min** – minimal average, **Max** – maximal average, **Ca** – calcium, **P** – phosphorus, **Mg** – magnesium, **Na** – natrium, **K** – potassium, **Cu** – copper, **Fe** - iron, **Mn** – manganese, **Zn** – zinc

3.7 Composition of fatty acids of grape by-products

The FA profile of grape pomace is well documented in scientific literature, but only a limited number of articles have been published on the content of FA in the grape stem and the grape bunch. The fatty acids of the grape by-products consist mainly of polyunsaturated fatty acids (PUFA), from 67.82% to 75.66% (Kolláthová et al., 2020). Kolláthová et al. (2020) reported the highest linoleic acid content (from 66.61% to 73.85%), especially in grape pomace. A similar linoleic acid content was reported in grape seed and grape oil (Fernandes et al., 2013, Yousefi et al., 2013, Hussein and Abdrabba, 2015, Ovcharova et al., 2016). Furthermore, Kolláthová et al. (2020) determined a higher content of α-linoleic acid (from 9.03% to 18.73%) in the fat of the grape stems. Then, oleic acid, as a monounsaturated fatty acid (MUFA), was the most abundant in all the by-products studied. However, Kolláthová et al. (2020) reported the highest amount of saturated fatty acids (SFA) in grape stems, from 18.45 to 32.88%, mainly palmitic (from 10.68 to 19.62%) and stearic acids (from 3.52 to 5.75%) compared to grape pomace. The high content of palmitic acid in pomaces may be due to the surplus of saturated compounds in their waxy structure (Gülcü et al., 2019). Lower levels of SFA were also reported in grape by-products in various studies (Tangolar et al., 2009; Gül et al., 2013, Mironeasa et al., 2016, García-Lomillo and Gonzáles-San José, 2017). For example, Yi et al. (2009) found on average 21.2% SFA, 14.4% MUFA and 62.7% PUFA in grape pomace. Ribeiro et al. (2015) determined the PUFA content 72.86% in grape pomace, with the predominance of linoleic (60.04%) and α-linolenic (13.64%) acids, followed by oleic (12.97%), palmitic (6.72%) and stearic acids (5%). The similar pattern was also observed in Kolláthová et al. (2020). On the contrary, Tsiplakou and Zervas (2008) and Gülcü et al. (2019) found a similar pattern of FA, except for linoleic acid. Additionally, Russo et al. (2017) reported that the grape stalk contained 21% palmitic, 4.6% stearic, 10.7% oleic, 35.4% linoleic, 13.4% α-linoleic and 11.3% behenic acids.

Table 8 Fatty acid profile of grape by-products (Kolláthová et al., 2020)

	Grape pomace		Grape stem	
	Mean ± SD			
FA in % fat^{-1}	**Min**	**Max**	**Min**	**Max**
Palmitic acid	7.69 ± 0.03	8.70 ± 0.03	10.68 ± 0.49	19.62 ± 5.85
Stearic acid	3.25 ± 0.01	4.03 ± 0.00	3.52 ± 0.21	5.75 ± 2.05
Oleic acid	9.80 ± 0.04	17.52 ± 0.02	9.43 ± 0.74	16.12 ± 0.12
Linoleic acid	66.61 ± 0.04	73.85 ± 0.09	36.06 ± 0.29	57.19 ± 1.21
α-linoleic acid	0.77 ± 0.01	1.81 ± 0.01	5.74 ± 0.13	18.73 ± 1.05

FA in % fat^{-1}	Grape pomace		Grape stem	
	Mean ± SD			
	Min	Max	Min	Max
Arachidic acid	0.23 ± 0.01	0.34 ± 0.01	1.21 ± 0.03	3.52 ± 0.07
Behenic acid	0.11 ± 0.00	0.24 ± 0.00	1.95 ± 0.08	5.73 ± 0.16
PUFA	75.66 ± 0.10	67.82 ± 0.04	47.57 ± 9.10	62.93 ± 1.15
MUFA	10.09 ± 0.05	17.95 ± 0.02	9.43 ± 0.74	16.61 ± 2.48
SFA	12.30 ± 0.01	13.37 ± 0.04	18.45 ± 0.73	32.88 ± 7.35
Ratio Σn3/n6	0.01 ± 0.00	0.02 ± 0.00	0.10 ± 0.00	0.48 ± 0.04
Ratio Σn6/n3	40.78 ± 0.27	89.59 ± 0.75	2.08 ± 0.18	9.96 ± 0.39

Abbreviations: **Min** – minimal average, **Max** – maximal average, **PUFA** – polyunsaturated fatty acids, **MUFA** – monounsaturated fatty acids, **SFA** – saturated fatty acids

The by-products of the winery production have an average nutritive value due to the higher lignin content, which could present a limiting factor for digestibility. Generally, there are differences between locations and cultivars. Grape pomace has a high ratio of CP, EE, and CF. The content of total sugars is affected by wine pressing technology. Grape pomace also has a high lignin content; however, there is a lower content of cellulose and hemicellulose. The grape stem is characterised by balanced content of CP, CF and NFE, with residual S after grape pressing, but there is low concentration of EE. In addition, the grape stem has a high concentration of H and C. Furthermore, the concentrations of minerals in the by-products of grapes are affected by the locality of origin and the variety of grapes. The different mineral content also resulted in different ratios between the minerals. However, the same concentration patterns in minerals content are observed in all by-products, with the highest concentrations of K, after that of Ca, P, Na and with the lowest Mg. The results of this experiment indicate a significant impact of the grape variety and location on the FA profile of grape by-products. Despite these differences, some similarities can still be found. Grape by-products are also a good source of PUFA, especially linoleic acid. However, the SFA content is limited. The grape stems were characterized by a high FA content, with the highest α-linoleic acid concentration.

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4 • SPECIFIC COMPOUNDS OF GRAPE BY-PRODUCTS

4.1 Polyphenols

Polyphenols are a diverse group of secondary metabolites of plants, well known for their antioxidant, cardioprotective, anticarcinogenic, antimicrobial, and anti-inflammatory characteristics (Tang et al., 2018; Colombo et al., 2019; Zhao et al., 2020). They are characterized by a very diverse structure, from simple molecules to highly polymerized compounds, and their classification is relatively complex (Thomas, 2017; Swallah et al., 2020). According to the content in grapes and grape pomace, they can be divided into *nonflavonoids* (phenolic acids, stilbenes) and *flavonoids* (flavonols, flavanols, anthocyanins).

Nonflavonoids

Phenolic acids in grapes and in grape by-products can be found in two forms. Hydroxycinnamic acids (p-coumaric, ferulic) and their derivatives (p-cutaric, feratric) are found mainly in grape skins (Kadouh et al., 2016; Cotea et al., 2018). Hydroxybenzoic acids (gallic, p-hydroxybenzoic, vanillic) are mainly present in grape seeds (Hassan et al., 2019). Gallic acid, as a precursor to hydrolyzable tannins, is the most abundant and important phenolic acid in grape pomace (Silva et al., 2018). The content of hydroxycinnamic acids is strongly influenced by the country of origin. Compared to the European varieties, these acids are prevalent in the East Asian and North American cultivars. The content of hydroxybenzoic acids in grapes between individual varieties is similar. Phenolic acids, in contrast to flavonoids, have a lower antioxidant capacity, while cinnamic acid derivatives have better antiradical properties (Silva et al., 2018; Hornedo-Ortega et al., 2020). As shown by the experiments on mice and rats, phenolic acids have anti-inflammatory properties and positively affect blood glucose levels and insulin resistance (Miranda et al., 2015). ***Stilbenes*** play a key role in protecting plants from environmental stress and resistance to pathogens (El Khawand et al., 2018). Resveratrol is the most common stilbene found in grape skins, especially of the red varieties (Németh et al., 2017). Resveratrol has recently been subject of interest due to its biological properties, including anticarcinogenic and protective effects in cardiovascular disease (Heber, 2011). In animal nutrition, its positive effects on indicators of liver profile, overall antioxidant status, and nutrient digestibility have been observed (Ahmed et al., 2013; Momchilova et al., 2014; Alagawany et al., 2015).

Flavonoids

Flavonoids have the potential to positively affect several biological processes in animals, which can improve their immunity, intestinal functions, reduce the risk of infectious diseases, and increase their performance and production (Kamboh et al., 2015; Brenes et al., 2016). Therefore, their higher proportion in grape by-products is desirable. ***Flavonols*** are natural yellow plant dyes found in the skin of grape berries (Peixoto et al., 2018). The most abundant flavonols in grapes and pomace are derivatives of quercetin, myricetin, kaempferol, and rutin (Favre et al., 2018; Tkacz et al., 2019). These compounds are considered to be strong antioxidants with a chemoprotective effect. Calderon-Montano et al. (2011) report that kaempferol and quercetin have a synergistic effect in reducing tumour cell proliferation, which means that combined supplementation with quercetin and kaempferol is more effective than the additive effects of individual flavonoids. Kaempferol also prevents atherosclerosis and myricetin reduces cholesterol and has anti-inflammatory effects (Alam et al., 2020). ***Flavanols*** are responsible for the sensory properties of wines such as bitterness and texture (Drappier et al., 2019). They generally occur in plants in the form of monomers (flavan-3-ol), dimers, oligomers (3-10 monomer units) or polymers (more than 10 monomer units). The main monomers are catechins, catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate. These monomer units condense easily to form tannins (proanthocyanidins). Condensed tannins represent a significant proportion of the biologically active substances found in the by-products of grape processing. They accumulate mainly in grape seeds, but they are also found in grape skins (Jordão and Cosme, 2017; Rousserie et al., 2019). Catechins are seen as the main carriers of the antioxidant capacity of grape pomace. Several authors have observed a positive correlation between catechin content in grape pomace extracts and their ability to scavange free radicals (Nagarajaiah et al., 2016; Del Pino-Garcia et al., 2017; Marchante et al., 2020). Epicatechin and catechin make up the majority of polyphenolic compounds in grape by-products (Gülcü et al., 2018; Balea et al., 2020). Grape seeds and bunches are the richest sources of these polyphenols (Özcan et al., 2017; Marchante et al., 2018). ***Anthocyanins*** are highly soluble plant pigments, directly responsible for the coloration of red grapes and wine berries (Hornedo-Ortega et al., 2020). Heras-Roger et al. (2016) reported that the reaction of anthocyanins with phenolic acids and flavonols results in more stable dyes in the co-pigmentation process. The main anthocyanins found in grape skins are derivatives of malvidin, peonidine, and petunidine (De Sales et al., 2018).

Approximately 60-70% of polyphenols in wine grapes remain in the pomace (Bordiga et al., 2019). Therefore, this by-product is one of the richest natural sources of biologically active compounds that receive significant attention due to their substantial health-promoting effects (Meini et al., 2019; Stuart et al., 2020). The content of total polyphenols is a key factor in the manifestation of the potential positive effect of grape pomace in animals (Antoniolli et al., 2015; Zhang et al., 2017). However, the amount and type of polyphenols in the pomace are affected by several factors, mainly grape variety, the proportion of seeds and peels, climate, viticultural practices, extraction and analytical methods, etc. (Gülcü et al., 2018; Salehi et al., 2019; Jimenez-Lopez et al., 2020). Hence, the comparison of results with the data from other studies is often complex.

Table 9 Overview of the polyphenolic composition of dried grape pomace (mg.kg^{-1} of dry matter)

Polyphenols	Amount
Total polyphenols[a]	12-154[1,2,3,4]
Total hydroxycinnamic acids	2-850[5,8]
Total hybroxybenzoic acids	29-1 292[5,8]
Gallic acid	40-380[4,5,6,11]
Resveratrol	6-86[1,5,7,11]
Total flavonoids[b]	1-19[2,7,11,12]
Total flavonols	30-630[1,8,17]
Qvercetin	13-251[1,8,10]
Myricetina	2-172[7,8]
Kaempferol	3-34[1,8,9]
Rutin	0,7-435[4,7,13]
Total flavanols	74-2 841[1,8]
Catechin	198-4 397[1,5,6,14]
Epicatechin	175-2 070[5,7,15,16]
Epicatechin gallate	10-45[1,8]
Gallocatechin	0-208[1,8]
Total anthocyanins[c]	176-29 850[1,8,17]
Malvidin	2-10 190[1,8,17]
Petunidin	0-1 340[1,8,17]
Peonidin	0-2 490[1,8,17]

[1]Marchante et al. (2018), [2]Kolláthová et al. (2019b), [3]Hanušovský et al. (2020), [4]Balea et al. (2018), [5]Fontana et al. (2016), [6]Yammine et al. (2020), [7]Brezoiu et al. (2019), [8]Lingua et al. (2016a), [9]Xu et al. (2015), [10]Mohamed Ahmed et al. (2020), [11]Casagrande et al. (2019), [12]Braga et al. (2016), [13]Gaafar et al. (2019), [14]Martins et al. (2016), [15]Jara-Palacios et al. (2020), [16]Tournour et al. (2015), [17]Hornedo-Ortega et al. (2020); [a] as gallic acid equivalent (mg GAE.g^{-1} of dry matter), [b]as quercetin equivalent (mg QE.g^{-1}od dry matter), [c]red grape pomace

4.2 Bioavailability and digestibility of polyphenols

If we are to explain the biological effects of polyphenols on animals, it is necessary to assume their bioavailability and their ability to reach target tissues (Abbas et al., 2017). In this regard, it is important to know the mechanisms by which they are absorbed, metabolized, and subsequently eliminated (Chamorro et al., 2017). There is considerable scientific debate in the absorption and metabolism of phenolics, and the results are inconsistent. The study of the absorption of polyphenols is restricted by their complex molecular structure. Most polyphenols are present in feed in a form that animals cannot absorb. These substances must first be hydrolyzed by digestive enzymes and by the present microflora. After their uptake, polyphenols are recognized by the system as xenobiotics; their bioavailability is thus relatively low compared to other nutrients (Brenes et al., 2016). Only about 5-10% of the total polyphenol intake is absorbed and metabolized by the body through biochemical reactions (Chiva-Blanch and Visioli, 2012; Landete, 2013; Gessner et al., 2017). Low molecular weight polyphenols are readily absorbed by passive diffusion in the small intestine (Manach et al., 2004; Majewska and Czeczot, 2009; Chamorro et al., 2019) and are rapidly released into the bloodstream for further distribution to organs or excretion through urine (Manach et al., 2004). Selected flavonoid metabolites are excreted in bile and reabsorbed from the intestine by enterohepatic circulation (Majewska and Czeczot, 2009), which can lead to a longer presence of polyphenols in the body. Higher molecular weight polyphenols (90-95% of their total intake) accumulate in the lumen of the colon, where they are subjected to the enzymatic activities of the intestinal microflora (especially βglucosidase) to form phenolic acids and other products (Monagas, 2010). All these metabolites, which originate through the action of microorganisms, enter the bloodstream or are excreted in the faeces (Majewska and Czeczot, 2009; Selma et al., 2009; Sánchez-Patán et al., 2012). However, they can also remain in the digestive tract and contribute to maintaining a healthy intestinal environment (Chamorro et al., 2019). Thus, intestinal microorganisms are responsible for the degradation of the complex structure of polyphenols into absorbable substances of low molecular weight, thereby directly affecting the bioavailability of phenolic compounds in feeds. Therefore, the biological activity of polyphenols in the body is caused not only by the initial compounds in food, but also by their low molecular weight metabolites formed in the degradation process by the intestinal microflora (Selma et al., 2009; Chamorro et al., 2017).

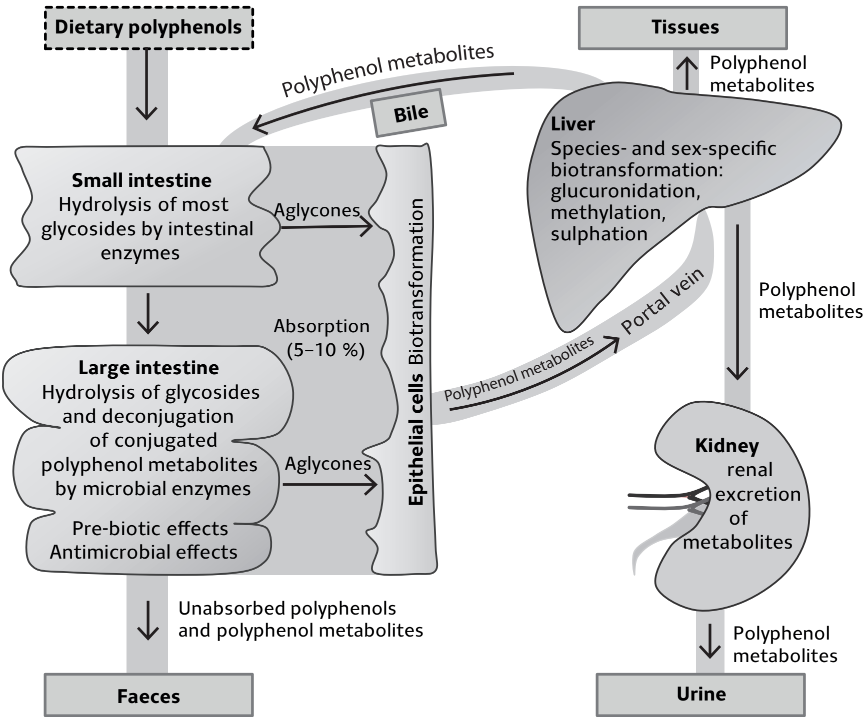

Figure 1 Absorption and metabolism of polyphenols in monogastric animals (Gessner et al., 2017)

4.3 Effect of polyphenols on the digestive tract of animals

Tannins are considered to be anti-nutritional factors that negatively affect the digestibility of nutrients, especially nitrogenous substances (Chamorro et al., 2017; Ebrahimzadeh et al., 2018a; Nardoia et al., 2020) and inhibit the effect of digestive enzymes (Cirkovic Velickovic and Stanic-Vucinic, 2018). However, current studies suggest that feeding an appropriate amount of these compounds may have a positive effect on overall health and performance of animals (Vinyard and Chibis, 2019; Buffa et al., 2020; Hossein-Vashan et al., 2020). Recently, there has been a growing interest in studying the interactions between polyphenols and intestinal microflora (Iqbal et al., 2020; Tabashsum et al., 2020; Wang et al., 2020). The prevailing view is that the intestinal microflora plays a crucial role in the potential health benefits of polyphenols. This is because they can metabolize them and thus produce even more bioactive substances of various physiological significance (Lillehoj et al., 2018; Kawabata et al., 2019; Luca et al., 2020). This was confirmed by Chamorro et al. (2019), when they detected in the faeces of poultry supplemented grape extract a higher amount and different nature of polyphenols compared to the group without supplementation. Undigested and unabsorbed polyphenols and their metabolites can affect intestinal microflora by accumulating in the digestive tract and stimulating or suppressing the growth of selected groups of microorganisms (Moreno-Indias, 2016; Hassan et al., 2019). According to Iqbal et al. (2020), polyphenols, on the one hand, suppress pathogenic bacteria and, on the other hand, promote beneficial bacteria by acting as prebiotics. A positive effect on the concentration of beneficial lactobacilli has been observed, while inhibiting the pathogenic bacterium E. coli in poultry and pigs when grape pomace and grape pomace extracts is fed (Verhelst, 2014; Ebrahimzadeh et al., 2018b; Kafantaris et al., 2018; Nardoia et al., 2020). Changes in intestinal morphology may affect nutrient absorption in animals. Ebrahimzadeh et al. (2018b) and Chamorro et al. (2019) report that villi elongation leads to improved nutrient absorption in the small intestine due to increased absorption surface (Kamboh et al., 2015). Wang et al. (2020) reported significant elongation of small intestinal villi with grape marc supplementation in pigs. The same results were obtained by Viveros et al. (2011), Hajati et al. (2015), and Yang et al. (2017) for poultry whose feed rations have been enriched with various polyphenol-rich wine by-products.

