

## Article

# Redefining the Use of Vinification Waste By-Products in Broiler Diets

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**Abstract:** In this study, the use of vinification by-products in broiler diets, as a sustainable and promising way of exploiting them, was examined. In particular, the potential use of ground grape pomace (GGP), wine lees extract (WYC) and grape stem extract (PE) in broiler diets was examined. Growth performance parameters, the weight of selected internal organs, meat quality traits, fatty acid profiles of breast meat and selected haematological parameters were determined. Two hundred and forty one-day-old broilers were assigned to four treatments with four replicate pens of fifteen broilers. There was one control treatment (CON), fed a basal diet, and the GGP, WYC and PE treatments, fed a basal diet supplemented with 25 g/kg GGP, 2 g/kg WYC and 1 g starch including 100 mg pure stem extract/kg PE, respectively. The duration of the experiment was 42 days. The average body weight gain during the starter, grower and finisher stages did not differ among treatments. Similarly, the feed intake, FCR and carcass yield did not show a significant difference. The weight of the internal organs was also similar among treatments. Some positive differences were observed in colour traits of meat and in haematological parameters. In the GGP group, saturated (SFAs) and unsaturated fatty acids (USFAs) were lower and higher, respectively, compared to the CON, WYC and PE groups. Vinification by-products seem to be a promising feed additive in broiler diets providing a sustainable approach to grape waste management without affecting broiler performance.

**Keywords:** broilers; grape pomace; grape stems; fatty acids; haematological parameters; meat quality; vinification by-products; waste



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## 1. Introduction

The global population is predicted to increase to 9.7 billion people by 2050 according to The United Nations [1]. As a result, more animal-origin products such as meat is likely to be necessary to cover consumers' high demands. However, the limitation of natural resources is projected to pose an extra obstacle in food production [2]. Meanwhile, the cost of conventional feedstuffs that farmers are facing nowadays is extremely high. Hence, searching for alternative feedstuffs that could be used in animal diets is necessary.

Several metric tons of agro-industrial biomass are wasted every year in the European Union [3]. The waste of this origin could be used in several sectors of the industry, including, but not limited to, animal diets, litter, organic fertilizers or as a source of heating and cooking [4]. Vineyards and wineries create high amounts of by-products since *Vitis vinifera* (the common grape vine) is considered to be the fruit crop with the highest production, exceeding 75 million tons per year, 41% of which is produced in Europe [5]. The majority of grape production is used for winemaking, while the Mediterranean area produces approximately 62% of the wine on a global scale [6]. By-products are produced during the

destemming of grapes and during the pressing and extracting procedure. The large quantities of grape waste produced annually pose a challenge for management techniques [7]. The wastes derived from wine making are mainly stems, in particular, the woody part of the grapevine, and the grape pomace, such as the skins, stems and seeds, that compose the solid residue of vinification [8]. Grape pomace accounts for 62% of the total amount of waste produced, while wine lees and stems account for 14% and 12%, respectively [9].

The biodegradation of this kind of waste in open fields, as a means of disposing of them, not only leads to a degradation of the environment and water resources [10], but also causes the waste of bioactive compounds present in these by-products [11]. The incorporation of grape by-products in broiler diets is a more sustainable approach regarding grape waste management compared to the aforementioned disposal methods, and may produce healthy products for human consumption, since winery by-products contain several bioactive compounds providing high added value for incorporation into animal diets [12,13]. A wide range of polyphenols, such as flavonoids, catechins and epicatechins, are dominant in these by-products [14]. Vinification by-products could be added to animal diets in varying levels depending on the antinutritional components present. A high crude fibre content along with antinutritional factors such as tannins present in grape pomace could have a negative effect on broiler performance [15].

This study was part of a project designed to assess the effects of adding vinification by-products (grape pomace, wine lees and stem extract) to broiler diets. Previously, we evaluated how vinification by-products affected the expression of genes involved in the oxidative status and total antioxidant capacity [16], and how the inclusion of grape by-products affected the transcriptional profiling of genes regulating the immune system in selected organs [17]. In the present study, the impact of these by-products in broiler performance, carcass yield, selected haematological parameters, the weight of internal organs, meat quality and breast fatty acid profiles of broilers was evaluated.

## 2. Materials and Methods

### 2.1. The Procurement of Grape By-Products

The process of acquiring vinification by-products and the compositional analysis was already presented in a previous article [16].

### 2.2. Broilers' Trial, Diets and Experimental Procedure

For the experimental trial, two hundred and forty as-hatched, one-day-old, Aviagen Ross 308 broilers were used. Broilers were provided from a commercial hatchery. The experimental trial lasted 42 days and conformed with the guidelines of the European Union Directive EU 63/2010 for the protection of animals used for scientific purposes and the Council of the European Union.

