Hindawi
Veterinary Medicine International
Volume 2024, Article ID 6658120, 9 pages
https://doi.org/10.1155/2024/6658120

Research Article

Inclusion of *Saccharomyces cerevisiae* var. *boulardii* RC009 and *Pediococcus pentosaceus* RC007 as a Probiotic Additive in Pigs' Postweaning Diets and Its Effect on Meat Composition, Carcass Characteristics, and Fatty Acids Profile after Slaughter

J. Parada,[1,2] **A. Magnoli**,[2,3] **V. Alonso**,[2,4] **L. Diaz Vergara**,[2,5] **M. Corti Isgro**,[1,2] **J. J. T. Posse**,[6] **M. A. Montenegro**,[2,5] **and L. Cavaglieri**[2,4]

[1]*Department of Animal Pathology, Faculty of Agronomy and Veterinary Medicine, National University of Rio Cuarto, Rio Cuarto, Cordoba, Argentina*
[2]*National Scientific and Technical Research Council (CONICET), Córdoba, Argentina*
[3]*Department of Animal Production, Faculty of Agronomy and Veterinary Medicine, National University of Rio Cuarto, Rio Cuarto, Cordoba, Argentina*
[4]*Department of Microbiology and Immunology, Faculty of Exact, Physical, Chemical and Natural Sciences, National University of Rio Cuarto, Rio Cuarto, Cordoba, Argentina*
[5]*Multidisciplinary Institute for Agrifood and Biotechnology Research and Transfer (IMITAB-CONICET), National University of Villa Maria, Cordoba, Argentina*
[6]*Department of Public Health, Faculty of Agronomy and Veterinary Medicine, National University of Rio Cuarto, Rio Cuarto, Cordoba, Argentina*

Correspondence should be addressed to J. Parada; jparada@ayv.unrc.edu.ar

Received 19 January 2023; Revised 19 January 2024; Accepted 27 January 2024; Published 20 February 2024

Academic Editor: Francesca Mancianti

The postweaning is recognized as one of the most challenging stages of pig growth that can affect their lifetime productivity. The aim of the study was to evaluate the inclusion of *Saccharomyces cerevisiae* var. boulardii RC009 and *Pediococcus pentosaceus* RC007 as a probiotic additive in pigs' postweaning diets and its effect on meat composition, carcass characteristics, and fatty acids profile after slaughter. The following three treatments (550 animals each) were included: T1: control balanced diet (CD), T2: CD with *S. boulardii* RC009 (1×109 CFU/kg feed), and T3: CD with *P. pentosaceus* RC007 (1×109 CFU/kg feed). The additive was administered throughout the postweaning phase (49 d), and then the pigs were moved to a fattening house where they no longer received probiotics in the feed. At 115 ± 5 kg, the animals were transferred to the slaughterhouse. Analyses of carcass composition, muscle depth, protein content, total fat and ash, drip and cooking water loss, and fatty acids were performed. Pigs consuming the probiotic additives had improvements in some of the production parameters. According to our results, we could observe that some modifications in meat composition after slaughter were observed in the group of pigs that consumed the probiotic additives during the postweaning, which could be considered as an improvement in meat quality. Pigs consuming probiotics had higher percentages of essential omega-3 fatty acids such as linolenic acid, and pigs consuming *S. cerevisiae* var. *boulardii* RC009 increased lean percentage and reduced the eicosanoic contents in meat. Also, an improvement in water retention capacity was observed in both groups treated with probiotics. While these results are promising, further studies are needed to evaluate the possible effect of these additives closer to slaughter, as well as their combined use.

1. Introduction

Pork is the most consumed meat worldwide, and consumers demand for safer pork is growing. The purpose of the swine production chain is to provide food for people (fresh, cured, or processed) so that it necessarily involves aspects that influence the pig's carcass quality. The new trends in food production are based on the safety, innocuousness, and stability of animal origin products integrated in the concept "One World One Health" which maintains that human health and animal health are interdependent and linked to the health of the ecosystems in which they exist [1]. Thus, the animal food is defined not only by its organoleptic attributes but also by the production process of these animals, involving health, welfare, and the environment.

Meat quality is a plural concept that differs depending on the segment of the meat chain. It can be related to its nutritional composition and organoleptic factors such as appearance, palatability, and safety. Consumers often agree that the most important quality criterion is the amount of muscle tissue (muscle) and the proportion of lean tissue [2]. Since consumers demand lean meat, with fewer calories and cholesterol, the industrialist wants to pay for more muscle and less fat. Another very important parameter to analyse the quality of meat is the water retention capacity, intimately linked to the pH decrease after *rigor mortis*. It is also important to analyse the nutritional value given by the percentage of protein, the contribution of calories, and minerals [3]. Although the beneficial and nutritional attributes of pigs are widely known, it should not be overlooked that the feed the animals receive can have an important influence on meat quality, and although the interrelation with other aspects of the production process (genetics, management, and slaughter) should be considered, it is known that feed plays a fundamental role [4]. Carcass composition traits have also been associated with the composition of the intestinal microbiota [5], which is closely related to the animal's diet and the way in which it can optimize the utilization of nutrients.

