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Effects of a Natural Polyphenolic Product from Olive Mill Wastewater on Oxidative Stress and Post-Weaning Diarrhea in Piglets

Georgios I. Papakonstantinou [1], Eleftherios Meletis [2], Konstantinos Petrotos [3], Polychronis Kostoulas [2], Nikolaos Tsekouras [1], Maria C. Kantere [1], Nikolaos Voulgarakis [1], Dimitrios Gougoulis [1], Leonidas Filippopoulos [4], Georgios Christodoulopoulos [5], Labrini V. Athanasiou [1] and Vasileios G. Papatsiros [1,*]

[1] Clinic of Medicine, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, Trikalon 224, 43100 Karditsa, Greece; geopapak@vet.uth.gr (G.I.P.); nitsekou@vet.uth.gr (N.T.); mkantere@uth.gr (M.C.K.); nvoulgarakis@uth.gr (N.V.); dgoug@vet.uth.gr (D.G.); lathan@vet.uth.gr (L.V.A.)
[2] Faculty of Public and Integrated Health, University of Thessaly, 43100 Karditsa, Greece; emeletis@outlook.com (E.M.); pkost@uth.gr (P.K.)
[3] Department of Agrotechnology, School of Agricultural Sciences, University of Thessaly, Geopolis Campus, Periferiaki Odos Larisas Trikalon, 41500 Larisa, Greece; petrotos@teilar.gr
[4] Hipra Hellas SA, 10441 Athens, Greece; leonidas.filippopoulos@hipra.com
[5] Department of Animal Science, Agricultural University of Athens, 75 Iera Odos Street, Botanikos, 11855 Athens, Greece; gc@aua.gr
* Correspondence: vpapatsiros@vet.uth.gr; Tel.: +30-2441-066012; Fax: +30-2441-066053

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Abstract: The present study aimed to investigate the effects of a commercial phytogenic feed additive (PFA) on the prevention of post-weaning diarrhea and oxidative stress in piglets. The concentrations of thiobarbituric acid reactive substances (TBARS) and protein carbonyls (CARBS) were investigated as biomarkers for oxidative damage, as were the health and performance parameters of weaned piglets. In total, 100 weaned piglets were divided into two groups: a control group (T1), which was fed regular weaning feed; an experimental group (T2), which was fed regular weaning feed supplemented with a phenolic feed additive (PFA) for 3 weeks. The TBARS and CARBS concentrations in plasma samples from 20 piglets per group were measured at 45 and 65 days of age. Fecal samples were collected from 24 weaned piglets per group using FTA ELUTE cards. Diarrhea score, body weight (BW) at weaning, and average daily weight gain (ADWG) were recorded. The TBARS ($p < 0.001$) and CARBS ($p = 0.001$) concentrations were significantly higher in the T1 group compared to those in the T2 group. The lowest diarrhea score was noted in the T2 group for the age groups of 45 ($p < 0.001$) and 65 days ($p = 0.008$). In conclusion, the use of a phenolic PFA in the current study had beneficial antioxidative and antimicrobial effects on weaned piglets, which improved their health and growth performance.

Keywords: piglets; antioxidant; polyphenol; olive; TBARS; protein carbonyls; BW; ADWG

1. Introduction

Weaning is a crucial stage in pig production and includes important challenges for pig welfare and growth performance [1,2]. During the weaning period, piglets are challenged with various environmental and psychosocial stress factors, resulting in decreased feed intake and growth performance in addition to increased morbidity and mortality rates [3,4]. Specifically, due to the transition from a milk-based diet to a solid feed diet, weaned piglets suffer from severe reductions in feed intake over the first days after weaning [5,6]. Furthermore, changes in feeding behavior and diet composition cause modifications to their gastrointestinal microbiota [7]. Consequently, weaned piglets often suffer from gastrointestinal disorders [8,9].

Weaning stress causes an increased oxidation process and the production of high concentrations of free radicals, which destroy the redox equilibrium in pigs [4,10–12]. This condition causes damage to epithelial cells and the morphology of the intestine, resulting in reductions in feed intake and growth rate, as well as the induction of diarrhea and inflammatory reactions [13,14]. Post-weaning diarrhea (PWD) is a multifactorial gastrointestinal disease occurring over the first 2 weeks after weaning due to several stress factors that are commonly enhanced by infection with specific pathogens, such as enterotoxigenic *Escherichia coli* (ETEC) [15,16]. However, PWD with no detection of ETEC is not unusual [17], and it has been reported that intestinal inflammation and diarrhea may be caused by intestinal dysbiosis in weaned piglets [18].

The use of zinc oxide (ZnO) as a feed additive has been commonly proposed for the prevention of PWD in piglets. Based on recent regulations from 2022, the application of dietary ZnO in weaning feed has been forbidden in the European Union (EU) to reduce the negative environmental consequences of pig manure in agricultural soils [19]. Due to modern consumer demands and public health awareness, restrictions on the use of antibiotics as growth promoters in pig diets have also been proposed and applied [20]. Phytogenic feed additives (PFAs) have mainly been proposed as potential alternatives to in-feed antibiotics, based on their antibacterial activity against both Gram-negative and Gram-positive bacteria [21–25]. In addition, PFAs have been reported to have potential antioxidant activity, thereby removing free radicals and protecting animals from oxidative damage [26,27]. The antioxidant properties of PFAs are mainly associated with phenolic compounds that react strongly with peroxyl radicals, which are produced by oxidized proteins and lipids [28,29].