Broilers were randomly assigned to 4 experimental treatments with four replicate floor pens of 15 broilers each. Feeding treatments were, namely, the control, GGP, WYC and PE groups, as previously described [16]. In the control group (CON), broilers were fed a basal diet based on corn and soybean meal; in the GGP group, broilers were fed a basal diet supplemented with 25 g/kg GGP; in the WYC group, broilers were fed a basal diet supplemented with 2 g/kg WYC; and in the PE group, broilers were fed a basal diet supplemented with 1 g starch including 100 mg of pure stem extract/kg (PE). Each pen (2 m<sup>2</sup>) was covered with wheat straw litter. The stocking density in each pen did not exceed 33 kg/m<sup>2</sup>, according to directive 2007/43/EC. The housing conditions (light and ventilation) were controlled. Every pen was provided with a heating infrared lamp for keeping the broilers warm, while the temperature was set to 32 °C for the first week and was gradually decreased every week. Diets were isoenergetic and isonitrogenous and were formulated according to the Aviagen recommendations for each growth phase, namely, the starter (0–10 days), grower (11–24 days) and finisher (25–42 days) phases. Feed and water were provided ad libitum. The composition of the diets for every growing phase, as

well as the determined and calculated analysis of the diets, was described in our previous work [16] (Tables S1 and S2).

### 2.3. Body Weight and Carcass Evaluation—Sampling

The initial body weight (BW) and body weight at the end of each growing phase, on the 10th, 24th and 42nd days, were recorded. The feed intake was also recorded for every growing phase, and the feed conversion ratio (FCR) was calculated at the end of each growing phase.

On the 42nd day, 32 broilers (8 per treatment and 2 per replicate pen) were randomly selected and sacrificed in order to assess the effect of vinification by-products on carcass yield. Samples of blood and selected internal organs (spleen, liver and bursa of Fabricius) were collected and their weight expressed as the % of the total body weight. Approximately 6 mL of whole blood was immediately transferred to heparin-containing tubes (170 units heparin; BD Vacutainer, Plymouth, UK) and stored in an icebox (Thomas Scientific, Swedesboro, NJ, USA) until its transfer to the Laboratory of Nutritional Physiology and Feeding. Then, the blood samples were centrifuged (SL16R, Thermo Fisher Scientific, Waltham, MA, USA) at 2500 rpm for 15 min at 4 °C to separate the plasma from the cells. The carcasses were kept for 24 h in a fridge at 4 °C and were weighed for the estimation of carcass yield. The pectoralis major breast muscle was removed and used for the measurement of the meat quality traits and the analysis of the fatty acid profiles.

### 2.4. Determination of Haematological Parameters and Internal Organ Weight

An automatic ABX Pentra 400 analyser (Horiba-ABX, Montpellier, France) was used for the determination of the haematological parameters. In particular, the serum of blood samples from 20 broilers was used to assess aspartate aminotransferase (SGOT-AST) (IU/L), alanine aminotransferase (SGPT-ALT) (IU/L), blood urea nitrogen (BUN) (mg/dL),  $\gamma$ -glutamyltransferase ( $\gamma$ -GT) (IU/L), alkaline phosphatase (SAP) (IU/L), cholesterol (CHOL) (mg/dL), fractions of albumins (ALB) (g/dL), total proteins (CP) (g/dL) and sfairines (SFAIR) (g/dL).

### 2.5. Meat Quality: $pH_{24}$ , Colour, Shear Force and Cooking Loss

The electrode of a pH meter (HI 99,163 Meat pH Temperature Meter, Hanna Instruments, Nufalau, Romania) was inserted into the breast muscle for the measurement of the pH 24 h postmortem. The meat colour was determined after remaining in room temperature for 30 min. A Miniscan XE (HunterLab, Reston, USA) was used and set to the L\*, a\*, b\* system (CIE, Commission Internationale de l'Éclairage, 1976), with white and black tiles as standard [18]. For the determination of the cooking loss, meat samples were weighed and placed in plastic bags and were cooked for 30 min at 85 °C in a water bath. Afterwards, the samples were left at room temperature under running tap water and weighed again to calculate the cooking loss (%). The shear force was determined as described by Cason et al. [19]. Every muscle was cut parallel to the muscle fibres in three strips of 1 cm<sup>2</sup> each using a Zwick Testing Machine (Model Z2.5/TN1S; Zwick GmbH & Co, Ulm, Germany) equipped with a shear blade (Warner-Bratzler G146; Instron, Grove City, PA, USA). The peak force measurements were calculated as N/mm<sup>2</sup>.

### 2.6. Fatty Acids in Breast Meat

Breast tissue samples were partially thawed at 4 °C and trimmed to remove any external adipose and connective tissue. The total fatty acids (FAs) were extracted and methylated directly, according to the method of O' Fallon et al. [20]. For the determination of the FA profile, an Agilent 6890 N gas chromatograph equipped with an HP-88 capillary column (60 m × 0.25 mm i.d. with 0.20  $\mu$ m film thickness, Agilent, Santa Clara, CA, USA) and a flame ionization detector (FID) was used. Each peak was identified and quantified using a 37-component FA methyl ester (FAME) standard mix (Supelco, Sigma-Aldrich, St. Louis, MO, USA).