4.4 Antioxidant activity of polyphenols

Oxidative stress arises from the imbalance between the concentration of free radicals and antioxidants in the body (Galano et al., 2016; Hellwig, 2019). If endogenous antioxidants from biological systems are not sufficient in the fight against free radicals, their deficiency can be compensated by exogenous antioxidants (Santos-Sánchez et al., 2019). These include polyphenols, of which flavonoids, in particular, have a remarkable ability to inactivate highly reactive forms of molecules (Zhong and Zhou, 2013; Lipiński et al., 2017). Several articles have been published on the antioxidant properties of wine by-products rich in phenolic compounds (Martins et al., 2016; Balea et al., 2018; Casagrande et al., 2019). The linear relationship between the total polyphenol content of grape pomace and its antioxidant activity has been confirmed by several authors (Marchante et al., 2018; Mohamed Ahmed et al., 2020). Shi et al. (2003) found that polyphenols in grapes have an antioxidant power 20 times stronger than vitamin E and 50 times stronger than vitamin C. The positive effect of feeding grape pomace and its extracts on antioxidant status was confirmed in poultry (Yang et al., 2017; Ebrahimzadeh et al., 2018a, b; Chamorro et al., 2017), pigs (Kafantaris et al., 2018; Chedea et al., 2019; Wang et al., 2019) and ewes (Buffa et al., 2020).

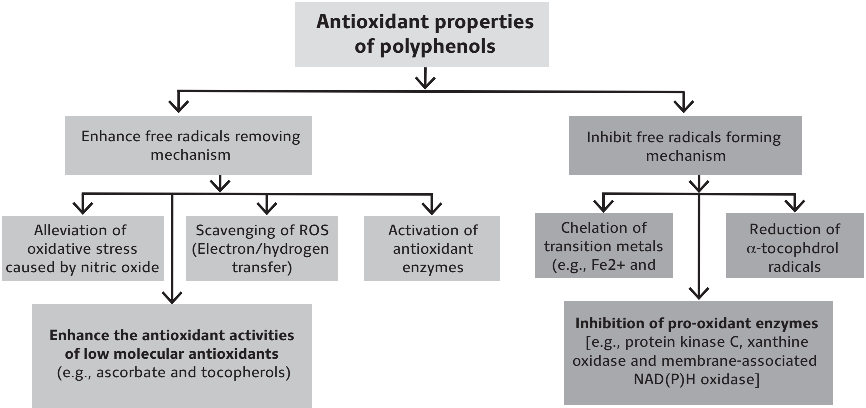

Figure 2 Antioxidant properties of polyphenolic compounds (Abdel-Moneim et al., 2020)

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5 • THE EFFECT OF GRAPE BY-PRODUCTS ON NUTRIENTS DIGESTIBILITY AND BIOCHEMICAL INDICATORS IN RUMINANTS

The addition of dried grape pomace affected the apparent digestibility of crude protein (Juráček et al., 2021). However, Guerra-Rivas et al. reported weak effects on ruminal degradation parameters of crude protein after feeding with grape by-products. Juráček et al. (2021) observed a lower digestibility coefficient of crude protein. This result corresponds with the findings of Bahrami et al. (2010). In addition, Ishida et al. (2015) found the effect of grape pomace feeding on the digestibility of crude protein. It can be assumed that this was due to a higher proportion of grape pomace from dry matter intake (24% of dry matter intake of wethers). Similarly, Abarghuei et al. (2010) and Jayanegara et al. (2012) determined lower crude protein digestibility in ruminants. However, differences in the digestibility of other nutrients (NFC, OM, and NDF) after the addition of grape pomace can be affected by its higher dosage (Juráček et al., 2021). Foiklang et al. (2016) reported higher digestibility of organic matter and NDF with a higher dosage of grape pomace in the daily diet. In contrast, Baumgärtel et al. (2007) observed the negative effect of grape pomace feeding on the digestibility of nutrients in sheep. However, Tayengwa et al. (2021) determined a higher digestibility of OM after feeding dried grape pomace in steers.

Table 10 Digestibility coefficients (%) of the different feeding groups (Juráček et al., 2021)

	Feeding Groups		
	C	GP1	GP2
CP	70.22[a] ± 1.19	72.17[b] ± 1.46	73.49[b] ± 0.98
EE	58.10 ± 3.45	63.03 ± 0.58	60.76 ± 2.69
CF	47.25 ± 4.04	50.25 ± 1.94	51.30 ± 1.14
NFE	67.31 ± 2.30	66.91 ± 1.44	69.85 ± 0.51
NFC	77.52[a] ± 1.07	77.78[a] ± 1.58	79.93[b] ± 0.16
OM	62.32[a] ± 2.83	62.93[a] ± 0.53	65.09[b] ± 0.62
ADF	49.49 ± 3.04	50.36 ± 0.24	51.37 ± 1.01
NDF	49.91[a] ± 3.83	51.40[a] ± 0.53	53.97[b] ± 1.19

Abbreviations: **C** – control, **GP1** – 1% addition of dried grape pomace from daily dry matter intake (DMI), **GP2** – 2% addition of dried grape pomace from daily DMI, **CP** – crude protein, **EE** – ether extract, **CF** – crude fibre, **NFE** – nitrogen free extract, **NFC** – nonfibre carbohydrates, **OM** – organic matter, **ADF** – acid detergent fibre, **NDF** – neutral detergent fibre. Different letters in the row indicate statistical differences (Tukey test, $p < 0.05$); data are presented as mean ± SD.

One of the factors that can affect the serum blood mineral profile is feeding (Pavlata et al., 2012). Minerals perform a number of important physiological functions, such as the effect on acid-base balance, osmotic pressure, adrenal function, normal heart function, and also the metabolism of proteins or carbohydrates (Persson and Luthman, 1974). However, Juráček et al. (2021) reported that the addition of dried grape pomace did not affect the serum P content in blood. However, the average P content in blood serum concentrations was higher than the upper limits of Tschuor et al. (2008). In contrast, Jelinek et al. (1984) determined a similar serum blood P content in rams, i.e., from 2.49 to 2.92 mmol.L^{-1}, affected by the age of the animals. Then, Chedea et al. (2017) did not find the effect of dried GP feeding on the serum blood P content in dairy cows. Subsequently, Juráček et al. (2021) determined the Ca content in the blood serum from 3.08 to 3.10 mmol.L^{-1} without the effect of GP on the blood Ca content after GP feeding. Merck (2019) reported a similar Ca content from 2.88 to 3.20 mmol.L^{-1}. Comparable serum Ca concentrations were also reported by Dias et al. (2010) and Kovacik et al. (2017), but over the limit reported by Schweinzer et al. (2017). Furthermore, Chedea et al. (2017) reported an effect of dried GP on Ca blood serum content in dairy cows (dosage 15% dried GP dose from DM). Similarly, Iannaccone et al. (2018) also reported a significant effect on the Ca content in Fresian calves after 10% addition of dried GP meal in concentrate. Juráček et al. (2021) found a ratio of Ca: P 1.07: 1 (C, GP1) and 1.13: 1 (GP2), which was also confirmed by Vrzgula et al. (1990). Juráček et al. (2021) then determined increased Mg concentrations (from 1.32 to 1.92 $mmol.L^{-1}$) after the GP feeding, which were higher than upper limits of 1.10 $mmol.L^{-1}$ reported by Tschuor et al. (2008) and 1.31 $mmol.L^{-1}$ determined by Merck (2019). Similarly, Šimpraga et al. (2013) determined the content of Mg in the range of 1.30–1.60 $mmol.L^{-1}$. Subsequently Juráček et al. (2021) reported a decrease in the Na + content after the addition of GP, but its content was in the 130.00-155.00 mmol.L-1 interval reported by Vrzgula et al. (1990). However, Kovacik et al. (2017) determined the lower Na^+ values in blood serum. The GP addition did not decrease significantly from 6.01 to 5.30 $mmol.L^{-1}$ the K^+ content (Juráček et al., 2021). According to Merck (2019), the reference range for K^+ in sheep is 3.90 to 5.40 $mmol.L^{-1}$. In contrast, Schuur et al. (2008) reported the K^+ interval from 4.60 to 6.50 $mmol.L^{-1}$. After that, Juráček et al. (2021) reported the higher ratio of Na and K after feeding by GP, which was similar compared to Vrzgula et al. (1990). 2% GP intake significantly increased Cl concentrations from 105.28 to 108.40 $mmol.L^{-1}$ (Juráček et al., 2021). Similar, Cl concentrations in blood serum were published by Vrzgula et al. (1990) and Tschour et al. (2008), but they were higher compared to Merck (2019). However, Kovacik et al. (2017) found higher concentrations of Cl^-.

The physiological range of glucose values was from 2.30 to 4.44 $mmol.L^{-1}$ (Tschour et al., 2008). However, Juráček et al. (2021) determined the decreasing effect of GP addition on glucose concentration (from 3.90 to 3.17 $mmol.L^{-1}$), which was also confirmed by Iannaccone et al. (2018). Similarly, Chedea et al. (2017) reported a decrease in glucose concentration after the addition of GP in dairy cows, and

Kolláthová et al. (2020) in horses. The decrease in glucose is probably related to the low energy value of GP Winkler et al. (2015). The decreased glucose content can also be associated with liver damage (cf. Jing et al., 2021). In contrast, Alba et al. (2019) reported statistically higher blood glucose after the addition of grape residue flour in lactating sheep. Juráček et al. (2021) reported a similar cholesterol content after GP feeding compared to the control group (1.01 mmol.L^{-1}). Similarly, Bahrami and Chekani-Azar (2010) and Alba et al. (2019) did not find differences in cholesterol concentrations after GP feeding. In addition to antioxidant activity, polyphenols have been shown to have several cardioprotective and atheroprotective effects, for example, by lowering plasma cholesterol levels (Iannaccone et al. 2018). Then, Juráček et al. (2021) determined the tendency to increase triglycerides (TG) after grape pomace feeding. Similarly, Chedea et al. (2017) described that GP feeding did not affect triglyceride values. However, Alba et al. (2019) reported an increasing effect of GP addition on TG concentrations in dairy sheep.

Changes in protein, albumin, and urea levels are needed to diagnose nitrogen metabolism disorders (Vrzgula et al., 1990). Juráček et al. (2021) reported increasing tendency in total protein content (from 74.45 to 77.25 g.L^{-1}), globulin (from 40.83 to 53.91 g.L^{-1}) and urea (from 6.36 to 6.52 mmol.L^{-1}) content and decreased albumin content (from 33.87 to 23.34 g.L^{-1}) after the addition of 2% grape pomace. In contrast, Alba et al. (2019) determined significantly lower TP, GLB, and urea after adding grape pomace residue in lactating sheep. They also similarly determined the same effect after GP feeding on albumin content. However, Bahrami and Chekani-Azar (2010) found no effect of GP addition on total protein serum content. The concentration of total proteins, albumin, globulin, and urea was in the reference range (Šimpraga et al., 2013). In contrast, Jelínek et al. (2003), Panev et al. (2013), and Carlos et al. (2015) revealed lower average total proteins in sheep.

Juráček et al. (2021) reported enzymatic profile indicators (AST, ALT, ALP) after the GP feeding comparable to Tschour et al. (2008)

and Rahman et al. (2018). Additionally, Juráček et al. (2021) determined the GGT values (from 0.14 to 0.20 βkat.L^{-1}) were under the limit recommended by Tschour et al. (2008), Lepherd et al. (2009) and Shek Vugrovecki et al. (2017). However, after GP feeding nonsignificantly lower AST values (from 2.02 to 1.26 βkat.L^{-1}) and higher ALT (from 0.34 to 0.41 βkat.L^{-1}), ALP (from 3.49 to 5.16 βkat.L^{-1}) and GGT (from 0.14 to 0.20 βkat.L^{-1}) values were observed. Furthermore, Chedea et al. (2017) found no effect of GP feeding in dairy cows on AST, ALP, and GGT. Similarly, Iannaccone et al. (2018) did not confirm the effect of GP addition on AST and ALT values in calves. Additionally, Nudda et al. (2015) described a nonsignificant effect of grape seed addition (300 g per day) on sheep AST and ALT parameters, but statistically significant higher ALP and lower GGT were observed. Finally, the decrease in AST values in lambs after feeding of GP was reported by Jin et al. (2016).

Table 11 Biochemical wether blood parameters (Juráček et al., 2021)

Parameters	Units	Feeding Groups		
		C	GP1	GP2
P	mmol.L^{-1}	2.89 ± 0.18	2.87 ± 0.16	2.75 ± 0.50
Ca	mmol.L^{-1}	3.09 ± 1.12	3.08 ± 0.42	3.10 ± 0.77
Mg	mmol.L^{-1}	1.69 ± 0.92	1.92 ± 0.96	1.32 ± 0.44
Na	mmol.L^{-1}	143.08 ± 2.96	135.13 ± 8.18	140.63 ± 1.96
K	mmol.L^{-1}	6.01 ± 1.16	5.62 ± 0.33	5.30 ± 0.06
Cl^-	mmol.L^{-1}	105.28^{a} ± 1.68	106.60^{a} ± 0.91	108.40^{b} ± 1.47
GLU	mmol.L^{-1}	3.90^{a} ± 0.30	3.17^{b} ± 1.05	3.26^{b} ± 0.35
CHOL	mmol.L^{-1}	1.01 ± 0.00	1.01 ± 0.00	1.01 ± 0.00
TG	mmol.L^{-1}	0.45 ± 0.06	0.53 ± 0.08	0.43 ± 0.07
TP	g.L^{-1}	74.45 ± 8.18	77.25 ± 6.01	66.25 ± 15.35
ALB	g.L^{-1}	33.87 ± 3.43	23.34 ± 10.15	29.41 ± 6.39
GLB	g.L^{-1}	40.83 ± 9.44	53.91 ± 12.97	46.50 ± 10.64
UREA	mmol.L^{-1}	6.36 ± 1.19	6.52 ± 0.86	5.63 ± 0.75
AST	μkat.L^{-1}	2.02 ± 0.79	1.26 ± 0.69	1.57 ± 0.28

Parameters	Units	Feeding Groups		
		C	GP1	GP2
ALT	μkat.L^{-1}	0.34 ± 0.14	0.40 ± 0.08	0.41 ± 0.04
ALP	μkat.L^{-1}	3.49 ± 1.51	4.34 ± 1.24	5.16 ± 1.37
GGT	μkat.L^{-1}	0.14 ± 0.08	0.20 ± 0.09	0.17 ± 0.06

Abbreviations: **C** – control, **GP1** – 1% addition of dried grape pomace from daily dry matter intake (DMI), **GP2** – 2% addition of dried grape pomace from daily DMI, **GLU** – glucose, **CHOL** – cholesterol, **TG** – triglycerides, **TP** – total protein, **ALB** – albumins, **GLB** – globulins, **AST** – aspartate aminotransferase, **ALT** – alanine aminotransferase, **ALP** – alkaline phosphatase, **GGT** – gamma glutamyl transferase. Different letters in row indicate statistical differences (Tukey test, $p < 0.05$); data are presented as mean ± SD.

The addition of GP to the daily diet of ruminants increased the digestibility of nutrients without a negative effect on the biochemical profile of the animals. The digestibility of crude protein, NFC, NDF, and OM in wethers was significantly higher at a higher dose of dried grape pomace (2% of GP). The addition of GP to the daily diet did not affect the nitrogen, enzymatic, mineral, and energetic profile of wether blood serum except Cl^- and glucose (2% of GP). Dried grape pomace in an amount of 2% dry matter in the diet can be considered as a suitable source of nutrients in the feeding of sheep, which should also improve the digestibility of diet crude protein, non-fibre carbohydrates, neutral detergent fibre and organic matter.

