Article

Productive Performance, Physiological Variables, and Carcass Quality of Finishing Pigs Supplemented with Ferulic Acid and Grape Pomace Subjected to Heat Stress Conditions

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Simple Summary: Phenolic compounds and sources rich in these secondary metabolites, have received great interest in the monogastric nutrition area and have been considered natural alternatives to the use of synthetic growth promoters in intensive swine production. The present study evaluated physiological variables, productive performance and carcass quality of finishing pigs supplemented with ferulic acid (FA) and grape pomace meal (GPM) for 31 days prior to slaughter under heat stress conditions. The inclusion of FA and GPM in the diet did not affect any of the physiological variables evaluated. On the contrary, the combined supplementation of FA and GPM affected feed intake (FI), while the FA addition modified hot and cold carcass yields. Likewise, GPM decreased the marbling degree of the carcasses. The results indicate that the combined supplementation of FA and GPM is favorable for carcass characteristics.

Abstract: The effect of individual and combined supplementation of FA and GPM on physiological variables, productive performance, and carcass characteristics of finishing pigs under heat stress conditions were investigated. Forty Yorkshire x Duroc pigs (80.23 kg) were individually housed and randomly distributed into 4 groups under a 2 x 2 factorial arrangement (n=10): Control (basal diet, BD); FA, BD + 25 mg FA; GPM, BD with 2.5% GPM; and MIX, BD with 25 mg FA and 2.5% GPM. Additives were supplemented for 31 days. The inclusion of FA or GPM did not modify rectal temperature and respiratory rate. An interaction effect ($P < 0.05$) was observed on FI, while average daily gain (ADG) and feed conversion (FC) were not affected by treatments ($P > 0.05$). The inclusion of FA improved hot and cold carcass weight and carcass yields, while the addition of GPM decreased the marbling ($P < 0.05$), and GPM tended to increase loin area ($P < 0.10$). GPM increased liver weight ($p < 0.05$). The addition of GPM and FA can improve some carcass characteristics under heat stress conditions. It is necessary to continue investigating different levels of inclusion of GPM and FA, to demonstrate their potential growth promoting effect in finishing pigs.

Keywords: Feedlot performance; grape pomace; ferulic acid; by-products; phenolic compounds; pork production.

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1. Introduction

Environmental stressors such as high temperatures and relative humidity represent one of the main challenges faced by swine production systems worldwide[1] . Heat stress is a significant environmental issue that negatively affects animal welfare indicators (hematological and biochemical parameters), productive performance, and generates economic losses for producers and industry [2]. Under environmental conditions that exceed thermoneutral zone in finishing pigs (18 to 25°C) induce heat stress, so the distribution of nutrients and energy from the diet is prioritized for the thermoregulatory function and the energetic contribution for the productive function is reduced, which causes changes in the behavior of the animals, decrease in productivity indexes, and sanitary imbalances [1]. This is considered a limiting factor in the meat supply to the population [2].

Therefore, to improve the productive performance of pigs during their finishing stage, intensive systems have resorted to various synthetic compounds such as β-adrenergics and antibiotics, with which it has been possible to modify the metabolism of the animals along with their zootechnical parameters such as ADG, FI, FC, and carcass quality [3]. However, their use has been restricted and banned in several countries in response to the risks they pose to public health and to changes in consumer purchasing trends [4]. These preferences have leaned towards feedstuffs free of synthetic compounds, which at the same time come from sustainable systems where animal welfare is guaranteed without neglecting meat quality and productivity [5]. In this sense, the addition of secondary plant metabolites (phytochemicals) in monogastric diets has been evaluated as an alternative to the use of traditional synthetic growth promoters and with this is possible to obtain simultaneous benefits in the animals [5,6].

Previous studies in pigs have demonstrated that phenolic compounds (PCs) such as FA and those present in various agro-industrial by-products such as grape pomace (GP), exert multiple biological activities (antioxidant, growth promoter, antimicrobial, immunomodulatory, etc.) and numerous action mechanisms [6,7]. FA has been considered an attractive strategy as a growth modulator in pigs. So far, different doses (12, 15, 25 and 100 ppm) of FA have been evaluated for a period of 28 days prior to slaughter and have reported an improvement on productive performance, transition in the type of muscle fibers, increased activity of antioxidant enzymes and reduction of malondialdehyde levels [6,8]. Respect to GP, this by-product is considered a rich source of PCs which, due to its antioxidant capacity, also presents a great potential to be included as a dietary additive. Recently, it has been shown that some of its PCs also have a potential as growth modulators, as it has been proven that they can also promote a transition of muscle fibers and regulate lipid metabolism [9,10]. Therefore, to enhance the beneficial effects of individual compounds, mixtures of extracts have been evaluated due to possible synergies that could occur between them [11]. Likewise, it can represent a strategy to counteract pre-slaughter stress in swine, guarantee an improvement in productivity without neglecting animal welfare and the meat quality.

However, no effects have been reported for the combined supplementation of grape pomace and ferulic acid against stressful stimuli and as a growth promoter [12]. Therefore, one of the challenges for animal nutrition and the pork industry is to find combinations of natural substances with which it is possible to generate a synergy of bioactive compounds through which simultaneous benefits are generated in the animal. Therefore, the aim of this study was to evaluate the effect of individual and combined supplementation of FA and GPM on productive performance, physiological variables, blood metabolites, and carcass characteristics in finishing pigs exposed to heat stress conditions.