Modern pig production demands the ideal combination of the reduced use of antibiotics, improvements in animal health as well as welfare, and increased profitability. This being the case, herd health programs need to measure and evaluate indicators for animal welfare and health, such as oxidative status. The oxidative status reflects the equilibrium between pro- and antioxidant molecules in animals [30]. Oxidative status has been reported to be an important health indicator for farm animals, as managing oxidative stress during various infectious diseases or under stress conditions (e.g., heat stress) improves health status [27,31]. Oxidative stress is also used as an indicator for imbalances between the production of reactive oxygen species (ROS) in organisms and the ability of antioxidant molecules to neutralize them [32]. Oxidative stress biomarkers, such as thiobarbituric acid reactive substances (TBARS) for lipid peroxidation and protein carbonyls (CARBS) for protein oxidation, are currently available for the design of epidemiological and clinical studies [33–36]. Plasma is easily obtainable from animals and is susceptible to the oxidation of both lipid and protein components. For this reason, plasma is considered to be an appropriate material for the in vivo investigation of oxidative stress biomarkers [37]. The plasma concentrations of TBARS and CARBS can be used as biomarkers for oxidative stress in pigs [27,32,38–40].

There have been limited published studies regarding the use of polyphenolic compounds derived from liquid olive oil byproducts (based on olive mill wastewater (OMWW) processing) as alternatives to antibiotics. Based on the results of previous studies, the present study aimed to investigate the possible beneficial effects of a phenolic PFA on the prevention of post-weaning diarrhea and oxidative stress in piglets. The criteria used to evaluate its effects were the clinical and growth performance of piglets, as well as the plasma indicators of oxidative status as biomarkers for health status.

2. Materials and Methods

2.1. Trial Farm/Animals

This study included 100 weaned piglets from a farrow-to-finish commercial pig farm, which were derived from the same batch of farrowing sows (Large White × Landrace, which are commercial hybrids of DanBred).

In the trial farm, artificial insemination was performed with purchased semen doses from a boar stub (Duroc breed). The inseminated ear-tagged sows were kept in individual stalls in a mating and gestation building until the 25th to 30th day of gestation, when they were moved to group housing. One week before the expected farrowing date, the sows were moved from the mating and gestation building to a farrowing building. Sows were housed without enrichment material (e.g., straw) in commercial farrowing crates, equipped with nipple drinkers and separate removable feeders for the sows and piglets. The routine herd health program of the trial farm included the administration of 75 μg of D-cloprostenol (Gestavet Prost®, Hipra, Amer, Girona, Spain) from 14.00 to 16.00 on the 114th day of gestation to synchronize the farrowing of all sows during working hours, allowing better sow and piglet support. Sows that had not farrowed by 05:30 the following day were given 10 IU of oxytocin. In addition, cross-fostering was allowed during the trial. Piglets were weighed 24 h after birth and assigned to a litter of 15 cross-fostered piglets.

Piglets were weaned at 25 days of age and transferred to the growing stage at 65 days of age. The vaccination program for weaned piglets included vaccinations against *Mycoplasma hyopneumoniae* and porcine circovirus type 2 (PCV2) at 18 days of age. All sows were routinely vaccinated against porcine reproductive and respiratory syndrome virus type 1 (PRRSV-1), Suid herpesvirus 1 (SHV-1), swine influenza (H1N1, H3N2), porcine parvovirus-1 (PPV-1), *Erysipelothrix rhusiopathiae*, *Escherichia coli* (*E. coli*), and Clostridia (*Clostridium perfringens type C*, *Clostridium novyi*, and *Clostridium difficile*). PWD history due to *E. coli* was detected in the trial farm based on microbiological and histopathological examinations. Routine sampling as part of the applied herd health management program of the trial farm showed that the farm was free of *Brachyspira* spp. *(Brachyspira hyodysenteriae* and *pilosicoli)* and *Salmonella* spp.

The experimental animals were housed in the same pens because the environmental exposure model was used in this study. All animals were housed under similar conditions (in terms of climate, ventilation, temperature, and humidity), and their pens were equipped with a fully automated watering system for the weaners. The indoor thermal environment of the farrowing and weaning pens of the trial farm had a climate control system for temperature and humidity, which was monitored hourly with a climate and management system (ARGOS S, MICROFAN B.V., Nederweert, the Netherlands) to measure temperature and relative humidity.

The feed was self-mixed and provided ad libitum to the piglets through the connected drinkers. During the suckling period, piglets were fed a high-quality commercial creep feed in the form of pellets based on highly digestible ingredients from the 7th to the 25th day of life (weaning day).