### 2.7. Statistical Analyses

The statistical analyses were performed using SPSS IBM software and the results were depicted as means and the standard error of means (SEM). For the broilers' growth performance, the experimental unit consisted of the replicate pen. The dietary effects were monitored using one-way ANOVA, followed by Tukey's test. Statistical significance was set at  $p \leq 0.05$ . Gender was not included in the statistical model as previously justified.

## 3. Results

### 3.1. Growth Performance Parameters and Carcass Yield

Broiler growth performance for every growing period is presented in Table 1. At the end of each of the three growing phases, namely, the starter (0–10 days), grower (11–24 days) and finisher (25–42 days), no differences were measured in body weight gain (ABG). Only the feed intake in the PE group during the grower phase (11–24 days) was higher by approximately 5.45, 7.35 and 8%, compared to the CON, GGP and WYC groups, respectively. Moreover, the BW on the 42nd day numerically increased ( $p > 0.05$ ) in the PE group by approximately 4, 3.9 and 3% in comparison with the CON, GGP and WYC groups, respectively. The FCR was slightly better ( $p = 0.049$ ) in the PE group compared with the CON group during the starter phase. No differences were observed for the carcass yield either.

**Table 1.** Broiler growth performance on starter, grower and finisher experimental periods among the four dietary treatments.

	Dietary Treatment				SEM	Significance
	CON	GGP	WYC	PE		
Initial BW (g)	44.08	44.08	44.79	44.50	0.540	0.824
Days 0–10						
ABG (g)	232.7	238.8	231.4	254.3	8.596	0.247
AFI (g)	288.7	287.5	285.7	292.9	7.895	0.925
BW 10 (g)	276.9	283.0	276.2	298.7	7.245	0.248
FCR	1.24 <sup>A</sup>	1.20 <sup>AB</sup>	1.24 <sup>AB</sup>	1.15 <sup>B</sup>	0.023	0.049
Mortality (%)	0	1.67	0	0	0.105	0.426
Days 11–24						
ABG (g)	952.0	926.8	931.4	991.4	23.569	0.256
AFI (g)	1195 <sup>B</sup>	1171 <sup>B</sup>	1164 <sup>B</sup>	1264 <sup>A</sup>	25.896	0.050
BW 24 (g)	1229	1210	1208	1290	27.495	0.215
FCR	1.26	1.26	1.25	1.28	0.008	0.399
Mortality (%)	0	0	3.33	1.67	0.224	0.248
Days 25–42						
ABG (g)	1747	1772	1797	1813	42.569	0.801
AFI (g)	2722	2737	2749	2857	61.598	0.463
BW 42 (g)	2976	2982	3005	3104	61.812	0.489
FCR	1.56	1.54	1.54	1.58	0.029	0.863
Mortality (%)	0	1.67	1.66	0	0.208	0.588
Carcass yield (%)	75.81	75.93	76.28	77.13	0.450	0.193

BW: body weight; AFI: average feed intake; FCR: feed conversion ratio (g feed/g gain); ABG: average body gain; SEM: pooled standard error of means; CON: control treatment; GGP: 25 g/kg ground grape pomace; PE: 1 g starch including 100 mg pure stem extract/kg; WYC: 2 g/kg wine yeast extract. Means with different capital superscript (A, B) in each row indicate significant difference ( $p \leq 0.05$ ).

### 3.2. Haematological Parameters and Weight of Internal Organs

As an indicator of broiler health, selected haematological parameters and the weight of specific internal organs were examined. Table 2 presents the analysed data. In particular, a significantly lower value of alanine aminotransferase (SGPT-ALT) was noted in the WYC

group with the wine lees extract. The remaining examined blood biochemical parameters were unaffected by the dietary treatments. Sfairines tended to be lower in the WYC treatment compared to the CON group, but no statistical significance was observed. Moreover, no differences were observed in the weight of the internal organs, as indicated in Table 2. The weight of the spleen, liver and bursa of Fabricius did not differ among treatments.

**Table 2.** Effect of diet on blood serum glutamate oxaloacetate transaminase (SGOT-AST) (IU/L), glutamate pyruvate transaminase (SGPT-ALT) (IU/L), urea nitrogen (BUN) (mg/dL),  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) (IU/L), alkaline phosphatase (IU/L), cholesterol (mg/dL), fractions of albumins (g/dL), CP (g/dL) and sfairines (g/dL).