2. Materials and Methods

2.1 Preparation of Grape Pomace Meal and Nutritional Components

Grape pomace (GP) from the Tempranillo cultivar with 48 h after pressing was supplied by an industrial wine production company in Ensenada, Baja California. The GP was dried in a convection oven (ENVIRO-PAK, model Micro-Pak, series MP500, Clackamas, OR, USA) at 60°C during 6 hours and 20 minutes to obtain a product with a moisture content of less than 10%. Dry GP was milled to a particle size of 1 mm in a Pulvex 200 equipment to obtain the GPM, which was packed in vacuum-

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sealed plastic bags, protected from light, and kept to 4 °C until use. The GPM was incorporated in the conventional feed at a proportion of 2.5%. The inclusion percentage was selected according to the total phenol content estimated in GPM and this did not exceed 1500 mg/kg feed of phenolic compounds per animal/day [13].

Moisture content (930.15), ash (942.05), protein (984.13), ethereal extract (920.39), and crude fiber (962.09) were determined according to the methods described by Association of Official Analytic Chemist (AOAC, 1990) [14]. Neutral detergent and acid detergent fiber were also quantified [15]. The nutrient content of GPM was 7.88 % moisture, 13.4% crude protein, 7.61% fat, 24.12% crude fiber, 28.43% acid detergent fiber (ADF), 40.48% Neutral detergent fiber (NDF), 7.09% ash and 2.56 Mcal/kg of metabolizable energy.

2.2 Antioxidant Capacity, Phenolic Compounds Content and Chemical Characterization of GPM

2.2.2 Obtaining extracts and measurement of phenolic content in *GPM*

Methanolic extracts of GPM were prepared[16] and used to quantify the total content of phenolic compounds, flavonoids, and antioxidant capacity. One g of GPM was homogenized in 10 mL of 80% methanol, subsequently sonicated for 30 min and centrifuged at 9400 rpm for 15 min. The supernatant was collected, filtered on Whatman N°1 paper and the residues were washed twice more with 80% methanol and stored at -20°C. The content of total phenolic compounds (soluble compounds) was estimated using the Folin Ciocalteu technique and gallic acid standard calibration curve. The absorbance was read at 765 nm using a FLUOstar Omega spectrophotometer (BMG Labtech, Durham, NC, USA). The TPC content was expressed as mg gallic acid equivalents (GAE)/g dw. Flavonoid content was determined by spectrophotometric methods [17]. Absorbance was measured at 512 nm on a FLUOstar Omega spectrophotometer (BMG Labtech). Flavonoid content was expressed as mg catechin equivalents (CE)/g dw (**Table 1**). Antioxidant capacity was determined by FRAP (ferric reducing antioxidant power), TEAC (trolox equivalent capacity) and DPPH methods and expressed in µmol of Trolox equivalents.

Table 1. Phenolic content and antioxidant capacity of GPM**.**

Variable	Value
Total phenols compounds, mg GAE/g	20.8
Flavonoids, mg/CE/g	11.3
Hydrolysable Tannins, mg GAE/g	3.34
Condensed Tannins, mg/CE/g	0.8
Anthocyanins, mg/CE/g	1.08
FRAP, µM TE/g	104.7
TEAC, µM TE/g	139.4
DPPH, µM TE/g	114.8

n=3, mg GAE/g (mg gallic acid equivalents/g); mg CE/g (mg catechin equivalents/g); µM TE/g (µmol Trolox equivalent/g); FRAP (Ferric reducing antioxidant power); TEAC (Trolox equivalent capacity); DDPH (2,2-diphenyl-1-picrylhydrazyl).

A qualitative characterization of phenolic compounds was carried out by thin layer chromatography using Nano-SIL NH2 UV254 (Macherey-Nagel 4 x 10 cm) aminated silica gel plates. A mixture of butanol: methanol: water: formic acid (50:25:20:1) was used as eluent phase and the phenolic compound standards: ferulic acid (FA), gallic acid (GA), chlorogenic acid (CGA), caffeic acid (CA), catechin (CAT), epicatechin (EC), and resveratrol (RES), were placed in different lanes together with the grape pomace samples (12µL). The chromatoplate was visualized with UV light (254 nm and 365 nm) and subsequently developed (p-anisaldehyde, sulfuric acid). The presence of CGA, CA, GA, EC, CAT, and a faint pattern of resveratrol was observed in the samples evaluated (**Supplementary Figure S1**).

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2.3. Animal Feeding Trial

All procedures involving handling and animal slaughter were conducted according to official Mexican standards (NOM-051-ZOO-1995, NOM-033-ZOO-1995, and NOM-062-ZOO-1999), and this study was approved by the ethics committee of the Universidad de Sonora. Respect to the supplemented additives, the GPM described in section 2.1 was used, while the FA was food grade with a high degree of purity (95%) and obtained from Laboratorios Minkab S.A de C.V. (Guadalajara, Jalisco, Mexico).

