







## Article

# The Effect of Dried Grape Pomace Feeding on Nutrients Digestibility and Serum Biochemical Profile of Wethers

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**Abstract:** The aim of this study was to find the effect of dried grape pomace (GP) feeding on the nutrients digestibility coefficients and biochemical parameters of sheep blood serum. The experiment was divided into three feeding periods—C (control), GP1 (1% grape pomace concentration), and GP2 (2% grape pomace concentration). Wethers in three groups in balance cages were housed for right feces collection. The C feed diet consisted of hay, ground wheat, soybean meal, mineral and vitamin lick. An experimental diet with 1% and 2% addition of GP from the daily dry matter intake was fed. After that, digestibility coefficients (in %) were calculated by the difference between nutrient intake and excretion. Furthermore, in the wethers' blood, biochemical parameters (mineral, energetic, nitrogen, and enzymatic profile) were analyzed. After the GP2 feeding, statistically significant higher digestibility of CP (crude protein), NFC (nonfiber carbohydrates), NDF (neutral detergent fiber), and OM (organic matter) was found. However, the addition of dried GP increased significantly the content of Cl<sup>-</sup> and decreased the value of glucose, nevertheless, their concentrations were within the reference interval. Parameters of the wethers' blood serum nitrogen and enzymatic profile were not affected by GP feeding. Dried grape pomace in an amount of 2% diet dry matter can be considered a suitable source of nutrients in sheep feeding, which in addition should improve the digestibility of diet crude protein.

**Keywords:** biochemical parameters; grape by-products; nutrition; sheep; utilization



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## 1. Introduction

The wine industry produces annually millions of tons of grape by-products, which are valuable resources of biologically active substances that have many potential uses, also in animal nutrition [1]. Grape pomace (GP) is a by-product from the wine industry and represents about 15–20% of the weight of the grape bunch [2]. The GP is a suitable feed additive for animal nutrition [3–8]. The product can be fed fresh, dried, or ensiled [9]. The nutritive value of grape pomace is variable depending on the grape-growing region, cultivar, technology of winemaking, and the proportion of seeds and pulp [10–12]. The GP is a source of health benefits: flavonoids with antioxidant and anti-inflammatory activity [13–15] that can improve rumen fermentation [16] and delay gas production [17]. Digestibility of crude protein, organic matter, and NDF (neutral detergent fiber) was increased in sheep receiving GP [18,19]. Many studies have focused on the biochemical profile of small ruminant's blood with impact on the effect of breed, age, gender, location, and season [20–24]. The effect of different dosages of GP on biochemical parameters of ruminants' blood in different

experiments was realized in dairy cows [25], in calves [26], or in sheep [27]. Our previous studies have analyzed the effects of various natural substances obtained as by-products of agricultural production on animal nutrient digestibility, health status, or reproductive efficiency [28–34]. These studies indicate the great potential of these products for use in animal nutrition, however, the GP addition in animal feeding has to be further examined. The hypothesis is that GP addition to the ruminants' daily diet will increase the nutrients digestibility without the negative effect on the animals' health. Based on the above, the aim of this study was to describe the effect of dried GP feeding on the nutrients digestibility coefficients and blood serum biochemical parameters of wethers.

## 2. Materials and Methods

### 2.1. The Materials Animals and Housing

Experiments were conducted at the Experimental Center of Livestock at the Department of Animal Husbandry (Slovak University of Agriculture in Nitra). The wethers were of Ile de France breed, obtained from the University farm in Kolinany (Slovak University of Agriculture in Nitra) with an average weight of  $34.05 \pm 1.97$  kg and age of 4 months. The study consisted of 3 groups: control—C, 1% grape pomace—GP1, and 2% grape pomace—GP2 (Table 1). During the preparatory time period, wethers were free housed in group without bedding in pens. Then, the wethers were housed in balance cages individually to monitor proper individual daily diet intake and feces collection in the balance period. The experiment complied with animal health care standards. The animals were under veterinary control and cared for by experienced animal caretakers during the whole experiment. The routine manipulation with animals during the experiment did not cause disproportionate and excessive stress. The conditions of animal care, manipulations, and use corresponded with the instructions of the Ethics Committee of the Slovak University of Agriculture in Nitra, Protocol No. 48/2013.