2.2. Experimental Material

The natural polyphenolic feed additive Medoliva® (Polyhealth S.A., Larissa, Greece) was added to the feed for weaned piglets (from 25 days to 65 days of life) at a dose of 1 kg/tonne. Medoliva® is a commercial natural product of olive fruit polyphenols encapsulated in maltodextrin (20% *w*/*w* polyphenolic compounds and large contents of hydroxytyrosol and tyrosol) derived from olive mill wastewater (OMWW) processing and based on a patented OMWW polyphenol powder [41–43].

2.3. Experimental Design

A total of one hundred (100) weaned piglets of the same batch were randomly assigned to one of two groups (Figure 1): (a) control group (T1): 50 weaned piglets were fed normal weaning feed; (b) experimental group (T2): 50 weaned piglets were fed normal weaning feed supplemented with a polyphenolic feed additive (Medoliva®, 1 kg/tonne final feed) for 40 days.

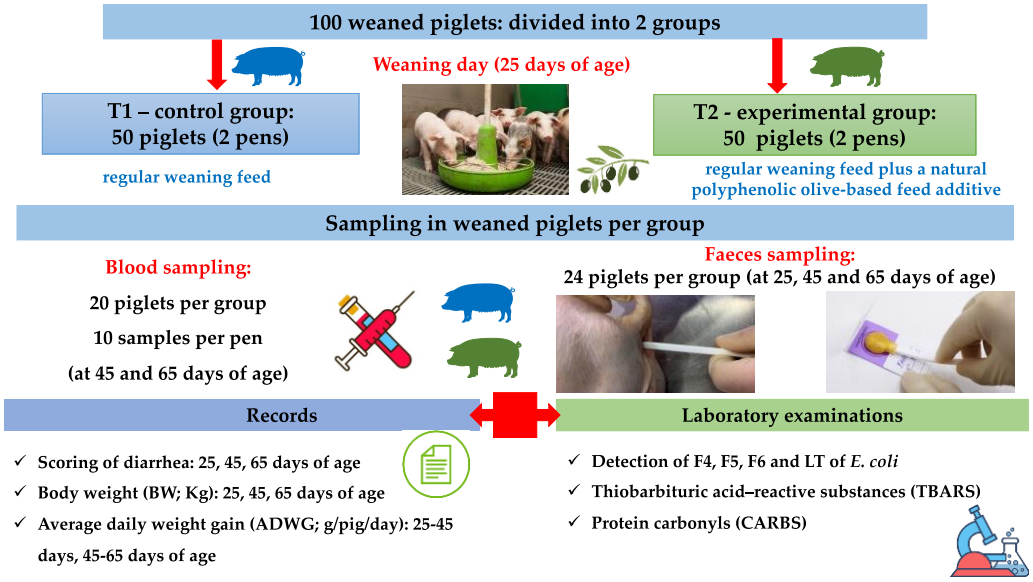

Figure 1. Flowchart of the trial design showing the experimental groups, sampling procedure, data recorded, and how the laboratory tests were performed.

All weaned piglets in the study were divided into two different groups of 50 piglets in the same room and 4 pens (2 pens × 25 piglets/pen), and piglets' ear ID tags were recorded. The sex ratio was 50/50, according to the available number of piglets (12 male and 13 female per pen, or vice versa in each pen). Each group included an equal distribution of piglets by BW: light (6.2–6.7 kg), medium (6.8–7.5 kg), and heavy (>7.5 kg). Each group included 16 light, 18 medium, and 16 heavy piglets. The selected piglets were derived from 20 litters, with an equal distribution of primiparous and multiparous sows from parity 1 to 5 (4 sows per parity). Thus, littermates were evenly distributed among groups, with equal numbers of piglets coming from sows of parity 1–5 per group based on an even distribution of their mean body weight (BW). No antibiotics were administrated in the feed or parenterally to the piglets during the trial period. Weaned piglets were housed in the same room with all-in all-out batch production. Piglets in the control group were housed in different pens than piglets in the experimental group. Piglets in each pen had no physical contact with piglets from another pen. All experimental pens were marked with a different color depending on the experimental group. The piglets' diet contained ZnO (2000 ppm) and amoxicillin (300 ppm) only one week before and one week after weaning.

All balanced weaning diets during the trial were produced in the farm's feed mill based on the same raw materials and offered the same contents for all groups (Supplementary File S1). Special measures (e.g., manufacture before the treatment feed) were taken for the control feeds to avoid contamination. The order of daily feeding in each pen was random for all piglets. A supplementary feed for weaned piglets with commercial premixes, containing vitamins, minerals, micro-/macroelements, and essential amino acids, was used according to the standards for recommended feed balance (Supplementary File S2).