2.3.1 Animals and Treatments

The study was carried out at the pig experimental unit of the Agriculture and Livestock Department (ALD) of the Universidad de Sonora, (UNISON) Hermosillo, during the months of May and June 2022. The average temperature and relative humidity during the study were 29.9± 3.01°C and 38.6±8.3%, respectively. Forty male pigs from commercial Duroc x Yorkshire cross breed identified with plastic earrings with an average live weight of 80.2±8.6 kg, which were individually housed in pens with drinking and feeding troughs. Animals were provided with ad libitum water and feed. The feeding test was carried out during the finishing stage for a period of 31 days, and 10 pens were randomly assigned to one of the following treatments under a completely randomized design with 2 x 2 factorial arrangement of treatments: Control (animals receiving basal diet, DB without additives); FA, DB + 25 mg FA/ kg; GPM, BD + 2.5% GPM/ kg; and MIX, BD + 25 mg FA + 2.5% GPM/kg). The basal diet was formulated to cover the nutritional requirements for the species and productive stage [18] (**Table 2**). The phenolic compound content and antioxidant capacity of each experimental diet are presented in the **Supplementary Figure S2 y S3**, respectively.

Table 2. Ingredients and chemical composition of experimental diets.

	Treatments*			
Ingredients	**Control**	**FA**	**GPM**	**MIX**
Wheat grain, %	76.2	76.2	73.7	73.7
Soybean meal, %	17.0	17.0	17.0	17.0
Vegetable oil, %	4.4	4.4	4.4	4.4
Premix[1], %	2.4	2.4	2.4	2.4
GPM, %	0.0	0.0	2.5	2.5
FA, mg/kg	--	25	--	25
Proximate Composition				
Crude protein, %	14.0	14.0	13.9	13.9
Moisture, %	11.9	11.9	11.9	11.9
Fat, %	7.0	7.0	7.0	7.0
Fiber crude, %	2.0	2.0	2.1	2.1
Ash, %	7.0	7.0	7.0	7.0
NFE, %	58.1	58.1	58.1	58.1

Control: animals receiving basal diet, DB without additives; FA: DB + 25 mg FA/ kg; GPM: BD + 2.5% GPM/ kg; and MIX: BD + 25 mg FA + 2.5% GPM/kg). The basal diet was formulated to cover the nutritional requirements for the species and productive stage. NFE: Nitrogen free extract. [1] Premix: Premix of amino acids, vitamins, and minerals. Each kilogram of feed provided 9.5 g dicalcium phosphate, 8.3 g limestone, 3.55 g sodium chloride, 2.3 g L-lysine, 0.5 g DL-methionine, 0.35 g L-threonine, 0.15 g L-tryptophan, 80 mg DL-tocopherol acetate, 2.2 g retinol acetate, 16.5 mg cholecalciferol, 4.4 mg sodium bisulfite, 242 mg choline, 33 mg niacin, 8.8 mg riboflavin, 24.2 mg D-pantothenic acid, and 0.04 mg vitamin B12.

2.4 Environmental Conditions and Physiological Variables

During the study, the environmental temperature and relative humidity of the herd were measured daily using a remote data logger (Hobo® Model U10-003, Onset Computer Corporation, MA) in which data were recorded every 20 min. From these variables, the temperature-humidity index (THI) was calculated using the following formula: THI = (0.8 x T) + ((RH /100) x (T- 14.4)) + 46.4, and the values obtained were classified into 4 categories: suitable (< 74), mild heat stress or alert zone (74≥78), moderate heat stress or danger zone (78≥82), and severe heat stress or emergency zone (≥82). These values were expressed as units THI [18].

Rectal temperature and respiratory frequency of the animals were measured twice a day (0800 h and 1500 h) and 3 times a week [1]. Rectal temperature was measured with a calibrated digital thermometer (accuracy ±0.2°C). Respiratory rate was determined visually by counting the movement of the flanks for a period of 15 seconds [1] and the values obtained were multiplied by 4 to calculate the number of breaths per minute (bpm).

2.5 Slaughter and Carcass Traits

At the end of the feeding trial, the pigs were slaughtered after fasting for 16 h and this process was carried out in accordance with the regulations (NOM-033-ZOO-1995) at the slaughterhouse of the ALD of the Universidad de Sonora. The pigs were electrically stunned prior to bleeding. Live weight at slaughter, hot carcass weight, and after refrigeration at 2 °C for 24 h, cold carcass weight, rib eye area (cm^2), backfat thickness (mm) at the 12th rib space were measured according to the United States Department of Agriculture (USDA) guidelines [19].

2.6 Blood Metabolites

Blood samples were collected from 5 pigs per treatment at the beginning and end of the study. Approximately 14 mL of blood was collected by jugular venipuncture from each animal in two vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), one tube with ethylenediaminetetraacetic acid (EDTA) and the other without anticoagulant and immediately placed on ice. For the hemogram test (red blood cells, hemoglobin, hematocrit, leukocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte distribution (RDW-CV), platelets, white blood cells, granulocytes, lymphocytes, and medium cells) the blood from the EDTA-containing tubes was used. The tubes that did not contain EDTA were centrifuged at 10,000 rpm for 10 minutes to separate the serum to the blood cells. The serum was stored at -20°C and the following blood parameters were evaluated: glucose, total protein, albumin, globulins, creatine kinase (CK), cortisol, growth hormone (GH), and insulin growth factor 1 (IGF-1). The parameters glucose, total protein, and albumin were quantified using corresponding laboratory kit following the manufacturer's instructions (RANDOX® Manual). The cortisol, GH, and IGF-1 were determined using enzyme-linked immunosorbent assay (Sigma-Aldrich®, St. Louis, MO, EE. UU).