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6 • THE EFFECT OF GRAPE BY-PRODUCTS ON WETHER BLOOD CELL COUNT PARAMETERS

Grape by-products are sources of many bioactive compounds, which have the potential to be positive in the feed ration for increasing their nutritional value (Ivanišová et al., 2019). In addition, some parts of plants can possess biochemical and antioxidant activity; the crucial thing is to understand the therapeutic effects on human or animal metabolism (Brindza et al. 2015). According to Westendarp (2006) tannins have various physiological effects - anti-irritant, antisecretolytic, antiphlogistic, antimicrobial or antiparasitic. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of the mouth and throat, or slightly injured skin. Studies with ruminants have also shown that the denaturing properties of tannins can possibly be used to improve the supply of protein to the small intestine. However, high tannin concentrations resulted in reduced animal performance and health disorders, both in ruminants and in monogastric animals (Westendarp, 2006). In the immune system, leucocytes create a part of cell-mediated immunity. Lymphocytes are responsible for specific immunity. One part of lymphocytes are memory T-cells, which protect the body against previously encountered pathogens (Kováčik et al. 2015). Erythrocytes from all mammals are anucleated, and most of them are in the shape of biconcave discs called discocytes (Harvey, 1997). Normally, mammalian erythrocytes circulate in blood for 2 to 5 months despite limited synthetic capacities and repeated exposure to mechanical and metabolic insults. Erythrocytes have three functions: transport of oxygen to tissues, transport of carbon dioxide to the lungs, and buffering of hydrogen ions H+ (Harvey, 2012). Haemoglobin is formed from hem, where it is bivalent iron, and from the protein (globin) part (Kováčik et al., 2015). The hemoglobin tetramer is capable of binding to four O2 molecules when fully oxygenated. Furthermore, hemoglobin is the main protein buffer in the blood. Haematocrit is characterized as a proportion between blood cells and blood volume. The other erythrocyte indices include the mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and red cell distribution width (RDWc) (Harvey, 2012). MCV represents the average volume of a single erythrocyte in femtoliters in a population of erythrocytes (Willekens et al., 2003). MCH is calculated by dividing the hemoglobin value (in grams per decilitre) by the RBC count (in millions of cells per microliter) and multiplying by 10. MCHC represents the average haemoglobin concentration within erythrocytes. RDWc is an electronic measure of anisocytosis or erythrocyte volume heterogeneity. Thrombocytes, blood platelets in mammals, are small round-to-oval anucleated cell fragments, thin discs when unstimulated, that form from proplatelet cylinders of megakaryocyte cytoplasm (Harvey, 2012). To thrombocytes, indices belong to platelet count (PLT) and mean platelet volume (MPV). MPV is the average volume of a single platelet recorded in femtoliters. Within the normal range of platelet count and MPV, there is an inverse correlation between platelet counts and MPV (Bessman et al., 1981). Normal physiological values of different blood parameters of animals are influenced by several factors such as age, sex breed, season, altitude, climatic conditions, life habits of the species and nutrition (Jelínek et al., 1996; Frelich et al., 2006; Tripathi et al., 2008). Evaluating blood parameters in animals used for experiment is essential for determining the positive or negative effects. During the entire experiment, the blood parameters of the given animals must be within the physiological range (Rolinec et al., 2013). Some components of plants or by-products obtained, e.g., by grape processing, are known to have the potential to influence the health status (Brindza et al., 2015; Ivanišová et al., 2019). Therefore, blood analysis is an appropriate tool to monitor the effects of changes in feed ration on the physiological reaction of animals (Rolinec et al. 2013). Bíro et al. (2019) and Rolinec et al. (2019) conducted an experiment with wethers fed with a diet containing dried grape pomace. The parameters of blood cell count were analysed. They tested two levels of grape pomace, 1 and 2%. The diets used in this experiment are shown in Table 12. The results of this experiment are shown in Table 13.

Table 12 Composition of diets of wethers fed in each of the experimental period (Bíro et al., 2019; Rolinec et al., 2019)

	Meadow hay	Wheat ground	Soybean meal	Dried grape pomace
1st time period (19 days)*	66%	23%	11%	-
2nd time period (12 days)*	66%	22%	11%	1%
3rd time period (12 days)*	66%	22%	11%	2%

* Blood samples were collected during the last day of each time period.

Table 13 Wether blood cell count parameters during the whole experiment (Bíro et al., 2019; Rolinec et al., 2019)

Mean	1st blood sampling	2nd blood sampling	3rd blood sampling	SEM
White blood cells (x $10^9.l^{-1}$)	9.18	9.63	9.16	0.281
Lymphocyte counts (x $10^9.l^{-1}$)	6.31	7.11	6.88	0.204
Medium size cells count (x $10^9.l^{-1}$)	0.05	0.05	0.05	0.001
Granulocyte counts (x $10^9.l^{-1}$)	2.82	2.47	2.24	0.228
Lymphocytes percentage (%)	68.83	73.96	75.09	1.701
Medium size cells (%)	0.50	0.50	0.50	β<0.000
Granulocytes (%)	30.68	25.58	24.48	1.698
Red blood cell count (x $10^{12}.l^{-1}$).	14.91	14.65	13.45	0.199
Haemoglobin (g.l^{-1})	152.6	150.4	135.9	1.850
Haematocrit (%)	35.82	33.67	31.56	0.425
Mean corpuscular volume (fl)	24.13	22.88	23.63	0.277

Mean	1st blood sampling	2nd blood sampling	3rd blood sampling	SEM
Mean corpuscular haemoglobin (pg)	10.25	10.29	10.10	0.053
MCHC ($g.l^{-1}$)	426.6	447.3	431.4	4.354
Red cell distribution width (%)	24.59	23.91	23.36	0.278
Platelet count (x $10^9.l^{-1}$)	505.9	597.4	448.4	51.120
Platelet percentage (%)	0.26	0.30	0.21	0.025
Mean platelet volume (fl)	5.23	5.09	4.60	0.086
Platelet distribution width (%)	26.58	26.33	24.78	0.366

SEM – standard error of the mean, **MCHC** – mean corpuscular haemoglobin concentration

The fundamental prerequisite for sheep performance is genetic (Gábor et al. 2006), however, nutrition is also very important (Gálik et al. 2014; Šimko et al. 2014). In feeding farm animals, it is possible to use some by-products produced during food processing. Currently, some experiments are aimed at detecting the properties of grape products and determining the effect of these on the performance of the farm animal. Due to its concentration of polyphenols, grape pomace has become an attractive research field, mainly in ruminant feeding. Peixoto et al. (2018) also declared that grape pomace is a source of phenolic compounds and diverse bioactive properties. Correddu (2014) fed dairy ewes with the addition of grape seeds to the feed ration and did not find an adverse effect on milk production traits and health status; moreover, he detected an immunomodulatory effect. On the other hand, high tannin concentrations could lead to a reduction in animal performance and health disorders, as well as in ruminants and monogastric animals (Westendarp, 2006). The positive or negative effect of changes in feed ration is expressed first in the blood of animals (Rolinec et al. 2013). As detected in this study, the count of leucocytes and lymphocytes was within the range of values published by other authors. Šoch et al. (2011) determined white blood cells in ewes from south Bohemia sampled at different altitudes, seasons and years in an interval of 6.47 to 9.57 x $10^9.l^{-1}$. Binev et al. (2006) published WBC in Ile de France breed with similar weight as it was in this study with a value of 8 x $10^9.l^{-1}$. After twelve days, consumption of the feed ration supplemented with dried grape pomace meal increased WBC from 9.18 to 9.63. A similar increase in white blood cell count was detected by Cash et al. (2016). They drenched katahdin lambs every 7 days with fermented Pinot Noir grape extract; at the start of the experiment, the white blood cell count was 9.95, after 63 days the WBC increased by 10.26. However, white blood cell count in control lambs and in lambs drenched every 14 days decreased from 9.56 to 7.97 x $10^9.l^{-1}$. According to Sehm et al. (2011) the increase of white blood cell count can be influenced by polyphenol rich feeding, which have the potential to positively influence the blood parameters, and the pattern of white blood cell mRNA gene expression of immunological marker genes. On the other hand, Correddu (2014) published a lower white blood cell count (8.5 x $10^9.l^{-1}$) in dairy sheep with a diet containing 300 g of grape seed per day/head compared to sheep in the control group (8.9 x $10^9.l^{-1}$). Supplementation of feed ration with 1% of dried grape pomace meal increased lymphocytes from 6.13 to 7.11 x $10^9.l^{-1}$ (Rolinec et al., 2019). However, supplementation with 2% dried grape pomace decreased lymphocyte count compared to 1% supplementation from 7.11 to 6.88 x $10^9.l^{-1}$ (Rolinec et al., 2019). Cash et al. (2016) detected decrease in lymphocytes after drenching of fermented Pinot Noir grape extract in lambs. Correddu (2014) also determined a lower percentage of lymphocytes of dairy sheep ingested in feed ration 300 g/day/head of grape seeds compared to the control group (53.7 vs 52.3%). However, other farm animals such as piglets (Sehm et al. 2011) and broiler chicks (El-Kelawy et al., 2018) showed an increased trend of lymphocyte percentage after dietary inclusion of fruit pomace or grape seeds, respectively. The widely accepted explanation for positive effects of condensed tannins (one of the sources is also grape pomace) on protein digestion and metabolism is that condensed tannins-protein complexes escape ruminal degradation resulting in greater protein availability in the lower tract. In order to confirm this explanation, however, further studies of complexes between condensed tannins and carbohydrates, endogenous proteins and microbial products and also the degradability of complexes between condensed tannins and proteins by ruminal microbes would need to be conducted (Reed, 1995). However, with the protection of dietary proteins against degradation in the rumen via condensed tannins, amino acid supply to the abomasum and small intestine increases, which results in improved nutritional status of the animals (Min and Hart 2003; Hoste et al. 2006). As published Heckendorn et al. (2007), the enhanced immune response can be mediated by improved protein availability. According to Rolinec et al. (2019), this may be the reason for the detected increase in white blood cells and lymphocytes after 12 days of supplementation of wether feed ration with dried grape pomace.

Similar results to the ones published by Bíro et al. (2019) for red blood cells and haemoglobin concentration (Table 13) were found also in the study by Soysal et al. (2011), where they researched the effect of the conventional and organic management system and genotype on the haematological parameters of sheep. They determined red blood cells in intervals from 12.9 to 15.5 x $10^{12}.l^{-1}$ and haemoglobin in intervals from 138 to 149 $g.l^{-1}$. Other authors published a lower count of red blood cells from 7.3 to 10.3 x $10^{12}.l^{-1}$ as well as of haemoglobin from 67 to 118 $g.l^{-1}$ in comparison with results of this study (Binev et al., 2006; Correddu, 2014; Moneva et al., 2016; Cash et al., 2016; Šoch et al., 2011). Haematocrit value from 33.67 to 31.56 % detected after ingestion of dried grape pomace meal (Table 13) was higher than the value detected by Correddu (2014), namely, 30.5 % for dairy ewes fed with the addition of 300 g per head/day of grape seeds, and by Paolini et al. (2003), i.e., 26 % for goats experimentally infected with *Haemonchus contortus* but fed with the addition of tannins. On the other hand, by lambs drenched with organic Pinot Noir fermented grape extract Cash et al. (2016) determined values of haematocrit similar to those in the study of Bíro et al. (2019). Rolinec et al. (2012) conducted an experiment with *in vivo* nutrients digestibility with Lacaune wethers and between the start and the end of the experiment detected a similar decrease in haemoglobin concentration from 154.8 to 143.5 $g.l^{-1}$. Red blood cells, haemoglobin concentration, and haematocrit decreased between the first and second blood sampling, as well as between the second and third blood sampling (Table 13). All three feed rations had enough copper but were slightly deficient in iron (data not shown). Bíro et al. (2019) hypothesised, that shortage of iron can be the reason for the decrease in RBC, HGB and HCT throughout the study. Harvey (1997) reported that iron deficiency usually results in anaemia in mammals. Vitseva et al. (2005) published that flavonoids derived from the purple grape have anti-thrombotic and anti-inflammatory properties. Bijak et al. (2013) determined that the polyphenol-rich extract of grape seeds acts as a prevention of thrombotic states. There have been too few experiments to determine the count of thrombocytes in sheep blood after ingestion of grape seeds. Correddu (2014) published that sheep in the group fed with the addition of 300 g of grape seed per head/day had a lower thrombocyte count, i.e., 643 vs. 729 x $10^9.l^{-1}$. A similar decrease in thrombocyte count was determined by Cash et al. (2016) after the use of organic Pinot noir fermented grape extract. Based on the results shown in Table 13, Bíro et al. (2019) concluded that ingestion of dried grape pomace in an amount tested in this study had no negative effect on sheep blood erythrocyte and thrombocyte parameters. Furthermore, Rolinec et al. (2019) claimed that the ingestion of dried grape pomace had no negative effect on the parameters of white blood cells.

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7 • THE EFFECT OF GRAPE BY-PRODUCTS ON THE NUTRITIONAL VALUE OF COW COLOSTRUM

Some by-products of the wine industry have a potential to improve the quality of animal products. Grape pomace is a by-product containing many biological substances with the potential to improve the nutritional quality of cow colostrum. The nutritional quality of the colostrum and the quality of the colostral fat is affected by the nutritional status of dry cows in the late state of pregnancy. For the newborn calf, ingesting colostrum in sufficient amounts is essential. In general, under colostrum we understand the udder secrete discharged for two or three days after calving. Primarily, colostrum provides energy and passive immunization to the newborn calf. Colostral protein and fat composition play an important role in health support and good performance. Other colostrum components, such as lactose, minerals, vitamins, and biologically active substances, make the colostrum a complete feed for the newborn calf. The most researched nutrient of cow colostrum is crude protein, whose concentration, as published by Mechor et al. (1992) is in positive correlation with immunoglobulin G concentration. Therefore, the components of colostrum crude protein have an essential effect on the passive immunization of the newborn calf. Contarini et al. (2014) reported that one of the least considered colostrum components is fat. Studies on colostrum fat have focused mainly on its total content in the first days after calving. The positive health effects of colostrum fat are promoted by oleic acid, n-3 fatty acids, or some poly unsaturated fatty acids (Haug et al., 2007). Some PUFA and n-3 FA play a very important role for the newborn calf, supporting the development of the emerging immune system and developing the brain and retina (Barceló-Coblijn and Murphy, 2009; Hill et al., 2011). Given the importance of colostrum for calf nutrition, the composition and quality of its fat fraction cannot be ignored and needs to be updated (Contarini et al., 2014). Grape pomace is a wine by-product, now used as a feed rich in several phytochemicals with a positive effect on health (Ivanišová et al., 2019). Scuderi et al. (2019) reported that grape pomace is rich in secondary compounds, including condensed tannins, and is used as a supplement in livestock feeding practices. They found that milk from cows fed with the addition of grape pomace was higher in immunomodulatory protein concentration; however, no effect on milk fat concentration was observed. According to Ianni's and Martino's study, (2020) grape pomace is a feed enriched by polyphenols and dietary fibre with the ability to induce beneficial effects on cow milk quality. First, a general increase in the concentration of polyunsaturated fatty acids was listed. Following these positive results of grape pomace on cow milk and the lack of information on the effect of grape pomace feeding on fatty acids and nutrient composition of cow colostrum, Rolinec et al. (2021) conducted a study aimed at determining the effect of grape pomace intake on nutritional quality and fatty acids of cow colostrum. The given cows were of the Slovak spotted breed. In this study, pregnant cows in the grape pomace group (GP) consumed a diet containing grape pomace powder for 7 days before calving. The amount of grape pomace powder (0.116 kg/cow/day) in the GP group was established according to the results of the previous studies conducted with ruminants, such as Bíro et al. (2019) and Rolinec et al. (2019). The calculated dietary intake per cow/day was 22.5 kg of fresh matter in the CON group and 22.62 kg of fresh matter in the GP group. The calculated dietary intake after calving was 43.25 kg of fresh matter per cow/day in both groups. The feeding scheme and the diet composition are shown in Table 14. Within 30 minutes after calving, the cows were milked with a vacuum milking machine. After milking and mixing, the first colostrum sample (0h) was taken. All the other colostrum samples were taken in a similar manner following this time schedule: 12th hour (12h), 24th hour (24h), 36th hour (36h), 48th hour (48h) and 60th hour (60h) after the first colostrum sampling. All colostrum samples were analysed for basal nutrients and fatty acid concentrations.

Table 14 Composition of the diets fed during the last 7 days before and after calving (Rolinec et al., 2021)

	During the last 7 days before calving		After calving
Feed (%)	**CON**	**GP**	**CON and GP**
Maize silage	57.77	57.47	48.60
Alfalfa silage	17.77	17.69	25.40
Whet straw	6.67	6.63	0.60
Rapeseed meal	5.33	5.31	5.50
Soybean meal	3.56	3.54	3.50
Barley grain	3.56	3.54	2.30
Maize grain	1.78	1.77	5.80
Wheat grain	1.78	1.77	5.20
Oat grain	1.07	1.06	-
Beet molasses	-	-	2.30
Mineral premix dry cow[1]	0.71	0.71	-
Mineral premix lactation[2]	-	-	0.80
Grape pomace powder	-	0.51	-

CON – control group, **GP** – grape pomace group

[1]-1 kg of mineral premix contains 4.0% Ca, 3.0% P, 4.0% Na, 10.0% Mg, 2 500 mg Cu, 10 000 mg Zn, 7 000 mg Mn, 50 mg Co, 200 mg I, 23 mg Se, 750 000 U.I. Vitamin A, 150 000 U.I. Vitamin D3, 7 000 mg Vitamin E, 140 mg Biotin, 25 000 mg Niacinamide (Mikrop Čebín, Czech Republic)

[2]-1 kg of mineral premix contains 18.0% Ca, 2.5% P, 9.0% Na, 8.0% Mg, 2 000 mg Cu, 5 000 mg Zn, 4 500 mg Mn, 25 mg Co, 120 mg I, 35 mg Se, 700 000 U.I. Vitamin A, 180 000 U.I. Vitamin D3, 3 000 mg Vitamin E, 80 mg Biotin (Mikrop Čebín, Czech Republic).