2.4. Sampling

Blood was collected via jugular vein puncture from 20 weaned piglets per group (10 samples per pen), restrained via a snout snare, at 45 and 65 days of age (same body weight per time and their ID ear tags were recorded). Blood was collected using S-Monovette® 9 mL, Lithium-Heparin (Sarstedt AG & Co. KG, Nümbrecht, Germany), and disposable 19G × 1.1/2″ (40 mm) needles (Nipro European HQ, Mechelen, Belgium). Plasma samples were obtained through centrifugation (5810 R, Eppendorf AG, Hamburg, Germany) at 3000× *g* for 15 min, at 4 °C, and samples of 1.5 ml collected in microcentrifuge tubes were stored at −80 °C until laboratory analyses.

In addition, fecal samples were randomly collected from weaned piglets (24 piglets per group, 12 samples per pen) at 25 and 45 days of age (the same piglets each time according to their ID ear tags). Two fecal samples were collected per animal; the first was collected using FTA ELUTE cards according to the manufacturer's specifications (Enterocheck®, Hipra, Amer, Girona, Spain), while the second was collected using swabs in an Amies transport medium (Transwab®, Corsham, Wilts, UK) and stored at 4 °C until analysis.

2.5. Laboratory Analysis

2.5.1. Oxidative Stress Biomarkers

Biomarkers of oxidative stress in the plasma of blood samples were determined as previously described [44,45]. A modified method, according to Keles et al. (2001), was used for (a) the determination of thiobarbituric acid reactive substances (TBARS) [45] and (b) the determination of protein carbonyls (CARBS) according to Patsoukis et al. (2004) [46].

2.5.2. Microbiological Examination

Fecal swabs were initially tested (12–24 h after collection) for the presence of *E. coli* by spreading them on ESBL-selective media (CHROMID® ESBL, BioMérieux, Marcy l'Etoile, France) and incubating the plates aerobically for 24–48 h at 37 °C. In addition, subcultures were cultured on both MacConkey agar and 5% sheep blood agar.

Simultaneously, the fecal samples were pooled on ELUTE cards (FTA-like) according to the manufacturer's guidelines (Enterocheck®, Hipra, Amer, Girona, Spain). The pooled samples were analyzed by a one-step multiplex polymerase chain reaction (PCR) to detect the genes encoding adhesion factors F4, F5, and F6, as well as the LT toxins of *E. coli*, using specific probes according to laboratory guidelines (Laboratorios Hipra, Amer, Girona, Spain) [47]. The results were classified as negative (−) based on the cycle thresholds (Ct) (>38.5 Ct value). The positive samples were classified into three categories according to the Ct value: pos (+): a low detectable quantity of genetic material (35–38.5 Ct value), pos (++): a moderate detectable quantity of genetic material (30–35 Ct value), and pos (+++): a large detectable quantity of genetic material (<30 Ct value).

2.6. Records

2.6.1. Clinical Observations

Clinical observations were performed daily on all experimental weaned piglets by experienced animal caretakers and 3–4 times per week by two swine veterinarians who spent at least 20–30 minutes in each pen. All clinical observations were based on a standardized grid and all observers were trained by a specialized academic veterinarian to limit the subjectivity of the data. At each clinical observation, the health status of all animals was recorded on a previously printed card, including the ear ID tag for each pen (Table 1). The scoring grid was based on the consistency of the feces and the health status of the piglets, and was scored daily on 5 levels [48,49]: 0 = healthy piglets (solid feces), 1 = disease onset (soft feces), 2 = mild disease (mild diarrhea with soft feces and rough hair coat), 3 = moderate disease (moderate diarrhea with soft feces, mild dehydration, and a rough hair coat), 4 = severe disease (severe diarrhea with liquid feces, severe dehydration, and a very rough hair coat). The mortality rate was also recorded.

2.6.2. Growth Performance Parameters

The live weight (BW; kg) of each piglet in the two groups was measured at 25 (day 0), 45, and 65 days. Average daily weight gain (ADWG; g/pig/day) was analyzed over two-time trial periods: (a) between 25 and 45 days; (2) between 45 and 65 days. The ADWG during the different trial periods was calculated as the difference between the initial and final BW divided by the duration of the phase. Data for dead or removed piglets were included in the calculation.

Table 1. Criteria for clinical observations in piglets during the trial.

Clinical Findings		
Score	**General Behavior**	**Gastrointestinal Signs**
0	No abnormalities	Physiological feces
1	Onset of illness	Soft feces
2	Mild depression, reluctance to move	Pasty feces or watery mild yellow diarrhea
3	Reduced general condition, extended resting	Watery moderate yellow diarrhea, reddened anal region
4	Strong depression, almost entirely resting	Watery severe yellow diarrhea

2.7. Statistical Analysis

Pearson's chi-squared test, [50] for the count data, was performed to examine statistically significant differences between the control group (T1) and the experimental group (T2), as well as to test for possible differences between different age groups within each group (T1 or T2), whereas the t-test was performed to examine differences in the variables measured on a continuous scale, such as body weight. Both tests were evaluated at a significance level of 0.05. Summary statistics and hypothesis testing were implemented in the R programming language [51].

3. Results

3.1. Mortality

According to the records of mortality, two piglets from the control group (T1) and three from the experimental group (T2) died during the trial period of 25–45 days. No statistically significant differences were found between the groups during the trial period of 25–45 days and 45–65 days.