At the farm, the weaning is a very stressful moment because of many factors like the separation of their mothers, the change in the kind of feed and environment [6]. These stress factors are also related to the occurrence of several intestinal and/or systemic pathologies that impact the farm performance indicators; their influence on intestinal postweaning pigs gut health impacts on lifetime gut health and, consequently, on animal performance until slaughter [7]. This encourages the use of antibiotics by producers to prevent the occurrence of diseases in the postweaning stage. It is known that the indiscriminate use of antibiotics generates resistance in pathogenic bacteria and represents a current threat to public health. An alternative developed in recent years to replace the nontherapeutic use of antibiotics and reduce the consequent impact on human health and the environment is the inclusion of probiotics as additives in the diet [8].

Méndez Palacios [9] concluded that there was an economic benefit when using both prebiotics and probiotics in the diet of pigs from weaning to finishing. Tufarelli [10] found that feeding probiotic blend enhanced growth performance and meat quality in growing-finishing pigs and also decreased faecal NH3-N and butyric acid levels, resulting in a viable approach to reduce animal excreta pollution.

Previous studies have demonstrated the probiotic properties of *S. cerevisiae* var. *boulardii* RC009 and *P. pentosaceus* RC007 isolated from the pig ecosystem. They were able to survive under gastrointestinal conditions and had beneficial properties such as aggregation/inhibition of pathogenic bacteria and the capacity to bind mycotoxins under gastrointestinal conditions [11, 12]. In addition, Poloni [13] showed that *S. cerevisiae var. boulardii* effectively counteracts the toxic effects of harmful aflatoxin B_1 (AFB_1) in livers. However, they did not demonstrate the influence of these strains on meat quality and other parameters related to lipid metabolism. Therefore, the study aimed to evaluate the inclusion of *S. cerevisiae* var. *boulardii* RC009 and *P. pentosaceus* RC007 as a probiotic additive in pigs' postweaning diets and its effect on meat composition, carcass characteristics, and fatty acids profile after slaughter.

2. Materials and Methods

The working protocol and the used techniques comply with the regulations of the Subcommittee on Animal Bioethics under the Ethics Committee of Scientific Research, as established in Resolution 376/22 of the Superior Council of the National University of Rio Cuarto.

2.1. Microorganisms. The probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* RC009 was isolated from the pig intestine. Morphological, biochemical, and molecular characterization was conducted according to Armando [11]. Species assignment was done using the Yeast Identification Database (https://www.yeast-id.com). The sequence comparisons were performed using the basic local alignment search tool (BLAST) program within the NCBI database and submitted to GenBank (ID #KF447149.1).

The probiotic lactic acid bacteria (LAB) *Pediococcus pentosaceus RC007* was isolated from feedstuff and characterized based on morphological, physiological, and biochemical tests. DNA extraction, polymerase chain reaction, and 16S rDNA sequencing were performed using the method proposed by Martínez [12]. DNA fragments were visualized after an electrophoretic run on 1.5% agarose gel stained with 0.5 μg ml^{-1} ethidium bromide, and gels were photographed using a MiniBIS Pro analyser (DNR Bio Imaging Systems, Jerusalem, Israel). The fragment sizes were measured by comparison with DNA 100-bp ladder (Invitrogen by Life Technologies, Buenos Aires, Argentina). For DNA sequencing of both strands, template DNA was sent to Macrogen Inc. (Seoul, Korea). Sequences were compared using the local alignment search tool (BLAST) program with the NCBI database (GenBank) (http://www.ncbi.nlm.nih.gov/BLAST/) and submitted to GenBank (ID #1,980,444).

2.2. Yeast and Lactic Acid Bacteria Biomass Production. Biomass of Saccharomyces cerevisiae var. boulardii RC009 was obtained from 24 h of culture in yeast-peptonedextrose (YPD) broth to which 1g PO4H2K/L was added in a BioFlo

2000 fermenter (New Brunswick Scientific Co., Inc, Enfield, CT, USA) operated at 4 × g, at 28°C, and 1.5 vvm of aeration. The pH value was adjusted to 5 with 6 M NaOH. The working volume was 4 L.

Pediococcus pentosaceus RC007 culture conditions were 3 L of optimized culture medium developed with commercial refinery syrup, stirring 4 × g at 37°C for 24 h, and 10% inoculum (v/v). The concentration of dissolved oxygen at the beginning of the experiment was 0%. Foam production was controlled by the addition of antifoam 289 (Sigma-Aldrich, St. Louis, MO, USA). The pH was maintained between 6.5 and 7 with the addition of 18 N H_2SO_4 or Na_2CO_3 20% w/v.

2.3. Probiotic Additives Formulation. The probiotic biomass obtained at the end of the fermentation of both microorganisms, LAB and yeast, was centrifuged at 1000 × g at 4°C for 10 min. The concentrated pellet was resuspended in the same volume of cryoprotectant (10% skim milk plus 5% yeast extract for the yeast and 10% skim milk only for LAB) and stored at −80°C. The viability of the lyophilized formula (1 g) was confirmed at the time of the trial.

2.4. Experimental Design. This experimental trial was carried out in the farm "Criadero de Cerdos de Aceitera General Deheza SA." The studies were developed with male and female pigs of the commercial genetic line Agroceres PIC (Camborough × 337) in the breeding herd, which received the following vaccines: at 21 and 42 days of age, commercial PCVM vaccine (Circovirus + Mycoplasma) and at 50 days of age, *A. pleuropneumoniae* vaccine. The animals were sexed and separated by weight similarity on the 1st day of the test. For the experience, a total of 1650 animals were used (50% male and 50% female) which were housed in three different confined facilities. The study was carried out with three consecutive weeks of weaning, and one treatment was applied to each of the weeks of weaning in weekly groups of 550 animals (full barn for each diet). From the first day of the trial, pigs were fed *ad libitum* with different experimental diets. Treatments were applied after weaning (21 days of age) and for the total rearing period (until 70 days of age). In the rearing shed, floors were entirely plastic and the environment was fully controlled by tunnel ventilation with heating gas heaters.