According to the results published by Rolinec et al. (2021), the colostrums of both groups of cows (CON and GP) meet the typical development of nutrients regarding time flow. In both groups, the concentration of solids, protein, casein, ash and solid-non-fat decreased between the first secret of the udder after calving and the colostrum sampled at the 60th hour after calving, while the lactose increased linearly ($P<0.001$) (Table 15 and 17). The fat concentration increased during the first 24 hours after calving and decreased in the period between 24 to 48 hours. The specific gravity value decreased within 36th hour after the calving and then remained alike. At all sampling times, protein concentration was not significantly higher ($P>0.05$) in the grape pomace group, compared to the control group. On the other hand, at all sampling times, the fat concentration was higher ($P>0.05$) in the control group, compared to the grape pomace group. At all sampling times, all the differences between the GP and CON groups were insignificant ($P>0.05$), except the somatic cell score at 12 h (Table 15). Saturated fatty acids were the group of fatty acids with the highest concentration in both groups of cows and at all sampling times. More valuable polyunsaturated fatty acids had the lowest concentration in the cow colostrum. At all sampling times, also, the saturated fatty acid colostrum concentration in the GP group was insignificantly lower ($P <0.05$), compared to the control group. During the first 60 hours after calving, oleic acid (C18:1) was the most abundant single FA in the. The concentration of n-3 fatty acids in both groups was highest in the first udder secrete (0h). The highest concentration of n-6 fatty acids was detected in the cows′ colostrum in the GP group at 0 h. At all sampling times, a nonsignificant effect ($P>0.05$) of dried grape pomace feeding during the last week of pregnancy on the fatty acid concentration of cows′ colostrum was detected (Table 16). With passing hours after calving, the polyunsaturated fatty acids linearly decreased in both groups ($P<0.05$) and the saturated fatty acids in both groups ($P<0.001$), while the monounsaturated fatty acids in the GP group ($P<0.05$) increased linearly (Table 17).

Table 15 Characteristics of cow colostrum during the experiment (%) (Rolinec et al., 2021)

Hours after calving	0h		12h		24h		36h		48h		60h	
Group	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**
Solids $\bar{x}$	22.1	23.0	19.1	18.8	16.6	15.6	15.7	14.7	14.4	14.0	14.2	14.6
SD	2.77	1.96	2.94	4.64	3.73	2.04	3.86	1.72	0.93	1.78	1.36	1.53
Fat $\bar{x}$	4.8	4.0	5.3	4.4	6.1	4.5	5.9	4.5	4.6	4.1	5.0	4.7
SD	1.98	1.36	1.65	2.89	4.92	1.44	4.40	1.25	0.89	1.44	1.46	1.69
Protein $\bar{x}$	13.4	15.2	9.4	10.6	5.7	6.4	4.9	5.2	4.7	4.8	4.4	4.7
SD	1.75	2.12	2.85	2.04	1.67	1.08	0.59	0.57	0.37	0.37	0.21	0.30
Lactose $\bar{x}$	2.2	2.1	3.0	2.5	4.0	3.7	4.1	4.1	4.3	4.2	3.9	4.4
SD	0.50	0.73	0.84	0.69	0.35	0.52	0.30	0.29	0.15	0.28	0.79	0.25
Ash $\bar{x}$	1.7	1.7	1.4	1.4	0.9	1.0	0.8	0.9	0.8	0.8	0.9	0.9
SD	0.27	0.23	0.30	0.30	0.23	0.15	0.07	0.15	0.06	0.18	0.17	0.14
Casein $\bar{x}$	12.1	13.9	8.3	9.4	4.7	5.5	4.0	4.3	3.9	4.0	3.6	3.8
SD	1.74	2.08	2.67	1.88	1.88	0.96	0.77	0.50	0.31	0.35	0.15	0.27
SNF $\bar{x}$	16.2	17.8	13.1	13.7	10.4	10.8	9.8	10.0	9.7	9.8	9.2	9.8
SD	1.34	1.75	2.04	2.12	1.47	0.91	0.74	0.47	0.37	0.23	0.50	0.35
SG $\bar{x}$	1048	1047	1040	1039	1036	1035	1035	1035	1035	1035	1035	1035
SD	4	9	6	7	2	0	0	0	0	0	0	0
SCS $\bar{x}$	3.8	3.3	4.2*	2.6*	3.3	3.5	3.7	3.3	3.3	3.5	3.5	3.2
SD	0.48	1.07	1.25	0.16	0.90	1.25	1.48	1.43	1.12	1.76	1.34	1.33

CON – control group of cows, **GP** – group of cows fed with the addition of dried grape pomace during last week of pregnancy, **SNF** – solid-non-fat, **SG** – specific gravity ($g.l^{-1}$), SCS – somatic cell score, **$\bar{x}$** – mean, **SD** – standard deviation, * within the hour, the values marked with * differ significantly between groups (P<0.05).

Sato et al. (1931) published cow colostrum quality parameters from his 18 experiments with different breeds. They determined the following intervals of nutrients for the colostrum sampled immediately after calving: total solids 17.36 to 30.00%; total protein 10.84 to 19.46%; casein 4.57 to 8.41%; fat 1.04 to 9.05% and lactose 1.05 to 2.56%. Even though these results were published in 1931, newer studies have confirmed high variability of colostrum nutrients. The production of nutrients by dairy cows is affected by genetics, but nutrition also plays a very important role (Trakovická et al., 2015; Imrich et al., 2021). The protein concentration of the colostrum sampled from dry cows fed with the addition of grape pomace immediately after calving was insignificantly higher compared to the control group (Rolinec et al., 2021) and higher than published by Mechor et al. (1992) for first milking after calving (12.5%), and by Puppel et al. (2019) in the review article (13.3%). A high concentration of solids and protein in the colostrum is highly desirable. As published by Mechor et al. (1992), the concentration of total solids and colostrum protein has a positive correlation with immunoglobulin G, responsible for the passive immunisation of newborn calves. According to Mechor et al. (1992), the addition of grape pomace powder could increase the concentration of colostrum proteins, and thus improve the immunological quality and health of newborn calves. Regarding the transfer of immunity from cows to calves, Franklin et al. (2005) highlighted the importance of dry cows feeding. They supplemented the dry cow diet with mannan-oligosaccharide and found a tendency to increase the concentrations of rotavirus antibodies in the colostrum of cows in the second and higher lactations. Scuderi et al. (2019) did not find a higher concentration of milk proteins in the cows fed with the addition of grape pomace, but found that a higher concentration of immunomodulatory proteins included bioactive proteins; this would be a feasible method to enhance the healthfulness of milk both for the milk-fed calf and the human consumer. In the study of Rolinec et al. (2021), at all sampling times, the concentration of colostral proteins was significantly higher in the group of cows fed with the addition of dried grape pomace. Therefore, the addition of grape pomace to the diet of pregnant cows could be seen as positive.

Table 16 Fatty acid concentration in cow colostrum (g.100 g-1 fat), (Rolinec et al., 2021)

Hours after calving	**0h**		**12h**		**24h**		**36h**		**48h**		**60h**	
Group	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**	**CON**	**GP**
PUFA x̄	11.3	11.0	7.3	8.1	7.5	5.0	7.1	5.2	6.3	6.2	7.3	6.5
SD	5.29	3.75	3.32	3.23	4.76	1.74	3.52	1.23	1.88	1.70	2.62	1.34
MUFA x̄	30.0	26.7	33.7	30.4	34.1	33.6	35.4	33.8	33.8	34.9	33.4	35.1
SD	5.26	10.64	4.08	6.45	3.74	3.66	5.74	3.92	6.17	5.50	8.74	3.71
SFA x̄	44.7	38.2	52.4	44.8	58.1	55.2	60.1	59.0	61.7	56.4	62.8	60.0
SD	14.51	12.44	7.40	12.25	12.63	9.70	8.61	6.41	5.58	8.40	4.49	4.00
PA x̄	24.9	21.6	23.7	21.8	19.3	21.7	19.4	21.6	22.3	18.2	22.6	20.3
SD	5.62	7.68	3.83	4.43	5.24	3.42	6.07	3.91	4.60	7.63	2.75	3.92
SA x̄	4.0	3.3	6.1	4.0	7.6	7.6	8.8	8.8	9.2	9.0	9.5	9.7
SD	0.87	4.50	2.19	2.44	1.53	1.62	1.14	0.95	1.47	2.03	2.17	1.73
OA x̄	35.8	38.5	33.7	35.5	31.7	32.3	31.8	30.3	29.6	31.6	28.9	31.1
SD	8.92	7.34	5.60	4.62	5.65	6.04	7.04	4.93	7.34	5.71	8.51	3.63
n6 x̄	2.5	4.7	1.2	2.4	2.3	1.6	2.6	2.2	2.9	2.5	3.5	3.0
SD	2.19	5.63	0.76	2.74	1.72	0.74	1.12	0.71	0.51	0.63	0.54	0.59
n3 x̄	1.6	1.5	1.3	1.3	1.4	1.2	1.4	1.1	1.4	1.3	1.4	1.4
SD	0.49	0.40	0.26	0.21	0.33	0.16	0.14	0.12	0.18	0.15	0.17	0.23
n6/n3 x̄	1.4	3.5	0.9	2.1	1.6	1.4	1.8	1.8	2.1	1.9	2.4	2.3
SD	1.15	5.15	0.51	2.81	1.11	0.64	0.75	0.59	0.32	0.60	0.38	0.56

CON – control group of cows, **GP** – group of cows fed with addition of dried grape pomace during last week of pregnancy, **PUFA** – polyunsaturated fatty acids, **MUFA** – monounsaturated fatty acids, SFA saturated fatty acids, **PA** – Palmitic acid (C16:0), **SA** – stearic acid (C18:0), **OA** – Oleic acid (C18:1), **n6** – fatty acids, **n3** – fatty acids, **n6/n3 ratio**, **x̄** –mean, **SD** – standard deviation, within the hour no significant difference between groups has been found.

Sato et al. (1931) published that cow colostrum is fairly constant in total ash content and specific gravity, which contrasts with the results published by Rolinec et al. (2021). However, Rolinec et al. (2021) also concluded that the percentage of total proteins, fat and lactose in the colostrum has wide fluctuation. Lactose concentration detected at 0h, 12h and 24 h after calving (Rolinec et al., 2021) was slightly lower than published by Nardone et al. (1997) and Puppel et al. (2019), but the concentration at 36 h after calving showed comparable results. Similarly to the findings published by Nardone et al. (1997), Rolinec et al. (2021) detected an increase in lactose concentration in both groups of cows (CON and GP) from 0 to 36 h after calving. For cows, the lowest lactose concentration in colostrum at 0 h followed by an increase with passing time is physiological. As reported by Zabielski et al. (1999), the concentration of lactose in the neonatal calf is lowest at birth and increases with time.

Table 17 Linear effects of the graded time from the calving on the nutrients concentration (Rolinec et al., unpublished results)

Item	Group	SEM	Linear	
			P-value	**Contrast**
Solids	**CON**	0.866	<0.001	-6.899
	GP	0.961	<0.001	-7.042
Fat	**CON**	0.697	0.754	-0.221
	GP	0.639	0.599	0.340
Protein	**CON**	0.664	<0.001	-7.469
	GP	0.475	<0.001	-8.406
Lactose	**CON**	0.243	<0.001	1.509
	GP	0.142	<0.001	1.700
Ash	**CON**	0.088	<0.001	-0.720
	GP	0.081	<0.001	-0.676
Casein	**CON**	0.621	<0.001	-7.066
	GP	0.443	<0.001	-8.060
SNF	**CON**	0.496	<0.001	-5.741
	GP	0.457	<0.001	-6.517
SG	**CON**	1.260	<0.001	-9.383
	GP	1.640	<0.001	-9.920
SCS	**CON**	0.539	0.270	-0.606
	GP	0.611	0.989	-0.009

Item	Group	SEM	Linear	
			P-value	Contrast
PUFA	CON	1.327	0.027	-3.102
	GP	0.865	0.020	-2.135
MUFA	CON	2.531	0.460	1.895
	GP	1.992	0.025	4.722
SFA	CON	3.587	<0.001	15.840
	GP	3.877	<0.001	18.029
PA	CON	2.122	0.340	-2.061
	GP	2.319	0.781	0.652
SA	CON	0.769	<0.001	4.378
	GP	1.084	<0.001	4.938
OA	CON	3.131	0.043	-6.634
	GP	2.359	0.006	-7.073
n6	CON	0.484	0.003	1.573
	GP	1.055	0.335	-1.034
n3	CON	0.127	0.411	-0.106
	GP	0.102	0.068	-0.194
n6/n3	CON	0.308	<0.001	1.277
	GP	0.985	0.339	-0.957

CON – control group of cows, **GP** – group of cows fed with the addition of dried grape pomace during last week of pregnancy, **SEM** – standard error of the mean, **SNF** – solid-non-fat, **SG** – specific gravity ($g.L^{-1}$), **SCS** – somatic cell score, **PUFA** – polyunsaturated fatty acids, **MUFA** – monounsaturated fatty acids, **SFA** saturated fatty acids, **PA** – Palmitic acid (C16:0), **SA** – stearic acid (C18:0), **OA** – Oleic acid (C18:1), **n6** – fatty acids, **n3** – fatty acids, **n6/n3** – ratio

Kehoe et al. (2007) analysed colostrum samples from 15 farms in Pennsylvania. They sampled the colostrum within 4 hours after calving and published the ash concentration at an average value of 0.5%, which is a three times lower value compared to the first colostrum sample (0h) published by Rolinec et al. (2021). Godden (2008) and Tsioulpas et al. (2007) published a lower concentration of ash in cow colostrum: 1.11 and 1.18%, respectively. The detected lower concentration of lactose, along with the higher concentration of ash compared to other studies, can be explained by the osmotic function of lactose in milk. Holt and Jenness (1984) stated that milk with a low level of lactose has an elevated level of inorganic elements. The specific gravity of the first udder secret after calving is in interval 1048 to 1072 $g.l^{-1}$ and decreased over time to average specific gravity of milk 1029 $g.l^{-1}$ (Madsen et al., 2004; Walstra et al., 2005; McGrath et al., 2016). The decrease in specific gravity is in negative correlation with an increase in lactose concentration (Madsen et al., 2004). The somatic cell count is affected mainly by the genetic, health status, and udder infection with microorganisms (Strapáková et al. 2016; Pecka-Kielb et al. 2016). In general, however, the somatic cell count of colostrum is higher compared to that of milk. Nguyen and Neville (1998) attributed the high somatic cell count in colostrum not to mastitic infection but to a physiological feature, i.e., the penetration of cells through leaky tight junctions between the mammary epithelial cells. In a review by McGrath et al. (2016), the somatic cell score of cow colostrum between 3.70 and 4.15 was published. The lower value of the somatic cell score detected by Rolinec et al. (2021) could be affected by the breed (Slovak spotted breed), which is not as predisposed to mastitis as, for example, Holstein Friesian cows. Contarini et al. (2014) listed a comprehensive description of the roles of colostral fat in newborn calves. Colostral fat supplies essential nutrients to provide energy, increase metabolism, and protect the newborn against microbial infection. Furthermore, fatty acid oxidation is useful to continue active gluconeogenesis to maintain glucose homeostasis (Hammon et al., 2012). The concentration of fat in the colostrum is very varied. The concentration of colostral fat directly at calving was published by Yaylak et al. (2018) for Simmental cows 8.50% and by Abd El-Fattah et al. (2012) for Holstein Friesian cows 8.04%. Also, Nardone et al. (1997) published higher values of fat of the first udder secrete (5.9 and 6.0%), compared to the result of Rolinec et al. (2021), 4.0 and 4.8%, respectively. On the other hand, results published by Nardone et al. (1997) show that at 24 hours after calving, lower values of colostral fat (3.8 and 2.9%) were found, compared to the values published by Rolinec et al. (2021), namely 4.5 and 6.1%. The average value of colostral fat published by Godden (2008) as well as Kehoe et al. (2007), i.e., 6.7%, was higher than that detected in all colostrum samples detected by Rolinec et al. (2021). Scuderi et al. (2019) determined the effect of including grape marc in dairy cattle rations, which is similar to the conclusions of Rolinec et al. (2021). Rolinec et al. (2021) took colostrum samples in 12-hour time periods, which was shorter compared to other studies. But the development of fatty acids concentration is comparable to that published by Contarini et al. (2014) for Holstein cows. Between 0 h and 60 h after calving, the concentration of saturated fatty acids and monounsaturated fatty acids increased, and the concentration of polyunsaturated fatty acids decreased (Table 16). Also, the colostrum of Holstein cows had the highest concentration of n-3 fatty acids in the first udder secrete after calving Contarini et al. (2014). Only in the second colostrum sample (12 h), the concentration of polyunsaturated fatty acids was higher in the GP group, compared to the control group, which contradicts the conclusions published by Ianni and Martino (2020). They published that ingestion of grape pomace increases concentration of polyunsaturated fatty acids in milk of dairy cows. Leiber et al. (2011) stated that the animal maintains a certain level of polyunsaturated fatty acids in the colostrum, regardless of the given diet or the level of adipose tissue stores. The significant effect of the different feeding systems on polyunsaturated fatty acids and saturated fatty acids was confirmed by Juráček et al. (2020). They found an identical proportion of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in milk of Simmental cows in silage feeding system, as detected by Rolinec et al. (2021), Table 16. This finding suggests that FA composition of colostrum is affected mainly with silage, i.e., the major ingredient in the TMR of cows. On the other hand, Rolinec et al. (2021) concluded that the addition of grape pomace fed in an amount of 0.116kg/cow/day to the diet of dry cows has the potential to increase the concentration of protein in colostrum. However, no significant effect of grape pomace addition into the diet of dry cows on analysed colostral nutrients and fatty acids has been observed.