**Table 1.** Experiment scheme.

Control		Grape Pomace Addition	
C (n = 8)		GP1 (n = 8)	GP2 (n = 8)
Preparatory period 14 days	Balance period 5 days	Preparatory period 7 days	Balance period 5 days

C—control group, GP 1—grape pomace 1% from daily dry matter intake, GP 2—grape pomace 2% from daily dry matter intake.

### 2.2. Feeding and Experimental Design

The composition of experimental and control daily diets are listed in Table 2. Grape pomace of the Pinot Gris variety (*Vitis vinifera* L.) was obtained from the academic vinery (Slovak University of Agriculture in Nitra). The nutrient content of feed components is shown in Table 3. During the whole experiment, animals were fed two times per day. Half of the daily diet was fed during the morning and another 50% was fed during the afternoon. Water, mineral and vitamin lick was accessible ad libitum. The concentration of biologically active substances (total polyphenols:  $27.38 \pm 1.38$  mg GAE/g—equivalent of gallic acid) was determined in a previous study [35]. The control (C) daily diet from meadow hay, ground wheat, soybean meal, and mineral and vitamin lick was formed. The preparatory period before C diet feeding was 14 days (Table 1). Following this, the experimental balance period lasted 5 days. Daily diet GP1 and GP2 consisted of meadow hay, ground wheat, soybean meal, mineral and vitamin lick, and dried GP (1 and 2% of daily dry matter intake, respectively). The preparatory period before experimental variant GP1 and GP2 lasted 7 days and the balance period 5 days. The difference between the experimental variants was only in the concentrations of dried GP in the diet.

**Table 2.** Feed rations used in the digestibility experiment.

Feeds (g)	Feeding Groups		
	C	GP1	GP2
Meadow hay	700.0	700.0	700.0
Ground wheat	118.6	118.6	118.6
Soybean meal	238.6	238.6	238.6
Grape pomace (dried)	-	10.3 *	20.6 **
Mineral and vitamin lick	ad libitum	ad libitum	ad libitum

\* 1% from daily dry matter intake, \*\* 2% from daily dry matter intake, mineral and vitamin lick (Jan Valasek, Ludrova, Slovakia) content was as follows: MnO (as Mn) 3100 mg, ZnO (as Zn) 4800 mg, Ca(IO<sub>3</sub>)<sub>2</sub> (as I) 125 mg, Se 31 mg, CoSO<sub>4</sub>·7H<sub>2</sub>O (as Co) 42 mg, vit. A 300,000 i.u., vit. D3 125,000 i.u., vit. E 100 mg, ash 95%, Ca 9.9%, P 5.0%, Na 13.7%, Mg 5.1% in 1 kg of dry matter.

**Table 3.** Chemical composition of feed components.

	Meadow Hay	Wheat	Soybean Meal	Grape Pomace
DM *	873.85	909.75	898.95	942.25
CP	69.12	125.86	484.85	98.70
EE	10.41	17.29	15.52	84.19
CF	388.29	31.55	52.04	183.98
ADF	459.15	43.56	103.9	380.87
NDF	697.17	116.77	117.03	459.67
NFE	478.45	805.79	377.89	593.42
NFC	169.56	720.57	312.89	317.72
OM	946.26	980.49	930.28	960.28
Ash	53.74	19.51	69.72	39.72
Ca	4.58	0.40	3.39	4.46
P	2.28	4.29	7.70	3.21
Mg	1.52	1.45	3.65	1.20
Na	0.30	0.20	0.30	0.26
K	12.82	5.07	24.86	12.89

DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, ADF: acid detergent fiber, NDF: neutral detergent fiber, NFE: nitrogen free extract, NFC: nonfiber carbohydrates, OM: organic matter, \* in g/kg of original matter, other nutrients in g/kg of dry matter.