2.5. Experimental Diets. Commercial pig feed was the regular provided by the establishment where the trial was carried out. It covers the nutritional requirements of the rearing stage in all phases as recommended by the National Research Council [13]. The composition to each phase of diets is described in Table 1. The phase 1 diet was administered from 21 days of age to 26 days of age, phase 2 diet from 26 to 34 days of age, phase 3 from 34 to 47 days, and phase 4 from 47 to 70 days of age.

In the treated diets, probiotic additives were incorporated in a concentration of 1×10^9 CFU/kg of feed, performing three treatments with 550 animals each; treatment 1: control diet (DC), treatment 2: DC with *S. boulardii* RC009, and treatment 3: DC with *P. pentosaceus* RC007.

Table 1: Centesimal composition and calculated values of diets provided to the animals in the experimental period.

Item	Unit	Phase 1	Phase 2	Phase 3	Phase 4
Dry matter	%	92.01	90.61	88.84	88.76
Crude protein	%	22.70	22.23	19.87	19.99
Metabolizable energy	Kcal	3,676.61	3,595.31	3,429.34	3,402.43
Total lysine	%	1.66	1.60	1.42	0.00
Dig lysine	%	1.55	1.50	1.31	1.19
Dig methioine	%	0.61	0.61	0.55	0.45
Dig cysteine	%	0.33	0.29	0.25	0.26
Dig met + cyst	%	0.93	0.90	0.88	0.00
Dig tryptophan	%	0.36	0.34	0.24	0.23
Dig threoine	%	1.01	0.97	0.84	0.77
Dig arginine	%	1.14	1.19	0.00	0.00
Dig valine	%	0.64	0.74	0.00	0.00
Crude fat	%	9.15	8.39	4.96	4.71
Crude fiber	%	1.91	2.39	3.49	4.00
Calcium	%	0.86	0.85	0.67	0.81
Total phosphorus	%	0.59	0.57	0.65	0.72
Available phosphorus	%	0.57	0.50	0.44	0.43
Lactose	%	15.00	7.50	0.00	0.00
Linoleic acid (C18 : 2)	%	2.71	3.07	2.45	0.00
Choline	mg/kg	735.00	735.00	593.22	315.00
Zinc	ppm	3,000.00	3,000.00	1,639.12	139.12
Copper	ppm	266.20	266.20	263.50	263.50
Selenium	ppm	0.40	0.40	0.34	0.34
Iron	ppm	90.39	90.39	75.47	75.45
Sodium	%	0.46	0.34	0.22	0.17
Chlorine	%	0.51	0.37	0.30	0.24
Ash	%	5.98	5.73	4.62	5.13

After the essay, pigs were transferred to a fattening house where they consumed the regular fattening diet without the addition of probiotics. Once finished the fattening stage (at 115 ± 5 kg and about 160 days hold), they were delivered to the slaughterhouse.

2.6. Carcass Composition and Muscle Thickness Determinations. At the end of fattening, the pigs were sent to slaughter at approximately 160 days of age. In each treatment, 50 pigs (half male and half female) were randomly selected. The transfer and slaughter protocol were the same for each treatment. The carcass weight (kg) was determined at the time of slaughter, and after that, thickness of *longissimus dorsi* muscle measured between the 10th and 12th rib was recorded using a manual caliper. Also, a sample of 50 g of this region of the *longissimus dorsi* muscle in 50 pigs from each treatment was taken to make determinations in the laboratory. Two different formulas were applied to determine the percentage of lean: Fat o Meater (FOM): $\textit{lean percent} = 51.691 - 0.214 \times (A) - 0.396 \times (B) + 0.136 \times (C)$; Hennesy: $\textit{lean percent} = 46.344 - 0.580 \times (B) + 0.232 \times (C)$, where (A) is fat thickness in mm at 10^a rib, (B) is fat thickness in mm at last rib, and (C) is muscle thickness in mm.

2.7. Meat Quality Determination. Protein content, total fat, and total ash were determined by the AOAC [14] method.

Drip loss analysis was performed according to the methodology described by Honikel [15].A meat fillet (20–50 g) was cut, weighed, hung on a hook, and placed in an inflated plastic bag, taking care that the meat does not touch the bag. The meat was conserved at 4°C for 24 h, and the excess liquid was dried with filter paper and the final meat weight was taken. The result was expressed as the percentage of moisture lost. The assay was done in triplicate.

In addition, cooking loss was determined according to the methodology described by Rezar [16]. The same meat samples used for drip loss were used to determine water loss by cooking. The samples were placed in plastic bags in a thermostatic bath at 75°C. The samples were cooked for 30 minutes and then removed from the bath and allowed to cool. Excess of moisture was removed with filter paper and the samples were weighed. The moisture loss was expressed as a percentage. All samples were evaluated in triplicate.