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8 • THE EFFECT OF GRAPE BY-PRODUCTS ON THE BLOOD OF SOWS AND THEIR NEWBORN OFFSPRING

In recent years, there has been a growing interest in feeding farm animals with innovative feeds, especially those rich in antioxidant compounds. The inclusion of agricultural by-products in the nutrition of farm animals is considered important due to the possible ecological pollution of the ecosystem during storage (Kafantaris et al., 2018, Brenes et al., 2016). According to the International Organisation of Vine and Wine (OIV), the global vineyard surface area is nearly 7.45 million ha, and the world grape production is approximately 73.10 million tons. In Europe, vineyard surface area is over 3.68 million ha, and the grape production is more than 29.56 million tons (OIV, 2018). Viniculture is an important agricultural activity in many countries and produces a large amount of grape pomace. These wine by-products are most often recovered by composting or free storage in open space, which can lead to environmental problems (Rondeau et al., 2013). The use of grape pomace as a feed or as a source of certain nutrients and biologically active substances has been studied by several authors (Kolláthová et al., 2020; Hanušovský et al., 2020; Ivanišová et al., 2019; Kafantaris et al., 2018). Various types of fruits, berries, and pomace contain a high concentration of polyphenols, which have been shown to have anticancer, antimicrobial, antioxidant, and immunomodulatory effects in vertebrates (Brindza et al., 2015; Sehm et al., 2011). Grape pomace is also a good source of nutrients such as Ca, P, Na, Mg, K, Cu, Fe (Hanušovský et al., 2020; Šimko et al., 2019). In general, calcium and phosphorus are most often lacking in the pig feed diet. Calcium and phosphorus are important for gestating sows for the development of fetuses and the integrity of the sow's skeleton. Grape pomace is also a source of iron, which is important in reducing the risks of anaemia in piglets (Svoboda et al., 2004; Estienne et al., 2019). Haematological parameters are an important diagnostic tool for assessing the health status of experimental subjects, but for the correct interpretation of the results of sows, it is necessary to consider the reproductive status and parity of sows and the time from birth of the piglets (Etim et al., 2014; Verheyen et al., 2007). In previous studies with ruminants, the ingestion of grape pomace in an amount of 1% of the diet increased platelet, white blood cell, and lymphocyte counts, which was considered an immunostimulatory effect (Rolinec et al., 2019), but slightly decreased red blood cell count and haemoglobin concentration (Bíro et al., 2019). In non-ruminants, Kolláthová et al. (2020) demonstrated the positive effects of grape pomace on nutrient digestibility, and Kafantaris et al. (2018) showed better growth capacity, antioxidant status, and meat quality in piglets. The parameters of blood cell count of newborn piglets are out of the physiological range, but leucocytes, lymphocytes, erythrocytes and haemoglobin also play an important role in the development of the immune system and the presence of anaemia (Reber et al., 2008; Svoboda et al., 2004). Grape pomace might be used as a nutrient source in sow diets. Ingestion of grape pomace affects the blood parameters of animals, but the question remains how the blood cell count of newborn piglets is affected when the diet of pregnant sows in the late stage of gravidity is supplemented with dried grape pomace at an amount of 1%. To answer this question, Mixtajová et al. (2020 and 2022) and Mixtajová and Rolinec (2020) published several articles about the effect of adding grape pomace to the diet of pregnant sows on energy, micromineral profile, and blood cell count, as well as on the blood cell count parameters of newborn piglets. The results of these articles are presented in Tables 19, 20, and 21. The composition of complete feed for lactating sows, as well as dried grape pomace fed in experiments by Mixtajová et al. (2020 and 2022) and Mixtajová and Rolinec (2020) is shown in Table 18.

Table 18 Concentration of nutrients in diets fed to sows in control and grape pomace sows (Mixtajová et al., 2020)

Group	Complete feed for lactating sow	Dried grape pomace
Dry matter ($g.kg^{-1}$)	893.25	941.4
Crude protein ($g.kg^{-1}$)	174.50	92.80
Ether extract ($g.kg^{-1}$)	20.75	79.25
Crude fibre ($g.kg^{-1}$)	46.70	172.60
Ash ($g.kg^{-1}$)	56.20	37.25
Nitrogen free extract ($g.kg^{-1}$)	595.10	559.50
Organic matter ($g.kg^{-1}$)	837.05	904.15
Starch ($g.kg^{-1}$)	408.70	14.30
Total sugars ($g.kg^{-1}$)	41.30	176.50
Non fibre saccharides ($g.kg^{-1}$)	508.00	299.05
Metabolizable energy pigs ($MJ.kg^{-1}$)	14.39	12.69
Ca ($g.kg^{-1}$)	8.78	4.27
P ($g.kg^{-1}$)	6.24	3.03
Mg ($g.kg^{-1}$)	2.59	1.08
Na ($g.kg^{-1}$)	2.97	0.25
K ($g.kg^{-1}$)	9.37	12.18
Cu ($mg.kg^{-1}$)	25.85	11.05
Fe ($mg.kg^{-1}$)	354.5	65.90
Mn ($mg.kg^{-1}$)	84.7	10.80
Zn ($mg.kg^{-1}$)	182.00	13.75

Table 19 Blood cell count parameters of sows fed from 108 days of gestation with the addition of dried grape pomace (Mixtajová and Rolinec, 2020; and Mixtajová et al., 2020)

	Last week of gestation		**First day after parturition**	
	CON	**GP**	**CON**	**GP**
Glucose (mmol.l^{-1})	4.21 ± 0.84	4.48 ± 1.18	4.34 ± 0.61	4.38 ± 0.67
Cholesterol (mmol.l^{-1})	1.18 ± 0.13	1.03 ± 0.12	1.47 ± 0.64	1.62 ± 0.62
Triglycerides (mmol.l^{-1})	0.56 ± 0.24	0.74 ± 0.28	0.61 ± 0.33	0.60 ± 0.32
P (mmol.l^{-1})	2.07 ± 0.23	2.16 ± 0.25	2.34 ± 0.17	2.71 ± 0.62
Ca (mmol.l^{-1})	2.36 ± 0.21	2.91 ± 0.34	2.45 ± 0.32	2.87 ± 0.75
Mg (mmol.l^{-1})	0.88 ± 0.06	0.98 ± 0.08	0.94 ± 0.14	0.86 ± 0.04
Cl (mmol.l^{-1})	113 ± 7.05	116 ± 3.55	108 ± 4.26	110 ± 3.39

CON – sows in control group, **GP** – sows fed from 108 days of gestation with the addition of grape pomace

After seven days of feeding sows with an addition of dried grape pomace to the diet of gestating sows, Mixtajová and Rolinec (2020) found no significant differences in glucose, cholesterol and triglycerides between the control and grape pomace group of sows. Furthermore, Mixtajová et al. (2020) concluded that dried grape pomace can be considered alternative source of nutrients for sows. No negative effects on the status of macroelements in the blood serum of experimental animals were found, with the level of incorporation of dried grape pomace in the feed diet in an amount of 1%. During the experiment, the chlorides level decreased in both groups; thus the reference values for this indicator were reached. However, further experiments are necessary to confirm the present results and try to determine the maximum incorporation rates for dried grape pomace in balance feeds. The effect of feeding in other categories of animals should also be monitored (Mixtajová et al. 2020).

Table 20 Blood cell count parameters of sows fed from 108 days of gestation with the addition of dried grape pomace (Mixtajová et al., 2022)

	Last week of gestation		First day after parturition	
	CON	**GP**	**CON**	**GP**
White blood cells ($x 10^9.l^{-1}$)	12.82 ± 3.64	11.28 ± 2.37	14.44 ± 2.61	12.07 ± 1.77
Lymphocytes count ($x 10^9.l^{-1}$)	5.43 ± 1.21	5.15 ± 1.41	7.06 ± 1.27	5.07 ± 0.80
Medium size cells count ($x 10^9.l^{-1}$)	0.49 ± 0.59	0.29 ± 0.45	0.46 ± 0.63	0.12 ± 0.06
Granulocytes count ($x 10^9.l^{-1}$)	6.90 ± 3.36	5.83 ± 1.71	6.96 ± 2.54	6.88 ± 1.13
Lymphocytes percentage (%)	45.39 ± 16.13	46.36 ± 10.06	50.08 ± 12.15	42.02 ± 3.51
Medium size cells (%)	3.83 ± 4.40	2.26 ± 3.03	3.14 ± 4.15	1.02 ± 0.51
Granulocytes (%)	50.78 ± 15.0	51.39 ± 7.91	46.78 ± 10.0	56.96 ± 3.10
Red blood cell count ($x 10^{12}.l^{-1}$)	6.10 ± 0.38	5.77 ± 0.72	5.36 ± 0.57	5.14 ± 0.54
Haemoglobin ($g.l^{-1}$)	108.67 ± 8.31	109.36 ± 11.4	98.75 ± 8.78	100.74 ± 10.9
Haematocrit (%)	33.97 ± 2.59	33.95 ± 3.12	32.47 ± 2.76	32.99 ± 3.63
Mean corpuscular volume (fl)	55.56 ± 3.05	59.00 ± 4.32	60.67 ± 3.23	64.31 ± 3.02
Mean corpuscular haemoglobin (pg)	17.81 ± 0.99	19.03 ± 1.30	18.44 ± 0.98	19.59 ± 0.99
MCHC ($g.l^{-1}$)	319.70 ± 6.02	322.30 ± 6.18	304.40 ± 8.10	304.70 ± 6.36
Red cells distribution width (%)	17.66 ± 0.92	17.63 ± 0.64	18.56 ± 0.69	18.42 ± 0.72
Platelet count ($x 10^9.l^{-1}$)	146.70 ± 59.4	145.10 ± 54.7	227.20 ± 70.9	248.30 ± 68.1
Platelet percentage (%)	0.15 ± 0.06	0.15 ± 0.06	0.21 ± 0.07	0.24 ± 0.08
Mean platelet volume (fl)	9.97 ± 0.76	10.53 ± 1.05	9.27 ± 1.05	9.49 ± 0.97
Platelet distribution width (%)	41.30 ± 1.34	42.54 ± 2.47	39.83 ± 2.43	40.63 ± 1.68

CON – sows in the control group, **GP** –sows fed for 108 days of gestation with the addition of grape pomace, **MCHC** – mean corpuscular haemoglobin concentration

Several authors reported a positive effect of grape pomace on health status, haematological parameters, and performance of animals Kolláthová et al., 2020; Nudda et al., 2019; Bíro et al., 2019; Rolinec et al., 2019; Kafantaris et al., 2018; Sehm et al., 2011). In the study by Mixtajová et al. (2022), the blood cell count parameters for the sows were within the reference values for the pigs published by Thorn (2010). However, the reference intervals for sows are more specific (Bhattarai et al., 2019; Sipos et al., 2011). The results of the blood cell count of sows (both sampling times, as well as both groups – Table 20) published by Mixtajová et al. (2022) also met these specific reference intervals. Similar changes in white and red blood cells parameters were also published by Ježek et al. (2018); Joksimović-Todorović et al. (2010) as well as by Thorn (2010). But these authors connected changes in blood cell count parameters to the parturition process and start of lactation. After 7 days of intake of a diet supplemented with grape pomace, Mixtajová et al. (2022) detected a higher count of platelets (248 x $10^9.l^{-1}$) in the blood of the sows. Increase in platelet count after intake of polyphenol-rich food was also published by Sehm et al. (2011).

Table 21 Blood cell count parameters of newborn piglets from sows fed from 108 days of gestation with the addition of dried grape pomace (Mixtajová et al., 2022)

Group	CON	GP
White blood cells (x $10^9.l^{-1}$)	3.34 ± 1.41	2.51 ± 0.87
Lymphocytes count (x $10^9.l^{-1}$)	2.25 ± 1.00	1.51 ± 0.49
Medium size cells count (x $10^9.l^{-1}$)	0.12 ± 0.21	0.07 ± 0.06
Granulocytes count (x $10^9.l^{-1}$)	0.97 ± 0.25	0.93 ± 0.63
Lymphocytes percentage (%)	71.38 ± 8.54	62.90 ± 18.44
Medium size cells (%)	3.60 ± 4.57	2.72 ± 2.15
Granulocytes (%)	25.03 ± 4.98	34.38 ± 18.44
Red blood cell count (x $10^{12}.l^{-1}$)	5.36 ± 0.26	4.57 ± 0.93
Haemoglobin (g.l^{-1})	97.58 ± 4.5	76.79 ± 15.69
Haematocrit (%)	34.38 ± 2.09	26.92 ± 5.32

Group	CON	GP
Mean corpuscular volume (fl)	64.08 ± 2.34	58.83 ± 2.46
Mean corpuscular haemoglobin (pg)	18.18 ± 0.81	16.76 ± 0.81
MCHC ($g.l^{-1}$)	283.60 ± 7.61	284.40 ± 7.10
Red cell distribution width (%)	17.03 ± 0.84	17.86 ± 1.73
Platelet count ($x\ 10^9.l^{-1}$)	44.17 ± 24.51	62.48 ± 55.22
Platelet percentage (%)	0.04 ± 0.02	0.05 ± 0.05
Mean platelet volume (fl)	7.82 ± 0.47	8.14 ± 1.44
Platelet distribution width (%)	37.78 ± 3.99	38.28 ± 2.70

CON – newborn piglet from sows in the control group, **GP** – newborn piglet from sows fed for 108 days of gestation with the addition of grape pomace, **MCHC** – mean corpuscular haemoglobin concentration

The blood cell count parameters of sows fed with the grape pomace supplementation generally reach physiological values, however, the blood of the newborn piglets of the sows in the grape pomace group was significantly worse than that of the sows of the control group, mainly in the count of white blood cells, lymphocytes and red blood cells, as well as in the haemoglobin concentration (Mixtajová et al., 2022 – Table 21). For comparison, Rolinec et al. (2015) detected in newborn piglets' blood 5.25 x $10^9.l^{-1}$ of white blood cells and 2.69 x $10^9.l^{-1}$ of lymphocytes. Much higher values of white blood cells for one day old piglets were published by Egeli et al. (1998) 9.89 $10^9.l^{-1}$. But this relatively high value can be attributed to passive immunisation and active transport of colostral white blood cells to the blood of neonates (Reber et al., 2008). Mixtajová et al. (2022) emphasised that the WBC count, mainly lymphocytes, of newborn piglets must be as high as possible; this is crucial within the frame of newborn piglets' own immunity connected with passive immunisation, which starts after the first colostrum intake. Therefore, the starting count of WBCs, mainly lymphocytes in the blood of newborn piglets, is very important. The newborn piglets of the sows fed last week of gestation with grape pomace addition suffered on anaemia, haemoglobin concentration under 80 $g.l^{-1}$ (Table 21). In the context of the statement made by Svoboda et al. (2004), the concentration 76.79 g.l^{-1} is dangerous. Svoboda et al. (2004) claimed that the concentration of haemoglobin decreased with time after birth, which increased the risk of death for piglets born with anaemia. The decrease in HGB concentration after birth can be caused by an increase in plasma volume. However, lower concentration of HGB directly at birth was - according to Mixtajová et al. (2022) – attributed to grape pomace intake by gestating sows. The higher platelet count in the blood of newborn piglets in the grape pomace group corresponds to the higher platelet count in the blood of sows after grape pomace intake. The decrease in hemoglobin concentration and the increase in platelet count was detected by Sehm et al. (2011) in piglets fed with a diet rich in polyphenols. According to these results, Mixtajová et al. (2022) concluded that supplementing the sow diet during the last week of pregnancy with dried grape pomace decreased the lymphocyte and red blood cell counts and the concentration of haemoglobin in the blood of newborn piglets. Low values of these parameters are undesirable in relation to the immature immune system of newborn piglets and to the risk of anaemia in neonatal piglets. Therefore, feeding pregnant sows with grape pomace cannot be recommended, particularly in the last stage of pregnancy.

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9 • THE EFFECT OF GRAPE BY-PRODUCTS ON NUTRIENTS DIGESTIBILITY AND BIOCHEMICAL INDICATORS IN HORSES

Kolláthová et al. (2019a) studied the effect of feeding dried grape pomace (DGP) in two different concentrations (200 g and 400 g) on selected haematological parameters in horses. The values of the monitored indicators were within the reference ranges. No significant differences were observed between the control and experimental groups of animals, suggesting that DGP supplementation did not affect the haematology of the horses. However, in experimental groups, a tendency for increased values for granulocyte percentage, platelet percentage, and mean platelet volume was detected compared to the control group. In contrast, total white blood cell count, lymphocytes count, lymphocyte percentage, and cell population of middle dimensions were higher in the control group. The granulocytes count, ideredhaemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were higher in the group fed 200 g of DGP, but in group E2 (400 g of DGP) they decreased again compared to the control group. The values of the remaining haematological parameters were practically unchanged in all groups. Similar conclusions were reached by Sehm et al. (2011) in a study of the effect of red grape pomace on piglet blood parameters. Also, Pascariu et al. (2017) and Kumanda et al. (2019) found no significant dietary effect of grape pomace on the haematology of broiler chickens, nor did Nudda et al. (2019) in ewes.