### 2.3. Blood Sampling and Analyses

Blood samples were collected from *vena jugularis externa* on the morning of the last day of the nutrition balance experiment in each variant. Sampling and analysis of blood were realized. For biochemical analysis of blood serum blood samples were centrifuged at  $1006 \times g$  for 30 min. Potassium (K), sodium (Na), and chloride (Cl) ions were analyzed by an EasyLite analyzer (Medica, Bedford, MA, USA) with an ion-selective electrode [36,37]. Blood serum concentrations of calcium (Ca), magnesium (Mg), phosphorus (P), triglycerides (TG), cholesterol (CHOL), glucose (GLU), total protein (TP), urea, albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), were determined using DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) kits on the Randox RX Monza analyzer (Randox Laboratories, Crumlin, UK) [37,38]. Globulin (GLB) was calculated mathematically by subtracting the serum levels of albumins from serum total proteins [32].

### 2.4. Feed and Feces Collection, Analysis and Determination of Digestibility

During the balance period once daily in the morning the rests and samples of feeds, daily diets, and feces were collected. The content of organic and inorganic nutrients was analyzed in the rests and samples of feeds and in pooled samples of feces for each animal for 5 days. Dry matter content (DM) was analyzed by gravimetric method at 103 °C, crude protein (CP) by Kjeldahl method, ether extract (EE) by gravimetric method according to the Soxhlet principle, crude fiber (CF) by gravimetric method as a residue insoluble in

acid and alkaline media after deduction of ash (Fibertec System, Tecator), acid detergent fiber (ADF) by gravimetric method as a residue after hydrolysis in acid detergent solution (Fibertec System, Tecator), neutral detergent fiber (NDF) by gravimetric method as a residue after hydrolysis in neutral detergent solution (Fibertec System, Tecator) and ash (A) by gravimetric method at 550 °C (muffle furnace) were determined. The content of organic matter (OM), nitrogen free extract (NFE), and nonfiber carbohydrates (NFC) were calculated according to formulas:

$$\text{OM} = \text{DM} - \text{A} \text{ (g/kg)} \quad (1)$$

$$\text{NFE} = \text{DM} - (\text{CP} + \text{EE} + \text{CF} + \text{A}) \text{ (g/kg)} \quad (2)$$

$$\text{NFC} = \text{DM} - (\text{CP} + \text{EE} + \text{NDF} + \text{A}) \text{ (g/kg)} \quad (3)$$

The content of Ca, Mg, Na, K was determined by High Resolution Continuum Source Atomic Absorption Spectrometer contraAA 700 (ANALYTIC JENA, Jena, Germany) and content of P by 6400 Spectrophotometer (JENWAY, Montreal, QC, Canada). In vivo apparent digestibility coefficients of CP, EE, CF, NFE, NFC, OM, ADF, and NDF in the diets (in %) were calculated as:

$$\text{In vivo digestibility coefficient} = [(\text{nutrient intake} - \text{nutrient excreted}) / \text{nutrient intake}] \times 100 \text{ (\%)} \quad (4)$$

### 2.5. Statistical Analysis

Statistical evaluation of results by IBM SPSS v26.0 was realized. For calculation of basic statistical characteristics (mean and standard deviation), determination of the significance of differences and comparison of the results between the control and experimental diets within the variables (Tukey Test). One-way ANOVA was performed at the level  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Nutrient Digestibility