2.8. Fatty Acids Analysis. The fatty acid profile of the meat samples from each treatment was determined. Fatty acid extraction was carried out following the methodology described by Ross [17] with some modifications. Samples of approximately 3 g of meat were taken, lipid extraction was carried out using 30 mL of a 2 : 1 chloroform/methanol solution (v/v) for 30 min with reflux, and saponification was carried out with 20 mL of 0.5 M NaOH (in methanol) and was refluxed at 100°C for 20 min; 10 mL of hexane were added, and it moved to a decanter ampoule and the organic phase was taken. It was dried over anhydrous sodium sulphate and filtered with a 0.45 μm filter. Fatty acids were detected by gas chromatography using a gas chromatograph (GC) (Agilent 7890A, United States) equipped with a flame ionization detector (FID), an automatic injector (Agilent 7693A, United States), and a ZB-Wax column (60 m × 0.25 mm × 0.25 μm). The chromatographic conditions were as follows: the injector and detector temperature were 250°C, and helium was used as carrier gas with a linear velocity of 1 mL.min-1. The gas chromatograph oven temperature was programmed to start at 180°C (maintained for 1 min), followed by a 2°C/min ramp to 240°C. The identification of fatty acid methyl esters (FAMEs) was achieved using a reference standard FAME mixture C14-C22 (Supelco Inc., Bellefonte, PA, USA). Fatty acids are expressed as a percentage of the sum of identified fatty acids. All samples were evaluated in triplicate.

The nutritional value of meat was evaluated by calculating health lipid indices such as polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA), PUFA *n*-6/*n*-3, and atherogenic (AI), thrombogenic (TI), and nutritive value (NVI) indices according to Ulbricht and Southgate [18] and Chen [19] using the following equations.

$$\mathrm{AI} = \frac{(C\,12:0 + 4 \times C\,14:0 + C\,16:0)}{\Sigma\ \text{unsaturated fatty acids (UFA)}}, \tag{1}$$

$$\mathrm{TI} = \frac{(C14:0 + C16:0 + C18:0)}{(0.5 \times \text{monounsaturated fatty acids (MUFA)} + 0.5 \times n-6\ \text{PUFA} + 3 \times n-3\ \text{PUFA} + n-3/n-6\ \text{PUFA})}, \tag{2}$$

$$\mathrm{NVI} = \frac{(C\,18:0 + C18:1)}{C\,16:0}. \tag{3}$$

2.9. Statistical Analysis. The productive parameters and the meat quality parameters ($n = 50$) were subjected to the analysis of the variance (ANOVA). The means were compared according to Fisher's minimum significant difference test (LSD) [20]. The analysis was carried out using the InfoStat program [21].

3. Results

3.1. Carcass Weight and Muscle Thickness. Table 2 shows the weight of the carcass in the treatments tested. The pig carcass is the whole body of the slaughtered animal as it appears after bleeding and evisceration operations, split in half, without bristles, hooves, genitals, or diaphragm. The results show that both carcass weight and muscle thickness are significantly improved in the probiotic treatments.

3.2. Meat Composition Determinations. The effects of different treatments on meat composition are reported in Table 3. The total protein content in meat is an indicator of its nutritional value. Treatment with *S. boulardii* significantly increases the total protein content although this effect was not observed in the treatment with *P. pentosaceus*. The mineral content (ash) of meat was not significantly different among treatment groups, and the meat intramuscular fat content decreased significantly in pigs consuming *S. boulardii*, while it increased in pigs consuming *P. pentosaceus,* with respect to the control group ($P < 0.05$) (Table 3).

The lean percentage in all treatments, both by the Fat O Meater method and by the Hennessy method, exceeded the 44% stipulated as the base value of lean tissue authorized for consumption according to the Secretariat of Agriculture,

Table 2: Carcass weight and *longissimus dorsi* muscle depth measured between the 10th and 12th rib in pigs of the three performed treatments.

Treatments	Carcass weight (kg)	Muscle depth (mm)
Control	99.8 (±9.5)[a]	81.7 (±9.6)[a]
S. boulardii	109.0 (±9.3)[b]	86.3 (±6.6)[b]
P. pentosaceus	108.2 (±8.4)[b]	83.4 (±8.6)[ab]

Means in the same column with different superscripts (a and b) are significantly different ($P < 0.001$), according to Fisher's protected LSD test. The standard deviation is enclosed in parenthesis.

Table 3: Proteins content, intramuscular fat, and ash determinations of the three performed treatments. Values are expressed as a percentage of dry matter (DM).

Treatments	Protein (% DM)	Intramuscular fat (% DM)	Ash (% DM)
Control	54.29 (±1.5)[b]	3.79 (±1.5)[b]	3.45 (±0.3)[a]
S. boulardii	61.60 (±2.1)[a]	3.36 (±1.8)[a]	3.74 (±0.5)[a]
P. pentosaceus	53.41 (±1.8)[b]	4.12 (±1.2)[c]	3.38 (±0.2)[a]

Means in the same column with different superscripts (a and b) are significantly different ($P < 0.001$), according to Fisher's protected LSD test. The standard deviation is enclosed in parenthesis.

Table 4: Lean percentage of the three performed treatments according to two different formulas: Fat o Meater (FOM) and Hennesy.

Treatments	Lean percentage	
	FOM	Hennesy
Control	56.77 (±1.66)[b]	57.42 (±2.46)[b]
S. boulardii	59.12 (±1.29)[a]	59.21 (±1.44)[a]
P. pentosaceus	56.70 (±1.42)[b]	57.47 (±1.90)[b]

Means in the same column with different superscripts (a and b) are significantly different ($P < 0.001$), according to Fisher's protected LSD test. The standard deviation is enclosed in parenthesis.