3.2. Clinical Scoring

The assessment of clinical diarrhea between groups at different times/ages is shown in Table 2. Statistically significant differences between control and experimental groups were found in 45- and 65-day age groups. (Table 2). In addition, statistically significant differences were found between all age groups in the control group, as well as between the 25- and 45-day age groups in the experimental group.

Table 2. Clinical diarrhea scoring (0 = firm feces, 1 = soft feces, 2 = mild diarrhea with soft feces, 3 = moderate diarrhea with soft feces, and 4 = severe diarrhea with liquid feces) between groups at different times/ages; minimum, maximum, and median.

Age →	**25 d**		**45 d**		**65 d**	
Group →	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Clinical Diarrhea Score						
0	28 (56)	26 (52)	12 (24)	31 (62)	17 (34)	33 (66)
1	4 (8)	6 (12)	6 (12)	12 (24)	17 (34)	16 (32)
2	12 (24)	10 (20)	10 (20)	6 (12)	10 (20)	1 (2)
3	1 (2)	6 (12)	11 (22)	1 (2)	1 (2)	0 (0)
4	5 (10)	2 (4)	11 (22)	0 (0)	5 (10)	0 (0)

F4, F5, and LT toxins of *E. coli* levels between groups at different times/ages (minimum, maximum, and median). No differences were observed between the control group (T1) and the experimental group (T2), as similar levels of the F4, F5, and LT toxins of *E. coli* were observed in piglets of both groups (Table 3).

Table 3. Observed frequencies (percentages) for clinical diarrhea scoring (0 = firm feces, 1 = soft feces, 2 = mild diarrhea with soft feces, 3 = moderate diarrhea with soft feces, and 4 = severe diarrhea with liquid feces) between groups at different times/ages.

Age	**25 d**		**45 d**		**65 d**	
Group	**Min–Max**	**Mean**	**Min–Max**	**Mean**	**Min–Max**	**Mean**
T1	0–4	1.02	0–4	2.06	0–4	1.2
T2	0–4	1.04	0–3	0.54	0–2	0.36
p-value	-	0.7	-	< 0.001	-	0.008

The observed frequencies of the scores of the F4, F5, and LT toxins of *E. coli* are shown in Table 4. Scores for the F6 toxin are not shown, because all animals, in the control group (T1) as well as the experimental group (T2), had a negative score (−).

Table 4. F4, F5, and LT toxins of *E. coli* values (0 = no detectable quantity of genetic material, 1 = low detectable quantity of genetic material, 2 = moderate detectable quantity of genetic material, and 3 = high detectable quantity of genetic material) between the control group (T1) and experimental group (T2) at different times/ages; minimum, maximum, and median. The lower part presents the observed frequencies (percentages).

Age		**25 d**				**45 d**			
Variable	**Group**	**Min–Max**		**Mean**		**Min–Max**		**Mean**	
E. coli F4	T1	0–3		1.5		0–3		1.67	
	T2	0–1		0.58		0–1		0.5	
	p-value	-		0.007		-		0.21	
E. coli F5	T1	0–1		0.5		0–1		0.42	
	T2	0–0		0		0–0		0	
	p-value	-		1		-		0.56	
E. coli LT	T1	0–2		0.16		0–0		0.00	
	T2	0–0		0		0–0		0.00	
	p-value	-		0.5		-		1	
Age		**25 d**				**45 d**			
Variable	**Group**	**Neg (−)**	**Pos (+)**	**Pos (++)**	**Pos (+++)**	**Neg (−)**	**Pos (+)**	**Pos (++)**	**Pos (+++)**
E. coli F4	T1	4 (33.5)	1 (8)	4 (33.5)	3 (25)	5 (42)	2 (16.5)	3 (25)	2 (16.5)
	T2	5 (42)	7 (58)	0	0	6 (50)	6 (50)	0	0
E. coli F5	T1	6 (25)	6 (25)	0	0	7 (58)	5 (42)	0	0
	T2	12 (100)	0	0	0	12 (100)	0	0	0
E. coli LT	T1	11 (92)	0	1 (8)	0	12 (100)	0	0	0
	T2	12 (100)	0	0	0	12 (100)	0	0	0

3.3. Growth Performance Parameters

BW values (Kg) between groups at 25, 45, and 65 days of age (minimum, maximum, standard deviation, and mean) are shown in Table 5. Statistically significant differences between the control group (T1) and the experimental group (T2) were found for BW at 45 and 65 days of age (Table 5).

The ADWG values (g) between groups at 25–45 and 45–65 days of age (minimum, maximum, standard deviation, and mean) are shown in Table 6. Statistically significant differences between the control group (T1) and the experimental group (T2) were found for the age groups of 25–45 and 45–65 days (Table 6). In addition, statistically significant differences were found between the age groups in the control group and the experimental group for BW and ADWG.

Table 5. Body weight (Kg) values between groups at different times/ages; minimum, maximum, standard deviation (sd), and mean.