	Dietary Treatment				SEM	Significance
	CON	GGP	WYC	PE		
SGOT-AST (IU/L)	752	938	871	979	110.45	0.577
SGPT-ALT (IU/L)	14.75 <sup>B</sup>	15.00 <sup>B</sup>	6.50 <sup>A</sup>	16.25 <sup>B</sup>	1.02	0.002
BUN (mg/dL)	1.81	2.27	1.81	2.38	0.18	0.290
$\gamma$ -GT (IU/L)	16.50	15.75	11.50	14.75	2.75	0.686
SAP (IU/L)	1351	1434	1693	1730	245.78	0.733
CHOL (mg/dL)	123	128.5	129.3	141.8	6.89	0.384
ALB (g/dL)	1.3	1.32	1.32	1.40	0.07	0.714
CP (g/dL)	3.15	2.97	2.72	3.22	0.19	0.225
SFAIR (g/dL)	1.85 <sup>T</sup>	1.65	1.40 <sup>T</sup>	1.82	0.15	0.083
Spleen (% of BW)	0.094 <sup>T</sup>	0.075 <sup>T</sup>	0.081	0.080	0.005	0.095
Liver (% of BW)	1.59	1.49	1.57	1.50	0.050	0.403
Bursa of Fabricius (% of BW)	0.071	0.063	0.065	0.053	0.006	0.192

SEM: pooled standard error of means; CON: control treatment; GGP: 25 g/kg ground grape pomace; PE: 1 g starch including 100 mg pure stem extract/kg; WYC: 2 g/kg wine yeast extract. Means with different capital superscript (A, B, T) in each row indicate significant difference ( $p \leq 0.05$ ).

### 3.3. Meat Quality Indices

In Table 3, the parameters of meat quality are summarized. The feeding grape by-products affected the colour trait of lightness. A higher value of  $L^*$  was observed in the groups fed diets containing ground grape pomace (GGP), wine lees extract (WYC) and grape stems extract (PE) compared to the control group, indicating a lighter meat colour. The colour factors  $a^*$  and  $b^*$  for the determination of redness and yellowness, respectively, did not differ. Physical traits, such as the  $pH_{24}$ , cooking loss and shear force, were similar among treatments.

**Table 3.** Carcass quality based on selected parameters among the four dietary treatments.

	Dietary treatment				SEM	Significance
	CON	GGP	WYC	PE		
Colour traits						
$L^*$	55.87 <sup>A</sup>	58.73 <sup>B</sup>	59.83 <sup>B</sup>	58.64 <sup>B</sup>	0.876	0.015
$a^*$	7.18	5.95	6.26	6.64	0.305	0.417
$b^*$	18.50	17.13	19.18	17.81	0.715	0.319
Physical traits						
$pH_{24}$	6.11	6.11	6.05	6.18	0.089	0.594
Cooking loss (%)	11.83	15.34	15.58	15.17	1.451	0.313
Shear force (100 N/mm <sup>2</sup> )	14.22	15.92	13.17	14.99	0.915	0.289

$L^*$ : lightness;  $a^*$ : redness;  $b^*$ : yellowness; SEM: pooled standard error of means; CON: control treatment; GGP: 25 g/kg ground grape pomace; PE: 1 g starch including 100 mg pure stem extract/kg; WYC: 2 g/kg wine yeast extract. Means with different capital superscript (A, B) in each row indicate significant difference ( $p \leq 0.05$ ).

### 3.4. Fatty Acids in Breast Meat

The percentage of individual fatty acids and of the main classes of fatty acids is presented in Table 4. No significant alterations were observed in the myristic, pentadecanoic, palmitoleic, margaric, stearic, C<sub>18:1</sub> trans, oleic,  $\alpha$ -linolenic,  $\gamma$ -linolenic, eicosadienoic, eicosatrienoic, arachidonic, docosadienoic, eicosapentanoic and docosahexaenoic fatty acids. The cis-vaccenic acid increased significantly in the CON, WYC and PE groups in comparison with the GGP group. Additionally, the linoleic acid had its highest value in the GGP group. Palmitic acid decreased in the breast meat of GGP-fed broilers compared to the other groups. As far as the main classes of fatty acids are concerned, saturated (SFAs) and unsaturated fatty acids (USFAs) were lower and higher, respectively, in the GGP group compared to the CON, WYC and PE groups. The index SFA/USFA was significantly lower in the group fed ground grape pomace. Moreover, the GGP group displayed an increased percentage of polyunsaturated fatty acids and a decreased value of the atherogenic index.