The apparent digestibility of crude protein was affected by dried grape pomace addition (Table 4). In the control group (C) a significantly lower digestibility coefficient of crude protein ( $p < 0.05$ ), compared to the GP1 and GP2 was observed. This result corresponds with findings that were reported by some authors [18,19]. According to Guerra-Rivas et al. [11] the diet fed to the sheep (control vs. grape pomace) had minor effects on ruminal degradation parameters of crude protein. Ishida et al. [39] found lower digestibility of crude protein of grape pomace in comparison to the control diet (65.69 vs. 75.14%). It can be assumed that this was due to a higher proportion of grape pomace from dry matter intake (24% from dry matter intake of wethers). This trend was also confirmed by Abarghuei et al. [40] and Jayanegara et al. [41]. Differences in the digestibility of other nutrients between the control and experimental group GP1 were not significant. However, the apparent digestibility of nonfiber carbohydrates (NFC), organic matter (OM), and neutral detergent fiber (NDF) of the diets significantly ( $p < 0.05$ ) increased by higher dose of dried grape pomace (C vs. GP2; NFC  $p = 0.018$ ; OM  $p = 0.022$ ; NDF  $p = 0.015$ ). This trend of increasing the digestibility of organic matter and NDF, with an increase in their intake, was also confirmed by Bahrami et al. [42] and Foiklang et al. [16]. On the contrary, Baumgartel et al. [43] observed decreasing nutrient digestibility between basal and test diet including grape pomace. After the addition of GP to the ruminants' diets, higher OM digestibility was found [44].

**Table 4.** Digestibility coefficients (%) of the different feeding groups.

	Feeding Groups		
	C	GP1	GP2
CP	70.22 <sup>a</sup> ± 1.19	72.17 <sup>b</sup> ± 1.46	73.49 <sup>b</sup> ± 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 <sup>a</sup> ± 1.07	77.78 <sup>a</sup> ± 1.58	79.93 <sup>b</sup> ± 0.16
OM	62.32 <sup>a</sup> ± 2.83	62.93 <sup>a</sup> ± 0.53	65.09 <sup>b</sup> ± 0.62
ADF	49.49 ± 3.04	50.36 ± 0.24	51.37 ± 1.01
NDF	49.91 <sup>a</sup> ± 3.83	51.40 <sup>a</sup> ± 0.53	53.97 <sup>b</sup> ± 1.19

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 fiber, NFE: nitrogen free extract, NFC: nonfiber carbohydrates, OM: organic matter, ADF: acid detergent fiber, NDF: neutral detergent fiber. Different letters in row indicate statistical differences (Tukey test,  $p < 0.05$ ); data are presented as mean ± SD.

### 3.2. Mineral Profile

The changes in feeding are manifested in blood serum mineral profile [45]. Minerals perform a number of important physiological functions, such as the effect on acid-base balance, osmotic pressure, adrenal function, normal heart function, but also the metabolism of proteins or carbohydrates [46–48]. The difference in the P content after the GP was not statistically significant (Table 5). However, average P concentrations were higher than the upper limits in comparison as previously reported [48–51]. On the other hand, Jelinek et al. [52] found in rams similar blood serum P content from 2.49 to 2.92 mmol/L (depending on age). Identically, Chedea et al. [25] did not describe a statistically significant effect of dried GP (15% concentrations) in dairy cows on blood serum P content. The Ca content was similar, after feeding of all examined diets and in the interval according to Merck [51] (2.88–3.20 mmol/L). Ca concentrations were also comparable with data reported by Dias et al. [20] and Kovacik et al. [37], but higher in comparison with Schweinzer et al. [53]. Similarly, Chedea et al. [25] reported an effect of dried GP on Ca content in dairy cows (diet contained 15% dried GP). Iannaccone et al. [26] also reported in Fresian calves (10% proportion of dried GP meal in concentrate) a significant effect on the content of Ca. A similar ratio of Ca:P 1.07:1 (C, GP1) and 1.13:1 (GP2) was found which is in consent with previously reported data [48]. Concentrations of Mg in experimental groups were higher than upper limits 1.10 mmol/L found by Tschour et al. [50] and 1.31 mmol/L Merck [51]. Simpraga et al. [21] determined the content of Mg 1.30–1.60 mmol/L, which was similar to GP2. The GP addition did not affect the content of Mg, which was also confirmed by Chedea et al. [25]. The Na<sup>+</sup> content was after the addition of GP lower in comparison with control variant but its content was in the interval 130.00–155.00 mmol/L reported by Vrzgula et al. [48]. However, the analyzed Na<sup>+</sup> values were lower than determined by Kovacik et al. [37]. The intake of GP decreased non-significantly the K<sup>+</sup> content. According to Merck [51], the reference range for K<sup>+</sup> is 3.90–5.40 mmol/L. The values found in our experiment were in the range reported by Tschour et al. [50] (4.60–6.50 mmol/L). The ratio of Na and K 23.81:1 (C), 24.04:1 (GP1), 26.53:1 (GP2) was found, thus similar compared to the recommendation of Vrzgula et al. [48]. The 2% GP intake increased the concentrations of Cl<sup>−</sup> ( $p < 0.05$ ), which we do not consider a negative effect, because the main problem for chlorides is mainly a decrease, which can cause digestive disorders [48]. However, in all groups, the Cl<sup>−</sup> concentrations in blood serum were in physiological range according to Vrzgula et al. [48] and Tschour et al. [50], but higher compared to Merck [51]. Kovacik et al. [37] found higher concentrations of Cl<sup>−</sup> compared in their study. The main factor that can influence the reduced mineral absorption in this type of dietary supplement is increased fiber intake [54], which is not confirmed by animals' in vivo studies, similar to our study.