2.7 Statistical Analysis

Each pig was considered as an experimental unit. For the analysis of variance (ANOVA) of productive performance, physiological variables, carcass characteristics, relative weight of organs, hormonal levels, biochemical and hematological parameters a 2 × 2 factorial arrangement under a completely randomized design was used. The model considered the fixed effect of additives (FA or GPM), and their interaction (FA x GPM). Initial and final body weight were considered in the model as covariates for productive performance and carcass quality, respectively. For blood and serum parameters, the estimated initial values of the same variables were used as covariates. When significant differences were detected, the comparison of means was performed using Tukey's test. The effect of

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the treatments was considered significant when p <0.05 and trends were considered at p < 0.10. All data were analyzed with the NCSS statistical program (version 2020, NCSS, Kaysville, Utah, USA).

3. Results

3.1. Quantification of Phenolic Compounds and Capacity Antioxidant of GPM

Table 1 shows the content of phenolic compounds, flavonoids and antioxidant capacity estimated for Tempranillo grape pomace. The average content of total phenols and flavonoids in grape pomace flour was 20.81 mg GAE/g, 11.30 mg CE/g respectively. The antioxidant activity by FRAP, TEAC and DPPH methods was 104.7; 139.4 and 114.8 μM TE/g respectively.

3.2 Physiologycal Variables

The average of environment temperature was 29.9°C, ranged from 20.41°C to 39.06°C, while the average relative humidity was 38.61% with a minimum and maximum of 19.8°C and 62.28°C, respectively (unpublished date). In this case, the average temperature exceeded the thermal comfort zone [20] of the finishing pigs (18-25°C). The THI in the present study ranged from 68.67 to 96.46 with an average of 76.02 units THI (unpublished date). The THI values indicate that the animals presented a mild to severe stress level during the entire study period, therefore they were within an alert (74-78) and emergency (>82) zone, in which they must prioritize their energy reserves for thermoregulation purposes.

According to **Table 3**, there was no effect of any of the additives (FA or GPM) or their interaction on the physiological variables (p >0.05). In the afternoon, the animals presented values of rectal temperature and respiratory rate higher than 39.3°C and 80 bpm respectively, which exceeded the normal values for these physiological variables and proves that the animals were under heat stress conditions during the study [1,21]. On the contrary, in the morning hours, both physiological variables remained within a normal range or thermoneutral zone [1].

Table 3. Rectal temperature and respiratory rate of finishing pigs supplemented with ferulic acid and grape pomace meal.

		Treatments					p- values		
		FA, mg		GPM, %			FA	GPM	FA x GPM
		0	25	0	2.5	SEM			
AM	RT, °C	38.65	38.7	38.69	38.66	0.004	0.521	0.611	0.931
	RR, bpm	51.9	50.59	53.26	49.23	2.30	0.694	0.232	0.963
PM	RT, °C	39.39	39.46	39.4	39.45	0.005	0.361	0.551	0.162
	RR, bpm	82.55	82.21	83.23	81.51	3.57	0.673	0.496	0.622

FA: ferulic acid; GPM: grape pomace meal; RT: rectal temperature; RR: respiratory rate. SEM: standard error of the mean.

3.4. Productive Performance of Pigs and Carcass Quality

The effect of individual and combined supplementation of FA and GPM on the productive performance of finishing pigs is presented in the **Table 4.** There was no significant effect of FA or GPM on final weight, ADG, and feed conversion (p >0.05). In contrast, there was an effect of the FA x GPM interaction on FI, which increased when only GPM was included in the diet, respect to Control and diet with two additives (p <0.05). Although there were no significant differences in ADG, this parameter increased by 10% in animals supplemented with GPM, and a gain of 3 kg in the final body weight was obtained, in comparison to Control.

Table 4. Productive performance of finishing pigs supplemented with ferulic acid and grape pomace meal.

Variable		**Treatments**					***p*- value**		
	FA	0		25 mg			FA	GPM	FA x GPM
	GPM	0	2.5%	0	2.5%	SEM			
IBW, kg		79.37	80.86	81.06	79.62	2.92	0.931	0.991	0.624
FBW, kg		116.03	119.09	116.28	116.62	1.38	0.432	0.331	0.231
ADG, kg		1.15	1.27	1.17	1.16	0.04	0.273	0.224	0.144
FI, kg DM/d		2.74a	2.99b	2.86ab	2.76a	0.08	0.461	0.344	0.038
FC, kg DM		2.39	2.38	2.40	2.39	0.07	0.552	0.617	0.692

FA: ferulic acid; GPM: grape pomace meal; IBW: initial body weight; FBW: final body weight; ADG: average daily gain; FI: feed intake; FC: feed conversion; DM, dry matter. Means with different letters, indicate significant differences ($p < 0.05$). SEM: standard error of the mean.