**Table 4.** The mean individual fatty acids (FAs) (% of total FA) in the breast meat of chickens fed the four diets.

Fatty Acids	Dietary Treatment					Significance
	CON	GGP	WYC	PE	SEM	
Myristic acid (C <sub>14:0</sub> )	0.325	0.311	0.313	0.320	0.010	0.986
Pentadecanoic acid (C <sub>15:0</sub> )	0.230	0.151	0.305	0.248	0.005	0.231
Palmitic acid (C <sub>16:0</sub> )	17.12 <sup>B</sup>	15.72 <sup>A</sup>	17.27 <sup>B</sup>	17.86 <sup>B</sup>	0.334	0.012
Palmitoleic acid (C <sub>16:1 n-7</sub> )	1.18	0.952	1.08	1.47	0.123	0.250
Margaric acid (C <sub>17:0</sub> )	0.117	0.122	0.104	0.076	0.018	0.394
Stearic acid (C <sub>18:0</sub> )	8.16	7.11	8.48	8.03	0.345	0.379
C <sub>18:1 trans</sub>	0.02	0.05	0.02	0.00	0.002	0.501
Oleic acid (C <sub>18:1 cis-9</sub> )	23.93	23.09	22.40	23.85	0.789	0.680
Cis-vaccenic acid (C <sub>18:1 cis-11</sub> )	1.693 <sup>B</sup>	1.427 <sup>A</sup>	1.811 <sup>B</sup>	1.587 <sup>AB</sup>	0.098	0.083
Linoleic acid (C <sub>18:2 n-6 cis</sub> )	32.16 <sup>B</sup>	37.03 <sup>A</sup>	31.28 <sup>B</sup>	31.17 <sup>B</sup>	1.815	0.013
$\alpha$ -linolenic acid (C <sub>18:3 n-3</sub> )	2.835	3.40	2.53	2.78	0.245	0.099
$\gamma$ -linolenic acid (C <sub>18:3 n-6</sub> )	0.20	0.26 <sup>T</sup>	0.22	0.26	0.021	0.394
Eicosadienoic acid (C <sub>20:2 n-6</sub> )	0.74	0.70	0.88	0.69	0.090	0.547
Eicosatrienoic acid (C <sub>20:3 n-6</sub> )	0.702	0.661	0.869	0.805	0.079	0.390
Arachidonic acid (C <sub>20:4 n-6</sub> )	9.04	7.70	10.54	9.56	1.456	0.608
Docosadienoic acid (C <sub>22:2 n-6</sub> )	0.164	0.137	0.235	0.157	0.035	0.131
Eicosapentanoic acid (C <sub>22:5 n-6</sub> )	0.791	0.696	0.940	0.818	0.102	0.581
Docosahexaenoic acid (C <sub>22:6 n-3</sub> )	0.532	0.435	0.567	0.465	0.095	0.764
Saturated fatty acids (SFAs)	25.96 <sup>B</sup>	23.42 <sup>A</sup>	26.48 <sup>B</sup>	26.31 <sup>B</sup>	0.502	0.024
Unsaturated fatty acids (USFAs)	73.99 <sup>B</sup>	76.55 <sup>A</sup>	73.39 <sup>B</sup>	73.62 <sup>B</sup>	0.789	0.022
SFA/UNFA	0.351 <sup>B</sup>	0.306 <sup>A</sup>	0.362 <sup>B</sup>	0.359 <sup>B</sup>	0.009	0.028
Monounsaturated fatty acids (MUFA)	26.82	25.52	25.32	26.90	0.986	0.655
Polyunsaturated fatty acids (PUFA)	47.17 <sup>A</sup>	51.02 <sup>B</sup>	48.07 <sup>A</sup>	46.72 <sup>A</sup>	0.963	0.013
Atherogenic Index (AI)	0.25 <sup>B</sup>	0.22 <sup>A</sup>	0.25 <sup>B</sup>	0.26 <sup>B</sup>	0.007	0.010

Atherogenicity index (AI) was calculated according to the equation (C<sub>12:0</sub> + 4 × C<sub>14:0</sub> + C<sub>16:0</sub>)/(PUFA + MUFA); SEM: pooled standard error of means; CON: control treatment; GGP: 25 g/kg ground grape pomace; PE: 1 g starch including 100 mg pure stem extract/kg; WYC: 2 g/kg wine yeast extract. Means with different capital superscript (A, B) in each row indicate significant difference ( $p \leq 0.05$ ).

## 4. Discussion

An assessment of the vinification by-products as feed additives was carried out in the present study aiming to redefine their use in broiler diets. Ground grape pomace (GGP), wine lees extract (WYC) and grape stem extract (PE) were added to the diet of broilers, and the results revealed that broiler performance, haematological parameters and meat quality were not negatively affected and, in some cases, were improved. The inclusion of 25 g/kg GGP, 2 g/kg WYC and 1 g starch including 100 mg pure stem extract/kg PE seemed to have no negative impact on the average body weight gain of broilers, indicating that the full body weight potential of broilers and carcass yield (%) can be achieved. Moreover,

the overall FCR did not differ among treatments, indicating a similar utilization of dietary nutrients. Thus, broilers fed the three different vinification by-products performed well, and no major differences were observed between the control and the experimental groups, indicating that by-products can be used in the poultry industry.