Table 5: Drip loss and cooking loss analysis of the three performed treatments.

Treatments	Water retention capacity	
	Drip loss (%)	Cooking loss (%)
Control	3.36 (±1.56)[a]	41.96 (±6.16)[a]
S. boulardii	1.81 (±1.11)[b]	26.17 (±9.43)[b]
P. pentosaceus	1.88 (±0.84)[b]	31.72 (±5.93)[b]

Means in the same column with different superscripts (a and b) are significantly different ($P < 0.001$), according to Fisher's protected LSD test. The standard deviation is in brackets.

Livestock, Fisheries, and Food (S.A.G.P. y A.). The treatment with *S. boulardii* had the highest lean percentage of the study, being significantly higher ($P < 0.001$) than the control group (Table 4).

3.3. Water Retention Capacity Analysis. The loss of moisture by dripping and cooking was evaluated in samples from *longissimus dorsi* muscle from animals in the three treatments. Moisture loss by dripping was greater in the control group, with respect to the pigs that consumed the probiotic treatments that had higher water-holding capacity (WRC). Moisture loss by cooking was also significantly different between control samples and probiotic treatments, with differences of about 30% less loss in the treated groups compared to the control (Table 5).

3.4. Fatty Acids Analysis. Fatty acid profiles and nutritional indices of the meat are presented in Table 6. The SFA percentage was significantly different ($P < 0.05$) among the three groups, except for the C18 : 00 (stearic acid) content, which did not differ among the three groups. The *P. pentosaceus* treatment resulted in significantly lower ($P < 0.05$) C14 : 00 percentage and no significant differences compared to the control for C16 : 00 (palmitic) and C22 : 0 (behenic). In contrast, *S. boulardii* treatment showed significantly lower ($P < 0.05$) C20 : 0 (eicosanoic) content. The total MUFA content was significantly lower ($P < 0.05$) in the *P. pentosaceus* treatment, showing no significant differences ($P < 0.05$) from the control for the *S. boulardii* treatment. C18 : 1 (n-9) trans (elaidic) was the most abundant MUFA in all samples, with levels between 18.04 and 30.9%. The total PUFA content was higher in the *P. pentosaceus* treatment. In all samples, C18 : 3 (n-3) cis (linolenic) was the major PUFA, with levels between 2.7 and 6.32%, whereas samples from pigs consuming *P. pentosaceus* had the highest content. The *P. pentosaceus* treatment had a significantly lower ($P < 0.05$) total trans and cis fatty acid percentage. The *P. pentosaceus* treatment resulted in a significantly lower ($P < 0.05$) content of trans and cis total fatty acids. For the control and *P. pentosaceus* treatments, no significant differences were found between trans and cis total fatty acid percentage, whereas for the *S. boulardii* treatment, the proportion of cis total fatty acid was significantly higher ($P < 0.05$) than the trans total fatty acid. In addition, the *S. boulardii* treatment resulted in a significantly lower PUFA/SFA ratio ($P < 0.05$), and no differences were found between the control and *P. pentosaceus* treatments. All treatments showed n-6 PUFA/n-3 PUFA ratios lower than the recommended value of 4, with *P. pentosaceus* treatment showing the lowest ratio. Of the meat health-promoting indices evaluated, only TI showed significant differences between the probiotic treatments, being significantly lower for *P. pentosaceus*, although without differences from the control. IA and NVI did not differ significantly among the three treatments.

Table 6: Effects of probiotics addition in pigs' postweaning diets on fatty acid profile and contents in meat samples (relative % fatty acids).