Regarding to carcass characteristics (**Table 5**), the FA x GPM interaction tended to be significant for hot carcass yields ($p < 0.10$), while the interaction between these additives was not significant for the other variables (HCW, CCW, backfat thickness, marbling, and CCW yields). The addition of FA improved both HCW and CCW yields ($p < 0.05$). It also tended to increase HCW and CCW ($p < 0.10$). The carcass marbling degree was reduced with the addition of GPM ($p < 0.05$) and this additive tended to increase the loin area ($p < 0.10$).

Table 5. Carcass traits of pigs supplemented with ferulic acid and grape pomace meal.

Variables		Treatments					**p-value**		
	FA	0		25 mg			FA	GPM	FA x GPM
	GPM	0	2.5%	0	2.5%	SEM			
HCW, kg		87	86.06	87.11	88.59	0.75	0.088*	0.728	0.123
CCW, kg		85.85	84.68	86.17	87.25	0.74	0.063*	0.956	0.152
HCW yields, %		82.41	81.21	82.62	83.61	0.53	**0.022**	0.851	0.056*
CCW yields, %		81.56	80.39	82.1	82.72	0.58	**0.020**	0.652	0.144
pH_{24}		5.52	5.51	5.5	5.5	0.04	0.741	0.913	0.841
Backfat thickness, mm		10.41	10.07	11.07	10.17	1.53	0.801	0.692	0.851
Marbling score		2.75	2.44	3.017	2.35	0.23	0.723	**0.049**	0.472
Loin area, cm^2		57.23	59.77	59.49	61.4	1.25	0.13	0.095*	0.822

FA: ferulic acid; GPM: grape pomace meal; HCW: hot carcass weight; CCW: cold carcass weight. Means with different letters, indicate significant differences ($p<0.05$). *Trends ($p<0.10$). SEM: standard error of the mean.

3.5. Relative organ weight of finishing pigs.

Table 6 presents the effect of individual and combined supplementation of ferulic acid and grape pomace on the relative organ weight of finishing pigs (% of FP). The FA x GPM interaction was significant for liver weight ($p < 0.05$), which decreased with the combined addition of additives, while

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the individual inclusion of GPM increased the percentage ratio for this organ (p <0.05). Likewise, the inclusion of FA tended to modify liver weight (p <0.10). On the contrary, there was no significant effect of the additives on the relative weight of the spleen, heart, lung, stomach, and kidneys (p >0.05).

Table 6. Relative weight of the organs of finisher pigs supplemented with grape pomace and ferulic acid.

Relative weight organ	Treatments						p-value		
	FA	0		25 mg			FA	GPM	FA x GPM
	GPM	0	2.5%	0	2.5%	SEM			
Liver, %		1.59ab	1.69a	1.60ab	1.50b	0.04	0.078*	0.982	**0.040**
Spleen, %		0.17	0.18	0.18	0.17	0.01	0.867	0.831	0.279
Heart, %		0.36	0.36	0.35	0.36	0.02	0.744	0.803	0.983
Lung, %		1.02	0.99	0.97	0.87	0.05	0.106	0.225	0.537
Stomach, %		0.52	0.57	0.52	0.50	0.02	0.138	0.657	0.138
Kidney, %		0.34	0.34	0.37	0.33	0.01	0.458	0.233	0.212

FA: ferulic acid; GPM: grape pomace meal; Means with different letters, indicate significant differences (p<0.05). *Trends (p<0.10). SEM: standard error of the mean.

3.6. Hormonal levels, hematological and biochemical parameters of finishing pigs.

The results of hematological and biochemical parameters of pigs supplemented with FA and GPM are presented in **Supplementary Table S1.** The values obtained were within the reference ranges reported for the species and its stage. The combined addition of FA and GPM tends to decrease MCV in finishing pigs (p <0.10), while GPM exerts a significant effect (p <0.05) for this variable. Likewise, the individual inclusion of GPM tends to increase erythrocyte, hemoglobin, and hematocrit values (p <0.10) in contrast to FA. On the contrary, there were no significant differences in most blood constituents (MCH, MCHC, RDW-CV, platelets, white blood cells, granulocytes, lymphocytes, and medium cells), biochemical variables (glucose, total proteins, albumins, globulins, albumin: globulin ratio, and CK), and hormones (cortisol, IGF-1, and GH) (p >0.05).

4. Discussion

The GP contains a highly variable proportion of pulp, skin, seeds, and stems, which together with the conditions of grape cultivation (ripening stage) and the production process determine its final composition. This by-product presents high variability in terms of nutritional quality and antioxidant potential. In general, grape pomace contains high amounts of soluble phenols, including flavonols (0.3-2.6 mg/g), anthocyanins (2.5 to 132 mg/g) and soluble proanthocyanins (1.2 to 68.5 mg/g) [1]. However, winemaking methods (fermentation, maceration, pressing) determine the concentration of phenolic compounds remaining in GP. Prolonged maceration times and high fermentation temperatures tend to increase the release of phenolic compounds in the wine, and the content of PCs remaining in the winemaking by-products is decreased [2,3]. In our study, the content of total phenolic compounds estimated for the Tempranillo variety (20.83 mg GAE/g) agrees with the ranges reported in other studies about red GPM [4], and exhibits considerable antioxidant capacity. Also, in this by-product we identify CGA, CA, GA, CAT, EC, and RES which exert multiple bioactivities in pigs (**Supplementary Figure S1**) and may help to explain the effects observed in this study.