The findings of the present study were in agreement with Kumanda et al. [21], who used red grape pomace in broiler diets at levels of 0, 2.5, 4.5, 5.5 and 7.5%. In their study, they reported no difference in the final body weight of broilers, while the FCR was found to be the lowest in the group with 7.5% of GP. In the same study, the group with the highest inclusion level had the lowest feed intake, and this was attributed to the high level of crude fibre. Many studies, such as those of Singh et al. [22] and Lau and King [23], determined a reduction in the feed intake of broilers as the inclusion level of grape pomace increased. Thus, low levels of grape by-products led to advantageous performances due to their lower fibre content. As a result, it is crucial to identify the optimum tolerance level of these by-products in broiler diets in accordance with antinutritional factors in order to maximize the nutrient utilization and growth performance of broilers.

The values of the assessed haematological parameters were within the normal range, indicating that no negative effect was induced in the broilers' health with the dietary inclusion. In the current study, serum SGPT-ALT significantly decreased in the group fed wine lees extract (WYC) compared to the CON, GGP and PE groups. Despite SGPT-ALT not seemingly being affected by diet, Erinle et al. [24] observed a decrease in this enzyme in broilers fed with 2.5% grape pomace compared to the controls, but with differences that were not statistically significant. SGOT-AST and SGPT-ALT are ideal indicators for the health status and normal function of the liver [25]. The results of the present study indicated that the health of dietary-supplemented broilers was maintained at high levels similar to that of the controls. Ebrahimzadeh et al. [26] did not detect any changes in the concentrations of total protein, glucose and cholesterol in the blood of broilers fed grape pomace. In the present study, the weight of the internal organs was similar for all groups, which suggested no alterations in the organ weight of broilers attributed to the dietary treatments. Similar results were reached by Kumanda et al. [21] and Aditya et al. [27].

The impact of the dietary inclusion of vinification by-products in meat quality characteristics was also evaluated. As far as the colour traits are concerned, the factor  $L^*$  was higher in the treatment groups in comparison with the CON group. Although Kasapidou et al. [28] did not observe any changes in lightness and yellowness after supplementing diets with 2.5, 5 and 10 g/kg grape pomace, redness seemed to be affected. However, the enhancement of broiler meat lightness when antioxidants such as isoflavones are added to their diets has previously been observed [29]. In a study conducted by Bennato et al. [30], the pH and cooking loss of broiler meat did not change, such as in the present study, while lightness did not change in the aforementioned study in contrast to ours when grape pomace was included in broiler diets. In the same study, a more intense red colour was observed in the experimental groups compared to the control group, and a tendency for more yellow meat was also noted.

Regarding the fatty acid profile of the meat, differences were observed in the main classes of fatty acids. The fatty acid profile of grape pomace was previously illustrated [16] and affected the muscle fatty acid profile in a more preferable way, as also indicated by the atherogenicity index. A great increase in the percentage of linoleic acid in the meat of the group that was dietary supplemented with ground grape pomace was observed, which was attributed to the fact that this was the major fatty acid present in this by-product [16,31–33]. The percentage of PUFA fatty acids also increased in the grape pomace group. The aforementioned alterations to the fatty acid profile could not be solely attributed to by-products, but also to other dietary ingredients, since oils such as soybean oil are often added to broiler diets in order to balance the energy content, resembling results that were also obtained from the study of Bennato et al. [30]. There is a constant effort to produce animal products that are enriched with polyunsaturated fatty acids and, especially, n-3

fatty acids [34]. The use of vinification by-products may be a feasible strategy to obtain these desired effects.

The utilization of vinification by-products as feed additives was investigated in the present study, aiming to reveal sustainable utilization strategies. By-products used as feed additives could boost the valorisation of waste and promote a circular economy model. The maintenance of broiler performance was the key finding of the present study, along with the improvement of meat PUFA in the case of GGP-fed broilers. However, combining the results of the present study with our previous work, which showed an improved oxidative status as an effect of the dietary supplementation with grape stems (PE) and wine lees extracts (WYC), the potential use of vinification by-products is promising.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su142315714/s1>, Table S1. composition (%) of the starting (0–10 d), growing (11–24 d) and finishing (25–42 d) phases of the control (CON), ground grape pomace (GGP), wine lees (rich in yeast cell walls) extract (WYC) and grape stems extract (PE) diets; Table S2. composition (%) and calculated analysis of the starting (0–10 d), growing (11–24 d) and finishing (25–42 d) phases of the control (CON), ground grape pomace (GGP), wine lees (rich in yeast cell walls) extract (WYC) and grape stems extract (PE) diets; Table S3. chemical composition (%) and fatty acid profile of ground grape pomace (GGP).

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data are presented within the article and Supplementary Materials.