Age	25 d			45 d			65d		
Group	**Min–Max**	**sd**	**Mean**	**Min–Max**	**sd**	**Mean**	**Min–Max**	**sd**	**Mean**
T1	6.7–8.1	0.28	7.1	11–17	1.31	13.3	25.7–29.3	0.52	27.13
T2	6.5–8.2	0.39	7.1	12.5–17.2	1.17	15.22	27.6–33.5	1.9	30.02
p-value	-	-	0.85	-	-	<0.001	-	-	<0.001

Table 6. Average daily weight gain (g) values between the control group (T1) and experimental group (T2) at different times/ages; minimum, maximum, standard deviation (sd), and mean.

Age	25–45 d			45–65 d		
Group	**Min–Max**	**sd**	**Mean**	**Min–Max**	**sd**	**Mean**
T1	234.5–351.0	26.24	332.6	625.0–655.0	7.29	641.8
T2	330.5–355.5	5.56	350.2	645.5–657.2	2.92	653.3
p-value	-	-	<0.001	-	-	<0.001

3.4. Assessment of Oxidative Stress Markers in Blood

Figures 2 and 3 show plasma TBARS and CARBS levels in the 45- and 65-day age groups, respectively. Statistically significant differences between the T1 and T2 groups were found for both TBARS and CARBS levels in plasma at 45 and 65 days of age (Table 7).

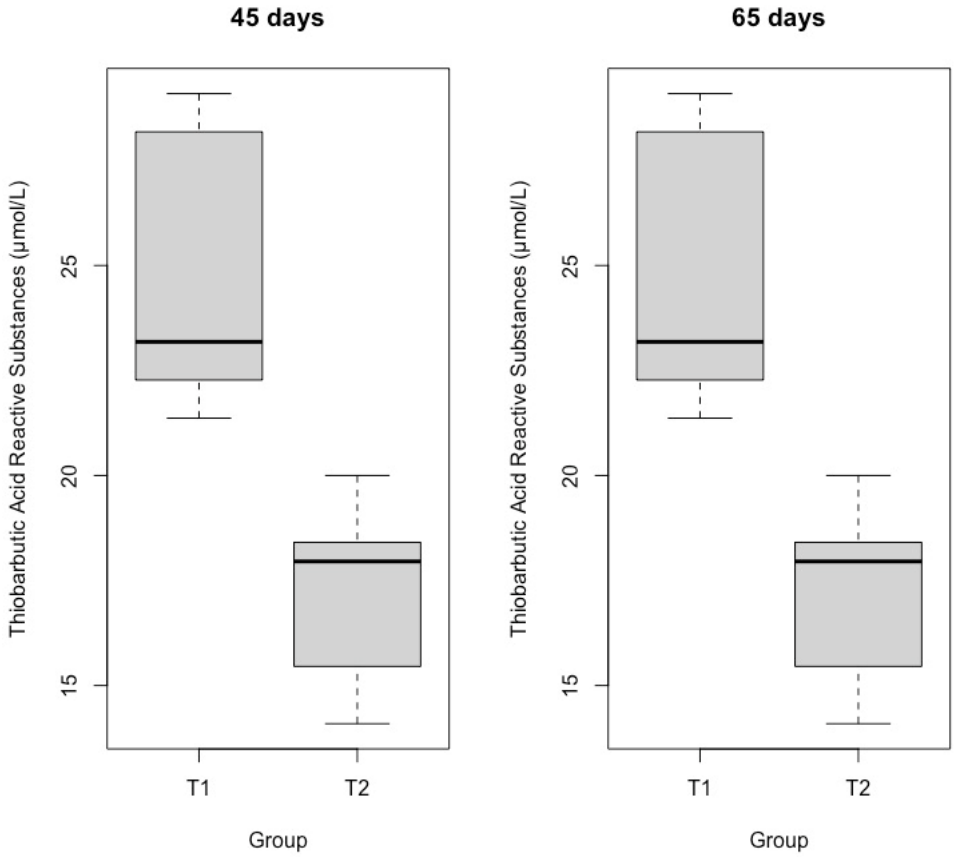

Figure 2. Boxplot of thiobarbutic acid reactive substance (µmol/L) levels in plasma between the control group (T1) and experimental group (T2) at different times/ages.