4.1. *Physiological Variables*

Rich sources in phytochemicals and especially PCs have received great interest as feed supplements to attenuate or minimize the stress to which pigs are subjected during their productive cycle

[22,23]. Its beneficial effect is attributed to the antioxidant potential per se of PCs and an improvement in mitochondrial function together with an increase in antioxidant enzyme activity [24,25]. In this context, under heat stress conditions and when the critical limit of the comfort zone is exceeded, animal's resort to certain mechanisms to minimize internal heat production and reach a homeostatic state [2]. Likewise, heat production and feed intake in pigs tends to decrease withing a range of 22.9°C to 25.5°C [26]. The room temperature and relative humidity fluctuations are responsible of increase respiratory rate and rectal temperature in finisher pigs. In this situation, pigs activate certain homeostatic mechanisms to adapt to some kind of stressor. Even, these adaptations usually have repercussions on productive performance parameters, among which feed intake has greatest impact [2].

In the present study, the inclusion of the additives (FA or GPM) did not affect the RF and RT, which can be attributed to PCs concentration, profile, dosage, antioxidant capacity, among others. Likewise, in other studies do not obtain changes in these variables with ferulic acid inclusion in small ruminants' diets under heat stress conditions [27]. However, the authors demonstrated that the inclusion of FA increases the deposition of fat reserves and could modulate the animal energy metabolism at temperatures higher than the thermoneutral zone. Even, other phytochemicals such as alkaloids can partially attenuate RF an RT in growing pigs by inhibiting the Na+/K+ ATPase pump activity (thermogenesis) [23]. In addition, other PCs such as resveratrol, quercetin, catechins, which were identified in grape pomace (**Supplementary Figure S1**) can modulate energy expenditure by inducing activity in brown adipose tissue and may act through different molecular mechanisms (SIRT1 activity, estrogen receptor stimulation, AMP-kinase signaling or sympathetic nerve activation) [28]. Although the use of natural antioxidants in monogastric diets can contribute to reduce the impact of environmental and increase their adaptive capacity [29] no changes were observed in our study. However, further research is needed to determine the possible mode of action of PCs as stress attenuating.

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

4.2. *Productive Performance and Carcass Traits*

To obtain higher lean tissue yields in pork carcasses, multiple growth promotion technologies such as β-adrenergics (ractopamine) have been employed to increase the efficiency of dietary nutrient utilization for protein deposition and lipolytic rate [30]**.** However, in view of the prohibition of its use in pig production, alternatives of vegetable origin have been sought. Similarly, there are multiple pure phenolic compounds and those embedded in agro-industrial by-products that could also act as growth modulators. In this sense, a study [6] suggested that FA can act similarly to ractopamine given that it has a structure analogous to catecholamines, especially noradrenaline. With the inclusion of FA in the diets of finishing pigs, improvements have been observed in productive parameters (ADG, FC, and FBW) and carcass characteristics (loin area, and backfat thickness) [6]. In our study, it was demonstrated that the combined inclusion of FA and GPM during the finishing stage modified the FI. The inclusion of GPM (2.5%) increased FI (9%), which can be associated with the presence of aromatic compounds from this by-product that stimulate intake and make the diet more palatable [13,31]. This result is considered attractive from a production and farm programming point of view (efficient use of facilities, less time spent per animal and higher slaughter weight in less time). Some fermentable sugars remaining in grape pomace could be related to a higher voluntary FI, due to the pigs' preference for sweet compounds [32]. Likewise, rich sources in PCs promote the secretion of saliva and digestive enzymes, improving nutrient absorption and utilization. Therefore, PCs have been used as feed additives to modify the organoleptic properties of monogastric diets and thus improve taste and palatability [13].

Hydroxycinnamic acids can contribute to the flavor profile through diverse mechanisms such as phenolic degradation that generates aromatic and taste compounds, impart flavor attributes, and can modify mechanisms of the Maillard reaction [33]. However, with high doses of PCs (1500 mg/kg), FI decreased in response to a strong dietary aroma and the sensitive palate (19000 buds) of pigs to bitter tastes[13,34]. This could explain the differences in FI observed with the combined inclusion of the two additives versus the individual inclusion of 2.5% GPM in the diet. This behavior may also be associated with a higher concentration of PCs imparting a strong aroma or some type of phenolic interaction that may be generated between the matrix and pure compounds [35]. In general, pigs show a greater response to bitter tastes and feed aversions than to preferences [34].Also, the inclusion of fibrous by-products in pigs diets tends to suppress FI [36,37]; however, in our study this situation did not occur, which can be attributed to the low content of NDF, ADF (degree lignification), hydrolysable tannins, condensed tannins of the GPM (Table 1) [12,38].