Fatty acid	Control	*S. boulardii*	*P. pentosaceus*	*P* value
C14 : 0	1.09 ± 0.31^{b}	1.27 ± 0.04^{b}	0.70 ± 0.07^{a}	0.023
C16 : 0	18.04 ± 4.56^{ab}	21.21 ± 2.56^{b}	12.99 ± 1.12^{a}	0.045
C18 : 0	7.80 ± 0.74^{a}	11.01 ± 2.73^{a}	9.95 ± 1.92^{a}	0.207
C20 : 0	6.96 ± 3.76^{b}	1.29 ± 0.61^{a}	14.19 ± 0.27^{c}	0.001
C22 : 0	5.55 ± 3.00^{a}	11.72 ± 1.51^{b}	10.42 ± 2.72^{ab}	0.051
ΣSFA	39.44 ± 1.16^{a}	46.49 ± 3.21^{b}	48.25 ± 3.71^{b}	0.022
C18 : 1 (*n*–9) trans	24.46 ± 5.02^{ab}	30.9 ± 3.59^{b}	18.04 ± 1.22^{a}	0.014
C18 : 1 (*n*–9) *cis*	14.13 ± 2.01^{b}	16.3 ± 1.50^{b}	8.28 ± 0.56^{a}	0.001
ΣMUFA	38.58 ± 7.03^{b}	47.28 ± 5.09^{b}	26.32 ± 1.79^{a}	0.007
C18 : 2 (*n*–6) trans	1.06 ± 0.15^{b}	1.30 ± 0.08^{c}	0.55 ± 0.08^{a}	0.001
C18 : 2 (*n*–6) *cis*	2.37 ± 0.16^{c}	0.26 ± 0.05^{a}	1.94 ± 0.16^{b}	<0.0001
C18 : 3 (*n*–3) *cis*	2.70 ± 1.49^{a}	3.30 ± 0.41^{a}	6.32 ± 1.15^{b}	0.015
ΣPUFA	6.14 ± 1.50^{a}	4.87 ± 0.45^{a}	8.80 ± 1.28^{b}	0.016
Σ*n*–3 PUFA	2.70 ± 1.49^{a}	3.30 ± 0.41^{b}	6.32 ± 1.15^{b}	0.015
Σ*n*–6 PUFA	3.44 ± 0.01^{c}	1.57 ± 0.04^{a}	2.48 ± 0.13^{b}	<0.0001
PUFA/SFA	0.15 ± 0.03^{b}	0.10 ± 0.01^{a}	0.18 ± 0.01^{b}	0.0102
Σtrans	25.52 ± 5.17^{ab}	32.20 ± 3.67^{b}	18.59 ± 1.25^{a}	0.012
Σ*cis*	19.20 ± 0.36^{b}	19.95 ± 1.87^{b}	16.54 ± 0.74^{a}	0.027
n–6 PUFA/*n*–3 PUFA	1.64 ± 1.06^{b}	0.48 ± 0.05^{ab}	0.4 ± 0.05^{a}	0.083
TI	0.88 ± 0.18^{ab}	0.96 ± 0.05^{b}	0.70 ± 0.03^{a}	0.070
AI	0.50 ± 0.07^{a}	0.50 ± 0.01^{a}	0.45 ± 0.03^{a}	0.333
NVI	2.61 ± 0.24^{a}	2.75 ± 0.04^{a}	2.81 ± 0.25^{a}	0.508

Means in the same row with a common letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$). Values are expressed as the means ± SD ($n = 3$). C14 : 0, miristic acid; C16 : 0, palmitic acid; C18 : 0, stearic acid; C20 : 0, arachidic acid; C22 : 0, behenic acid; C18 : 1 ($n-9$) trans, elaidic acid; C18 : 1 ($n-9$) cis, oleic acid; C18 : 2 ($n-6$) trans, linolelaidic acid; C18 : 2 ($n-6$) cis, linoleic acid; C18 : 3 ($n-3$) *cis*, linolenic acid. SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. TI: thrombogenic index. AI: atherogenic index. NVI: nutritive value.

4. Discussion

Pork quality is a broad concept that includes, among others, factors related to the nutritional and organoleptic characteristics of the product and its food safety. However, from an industrial point of view, several variables determine the degree of suitability of the product for industrial processing. In this study, pork composition, carcass characteristics, and fatty acid profile in slaughter pigs were evaluated after the inclusion of different probiotics in the postweaning phase. The postweaning is recognized as one of the most challenging stages of pig growth that can affect their lifetime productivity [7, 22]. It was observed that the use of probiotic additives in the early growth stage had a positive influence on production parameters such as carcass weight and muscle depth after slaughter. These improvements in growth performance with the addition of probiotics in feed have been previously reported [10, 23, 24] and were associated with improved intestinal health and utilization of nutrients released by the probiotic.

Pork quality is affected by multiple factors throughout the meat chain. It is currently recognized that one of the most important quality criteria for the industry is the muscle percentage or lean production. In the present study, a significant increase in the proportion of lean meat was observed in pigs consuming *S. boulardii*, which is a desirable attribute for producers, consumers, and industrialists. These results are in agreement with Dávila-Ramirez [25], who observed an increase in lean percentage in pigs, under stress conditions, supplemented with *S. boulardii*. However, beyond the interest in obtaining lean meats, some authors indicated that genetic improvements to increase lean production in pigs lead to an unfavorable pattern of fatty acids in fat depots [26]. This pattern is characterized by increased C18 : 2 *n*-6 *trans* ratios [27] as shown by our results in animals treated with *S. boulardii*.

The World Health Organization recommends reducing the intake of saturated fats, which are associated with the production of cholesterol and the development of coronary heart disease. Instead, it recommends replacing them with the consumption of certain PUFAs that are considered essential, as they are not synthesized by the human body. In the present study, *P. pentosaceus* supplementation increased the levels of C18 : 3 (*n*-3) (linolenic) and consequently the total PUFA percentage. These results agree with those observed by Chang [28] and Grela [29] for the probiotic supplement in pig diets with *Lactobacillus plantarum* and a mixture of microorganisms (*Lactococcus lactis* IBB500, *Carnobacterium divergens* S1, *Lactobacillus casei* ŁOCK 0915, *Lactobacillus plantarum* ŁOCK 0862, and *S. cerevisiae* ŁOCK 0141, respectively). The values obtained for the n-6 PUFA/n-3 PUFA ratio for the three treatments are low and are within the limits established by the World Health Organization to promote health and minimize cardiovascular disease risks. Zhimei [30] also obtained low values of the n-6/n-3 ratio for pigs treated with a control diet and supplemented with the probiotic *L. reuteri 1* in pigs. On the contrary, Grela [27] and Chang [26] obtained very high values of this relationship for both, the control and supplemented with probiotics diet.

Only for the *S. boulardii* treatment, significantly higher total trans fatty acid percentages than cis fatty acids were observed. This could be due to changes in the gut microbiota induced by the yeast modifying the biohydrogenation of PUFA or resulting in the inhibition of isomerases.