**Table 5.** Biochemical wether blood parameters.

Parameters	Unit	Feeding Groups		
		C	GP1	GP2
P	mmol/L	2.89 ± 0.18	2.87 ± 0.16	2.75 ± 0.50
Ca	mmol/L	3.09 ± 1.12	3.08 ± 0.42	3.10 ± 0.77
Mg	mmol/L	1.69 ± 0.92	1.92 ± 0.96	1.32 ± 0.44
Na	mmol/L	143.08 ± 2.96	135.13 ± 8.18	140.63 ± 1.96
K	mmol/L	6.01 ± 1.16	5.62 ± 0.33	5.30 ± 0.06
Cl <sup>-</sup>	mmol/L	105.28 <sup>a</sup> ± 1.68	106.60 <sup>a</sup> ± 0.91	108.40 <sup>b</sup> ± 1.47
GLU	mmol/L	3.90 <sup>a</sup> ± 0.30	3.17 <sup>b</sup> ± 1.05	3.26 <sup>b</sup> ± 0.35
CHOL	mmol/L	1.01 ± 0.00	1.01 ± 0.00	1.01 ± 0.00
TG	mmol/L	0.45 ± 0.06	0.53 ± 0.08	0.43 ± 0.07
TP	g/L	74.45 ± 8.18	77.25 ± 6.01	66.25 ± 15.35
ALB	g/L	33.87 ± 3.43	23.34 ± 10.15	29.41 ± 6.39
GLB	g/L	40.83 ± 9.44	53.91 ± 12.97	46.50 ± 10.64
UREA	mmol/L	6.36 ± 1.19	6.52 ± 0.86	5.63 ± 0.75
AST	μkat/L	2.02 ± 0.79	1.26 ± 0.69	1.57 ± 0.28
ALT	μkat/L	0.34 ± 0.14	0.40 ± 0.08	0.41 ± 0.04
ALP	μkat/L	3.49 ± 1.51	4.34 ± 1.24	5.16 ± 1.37
GGT	μkat/L	0.14 ± 0.08	0.20 ± 0.09	0.17 ± 0.06

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.