Table 22 Effect of dried grape pomace on selected hematological parameters of horses (Kolláthová et al., 2019a)

Parameters	Control	E1	E2	Reference ranges
	Mean ± Standard deviation			
WBC ($10^9.l^{-1}$)	7,26 ± 1,00	6,91 ± 1,99	6,22 ± 0,72	5,60 – 12,10[1,2,3]
LYM ($10^9.l^{-1}$)	2,72 ± 0,88	1,74 ± 0,66	2,09 ± 0,53	1,20 – 5,10[1,2,3]
MID ($10^9.l^{-1}$)	0,25 ± 0,24	0,04 ± 0,01	0,19 ± 0,20	0,20 – 0,40[6]
GRA ($10^9.l^{-1}$)	4,29 ± 0,52	5,13 ± 1,46	3,94 ± 0,49	2,30 – 9,50[6]
LY% (%)	36,92 ± 8,28	24,64 ± 6,04	33,23 ± 5,51	17,00 – 68,00[6]
MI% (%)	3,12 ± 2,94	0,58 ± 0,01	3,42 ± 3,61	2,10 – 4,40[6]
GR% (%)	59,96 ± 10,87	74,78 ± 6,04	63,35 ± 3,35	22,00 – 80,00[6]
RBC ($10^{12}.l^{-1}$)	7,80 ± 0,45	7,93 ± 0,68	7,82 ± 0,88	6,00 – 12,00[1,2,3]
HGB ($g.l^{-1}$)	123,31 ± 5,56	126,60 ± 10,21	119,23 ± 7,83	100,00 – 161,00[1,2,3]
HCT (%)	33,30 ± 2,34	33,69 ± 2,30	32,85 ± 2,49	32,00 – 53,00[2,3]
MCV (fl)	42,76 ± 3,30	42,64 ± 3,25	42,24 ± 3,41	34,00 – 58,00[1,2,3]
MCH (pg)	15,84 ± 0,83	16,01 ± 1,14	15,34 ± 1,24	13,00 – 19,00[1,2,3]
MCHC ($g.l^{-1}$)	370,90 ± 11,81	375,56 ± 8,62	363,20 ± 6,04	310,00 – 393,00[1,2,3]
RDWc (%)	19,72 ± 0,71	19,74 ± 0,89	19,79 ± 0,71	17,00 – 24,00[4,6]
PLT ($10^9.l^{-1}$)	79,61 ± 59,51	88,05 ± 19,82	81,85 ± 21,28	100,00 – 600,00[2,3,4]
PCT (%)	0,07 ± 0,03	0,06 ± 0,02	0,06 ± 0,01	0,05 – 0,22[4,5]
MPV (fl)	4,81 ± 3,24	6,96 ± 0,50	7,04 ± 0,24	4,00 – 9,30[3,4,5]
PDWc (%)	36,00 ± 3,21	36,57 ± 1,75	37,03 ± 0,34	26,00 –74,00[5,6]

WBC – total white blood cell count, **LYM** – lymphocytes count, **MID** – cell population of middle dimensions including monocytes and eosinophils, **GRA** – granulocytes count, **LY%** – lymphocyte percentage, **MI%** – cell population of middle dimensions including monocytes and eosinophils percentage, **GR%** – granulocytes percentage, **RBC** – red blood cell count, **HGB** – hemoglobin, **HCT** – hematocrit, **MCV** – mean corpuscular volume, **MCH** – mean corpuscular hemoglobin, **MCHC** – mean corpuscular hemoglobin concentration, **RDWc** – red cell distribution width, **PLT** – platelet percentage, **PCT** – thrombocyte count, **MPV** – mean platelet volume, **PDWc** – platelet distribution width. [1]Rossdales (2016); [2]Adla (2019b); [3]Merck (2019); [4]US Davies (2001); [5]Satué et al. (2017); [6]Human (2004)

The effect of feeding different doses of DGP on the biochemical markers of equine blood serum was studied by Kolláthová et al. (2020). Although an increase or decrease in some parameters was recorded, the parameters were all in the reference ranges. Of the macroelements, a higher K concentration was detected when feeding 400 g of DGP. This is in line with the higher content of this element in the pomace (Hanušovský et al., 2019, Šimko et al., 2019). An increasing tendency was also observed for Mg and Cl^-. The serum Ca concentration in the experimental groups was lower, while the concentrations of P and Na were practically unaffected by the DGP consumption. Chedea et al. (2019) also observed a decrease in Ca content and increased serum P levels in piglets fed 5% grape pomace. Davies et al. (2009) found no effect on serum Na, Cl^-, Mg, and K concentration when grape seed extract was fed to racehorses in the amount of 50-150 $mg.kg^{-1}$ of BW. Voicu et al. (2014) detected elevated serum levels of Mg in fattening cattle as a consequence of DGP supplementation, while P and Ca concentrations remained unchanged. Chedea et al. (2017) reported an increased tendency for Ca content and unchanged Mg and P levels in blood serum in dairy cows receiving 15% DGP in the feed ration. Most of the parameters of enzymatic, energy and protein profile of horses were not significantly affected by the addition of DGP to their feed rations (Kolláthová et al., 2020). Pistol et al. (2017) and Taranu et al. (2018) reported the same results in pigs supplemented with 5% grape seed. A decrease in total protein concentration was observed between horses fed 200 g and 400 g of DGP (Kolláthová et al., 2020). Kara et al. (2016) and Voicu et al. (2014) did not report this phenomenon for laying hens and fattening cattle. The 400 g dose of DGP significantly reduced the activity of the ALT enzyme compared to the C group. The opposite findings were made by Yang et al. (2017) as a result of adding proanthocyanidin grape extract to poultry feed rations. Furthermore, a tendency for higher concentrations of bilirubin, cholesterol and urea was observed in the experimental groups of horses (Kolláthová et al., 2020). Increases in urea and cholesterol levels have also been found in cattle. In the case of cholesterol, this phenomenon is attributed to the higher fat content of the feed rations (Voicu et al., 2014; Chedea et al., 2017). In contrast, in horses fed DGP a decreasing tendency of AST, glucose, and triglycerides was found (Kolláthová et al., 2020). Daviest et al. (2009) observed a decrease in serum glucose concentration in racehorses after administration of grape seed extract at 100 and 150 mg.kg^{-1} of BW. According to Pinent et al. (2004), the reason for this is that proanthocyanidins in grape by-products may delay intestinal glucose absorption or produce an insulin-like effect. DGP supplementation did not affect albumin and globulin levels, which is consistent with the results of experiments with cattle and poultry (Voicu et al., 2014; Chedea et al., 2017).

Table 23 Effect of dried grape pomace on selected blood serum parameters of horses (Kolláthová et al., 2020)

Parameters	Control	E1	E2	Reference ranges
	Mean ± Standard deviation			
Ca (mmol.l^{-1})	2,85 ± 0,30	2,62 ± 0,23	2,70 ± 0,12	2,55 – 3,30[1,2,3]
P (mmol.l^{-1})	0,89 ± 0,10	0,92 ± 0,21	0,87 ± 0,33	0,49 – 1,90[1,2,3]
Mg (mmol.l^{-1})	1,19 ± 0,46	1,20 ± 0,33	1,26 ± 0,24	0,58 – 1,00[1,2,3]
Na (mmol.l^{-1})	134,75 ± 2,22	134,75 ± 3,39	133,85 ± 0,95	128,00 – 144,00[1,2,3]
K (mmol.l^{-1})	3,88 ± 0,31[a]	4,24 ± 0,47[ab]	4,43 ± 0,20[b]	2,90 – 5,00[1,2,3]
Cl$^-$ (mmol.l^{-1})	102,38 ± 1,81	104,73 ± 1,93	103,40 ± 1,42	95,00 – 109,00[1,2,3]
GLU (mmol.l^{-1})	3,47 ± 0,14	3,36 ± 0,48	3,36 ± 0,30	3,44 – 7,43[1,2,3]
TAG (mmol.l^{-1})	0,49 ± 0,46	0,28 ± 0,12	0,29 ± 0,13	0,16 – 1,20[2,4]
TP (g.l^{-1})	65,48 ± 7,69[ab]	70,03 ± 1,89[a]	66,62 ± 1,50[b]	56,00 – 76,00[2,3,4]
Urea (mmol.l^{-1})	2,04 ± 0,42	2,90 ± 0,60	2,49 ± 0,62	2,50 – 10,00[1,2,3]
BILI (ˇmol.l^{-1})	25,43 ± 8,97	33,98 ± 9,20	34,11 ± 14,25	0,00 – 57,00[1,2,3]
CHOL (mmol.l^{-1})	1,47 ± 0,66	1,60 ± 0,35	1,64 ± 0,52	1,90 – 3,70[1,2,4]
AST (μkat.l^{-1})	4,36 ± 0,53	4,29 ± 0,55	3,87 ± 0,44	1,70 – 9,92[1,2,3]
ALP (μkat.l^{-1})	1,34 ± 0,23	1,37 ± 0,51	1,33 ± 0,52	1,17 – 5,48[1,4]
ALT (μkat.l^{-1})	0,20 ± 0,03[a]	0,25 ± 0,05[a]	0,15 ± 0,03[b]	<0,53[2,4]
ALB (g.l^{-1})	30,27 ± 4,65	28,11 ± 1,25	29,42 ± 2,13	24,00 – 41,00[2,3,4]
GLB (g.l^{-1})	35,21 ± 9,04[a]	41,92 ± 2,00[a]	37,20 ± 2,65[b]	18,00 – 48,00[2,3]

GLU – glucose, **TAG** – triglycerides, **TP** – total protein, **BILI** – bilirubin, **CHOL** – cholesterol, **AST** - aspartate aminotransferase, **ALP** – alkaline phosphatase, **ALT** – alanine aminotransferase, **ALB** – albumins, **GLB** – globulins. [1]Adla (2019a); [2]Rossdales (2016); [3]Merck (2019); [4]Tučková a Lepejová (2011)

Kolláthová et al. (2020) found an increased plasma iron reduction capacity (FRAP) in horses administering DGP. This fact is probably related to the higher content of polyphenols, especially catechins, in the feed rations of horses. 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. 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 enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) in horses was not affected by DGP consumption, as documented by Kolláthová et al. (2020, 2021?). Kerasioti et al. (2017) reported similar results for SOD in sheep fed grape pomace silage. 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 by increasing 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 a better redox status in multiparous sows during late gestation and lactation after dietary administration of grape seed polyphenols (increased activity of SOD and GPx). The GPx and SOD activities reflect the efficiency of the first-line antioxidant defence system of cells and tissues against the damaging effects of free radicals. Hence, an increased level of these enzymes in blood or tissues is desirable (Surai, 2016). Hao et al. (2015) and Fan et al. (2015) detected a significant increase in 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 to Alía et al. (2013), the increased

TAS is a result of intestinal absorption of polyphenolic compounds and their ability to reach the target organs and plasma. However, Goñí 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 defences, is omissible (Gladine et al., 2007). In this regard, the improvement of TAS may be limited by the amount of GP in feed rations.

Table 24 Effect of different levels of dried grape pomace on antioxidant mechanisms of horses (Kolláthová, 2020; Kolláthová et al., 2021)

Parameter	Control	E1	E2
	Mean ± Standard deviation		
FRAP (μM.Fe^{2+})	258,04 ± 39,60[a]	329,02 ± 32,49[b]	276,21 ± 70,04[ab]
SOD (U.g^{-1} TP)	46,55 ± 11,19	42,54 ± 1,46	45,58 ± 6,63
GPx (U.g^{-1} TP)	20,53 ± 10,31	18,92 ± 6,49	16,02 ± 1,84

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

Kolláthová et al. (2020, 2021) and Kolláthová (2020) reported slightly increased digestibility coefficients of nutrients when supplementing 200 g of DGP. This phenomenon may have been due to the presence of polyphenolic compounds in grape pomace, which can optimally have a positive effect on nutrient digestibility (Makkar, 2003) by modifying intestinal morphology and intestinal microflora (Yang et al., 2017; Wang et al., 2020). According to Viveros et al. (2011), the action of polyphenols slows down the microbial degradation of nutrients, which should make nutrients available for longer use. However, Kolláthová et al. (2020) further state that feeding 400 g of DGP to horses lowered the digestibility of all nutrients. This undesirable reduction in digestibility could be related to higher amounts of condensed tannins in feed rations, which are known to form indigestible complexes with certain nutrients (Baumgärtel et al. 2007; Nistor et al., 2014). Lastly, the inclusion of DGP in feed rations did not reduce feed intake in horses (Kolláthová et al., 2020). These observations are consistent with the results of Davies et al. 2009, who fed horses with grape seed extract. Ishida et al. (2015), Aditya et al. (2018) and Reyes et al. (2020) made the same findings in rams and broilers. At present, only a limited amount of information is available on the use of grape by-products in horse nutrition, and information from experiments with other animal species is inconsistent. Davies et al. (2009) investigated the effect of grape seed extract on digestion in racehorses, finding that supplementation with the product increased faecal pH. This result is probably related to the influence of the bacterial microflora in the back of the horse colon. The improvement in digestibility of CP, ADF and NDF due to the inclusion of grape pomace powder was reported by Foiklang et al. (2016) in dairy steers. According to Bahrami et al. (2010), the digestibility of DM, OM, CP and NDF was improved by increasing the DGP content with the highest values observed for the inclusion of 10% DGP in the 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 exact opposite results for sheep. Vinyard and Chibisa (2019) also observed decrease in DM, ADF and NDF digestibility as an effect of grape pomace feeding on 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 in their diets, but Lichovnikova et al. (2015) stated that grape pomace increased the apparent ileal digestibility of several amino acids in broilers. On the other hand, Nardoia et al. (2020) reported lower digestibility of CP in broilers fed fermented and unfermented grape skins.

Table 25 Effect of dried grape pomace on apparent digestibility of nutrients in horses (Kolláthová et al., 2020; Kolláthová, 2020)

	Control	**E1**	**E2**
Nutrients	**Mean ± Standard deviation**		
Dry matter	70,47 ± 9,40	71,14 ± 4,06	66,47 ± 9,51
Crude protein	72,91 ± 7,01	72,81 ± 4,53	72,55 ± 7,38
Crude fat	68,97 ± 7,80	66,50 ± 3,44	70,65 ± 8,11
Crude fibre	54,00 ± 15,52	55,98 ± 7,16	45,00 ± 13,82
NFE	78,55 ± 6,07	78,19 ± 3,34	76,41 ± 6,12
Organic matter	71,85 ± 8,52	72,21 ± 4,28	68,00 ± 8,35
Starch	99,29 ± 0,43	98,95 ± 0,50	99,14 ± 0,81
Total sugar	98,97 ± 0,51	99,21 ± 0,41	98,98 ± 0,83
NSC	87,59 ± 3,06	87,05 ± 1,22	86,49 ± 3,39
ADF	50,69 ± 16,24	52,16 ± 7,91	38,55 ± 15,66
NDF	57,22 ± 13,85	58,25 ± 7,20	50,27 ± 12,79
Cellulose	56,75 ± 13,87	57,57 ± 7,04	47,87 ± 10,86
Hemicellulose	66,84 ± 10,53	67,31 ± 6,37	67,92 ± 8,85

NFE – nitrogen free extract, **NSC** – non-structural carbohydrates, **ADF** – acid detergent fibre, **NDF** – neutral detergent fibre

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10 • THE EFFECT OF GRAPE BY-PRODUCTS ON GEESE FATTENING: PERFORMANCE AND FATTY ACID COMPOSITION

Nowadays, the consumption of goose meat has increased throughout the world, mainly in China; a slow increase is detectable in European countries, too (Nemati et al., 2020; Liu et al., 2013). The production of goose meat is aimed at economic efficiency and high meat quality with low fat concentration (Biesiada-Drzazga, 2014). Consumers of goose meat also require balanced ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), (Tao, 2015). Fatty acids in the body are primarily a source of energy, a component of cell membranes, influence cellular metabolism, hormone function; and play an important role in intracellular signalling pathways and gene expression (Calder, 2015). As published in in Thapa (2020), not all fatty acids can be produced endogenously in the body, they need to be ingested through a diet. Poultry products belong to human nutrition to the main sources of nutrients and fatty acids. Alagawany et al. (2019) confirm that the concentration of fatty acids concentration of animal products, including goose, can be easily affected by nutrition sources. Grape pomace as a by-product of the wine industry is a source of essential fatty acids, polyphenols, and substances with antioxidative properties (Hosseini-Vashan et al., 2020; Yu and Ahmedna, 2013).

Grape pomace is produced as a by-product of wine; fruit juice production (Gómez-Brandón et al., 2019) represents approximately 20% weight of processed grapes (de Souza et al., 2015). They consist of skin, seeds, and stems left as residues after grapes have been pressed (Russo et al., 2017). Apart from phenols, proteins, minerals, grape pomace is also a rich source of essential fatty acids. In grape pomace, the kernels are the main source of grape oil. Linoleic acid is the dominant fatty acid with a portion of 70% of all fatty acids (Gómez-Brandón et al., 2019). The skin of the grape contains high concentration anthocyanins (Yu and Ahmedna, 2013). Red grape varieties are typical for the presence of resveratrol bearing anticancer, antioxidant, cardioprotective, and anti-inflammatory properties (Ko et al., 2017; Sanders et al., 2000; Yu and Ahmeda, 2013). Zhang et al. (2017) reported an increase of live weight, improves animal growth, and reduces stress after feeding poultry with the addition of resveratrol. Azizi et al. (2018) found a positive effect of adding grapes to poultry diet on the quality of animal products and health. The inclusion of grape pomace likewise increases feed intake during pre-fattening and fattening periods and reduces blood triglyceride and cholesterol concentrations in fattening poultry (Hosseini-Vashan, et al., 2020). Drotárová et al. (2022) performed a feeding experiment with the addition of grape pomace to the daily diet of goose during fattening. The results of this experiment are presented in Tables 26 and 27.

After a 49-day feeding experiment Drotárová et al. (2022) observed higher live weight, average daily gain, carcass weight, and liver weight of geese fed diet supplemented with 1% dried grape pomace. However, from all these positive results, only liver weight (125.2 vs 102.2 g) was significant. They attributed better feeding performance and liver weight to increased polyphenol intake in the experimental group (Table 26). Zhang et al. (2017) also detected a positive effect of feeding a higher dose of resveratrol to the performance of fattened broilers. Wang et al. (2010) also observed positive results with grape meal feeding. The inclusion of 9% grape meal in the feed mixture positively affected the average daily gain of geese, similar results were also observed by Haščík et al. (2021) with the inclusion of 3% grape pomace in the broiler feed mixture. Improvements in growth and fattening parameters were also noted by Pascariu et al. (2017). Statistically significant results of the inclusion of grape pomace in the diet of broiler chickens were also reported by Brenes et al. (2008). Increased feed intake with statistical significance at the time of pre-fattening and fattening was observed by Zhang, et al. (2017).