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## References

1. United Nations News. Global Perspective Human Stories. Available online: <https://news.un.org/en/story/2022/07/1122272> (accessed on 5 October 2022).
2. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: The challenge of feeding 9 billion people. *Science* **2010**, *327*, 812–818. [CrossRef]
3. FUSIONS. *Estimates of European Food Waste Levels*; IVL Swedish Environmental Research Institute: Stockholm, Sweden, 2020.
4. Ong, K.L.; Kaur, G.; Pensupa, N.; Uisan, K.; Lin, C.S.K. Trends in food waste valorization for the production of chemicals, materials and fuels: Case study South and Southeast Asia. *Bioresour. Technol.* **2018**, *248*, 100–112. [CrossRef]
5. FAO-OIV. Focus on Table and Dried Grapes. 2016. Available online: <http://www.fao.org/3/a-i7042e.pdf> (accessed on 15 October 2022).
6. FAO. STAT-FAO Statistical Database. 2010. Available online: <http://faostat3.fao.org> (accessed on 25 August 2021).



7. Skuras, D.; Psaltopoulos, D. A broad overview of the main problems derived from climate change that will affect agricultural production in the Mediterranean area. In *Building Resilience for Adaptation to Climate Change in the Agriculture Sector*; FAO: Rome, Italy, 2012; pp. 217–260.
8. Brenes, A.; Viveros, A.; Chamorro, S.; Arija, I. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. *Anim. Feed Sci. Technol.* **2016**, *211*, 1–17. [[CrossRef](#)]
9. Naziri, E.; Nenadis, N.; Mantzouridou, F.T.; Tsimidou, M.Z. Valorization of the major agrifood industrial by-products and waste from Central Macedonia (Greece) for the recovery of compounds for food applications. *Food Res. Int.* **2014**, *65*, 350–358. [[CrossRef](#)]
10. Filippi, K.; Georgaka, N.; Alexandri, M.; Papapostolou, H.; Koutinas, A. Valorisation of grape stalks and pomace for the production of bio-based succinic acid by *Actinobacillus succinogenes*. *Ind. Crops Prod.* **2021**, *168*, 113578. [[CrossRef](#)]
11. Ferri, M.; Vannini, M.; Ehrnell, M.; Eliasson, L.; Xanthakis, E.; Monari, S.; Sisti, L.; Marchese, P.; Celli, A.; Tassoni, A. From winery waste to bioactive compounds and new polymeric biocomposites: A contribution to the circular economy concept. *J. Adv. Res.* **2020**, *24*, 1–11. [[CrossRef](#)] [[PubMed](#)]
12. Georganas, A.; Giamouri, E.; Pappas, A.C.; Papadomichelakis, G.; Galliou, F.; Manios, T.; Tsiplakou, E.; Fegeros, K.; Zervas, G. Bioactive Compounds in Food Waste: A Review on the Transformation of Food Waste to Animal Feed. *Foods* **2020**, *9*, 291. [[CrossRef](#)] [[PubMed](#)]
13. Leyva-López, N.; Lizárraga-Velázquez, C.E.; Hernández, C.; Sánchez-Gutiérrez, E.Y. Exploitation of Agro-Industrial Waste as Potential Source of Bioactive Compounds for Aquaculture. *Foods* **2020**, *9*, 843. [[CrossRef](#)] [[PubMed](#)]
14. Dorri, S.; Tabeidian, A.S.; Toghyani, M.; Jahanian, R.; Behnamnejad, F. Effect of different levels of grape pomace on blood serum and biochemical parameters of broiler chicks at 29 and 49 days of age. In Proceedings of the 1st International and 4th National Congress on Recycling of Organic Waste in Agriculture, Isfahan, Iran, 26–27 April 2012.
15. Van Niekerk, R.F.; Mnisi, C.M.; Mlambo, V. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. *Br. Poult. Sci.* **2020**, *61*, 566–573. [[CrossRef](#)]
16. Mavrommatis, A.; Giamouri, E.; Myrtsi, E.D.; Evergetis, E.; Filippi, K.; Papapostolou, H.; Koulocheri, S.D.; Zoidis, E.; Pappas, A.C.; Koutinas, A.; et al. Antioxidant Status of Broiler Chickens Fed Diets Supplemented with Vinification By-Products: A Valorization Approach. *Antioxidants* **2021**, *10*, 1250. [[CrossRef](#)]
17. Mavrommatis, A.; Simitzis, P.E.; Kyriakaki, P.; Giamouri, E.; Myrtsi, E.D.; Evergetis, E.; Filippi, K.; Papapostolou, H.; Koulocheri, S.D.; Pappas, A.C.; et al. Immune-Related Gene Expression Profiling of Broiler Chickens Fed Diets Supplemented with Vinification Byproducts: A Valorization Approach II. *Animals* **2021**, *11*, 3038. [[CrossRef](#)] [[PubMed](#)]