### 3.3. Energetic Profile

The glucose values (Table 5) were in physiological range 2.30–4.44 mmol/L [48,50,51]. However, glucose value decreased after the addition of GP (1% GP by 18.72%; 2% GP by 16.41%), but statistically significant ( $p < 0.05$ ) only in GP2, that was also confirmed by Iannaccone et al. [26]. The concentrations of 1% GP also non-significantly decreased glucose concentration in an experiment of Chedea et al. [25] and Kollathova et al. [8]. The decrease in glucose is probably related to the low energy value of GP [10]. Decreased glucose content is also associated with liver damage [48,55], which in our case can be refuted based on the results of liver enzymes. On the other hand, Alba et al. [27] determined statistically higher blood glucose after the addition of grape residue flour (2% from concentrate) in lactating dairy sheep compared to a recent study. The cholesterol concentrations in the wethers' blood serum were in all groups very similar. Bahrami and Chekani-Azar [42] and Alba et al. [27] found no statistically significant differences in cholesterol concentrations after GP feeding. Slightly lower cholesterol values in blood serum compared to physiological range ([49]: 1.05 mmol/L) were found. In addition to antioxidant activity, polyphenols have been shown to have several cardioprotective and atheroprotective effects, including lowering plasma cholesterol levels [26]. The concentration of triacylglycerides (TG) in GP2 was the highest but statistically non-significant. Similar results were also reported by Chedea et al. [25], where GP feeding has not affected the values of triacylglycerides. On the other hand, Alba et al. [27] after feeding grape pomace confirmed a statistically significant increase in TG in dairy sheep as a consequence of increased fat intake from grape pomace.

### 3.4. Nitrogen Profile

Changes in protein, albumin, and urea levels are needed to diagnose disorders of nitrogen metabolism [48]. The highest but statistically non-significant content of total proteins ( $p = 0.380$ ), globulin ( $p = 0.548$ ), and urea ( $p = 0.564$ ) in GP1 was found (Table 5). However, in the control, the highest albumin content was observed but statistically non-significant ( $p = 0.154$ ). After the GP addition, a narrower ratio between albumin and globulin (C 0.83/1; GP1 0.43/1; GP2 0.63/1) was observed. Alba et al. [27] reported statistically significant lower TP, GLB, and urea after grape residue flour (2% from concentrate)

addition in lactating dairy sheep in comparison with the present study. Alba et al. [27] also determined similar results after GP feeding on albumin (statistically non-significant). Bahrami and Chekani-Azar [42] found no significant effect of GP on the content of total proteins. The concentration of total proteins, albumin, globulin, and urea were in the reference range [21,56]. In contrast to our results, Panev et al. [57], Carlos et al. [58], and Jelinek et al. [59] reported lower average total proteins in wethers, in Morada Nova sheep, as well as generally in sheep.

### 3.5. Enzymatic Profile

Enzymatic profile indicators (AST, ALT, ALP) (Table 5) were in physiological range according to Tschour et al. [50]. Determination of enzyme activity is necessary in order to exclude hepatopathy. Furthermore, AST and ALT values were comparable with Rahman et al. [60]. The GGT values were under the limit recommended by Tschour et al. [50], Lephherd et al. [61], and Shek Vugrovecki et al. [24], but in accordance with reference values according to Al-Hadithy et al. [62]. After the GP feeding non-significant lower AST ( $p = 0.512$ ) values and higher ALT ( $p = 0.490$ ), ALP ( $p = 0.124$ ) and GGT ( $p = 0.857$ ) values were observed. Similarly, Chedea et al. [25] did not confirm the effect of GP feeding in dairy cows (diet contained 15% dried GP) on AST, ALP, and GGT. Iannaccone et al. [26] also did not find the effect of GP addition in calves (10% dried GP meal in concentrate) on AST and ALT values. In the study of Nudda et al. [63] the effect of grape seeds addition (300 g per day) on sheep AST and ALT parameters was not found but statistically significant higher ALP and lower GGT were observed. Comparable with the present study, a decrease in AST values in the lambs after feeding of GP (5%, 10%, and 20% in dry matter) with the lowest value in variant with 5% addition, was reported by Jin et al. [64].

## 4. Conclusions

The GP addition to the ruminants' daily diet increased the digestibility of nutrients without the negative effect on the biochemical profile of 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 into the daily diet did not affect the nitrogen, enzymatic, mineral, and energetic profile of wethers blood serum except  $\text{Cl}^-$  and glucose (2% of GP). Dried grape pomace in an amount of 2% diet dry matter can be considered as a suitable source of nutrients in sheep feeding, which in addition should improve the digestibility of diet crude protein.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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