Table 26 Parameters of goose fattening with diet containing dried grape pomace (Drotárová et al., 2022)

Parameter	Day of the experiment	Group	Mean ± S.D.
Live weight (g)	0th day	E	1857 ± 196
		C	1872 ± 248
	14th day	E	3416 ± 370
		C	3346 ± 333
	32nd day	E	5010 ± 643
		C	4890 ± 504
	49th day	E	5752 ± 752
		C	5579 ± 770
Average daily gain during experiment (g)		E	79.5 ± 15.23
		C	75.6 ± 15.75
Feed consumption per kg of live weight gain (kg)		E	2.94
		C	2.94
Carcass weight (g)		E	3775 ± 554
		C	3708 ± 545
Carcass yield (%)		E	65.6 ± 3.59
		C	66.4 ± 2.24
Liver weight (g)		E	125.2 ± 18.77
		C	102.2 ± 18.64

E – experimental group with 1% grape pomace addition; **C** – control group without grape pomace; **S.D.** – standard deviation; * mean values between groups differ significantly at $P<0.0$

In terms of fatty acid concentration in abdominal fat of geese fed with the addition of grape pomace, Drotárová et al. (2022) reported an insignificant higher concentration of C14:0 (+19.2%) C18:1 cis9 (+3.0%) and C20: 4 n6 (+3.6%) compared to the control group (Table 27). On the other hand, the abdominal fat of the experimental geese had an insignificant lower concentration of C16: 0 (-4.0%), C17: 0 (-8.3%), C18: 3 n3 (-8.9%) compared to the control group. Also, changes in SFA, MUFA, and PUFA were without statistical significance (Table 27). In contrast, Karadağoğlu et al. (2020) found a higher concentration of linolenic acid (n-3 C18:3), arachidonic acid (n-6 C20:4), eicosapentaenoic acid (n-3 C20:5), and docosahexaenoic acid (n-3 C22:6) in the breast meat of broiler chickens fed supplemental grape seed extract. Also, Cartoni Mancinelli et al. (2019) determined higher long-chain fatty acids in geese meat raised in vineyard pasture (system with organic grape production). Drotárová et al. (2022) concluded that grape pomace has the potential to improve fattening performance of geese and change the concentration and ratio of fatty acids in goose products. However, further experiments are needed to determine the suitable physical form (grape pomace, skin, grape seed – dried or extract) and the concentration which can be used in geese feeding.

Table 27 Fatty acid composition of abdominal fat of geese
(Drotárová et al., 2022)

Fatty acid	Name of fatty acid	Group	Mean ± S.D.
C 14:0	myristric acid	E	0.31 ± 0.04
		C	0.26 ± 0.04
C 16:0	palmitic acid	E	20.41 ± 1.31
		C	21.27 ± 1.03
C 17:0	heptadecanoic acid	E	0.11 ± 0.02
		C	0.12 ± 0.01
C 18:0	stearic acid	E	6.50 ± 0.64
		C	6.50 ± 0.43
C 18:1 cis 9	oleic acid	E	45.73 ± 2.62
		C	44.41 ± 1.96
C 18:2 cis 6	linoleic acid	E	19.08 ± 2.40
		C	19.09 ± 0.95

Fatty acid	Name of fatty acid	Group	Mean ± S.D.
C 18:3 n-3	linolenic acid	E	3.19 ± 0.75
		C	3.50 ± 0.64
C 20:4 n-6	eicosenoic acid	E	0.29 ± 0.03
		C	0.28 ± 0.02
ΣMUFA	monounsaturated fatty acids	E	47.8 ± 2.70
		C	46.63 ± 1.99
ΣPUFA	polyunsaturated fatty acids	E	22.40 ± 3.17
		C	22.74 ± 1.52
ΣSFA	saturated fatty acids	E	27.30 ± 1.08
		C	28.20 ± 1.27
Σn3/Σn6	ratio n-3/n-6 fatty acids	E	0.16 ± 0.02
		C	0.18 ± 0.03
Σn6/Σn3	ratio n-6/n-3 fatty acids	E	6.18 ± 0.74
		C	5.63 ± 0.85

E – experimental group with 1% of grape pomace addition; **C** – control group without grape pomace; **S.D.** – standard deviation

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11 • THE EFFECT OF GRAPE BY-PRODUCTS ON DOG BLOOD PARAMETERS

The intake of grapes and raisins by dogs is associated with severe reactions and may even be fatal. On the other hand, by-products of the wine industry (grape pomace, skin, and seeds) contain a wide range of bioactive compounds with antioxidant and antimicrobial properties (Brenes et al., 2016; Gálik et al. 2019; Ivanišová et al., 2019). In addition, Brindza et al. (2015) concluded that plant antioxidants are beneficial for improving dog health. However, studies by Mazzaferro et al. (2004), Morrow et al. (2005), and Eubig et al. (2005) reported acute renal failure in dogs after consuming grapes, raisins or both. Vomiting, diarrhoea, lethargy, and anuria were common clinical signs. On the other hand, Martineau et al. (2016) concluded that long-term consumption of a pet-specific blend of a polyphenol-rich extract from grape and blueberry was not associated with renal or hepatic injury. They considered this extract safe. Rolinec et al. (2020) conducted a study with bitches of the American Staffordshire terrier breed. The amount of dried grape pomace consumed by the dogs during the experiment was 1% of the daily diet. The blood indices after the three-week experiment are shown in Table 28.

Table 28 Blood parameters of dogs fed with a diet containing 1% dried grape pomace for three weeks (Rolinec et al., 2020)

Mean ± S.D.	Control diet	Diet with 1% dried grape pomace
White blood cells (x $10^9.l^{-1}$)	$9.26^a \pm 0.50$	$12.0^b \pm 1.24$
Lymphocyte counts (x $10^9.l^{-1}$)	$3.56^a \pm 0.59$	$5.82^b \pm 0.37$
Medium size cells count (x $10^9.l^{-1}$)	0.56 ± 0.19	0.43 ± 0.07
Granulocytes count (x $10^9.l^{-1}$)	5.13 ± 0.19	5.78 ± 1.52

Mean ± S.D.	Control diet	Diet with 1% dried grape pomace
Red blood cell count (x 10^{12}.l^{-1})	8.78 ± 0.27	8.94 ± 0.19
Haemoglobin (g.l^{-1})	201 ± 7.00	206 ± 2.65
Haematocrit (%)	58.9 ± 1.75	59.9 ± 0.95
Mean corpuscular haemoglobin (pg)	22.9 ± 0.23	23.1 ± 0.34
Platelet count (x 10^{9}.l^{-1})	423 ± 7.83	407 ± 19.06
Platelet percentage (%)	0.45 ± 0.02	0.46 ± 0.04
Total protein (g.l^{-1})	67.5 ± 1.88	67.4 ± 4.86
Albumin (g.l^{-1})	37.2 ± 1.72	36.2 ± 2.18
Creatinine (μmol.l^{-1})	129 ± 1.50	121 ± 6.85
Glucose (mmol.l^{-1})	5.38^{a} ± 0.05	5.50^{b} ± 0.02
Cholesterol (mmol.l^{-1})	7.24 ± 0.07	7.15 ± 0.21
Lipase (μkat.l^{-1})	0.43 ± 0.04	0.41 ± 0.02
Calcium (mmol.l^{-1})	2.53 ± 0.04	2.55 ± 0.07
Phosphorus (mmol.l^{-1})	1.36 ± 0.13	1.29 ± 0.09

Within a row, the means marked with different superscripts differ significantly ($P<0.05$).

In this study, a higher value of white blood cells, lymphocytes, as well as glucose concentration were determined in the blood of dogs fed with a diet containing 1% dried grape pomace for three weeks. Compared to the reference intervals for dog blood (which is 3.4 x 10^{9}.l^{-1} according to Harvey et al. (2012) and 4.8 x 10^{9}.l^{-1} according to the upper reference value of the Abacus Junior Vet haematological apparatus), the lymphocyte count in dogs from the experimental group is high. The increase of LYM within the reference interval can be considered stimulating for the immune system; however, an increase above the reference interval cannot. Similar conclusions can also be assigned to the effect of grape pomace on RBC count and HGB concentration. After feeding the control diet, RBC and HGB were at the upper value of the reference interval; what's more, feeding the GP diet increased both parameters above the reference interval. Eubig et al. (2005) published a retrospective evaluation of 43 dogs with clinical or health problems after the ingestion of grapes or raisins. The output of the study was as follows: decreased urine input, ataxia, weakness. In general, hypercalcemia and hyperphosphatemia were present in 90% or 62% of dogs, respectively. None of these findings were detected in this study (Table 28). Furthermore, Morrow et al. (2005) detected canine renal pathology associated with grape or raisin ingestion. In that study, the dogs were exposed to different amounts of raisin (3 to 30 g. kg^{-1} of dry feed matter) and grapes (4 $g.kg^{-1}$ of dry feed matter in unknown amount). Dogs in the Morrow et al. (2005) study have detected creatinine in an interval from 189 to 982 $\mu mol.l^{-1}$, which indicate serious failure in renal function. The creatinine concentration detected in this study (both groups) is within an interval of 114 to 130 $\mu mol.l^{-1}$, i.e., above the upper limit of physiological optimum (35 to 106 $\mu mol.l^{-1}$) of dogs published by Škorová, 2021. However, the average creatinine concentration in dogs fed with the addition of dried grape pomace was lower, compared to the control group. The results of this study suggest that feeding dogs with dried grape pomace is not as dangerous as feeding with grapes and raisins, as concluded by Eubig et al. (2005) and Morrow et al. (2005). However, also the dose of 1% dried grape pomace in the diet increased some blood parameters above the physiological range. Other parameters of blood cell count, as well as biochemical parameters, were within published reference intervals for dogs (Slanina et al., 1991; Harvey, 2012; Škorová, 2021). Based on the differences published in articles Eubig et al. (2005); Morrow et al. (2005) and results of this study, it can be hypothesized that the physical form (grapes, dried grape pomace, raisins) plays a very important role in the manifestation of clinical signs and blood parameters. At any rate, the feeding of dogs with grape-by products (or dried grape pomace) is questionable and further research in this field is necessary.

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12 • CONSERVATION OF GRAPE BY-PRODUCTS

Grape pomace in animal nutrition can either be used fresh (Molina-Alcaide et al., 2008) or preserved (dried, or ensiled) (Bahrami et al., 2010; Caetano et al., 2019). Grape pomace can also be used as a silage additive (Li et al., 2017). One of the ways of influencing the quality of silages is the addition of silage additives (Muck et al., 2018). Urea as a silage additive can affect the nutritional value (mainly protein value), fermentation, and stability of silage during aerobic exposure (Yitbarek and Tamir, 2014). In the research by Vašeková et al. (2019), the addition of urea (in a dose of 0.2 %) affected dry matter and crude protein ($P<0.05$) in the silage of grape pomace. The addition of urea significantly increased the crude protein content (148.03 vs. 131.64 $g.kg^{-1}$ of DM). This conclusion was also confirmed by the results published by Dinić et al. (2015). Likewise, Winkler et al. (2015) observed a significantly higher ($P<0.05$) crude protein content ($P <0.05$) in grape pomace silage with the addition of a chemical additive containing urea. The effect of urea addition on the crude fibre and crude fat was not confirmed (Vašeková et al., 2019), which is consistent with the findings of Dinić et al. (2015). Grape pomace is characterized by a moderate mineral content (Chikwanha et al., 2018), while the addition of urea reduces the ash content (Vašeková et al., 2019). According to Vašeková et al. (2019), the urea content decreases ($P <0.05$) the total sugars and nitrogen-free extract in the silage of grape pomace. The results in sugar content do not correspond to the findings reported by Winkler et al. (2015). The differences in starch content between variants were significant ($P<0.05$) which confirmed that urea positively affects starch content in grape pomace silage (Vašeková et al., 2019).

The higher starch content in silage is probably related to the inhibitory effect of urea on undesirable microflora. According to Vašeková et al. (2019), the addition of urea has a positive effect (P<0.05) on the organic matter content of the grape pomace silage. Furthermore, Vašeková et al. (2019) did not observe any effect of urea on the energy value of the silage, which is inconsistent with the findings of Yaghoubi et al. (2014). Generally, grape pomace has a low energy value, similar to the straw of dense-sowing cereals (Winkler et al., 2015). The results of Vašeková et al. (2019) confirmed the effect of urea addition (P<0.05) on the PDIN content (81.58 g with urea vs. 72.53 in control) and PIIE content (48.40 g with urea vs. 43.77 in control). The experiments by Phesatcha and Wanapat (2016) and Kang et al. (2018) confirmed the positive effect of urea on the content of crude protein and PDIN. According to Vašeková et al. (2019), grape pomace silage is a feed with protein character (PDIN/NEL=22.18 in control and 24.95 in silage with urea).

Table 29 Nutrient content and nutritive value of grape pomace silage (Vašeková et al., 2019)

Parameters	Units	Control - without additive	With urea (in a dose of 0.2 %)
Dry matter	$g.kg^{-1}$	$366.60^{a} \pm 7.90$	$386.88^{b} \pm 10.20$
Crude protein	$g.kg^{-1}$ of DM	$131.64^{a} \pm 3.53$	$148.03^{b} \pm 2.51$
Crude fat	$g.kg^{-1}$ of DM	108.96 ± 6.33	113.15 ± 6.40
Crude fibre	$g.kg^{-1}$ of DM	206.00 ± 11.47	200.51 ± 6.55
Ash	$g.kg^{-1}$ of DM	$50.13^{a} \pm 0.97$	$47.04^{b} \pm 0.33$
Nitrogen free extract	$g.kg^{-1}$ of DM	$503.27^{a} \pm 4.60$	$491.29^{b} \pm 10.05$
Starch	$g.kg^{-1}$ of DM	$51.97^{a} \pm 2.33$	$57.31^{b} \pm 3.29$
Total sugars	$g.kg^{-1}$ of DM	$15.59^{a} \pm 0.87$	$13.85^{b} \pm 1.02$
Organic matter	$g.kg^{-1}$ of DM	$949.87^{a} \pm 0.97$	$952.96^{b} \pm 0.33$
PDIN	$g.kg^{-1}$ of DM	$72.53^{a} \pm 1.96$	$81.58^{b} \pm 1.39$
PDIE	$g.kg^{-1}$ of DM	$43.77^{a} \pm 1.01$	$48.40^{b} \pm 0.59$
NEL	$MJ.kg^{-1}$ of DM	3.27 ± 0.01	3.27 ± 0.01
NEG	$MJ.kg^{-1}$ of DM	2.51 ± 0.01	2.51 ± 0.01

Abbreviations: **DM** – dry matter, PDIN, **PDIE** – protein digestible in the intestine of ruminants, **NEL** – net energy of lactation, **NEG** – net energy of gain, values with the different indexes in a row are significant at P<0.05, data are presented as mean ± SD.

The results of Bíro et al. (2020b) confirmed that the Ca content was not affected by adding urea in the grape pomace silage of, but a significant (P<0.05) effect (P <0.05) was detected in P. Furthermore, the Ca / P ratio was 1.36/1 in silage without additive and 1.39/1 in silage with urea. Heuzé and Tran (2017) reported a higher content of Ca (6.10 g.kg^{-1} of DM) than results of Bíro et al. (2020b). Compared to corn silage, grape pomace silage has a higher value of Ca (Juráček et al., 2019b). Grape pomace silage is characterized by a higher content of P compared to corn, alfalfa and grass silage (Bíro et al., 2020b; Skalická et al., 2013). The Mg content in grape pomace silage in the research of Bíro et al. (2020b) was found between 1.29 and 1.33 g.kg-1 of DM with the highest content (P>0.05) in urea silage. However, Heuzé and Tran (2017) reported a lower Mg content (1.20 g.kg^{-1} of DM). A statistically significant decrease in Na and an increase in K content was observed between control and urea silage with urea (Bíro et al., 2020b). The wider ratio K/Na (41.18/1) was found in control silage and the narrower ratio (28.17/1) in silage with urea addition (Bíro et al., 2020b). Heuzé and Tran (2017) reported a lower Na content (0.20 g.kg^{-1} of DM) and a higher K content (19.40 g.kg^{-1} of DM) in grape pome silage compared to the results of Bíro et al. (2020b). Tayengwa and Mapiye (2018) observed an even higher content of Na and K (Na 0.70 g and K 20.40 g.kg^{-1} of DM). The addition of urea did not affect (Pβ0.05) the content of Cu, Mn, Zn and Fe (Bíro et al., 2020b). Heuzé and Tran (2017) reported a higher value of Cu (34.00 mg.kg^{-1} of DM) in comparison with the results published by Bíro et al. (2020b). The content of Zn was found between 24.63 and 23.72 mg.kg^{-1} in the DM silage of grape pomace in the experiment of Bíro et al. (2020b). Similarly, Šimko et al. (2019) determined the Zn content in the grape pomace from 16.42 to 28.99 mg.kg^{-1} of DM.

Table 30 Macro- and micro-element content of grape pomace silage (Bíro et al., 2020b)

Parameters	Units	Control - without additive	With urea (in a dose of 0.2 %)
Ca	$g.kg^{-1}$ of DM	5.50 ± 0.33	5.45 ± 0.07
P	$g.kg^{-1}$ of DM	4.03^{a} ± 0.08	3.92^{b} ± 0.08
Mg	$g.kg^{-1}$ of DM	1.29 ± 0.04	1.33 ± 0.01
Na	$g.kg^{-1}$ of DM	0.39^{a} ±0.11	0.48^{b} ± 0.03
K	$g.kg^{-1}$ of DM	16.06^{a} ± 1.90	13.52^{b} ± 1.03
Cu	$mg.kg^{-1}$ of DM	16.97 ± 0.64	18.47 ± 2.08
Fe	$mg.kg^{-1}$ of DM	107.33 ± 6.50	112.33 ± 8.29
Mn	$mg.kg^{-1}$ of DM	14.07 ± 0.39	14.32 ± 0.32
Zn	$mg.kg^{-1}$ of DM	24.63 ± 3.54	23.72 ± 2.05

Abbreviations: **DM** – dry matter, values with the different index in a row are significant at P<0.05, data are presented as mean ± SD.