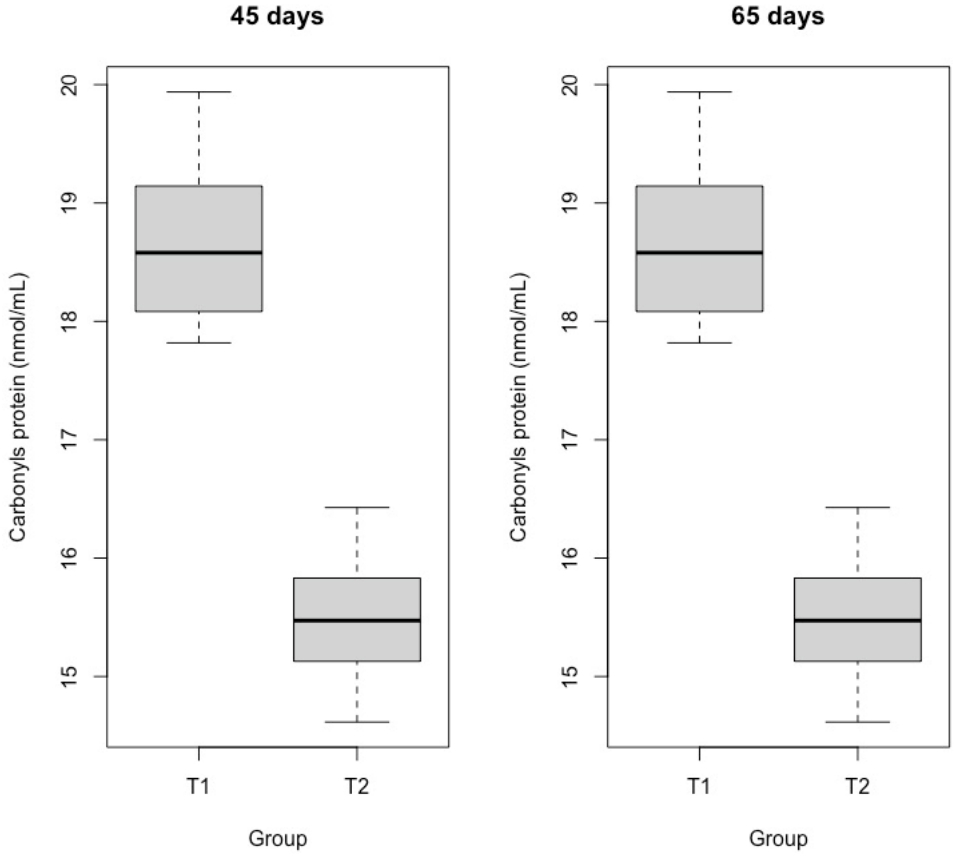

Figure 3. Boxplot of carbonyls protein (nmol/mL) levels in plasma between the control group (T1) and experimental group (T2) at different times/ages.

Table 7. Thiobarbutic acid reactive substance (µmol/L) and protein carbonyls (nmol/mL) levels in plasma between the control group (T1) and experimental group (T2) at different times/ages; minimum, maximum, standard deviation (sd), and mean.

Age		**45 d**			**65 d**		
Variable	**Group**	**Min–Max**	**sd**	**Mean**	**Min–Max**	**sd**	**Mean**
TBARS (µmol/L)	T1	18.28–19.94	0.61	19.18	17.82–18.88	0.41	18.18
	T2	14.62–15.58	0.42	15.09	15.13–16.43	0.48	15.86
	p-value	-	-	<0.001	-	-	<0.001
CARBS (nmol/mL)	T1	21.82–29.09	3.41	26.18	21.36–23.64	0.87	22.64
	T2	14.09–17.73	1.41	15.55	18.18–20.00	0.73	18.73
	p-value	-	-	0.001	-		<0.001

4. Discussion

During the weaning period, piglets are exposed to the effects of nutritional, psychological, environmental, and social stressors [1,52]. Post-weaning stress is usually associated with decreased feed intake and growth performance in addition to increased susceptibility to infections [6,7]. PWD is considered a major health problem with a significant economic impact due to decreased BW and ADWG as well as increased morbidity and mortality rates [53,54]. Many studies have focused on investigating the most ideal prevention strategy for PWD. In view of the increasing resistance to antibiotics and the limitation of their use in pig diets [19,20], pig nutrition plays a key role in future prevention strategies [55,56]. For example, previous studies reported the beneficial effects of adding vegetable oils to weaners' diets against pathogens, including *E. coli*, which can cause gastrointestinal diseases [21,22]. The results of these studies are consistent with our results, as we found that the incidence of diarrhea was significantly lower in the treated group. In addition, several

studies have shown that the addition of vegetable oils to the diet leads to an increase in BW and ADWG [26,56,57]. The present study confirmed these results, as piglets in the T2 group had higher BW and gained more ADWG. In addition, several studies have demonstrated the anti-inflammatory, antimicrobial, and antioxidant effects of herbal products from various plants and herbs, such as *Origanum vulgaris*, *Allium sativum*, *Macleaya cordata*, *Emblica officinalis*, *Foeniculum vulgare*, *Citrus sinensis*, *Andrographis paniculate*, *Glycyrrhizia glabra*, *Tinospora cordifolia*, *Capsicum annuum*, and *Curcuma longa* [23–25,27,58].

In addition, the weaning of piglets is known to be an extremely stressful condition [59], generating high concentrations of free radicals, which lead to severe oxidative damage [10]; however, feed supplements containing antioxidants have been suggested to reduce the negative effects of oxidative stress on pig health [10,27,60]. The results of the current study support these previous reports, as the tested polyphenolic olive PFA exhibited potent antioxidant activity in weaning pigs. In addition, a previous study found that the addition of the tested PFA to the diet of broiler chickens improved their redox status, resulting in decreased lipid peroxidation, as evidenced by decreased TBARS levels in plasma and tissues [42]. Previous studies have also reported that phenolic PFA can increase weanling resistance to stressors and improve meat's growth performance as well as oxidative stability [61,62]. Similar effects on the reduction in TBARS and CARBS have been observed in studies with polyphenolic PFA OMWW in pigs, poultry, and small ruminants [42,43,63]. These findings are consistent with the present results, as it was found that feeding weaned piglets with polyphenolic PFA can significantly reduce oxidative-stress-induced damage to proteins and lipids, as evidenced by the reduction in CARBS and TBARS levels, respectively.