18. Mavrommatis, A.; Zografaki, M.-E.; Marka, S.; Myrtsi, E.D.; Giamouri, E.; Christodoulou, C.; Evergetis, E.; Iliopoulos, V.; Koulocheri, S.D.; Moschopoulou, G.; et al. Effect of a Carotenoid Extract from *Citrus reticulata* By-Products on the Immune-Oxidative Status of Broilers. *Antioxidants* **2022**, *11*, 144. [[CrossRef](#)] [[PubMed](#)]
19. Cason, J.A.; Lyon, C.E.; Papa, C.M. Effect of Muscle Opposition during Rigor on Development of Broiler Breast Meat Tenderness. *Poult. Sci.* **1997**, *76*, 785–787. [[CrossRef](#)]
20. O’Fallon, J.V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. *J. Anim. Sci.* **2007**, *85*, 1511–1521. [[CrossRef](#)] [[PubMed](#)]
21. Kumanda, C.; Mlambo, V.; Mnisi, C.M. From landfills to the dinner table: Red grape pomace waste as a nutraceutical for broiler chickens. *Sustainability* **2019**, *11*, 1931. [[CrossRef](#)]
22. Singh, A.K.; Berrocoso, J.F.D.; Dersjant-Li, Y.; Awati, A.; Jha, R. Effect of a combination of xylanase, amylase and protease on growth performance of broilers fed low and high fiber diets. *Anim. Feed Sci. Technol.* **2017**, *232*, 16–20. [[CrossRef](#)]
23. Lau, D.W.; King, A.J. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *J. Agric. Food Chem* **2003**, *51*, 1602–1607. [[CrossRef](#)]
24. Erinle, T.J.; Oladokun, S.; MacIsaac, J.; Rathgeber, B.; Adewole, D. Dietary grape pomace—effects on growth performance, intestinal health, blood parameters, and breast muscle myopathies of broiler chickens. *Poult. Sci.* **2022**, *101*, 101519. [[CrossRef](#)]
25. Zhang, X. Application of total bile acid, ALT and AST in serum. *Jilin Med. J.* **2011**, *32*, 4840–4841.
26. Ebrahimzadeh, S.K.; Navidshad, B.; Farhoomand, P.; Mirzaei Aghjehgheshlagh, F. Effects of grape pomace and vitamin E on performance, antioxidant status, immune response, gut morphology and histopathological responses in broiler chickens. *S. Afr. J. Anim. Sci.* **2018**, *48*, 324–336. [[CrossRef](#)]
27. Aditya, S.; Ohh, S.J.; Ahammed, M.; Lohakare, J. Supplementation of grape pomace (*Vitis vinifera*) in broiler diets and its effect on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality. *Anim. Nutr.* **2018**, *4*, 210–214. [[CrossRef](#)] [[PubMed](#)]
28. Kasapidou, E.; Soddidou, E.N.; Zdragas, A.; Papadaki, C.; Vafeas, G.; Mtianga, P. Effect of grape pomace supplementation on broiler meat quality characteristics. *Eur. Poult. Sci.* **2016**, *80*, 135–142.
29. Salami, S.A.; Majoka, M.A.; Saha, S.; Garber, A.; Gabarrou, J.-F. Efficacy of Dietary Antioxidants on Broiler Oxidative Stress, Performance and Meat Quality: Science and Market. *Avian Biol. Res.* **2015**, *8*, 65–78. [[CrossRef](#)]
30. Bennato, F.; Di Luca, A.; Martino, C.; Ianni, A.; Marone, E.; Grotta, L.; Ramazzotti, S.; Cichelli, A.; Martino, G. Influence of grape pomace intake on nutritional value, lipid oxidation and volatile profile of poultry meat. *Foods* **2020**, *9*, 508. [[CrossRef](#)]
31. Ianni, A.; Martino, G. Dietary Grape Pomace Supplementation in Dairy Cows: Effect on Nutritional Quality of Milk and Its Derived Dairy Products. *Foods* **2020**, *9*, 168. [[CrossRef](#)]

32. Manso, T.; Gallardo, B.; Salvá, A.; Guerra-Rivas, C.; Mantecón, A.R.; Lavín, P.; De la Fuente, M.A. Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk composition. *J. Dairy Sci.* **2016**, *99*, 1111–1120. [[CrossRef](#)]
33. Tsiplakou, E.; Zervas, G. The effect of dietary inclusion of olive tree leaves and grape marc on the content of conjugated linoleic acid and vaccenic acid in the milk of dairy sheep and goats. *J. Dairy Res.* **2008**, *75*, 270–278. [[CrossRef](#)]
34. Bennato, F.; Ianni, A.; Innosa, D.; Grotta, L.; D'Onofrio, A.; Martino, G. Chemical-nutritional characteristics and aromatic profile of milk and related dairy products obtained from goats fed with extruded linseed. *Asian Australas. J. Anim. Sci.* **2020**, *33*, 148. [[CrossRef](#)]