The dry matter content significantly affects the quality of the silage fermentation process (Borreani et al., 2018). The addition of urea (in a dose of 0.4 %) affected the content of dry matter of the grape pomace silage (P<0.05) in the results of Juráček et al. (2019a). Furthermore, the silage of grape pomace without additives and with urea had a higher dry matter content than 30 %, which is the minimum content that inhibits the production of silage effluents (Bíro et al., 2020a). The dry matter losses were significantly (P<0.05) affected by the urea addition (11.20%) compared to the control treatment (19.10%) (Juráček et al., 2019a). These results correspond to the finding reported by Sousa et al. (2008). Köhler et al. (2013) observed different dry matter losses depending on the type of silage (2-26 % in grass silage, 6-15 % in alfalfa silages, and 4-19 % in maize silage). Compared to the control, in urea silage, a significantly different (P<0.05) lactic acid content (P <0.05) was found in the experiment of Juráček et al (2019a). A similar effect was reported by Sousa et al. (2008). Conversely, Doležal et al. (2017) observed a lower lactic acid content after the addition of urea. The effect of urea on the content of acetic acid was insignificant, with a higher content in the control (Juráček et al., 2019a). These results correspond to the findings reported by Doležal et al. (2017). The acetic acid content in silage is also affected by the dry matter content (Kung et al., 2018), while the acetic acid content decreases as the dry matter content increases, as confirmed also by the results of Juráček et al. (2019a). The optimal ratio of lactic to acetic acid in silage is greater than 3.0:1.0 (Jalč et al., 2010). In the research of Juráček et al. (2019a), a lower lactic to acetic acid ratio was observed in control (4.9: 1.0) *vs.* silage with urea (5.7: 1.0). These results were also confirmed by Doležal et al. (2017) in maize silage with addition of urea (in a dose of 0.5 %). The silage of grape pomace of both variants did not contain undesirable butyric acid (Juráček et al., 2019a). In quality silage, the optimal proportion of lactic acid from the total acid content should be between 65-70% (Kung and Shaver, 2001). Compared to the control silage (83.10%), a higher proportion of lactic acid was found in silage with the addition of urea (85.10%) in the experiment of Juráček et al. (2019a). These findings are consistent with the results of Doležal et al. (2017). The addition of urea did not affect ($P>0.05$) pH value ($P> 0.05$) in the grape pomace silage (Juráček et al., 2019a). These results correspond to the findings reported by Yaghoubi et al. (2014). The pH values of both variants were below 4.5 (Juráček et al., 2019a), which indicates a well-preserved silage with a range of dry matter content of 30-40 %, as described by Bíro et al. (2020a). The desired concentration of alcohols in silage is below as 2 % (Mitrík, 2006), while a higher content of alcohols is related to the activity of yeasts (Shaver, 2013). In the research of Juráček et al. (2019a), the alcohol content in the silage of grape pomace in both variants was observed to be higher than 2%. Furthermore, the alcohol content was significantly ($P<0.05$) affected ($P <0.05$) by the addition of urea compared to the control. These results correspond to the findings reported by Sousa et al. (2008) in sugarcane silages with urea addition (in a dose of 1 %). According to Doležal et al. (2012), acidity of water extract is a considerable parameter as regards silage feeding. The addition of urea affected

(P<0.05) the acidity of the water extract and the proteolysis of the silage of grape pomace silage (Juráček et al., 2019a). Obviously, the higher proteolysis was determined in the urea-containing silage of grape pomace. The content of fermentation products in silage affects the addition of silage additives and the content of dry matter (Bencová, 1999). In the experiment of Juráček et al. (2019a), the highest content of fermentation products was found in grape pomace silage with urea was found (P<0.05).

Table 31 Fermentation parameters of grape pomace silage (Juráček et al., 2019a)

Parameters	Control - without additive	With urea (in a dose of 0.4 %)
Dry matter ($g.kg^{-1}$)	366.60^{a} ± 7.90	391.98^{b} ± 15.09
Dry matter losses %	19.10^{a} ± 1.79	11.20^{b} ± 3.31
Lactic acid ($g.kg^{-1}$ of DM)	16.78^{a} ± 2.13	18.79^{b} ± 1.00
Acetic acid ($g.kg^{-1}$ of DM)	3.41 ± 0.90	3.28 ± 1.31
Butyric acid ($g.kg^{-1}$ of DM)	ND	ND
pH value	4.15 ± 0.01	4.21 ± 0.04
Degree of proteolysis %	1.50 ^{a} ± 0.07	2.39^{b} ± 0.21
Alcohols ($g.kg^{-1}$ of DM)	69.14^{a} ± 7.09	56.91^{b} ± 4.25
Acidity of water extract*	458.83^{a} ±16.42	494.83^{b} ± 12.67
Fermentation products ($g.kg^{-1}$ of DM)	91.26^{a} ± 9.54	80.86^{b} ± 5.54

Abbreviations: **DM** – dry matter, *mg KOH.100g^{-1} of silage, values with the different index in a row are significant at P<0.05, data are presented as mean ± SD.

Grape pomace silage is a feed with protein character, with a crude protein content of 13 % (without additive) and containing more than 20% crude fibre. The addition of urea affects the nutritive value of grape pomace silage by increasing the content of crude protein and PDIN, inhibiting dry matter losses, starch, and organic matter losses. However, silage with urea is characterized by a lower total sugar content. The urea also affects the fermentation parameters of the grape pomace silage. Silage with urea is characterized by a higher content of lactic acid, a higher degree of proteolysis, and a lower content of alcohols. Compared to alfalfa, grass, and corn silage, grape pomace silage has a higher phosphorus content. The addition of urea as a silage additive affects the mineral profile of the silage of grape pomace.

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• RESUME IN SLOVAK

Kŕmne aditívne látky sú neodmysliteľnou súčasťou modernej výživy zvierat. V minulosti boli spájané hlavne s rastovými stimulačnými aditívami na báze antibiotík. Z dôvodu reziduálnych účinkov sa od nich postupne upustilo a v súčasnosti sa nesmú plošne skrmovať ako kŕmne aditíva. Problémom bola hlavne antibiotická rezistencia u zvierat a neskôr aj v humánnej populácii. Používanie kŕmnych aditívnych látok je v rámci európskeho spoločenstva limitované krmivárskym právom, ktoré určuje podmienky uvádzania na trh a ich využívanie vo výžive zvierat. V súčasnej výžive zvierat sa najmä z hľadiska ekonomickej efektívnosti, udržateľnosti a environmentálnej akceptovateľnosti opäť výraznejšie presadzujú vedľajšie produkty pochádzajúce z agropotravinárskeho priemyslu. Okrem tradičných minoritných surovín sa hľadajú možnosti nutričného zhodnotenia aj iných medziproduktov. Pomerne veľké množstvo vedľajších produktov vzniká aj pri priemyselnom spracovaní hrozna, hlavne hroznové výlisky a strapiny. Predovšetkým hroznové výlisky majú pomerne zaujímavý obsah viacerých organických látok, minerálnych látok a špecificky účinných zlúčenín, ktoré majú potenciál využitia vo výžive zvierat. Nutričná kvalita vedľajších produktov spracovania hrozna závisí od viacerých faktorov, hlavne však od obsahu živín v pôvodnej surovine a od technológie spracovania. Hroznové výlisky sú typické vyšším obsahom dusíkatých látok, hrubého tuku, hrubej vlákniny, ako aj jej frakcií ADV a NDV. Z hľadiska obsahu minerálnych látok sú hroznové výlisky bohaté hlavne na vápnik, fosfor a horčík. Z mikroprvkov má vyššie zastúpenie železo. Zaujímavý môže byť obsah draslíka. Viaceré práce publikované vo svete poukazujú na výnimočný profil mastných kyselín vo vedľajších produktoch spracovania hrozna. Predovšetkým zastúpenie polynenasýtených mastných kyselín je pozitívne, nakoľko sa na celkovom tuku vo výliskoch podieľajú aj viac ako 70 %-ami. Hroznové strapiny sú zaujímavé aj tým, že ich tuk je tvorený vo veľkej miere polynenasýtenými mastnými kyselinami, v ktorých má pomerne veľké zastúpenie aj kyselina α-linolénová. To pravdepodobne spôsobuje aj priaznivejší pomer medzi n-6 a n-3 nenasýtenými mastnými kyselinami v porovnaní s hroznovými výliskami. Vedľajšie produkty spracovania hrozna sú zdrojom aj viacerých biologicky aktívnych látok, ktorých zastúpenie je významné hlavne v hroznových výliskoch. Ich obsah je však veľmi variabilný a môže sa pohybovať od niekoľkých gramov až po desiatky, stovky. Obsahom v hroznových výliskoch sú typickými polyfenolmi resveratrol, qvercetín, katechín, epikatechín a pod. Tieto látky majú pozitívny, stimulačný a protektívny efekt na vnútorné prostredie zvieraťa, dôležitým momentom ich prejavu je výška aplikačnej dávky. Poly fenoly majú značnú antioxidačnú aktivitu. Práce publikované vo svete, ale aj práce autorského kolektívu tejto monografie poukazujú na pozitívny vplyv nutričnej suplementácie kŕmnych dávok prežúvavcov hroznovými výliskami. Zaradením tejto kŕmnej suroviny možno zvýšiť stráviteľnosť živín, hlavne dusíkatých látok a tuku. A to bez negatívneho vplyvu na zdravotný stav zvierat. Nie sú známe ani negatívne vplyvy hroznových výliskov na hematologické a biochemické parameter krvi prežúvavcov. Z hľadiska vplyvu zaradenia hroznových výliskov do kŕmnych diet dojníc možno dosiahnuť mierne zvýšenie obsahu bielkovín a nižší obsah tuku v mledzive. Výrazné rozdiely v zastúpení živín sú však menej pravdepodobné. Vo výžive ošípaných nie je vhodné skrmovať vo väčšom množstve hroznové výlisky pri prasniciach, najmä v poslednej fáze prasnosti. Publikované práce totiž poukazujú na možný negatívny prejav, nižšiu sérovú koncentráciu lymfocytov a červených krviniek, ako aj hemoglobínu u narodených prasiatok. Zaradenie obmedzeného množstva hroznových výliskov do kŕmnych diet športových koní má pozitívny vplyv na niektoré antioxidačné mechanizmy v organizme, avšak hlavne na vyššiu stráviteľnosť živín kŕmnej dávky. Publikované práce poukazujú na to, že racionálne zaradenie hroznových výliskov do kŕmnej dávky môže zvýšiť stráviteľnosť sušiny, organickej hmoty, ale najmä vlákniny a jej frakcií. Je však potrebné zdôrazniť, že hroznové výlisky obsahujú aj viaceré antinutričné látky, ktorých vyššia koncentrácia v krmive môže pôsobiť depresívne na trávenie a znižovať využiteľnosť viacerých živín. Autori tejto monografie realizovali aj experiment s hroznovými výliskami vo výžive výkrmových husí. Zistili však negatívny vplyv na priemernú živú hmotnosť husí na konci výkrmu, ako aj tendenciu nižších priemerných denných prírastkov živej hmotnosti. Na druhej strane však zistili tendenciu vyššej jatočnej výťažnosti. Zároveň zistili tendenciu vyššieho zastúpenia kyseliny linolénovej v tuku a tendenciu vyššieho zastúpenia polynenasýtených mastných kyselín v tuku. Hroznové výlisky sú využiteľné vo výžive zvierat v podobe čerstvého krmiva, ale aj konzervovaného krmiva. Konzervované môžu byť sušením, ale aj silážovaním. Hroznové výlisky môžu byť zároveň využiteľné aj ako silážne aditívum. Pri silážovaní hroznových výliskov sa ako vhodné silážne aditívum javí močovina a to najmä z hľadiska protektívneho vplyvu na obsah dusíkatých látok. Nedochádza k takému rozkladu ako v prípade, keď sú hroznové výlisky zasilážované bez aplikácie močoviny. Podobný ochranný efekt môže mať močovina aj z hľadiska obsahu tuku v silážovaných hroznových výliskoch. Z hľadiska výsledku fermentačného procesu, aplikácia močoviny pri silážovaní hroznových výliskov znižuje straty sušiny, stimuluje produkciu kyseliny mliečnej za súčasného tlmenia produkcie kyseliny octovej a maslovej. Avšak, v silážovaných výliskoch v prídavkov močoviny môže byť vyšší stupeň proteolýzy v porovnaní s kontrolným variantom.

Akokoľvek, vedľajšie produkty spracovania hrozna, najmä hroznové výlisky majú potenciál nutričného zhodnotenia vo výžive zvierat s preferenciu potravinových. Hroznové výlisky sú cenným zdrojom viacerých živín, minerálnych látok a špecificky účinných zlúčenín, ktoré majú pozitívny vplyv na zdravotný stav a úžitkovosť zvierat. Je však potrebné detailnejšie analyzovanie obsahových látok, možnej úpravy pre skrmovaním (napríklad využitím nanotechnológií), ale najmä dostatočného testovania s cieľom kvalifikovaného odhadu tolerovateľného zastúpenia v kŕmnych diétach zvierat.

• LIST OF AUTHORS

Prof. Ing. Branislav Gálik, PhD. is the Head of the Institute of Nutrition and Genomics as a Full Professor. His professional orientation is non-ruminant nutrition (with a special preference in horses and poultry). In this field, he has been interested in feed science and diversity, as well as feed additives and feed utilization. He has also been dealing with feed additives from the environmental point of view. Currently, his research focuses on the nutritional potential of different by-products of the agro-food industry in livestock nutrition, as well as insects used as alternative protein source in animal nutrition. As author and co-author, he has published more than 60 papers in journals indexed in databases Web of Science and/or Scopus.

Ing. Renata Kolláthová, PhD. works at the AgroBioTech Research Centre of the Slovak University of Agriculture in Nitra (Slovak Republic) as a postdoctoral scientific researcher. During her doctoral studies, she focused on a complex analysis of the nutritional value, the content of biologically active substances and *in vitro* digestibility of grape pomace, as well as on the possibilities of its use in equine nutrition (i.e., effect on the digestibility of nutrients, haematological and biochemical parameters). Currently, she deals mainly with the mineral content determination of various biological materials by the ICP-OES method (inductively coupled plasma - optical emission spectrometry).

Doc. Ing. Michal Rolinec, PhD. works at the Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra (Slovak Republic) as an Associate Professor. His professional orientation is non-ruminant nutrition, esp. swine nutrition, colostrum quality, antimicrobial resistance of bacteria and nutrition of companion animals. In those fields, he has been interested in the nutritional and immunological quality of colostrum and milk, and the passive immunisation of the neonates. Currently, he researches the effects of nutrition on the gastrointestinal microbiome of animals and the use of insects in animal nutrition, as a part of milk-replacers for animals. As author and co-author, he has published more than 39 papers in journals indexed in the Web of Science and/or Scopus databases.

Prof. Ing. Miroslav Juráček, PhD. works at the Department of Animal Nutrition, Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra (Slovak Republic) as a Full Professor. His professional field is ruminant nutrition, quality of animal products, feed conservation, and quality of feed. Currently, his research activities focus on the conservation of feed to assess the impact of silage additives on fermentation characteristics, aerobic stability, digestibility, nutritional value, and hygienic quality. He focuses on the contamination of feed by mycotoxins and their influence on the quality of animal products. His papers as author and co-author have been cited more than 230 times in journals indexed in databases Web of Science and Scopus.

Ing. Ondrej Hanušovský, PhD. is a university teacher and Assistant Professor at the Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources (Slovak University of Agriculture). Professionally, he focuses on innovative methods of internal environment monitoring of high yield dairy cows with e-boluses. Furthermore, he deals with ruminant nutrition and utilisation of grape by-products in animals. As author and co-author, he has published 39 papers in journals indexed in Web of Science and Scopus with 43 citations.

Prof. Ing. Milan Šimko, PhD. works at the Department of Animal Nutrition, Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra (Slovak Republic) as a Full Professor. His professional field is ruminant nutrition, quality of animal products and quality of feed. Currently, his research activities focus on the quality of feed to assess the impact of the digestibility of nutrients and milk quality. His works as author and co-author have been cited more than 240 times in journals indexed in the Web of Science and Scopus databases.

Ing. Luboš Zábranský, PhD. works at the Department of Animal and Husbandry of the Faculty of Agriculture, University of South Bohemia in České Budějovice (Czech Republic) as Assistant Professor. His professional orientation is ruminant nutrition (with a preference in dairy calves). He is interested in feed science and diversity, as well as feed additives and their utilization. For a long time, he has been researching feed additives from the environmental point of view. Actually, he solves the improvement of health and nutrition through synbiotic feed additives in dairy calves and the improvement of feed conversion and growth gains in fattening chickens, using environmentally friendly antibiotic-free feed additives. As author and co-author, he has published 12 papers in journals indexed in databases Web of Science or Scopus, is a co-author of 4 patents, and is researcher or co-researcher of 5 grants granted by the National Agency for Agricultural Research.

Gálik 0,5 VA
Kolláthová 1,5 VA
Rolinec 1,5 VA
Juráček 1,5 VA
Hanušovský 1,5 VA
Šimko 1 VA
Zábranský 0,5 VA

GRAPE BY-PRODUCTS

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Branislav Gálik
Renata Kolláthová
Michal Rolinec
Miroslav Juráček
Ondrej Hanušovský
Milan Šimko
Luboš Zábranský

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