Minimizing oxidative stress in pigs in intensive production systems is essential for optimizing health and productivity, which contributes to achieving One Health and environmental sustainability goals in pork production [19]. As mentioned earlier, the weaning of piglets is a stressor that causes oxidative stress and the subsequent manifestation of intestinal disorders [10,31]. In particular, the increased oxidative stress in weaned pigs may destroy their redox balance and consequently damage their epithelial cells as well as intestinal morphology and structure [64]. Oxidative stress could thus lead to PWD, which is the main cause of a reduced growth rate [16]. Liu et al. (2014) reported that the addition of PFA to the diet can act as an antioxidant and remove free radicals, which protects pigs from oxidative damage [26]. Our study confirmed the above results as shown by the decrease in the plasma concentrations of TBARS and CARBS between the control group (T1) and the experimental group (T2). In addition, our study showed that the administration of polyphenolic PFA in the weaning diet resulted in improved clinical performance of weaned piglets, as piglets in the T2 group had a lower frequency of PWD than piglets in the control group. Therefore, it is reasonable to assume that the reduction in oxidative stress in weaned piglets, achieved by the addition of PFA, also improved their health status.

Since the ban on the use of antibiotics as growth promoters in the EU feed industry, research interest in natural feed additives, such as phenol additives, has increased. The research community has focused on natural phenolic compounds as potential alternatives to antibiotics and as natural antioxidant sources for feed additives in swine production [65,66]. Our study provides new information on the use of polyphenolic compounds obtained from liquid olive oil byproducts (based on the processing of olive mill wastewater) as alternatives to antibiotics. In addition, the use of the tested additive helps to reduce pollution from the disposal of olive mill wastewater into the environment (soil or waterways), an important environmental problem in Mediterranean countries and the protection of ecological systems [66]. For the prevention of PWD in piglets, the wide use of ZnO as a feed additive in weaning diets is a common practice [67]; however, most of the ZnO used in pig diets is disposed of as manure, which leads to the severe metallization of soil, accumulation in pork, and increased antimicrobial resistance [68]. Since June 2022, the EU has banned the use of high-dose ZnO in pig feed. In this direction, various alternative feeding strategies are proposed and investigated for the purpose of maintaining farm productivity and reducing ZnO excretion in pig manure through the strategic use of high doses of ZnO, both of which

are of great importance for modern environmentally friendly pig production systems [19]. Therefore, new strategies and alternative products for the prevention of PWD are urgently needed. Based on our results, the tested polyphenolic feed additive could be proposed as an alternative method to administer ZnO in weaning diets for the prevention of PWD. Further studies are needed to investigate possible dosages as well as the duration of application under field and experimental conditions; however, a shortcoming of our study was the investigation of the possible beneficial effects of the tested PFA at different dosages and production stages, including the finishing stage as well as the duration of the trial period (no external funding to support our trial). In addition, antibiotics were used during the trial at the weaning stage, which has an effect on reducing diarrhea. However, it is important to evaluate the additional effect of using the tested PFA under standard field conditions in commercial pig farms. In addition, future researchers could investigate the effects of PFA on the bioactivity of microbiota. In addition, the bioavailability and action of microbiota are essential mimetic factors associated with the prevention of diarrhea and oxidative stress [69]. It is suggested that the indirect regulation of gut microbiota composition can be considered a biological mechanism for antioxidant natural products. The composition of the gut microbiota is directly related to the production of ROS. For this reason, ROS can cause serious damage to the gut [70]. Previous studies reported that natural products with antioxidant properties can alter the abundance and composition of the gut microbiota, which ultimately decrease the production of ROS by activating antioxidant enzymes and signaling pathways [71,72].

5. Conclusions

In conclusion, our study revealed the beneficial effects of polyphenolic olive PFA on the antioxidant properties of weaned piglets, due to the reduced plasma concentrations of TBARS and CARBS.

In conclusion, our study demonstrated that a polyphenolic olive PFA has important antimicrobial and antioxidant properties for weaned piglets that improve their health status and growth performance, including a reduced diarrhea score, decreased plasma concentrations of TBARS and CARBS, and improved BW as well as ADWG parameters. Further studies are needed to investigate the beneficial effects of polyphenol addition and supplementation at different doses and stages of production, as well as the duration of the trial period. In addition, future studies could investigate the effects of PFA on the bioactivity of the gut microbiota.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13071356/s1, File S1: Diet composition and nutrient content of weaning feed during the trial period, File S2: (Footnote): Premix of vitamins/minerals.

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