The high SFA values, for C20 : 0 (arachidic acid), but particularly for the surprisingly high values of C22 : 0 (behenic acid), found in the study for the treatments have previously been linked to diet, genetic breed, and the weight and age of the animals. A diet rich in cereals and other carbohydrate-rich feeds may increase the levels of saturated fatty acids in meat [31]. Pigs-fed diets high in corn or other grains tended to have more saturated fats. Some pig breeds tend to produce meat with higher saturated fat levels [32]. Genetic makeup influences fat deposition and metabolism in animals. In addition, heavier and older pigs tend to have a higher saturated fat percentage because they have more time to deposit fat in the body [33]. However, since many of the variables were the same for all the groups under study, this difference in the formation and deposition of fatty acids in the muscle could be associated with modifications in the intestinal microbiota as has been previously proposed [34].

The nutritional value of pork meat can be improved by decreasing the SFA level because SFA are related to the incidence of cardiovascular heart diseases [35]. In our study, we demonstrated that dietary supplementation with *S. boulardii* reduced the C20 : 0 (eicosanoic) amount in meat. Similar results were obtained by Zhu [36], who evaluated the effects of the addition of probiotics (a mixture of *L. plantarum* and *S. cerevisiae*) to the diet of Bama Mini-pigs on carcass traits and meat quality. In addition, the values obtained for the health-promoting indices of meat were those expected for pigs TI = 1.12 and AI = 0.47 [37, 38] and were similar to those obtained by Grela [29] for pigs fed with a control diet and supplemented with a combination of probiotics. On the other hand, there was a slight increase in the levels of trans fatty acids found in the pigs consuming the probiotics; it can be considered a negative effect from a health point of view. Further studies are needed to evaluate the significance of this effect.

In the present study, the ash content was not modified in pigs receiving probiotics, in contrast to that reported by Chang [28] who found that dietary supplementation with probiotics significantly reduced the ash content although they also found an increase in the protein level.

For both the industry and the consumer of fresh pork, the water-holding capacity of muscle tissue has a central influence. For the industry, loss of WRC reduces the sausage yield, while abundant loss of liquid during cooking generates consumer rejection. This loss of liquid retention capacity is associated with the rate of post-mortem pH decrease. In our results, we observed that the water retention capacity was better in pigs that consumed the probiotic additive in the early stages of growth. Many causes associated with the management or slaughter can generate these pH changes. Although in the present study, the animals were slaughtered at the same age under the same protocol, and the possible effect of slaughtering with a period of 7 days between groups should be considered in the interpretation.

Some modifications in meat composition after slaughter were observed in the group of pigs that consumed the probiotic additives during the postweaning, which could be considered as an improvement in meat quality. While these results are promising, further studies are needed to evaluate the possible effect of these additives closer to slaughter, as well as their combined use.

5. Conclusion

The group of pigs who were given probiotic supplements during their postweaning period showed some changes in their meat composition after slaughter, which may be interpreted as an improvement in the quality of pork. Pigs fed with probiotics showed higher percentages of essential omega-3 fatty acids, such as linolenic acid, and pigs fed with *S. cerevisiae* var. boulardii RC009 showed a decrease in eicosanoic contents in meat and an increase in lean percentage but it also increased the levels of trans fatty acids. In addition, both probiotic-treated groups showed an improvement in the capacity to retain water [39].

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We appreciate the collaboration of Manuel González and Germán Mondino from Aceitera General Deheza farm (AGD) (Santa Eufemia, Córdoba, Argentina). The authors thank BIOFEED TECH SAS for the industrial scale production of the tested probiotics. This study was supported by the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación, PICT under grants no. 3089/18 and PICT-2020-SERIEA-01733.

References

[1] L. Xu, X. Yang, L. Wu, X. Chen, L. Chen, and F. S. Tsai, "Consumers' willingness to pay for food with information on animal welfare, lean meat essence detection, and traceability," *International Journal of Environmental Research and Public Health*, vol. 16, no. 19, p. 3616, 2019.

[2] I. Chernukha, E. Kotenkova, V. Pchelkina et al., "Pork fat and meat: a balance between consumer expectations and nutrient composition of four pig breeds," *Foods*, vol. 12, no. 4, p. 690, 2023.

[3] S. Rajic, S. Simunovic, V. Djordjevic, M. Raseta, I. Tomasevic, and I. Djekic, "Quality multiverse of beef and pork meat in a single score," *Foods*, vol. 11, no. 8, p. 1154, 2022.

[4] I. Argemí-Armengol, D. Villalba, M. Tor et al., "The extent to which genetics and lean grade affect fatty acid profiles and volatile compounds in organic pork," *PeerJ*, vol. 7, no. 7, Article ID e7322, 2019.

[5] P. Khanal, C. Maltecca, C. Schwab, J. Fix, and F. Tiezzi, "Microbiability of meat quality and carcass composition traits in swine," *Journal of Animal Breeding and Genetics*, vol. 138, no. 2, pp. 223–236, 2021.

[6] D. M. Weary, J. Jasper, and M. J. Hotzel, "Understanding weaning distress," *Applied Animal Behaviour Science*, vol. 110, no. 1-2, pp. 24–41, 2008.

[7] S. McOrist and K. H. Mellits, "The important lifetime effects of intestinal gut health of pigs at weaning," *The Veterinary Journal*, vol. 184, no. 3, pp. 253-254, 2010.