Regarding FA supplementation, recent studies showed that the addition of FA (25 mg) in the diet of finishing pigs (Landrace x Yorkshire) improve their productive performance (FC and ADG) [6]. On the contrary, in our research, when evaluating the same dose of FA (25 mg) in crossbreeds (Yorkshire x Duroc), it did not affect the productive performance parameters. Similar results were reported by Herrera et al. [39], who evaluated low doses of FA (12-15 mg) in finishing pigs (Landrace Yorkshire Duroc) and their results were attributed to an insufficient dose to generate an effect on pigs' performance. Likewise, multiple factors, such as purity, vegetable origin of the FA evaluated [6,39] as well the experimental conditions (ambient temperature and relative humidity) joint to evaluation period, which determine nutrient utilization in pigs and may partially explain the differences between our results with Valenzuela-Grijalva research [6]. Even, previous studies [40–42] have shown that breed and genetic line influence growth rate and productive performance parameters, such as feed conversion and feed intake. In this context, the Landrace and Yorkshire breeds are more efficient than Duroc breed in terms of ADG and FCR during the finishing stage. Also, this breed tends to deposit more fat than protein in the finishing stage [42]. This could explain the results reported in our study and in other works [39], in which crosses with Duroc were used and no effect of FA.

Conversely, we observed that FA (25 mg) improved cold carcass and hot carcass yield, as well as tended to increase hot and cold carcass weights. These results demonstrate that pigs supplemented with FA were more efficient in utilizing dietary nutrients and depositing lean tissue. Also, these help to support and complement previous research in which lower back fat deposition (6.44 mm), increased loin area (52.45 cm^2), higher lean efficiency (59.66%) and carcass efficiency (89.2%) were obtained with the inclusion of 15 mg and 25 mg [6,39] In this sense, it has been proposed that FA could act in a similar way to β-adrenergic (ractopamine) to favor protein deposition and increase lean mass in muscle. This effect has been attributed to a similarity between FA and noradrenaline structures [43] , thus favoring its recognition by adrenergic receptors at the membrane level. Furthermore, it has been suggested that FA has an affinity for β2-adrenergic receptors and increases the expression of β2-AR transcripts in a manner like ractopamine, suggesting a stimulation of β-ARs together with a modification of mRNA patterns [6]. β-adrenergics act as dietary nutrient partitioning agents that allocate part of dietary energy to protein synthesis and promote adipose tissue lipolysis, conversely, they minimize the rate of protein degradation along with lipogenesis. Also, it has been shown that supplementation of 25 ppm FA in finishing pigs increases the proportion of intermediate muscle fibers (19%) and decreases the proportion of glycolytic fast fibers (77%) [6], which has been correlated with an increase in insulin sensitivity [7]. In this context, it has been suggested that FA and other PCs (chlorogenic acid, resveratrol, quercetin) can modulate the expression of β2 AR receptors [9,45] by activating G protein and mTOR (main regulators of cellular metabolism and protein synthesis) and regulate the transition muscle fibers through different signaling pathways (SiRT1 1/AMPK, adiponectin). This effect has been associated with a decrease in LDH (lactate dehydrogenase) activity and glycolytic fast fibers while increase gene expression of oxidative slow fibers and SDH (succinate dehydrogenase) activity [7,44,45]

Respect to dietary inclusion of GPM, a reduction in marbling degree and a trend to increase loin area was observed. The reduction in intramuscular fat was also observed with other agro-industrial by-products (tomato, avocado meal, and citrus residues) which has been associated to its proximal composition that generate a nutrient contribution to the diet [37,46]. This results, can be related to

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some PCs (chlorogenic acid, caffeic acid, catechin, epicatechin, resveratrol) previously identified in GPM (**Supplementary Figure S1**) and in other research [44,47]. Likewise, recently has been demonstrated that the inclusion of PCs such as resveratrol, and flavonoids (quercetin derivatives and anthocyanins) from mulberry in finishing pigs' diets [44,45,48] modifies fat metabolism and reduce backfat deposition. In this sense, it has been proposed that these compounds can modulate some genes involved in fat metabolism, inhibit fatty acid synthesis (chlorogenic acid, caffeic acid, quercetin) and acting at the level of adiponectin receptors[44,48]. Also, as previously mentioned, these PCs may regulate the transition of muscle fibers and thus explain the trend obtained in loin area. Even, Zeng et al. [49] suggest that mulberry leaves (flavonoids) may have a nutrient partitioning effect, and modify the proportion of type II a muscle fiber in finishing pigs [50] In this context, the GPM evaluated also contain compounds that could exert this modulatory effect in finishing pigs; however, future research should be conducted to corroborate this assumption and will also be corroborated by evaluating the fatty acid profile of meat supplemented with GPM or FA.

4.3. Relative Organ Weights of Finishing Pigs

The internal organs of monogastric can undergo multiple metabolic and structural changes in response to dietary supplementation of certain additives, phytochemicals, or growth promoters [51], which can be reflected in their relative weight and influence the final pig weight joint to its carcass yields[52]. It has been proven that secondary metabolites such as PCs could act similarly to traditional synthetic promoters (β-adrenergics) and can cross various tissues depending on their structure. In monogastric, usually most [6] of these PCs are metabolized in the liver and intestine, where their conjugation takes place [51,53]. Therefore, previous research has evaluated the effect of multiple PCs and growth promoters on the relative weight of visceral organs involved in their metabolism and which also possess specific receptors for β-adrenergics, which could help to support the results of future research. From these studies, diverse and inconsistent results have been obtained, whose effects could be attributed to the doses evaluated [54,55] and it has even been hypothesized that the size of the organs involved in PCs metabolism could reflect some effect of these as a possible growth promoter [52,55].

In the present study, the inclusion of FA and GPM in the finishing diets modified the relative liver weight in pigs, which increased with individual supplementation of GPM and decreased with the inclusion of both additives. These results could be partially supported by the research of Njoku et al. [56] who reported an increase in relative liver weight as a function of FI and attributed this to chemical changes occurring in this organ during FI. It has also been shown that FI stimulates the growth of visceral organs, determines the distribution and use of animal protein [57]. Therefore, in our research, the increase observed in the relative weight of the liver can be explained by the higher FI obtained with the GPM. In contrast, Dávila-Ramírez et al. [52] did not observe a significant effect on organ weight in finishing pigs supplemented with a commercial mixture of plant extracts (protorgan). Likewise, other studies evaluated the inclusion of 2% tannic acid in a murine model, with which no changes were obtained in the relative weight of the liver and intestine [51].

Regarding FA and its structural similarity to some growth-promoting compounds, Aalhusi et al. [54] demonstrated that the inclusion of ractopamine hydrochloride in finishing pigs decreased liver weight but did not exert significant changes in lung and heart size. On the contrary, Bergstrom et al. [55] reported an increase in liver and heart weight as a function of ractopamine dose. Nevertheless, the authors do not explain the changes obtained in the relative weight of the liver. On the other hand, recent studies demonstrated that maternal supplementation with polyphenols exerts significant changes in the weight of the adrenal glands (not evaluated) and spleen in their progeny during the finishing stage (180 days) [58]. This information could be considered for future research with which it may be possible to prove a possible promoter effect in the adrenal glands.

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4.4. Hematological and Biochemical Variables and Hormone Levels

One of the most common physiological responses exhibited by animals under heat stress conditions is the decrease in hematocrit and hemoglobin content due to erythrocyte lysis and reduced erythropoiesis, which is caused by the increase in oxygen partial pressure and respiratory rate [59,60]. Likewise, under these conditions the platelet content is modified. In this sense, previous studies have shown that the use of non-conventional feed sources rich in PCs can positively modify some hematological and biochemical parameters in monogastric under stressful situations [22,61]. Therefore, blood metabolites have been considered as indicators of nutritional metabolism and health of animals [62]. In the present study, the values obtained to hormonal levels, hematological and biochemical parameters were within the reference ranges reported for the species and its stage [63].

However, it is shown that the joint addition of FA and GPM decreases MCV, while erythrocyte, hematocrit and hemoglobin values tend to increase with GPM addition. Similar results were reported in other investigations [64,65] in which mixtures of herbal extracts were supplemented in finishing pig's diets and an increase in red cell content was observed. These changes could reflect an improvement in the respiratory capacity of monogastric [66]and this information may help to support the trend presented with grape pomace on some hematological variables.

On the contrary, our experimental results differ from the study of Nicolás-López et al. [22,67], who demonstrated that the inclusion of FA in diets for finishing sheep tends to increase MCV and decrease erythrocyte content along with platelet count. In this sense, it has been proposed that FA can generate slight alterations in red cell and platelet content due to a reduction in activity of the thyroid gland, which exerts great influence under heat stress conditions [22]. Likewise, it has been reported that this compound can increase the release of erythropoietin in the kidney, modulate erythropoiesis and exert a cytoprotective. Therefore, it is convenient to expand research in swine due to the importance of this phase in their health. Likewise, other factors such as the final live weight of the animal may influence these hematological variables since this parameter tends to affect the animal's response to stressful situations.

Although it has been reported that PCs inclusion in monogastric diets could have an influence on the hypothalamic-pituitary-adrenal gland axis [68], no significant effect on cortisol levels was observed in our study. Similar results were reported by Dávila-Ramirez et al. [52], who also did not observe an effect of the inclusion of herbal extracts in finishing pigs. Likewise, no significant changes were observed in the levels of growth hormone and insulin-like growth factor. On the contrary, in other studies, with the inclusion of herbal extracts in growing pigs, an increase in IGF-1 levels was reported [69,70]. The differences between these results and our study can be attributed to multiple factors such as biological variability, number of animals, growth stage, dose of the compound evaluated, experimental conditions, among others. These results demonstrate that the inclusion of GPM does not exert negative effects on blood metabolites, since they are within the reference values established for the species and for its stage.

5. Conclusions

Dietary GPM supplementation in finishing pigs exposed to heat stress conditions can improve feed intake and some carcass traits (loin area), modify marbling degree and relative liver weight. Although physiological variables were not modified, this source of PCs can be considered suitable as a sensory and zootechnical additive in pig production, it could also represent as a promising alternative to maintain pig performance during to environmental changes. While dietary FA supplementation in finishing phase improve some carcass traits like carcass weight and cold carcass yield, which may suggest an improvement in lean tissue deposition with lower feed intake and possible effect on growth modulation. However, further research is required to clarify these results obtained with a greater number of pigs. Likewise, the results of this research contribute to expand the information available on the use of PCs as phytogenic additives in swine production.

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Data Availability Statement: The data sets analyzed in the present study are available from the corresponding authors upon request.

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