[8] J. Parada, A. Magnoli, M. C. Isgro et al., "In-feed nutritional additive probiotic *Saccharomyces boulardii* RC009 can substitute for prophylactic antibiotics and improve the production and health of weaning pigs," *Veterinary World*, vol. 16, no. 5, pp. 1035–1042, 2023.

[9] N. Méndez-Palacios, M. Mendez-Mendoza, F. Vazquez-Flores, J. G. Castro-Colombres, and J. E. Ramirez-Bribiesca, "Productive and economic parameters of pigs supplemented from weaning to finishing with prebiotic and probiotic feed additives," *Animal Science Journal*, vol. 89, no. 7, pp. 994–1001, 2018.

[10] V. Tufarelli, A. M. Crovace, G. Rossi, and V. Laudadio, "Effect of a dietary probiotic blend on performance, blood characteristics, meat quality and faecal microbial shedding in growing-finishing pigs," *South African Journal of Animal Science*, vol. 47, no. 6, pp. 875–882, 2017.

[11] M. R. Armando, C. A. Dogi, R. P. Pizzolitto et al., "*Saccharomyces cerevisiae* strains from animal environment with in vitro aflatoxin B1 binding ability and anti-pathogenic bacterial influence," *World Mycotoxin Journal*, vol. 4, no. 1, pp. 59–68, 2011.

[12] M. P. Martinez, M. L. Gonzalez Pereyra, M. G. Fernandez Juri, V. Poloni, and L. Cavaglieri, "Probiotic characteristics and aflatoxin B1 binding ability of Debaryomyces hansenii and Kazaschtania exigua from rainbow trout environment," *Aquaculture Research*, vol. 49, no. 4, pp. 1588–1597, 2018.

[13] V. Poloni, A. Magnoli, A. Fochesato et al., "A *Saccharomyces cerevisiae*RC016-based feed additive reduces liver toxicity, residual aflatoxin B1 levels and positively influences intestinal morphology in broiler chickens fed chronic aflatoxin B1-contaminated diets," *Animal Nutrition*, vol. 6, no. 1, pp. 31–38, 2020.

[14] AOAC, *Official Methods of Analysis of AOAC International*, AOAC International, Gaithersburg, MD, USA, 19th edition, 2012.

[15] K. O. Honikel, "How to measure the water-holding capacity of meat? Recommendation of standardized methods," in *Evaluation and Control of Meat Quality in Pigs*, P. V. Tarrant, G. Eikelenboom, and G. Monin, Eds., Springer, Dordrecht, Netherlands, 1987.

[16] V. Rezar, J. Salobir, A. Levart et al., "Supplementing entire male pig diet with hydrolysable tannins: effect on carcass traits, meat quality and oxidative stability," *Meat Science*, vol. 133, pp. 95–102, 2017.

[17] G. R. Ross, C. P. Van Nieuwenhove, and S. N. González, "Fatty acid profile of pig meat after probiotic administration," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 23, pp. 5974–5978, 2012.

[18] T. L. V. Ulbricht and D. A. T. Southgate, "Coronary heart disease: seven dietary factors," *The Lancet*, vol. 338, no. 8773, pp. 985–992, 1991.

[19] Y. Chen, Y. Qiao, Y. Xiao et al., "Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers and spent hens," *Asian-Australasian Journal of Animal Sciences*, vol. 29, no. 6, pp. 855–864, 2015.

[20] G. P. Quinn and M. J. Keough, *Experimental Design and Data Analysis for Biologists*, Cambridge University Press, Cambridge, UK, 2002.

[21] J. A. Di Rienzo, F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C. W. Robledo, *InfoStat Versión*, Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina, 2020.

[22] D. Shin, S. Y. Chang, P. Bogere, K. H. Won, J. Y. Choi, and Y. J. Choi, "Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs," *PLoS One*, vol. 14, no. 8, Article ID e0220843, 2019.

[23] R. Dowarah, A. K. Verma, N. Agarwal, and P. Singh, "Efficacy of species-specific probiotic Pediococcus acidilactici FT28 on blood biochemical profile, carcass traits and physicochemical properties of meat in fattening pigs," *Research in Veterinary Science*, vol. 117, pp. 60–64, 2018.

[24] S. Wang, B. Yao, H. Gao et al., "Combined supplementation of *Lactobacillus fermentum* and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation, and modulated intestinal microbiota in weaned pigs," *BMC Veterinary Research*, vol. 15, no. 1, p. 239, 2019.

[25] J. L. Dávila-Ramírez, M. R. Carvajal-Nolazco, M. J. López Millanes et al., "Effect of yeast culture (*Saccharomyces cerevisiae*) supplementation on growth performance, blood metabolites, carcass traits, quality and sensorial traits of meat from pigs under heat stress," *Animal Feed Science and Technology*, vol. 267, Article ID 114573, 2020.

[26] V. V. Almeida, J. P. M. Silva, A. P. Schinckel et al., "Effects of increasing dietary oil inclusion from different sources on growth performance, carcass and meat quality traits, and fatty acid profile in genetically lean immunocastrated male pigs," *Livestock Science*, vol. 248, Article ID 104515, 2021.

[27] J. D. Wood, M. Enser, A. V. Fisher et al., "Fat deposition, fatty acid composition and meat quality: a review," *Meat Science*, vol. 78, no. 4, pp. 343–358, 2008.

[28] S. Y. Chang, S. A. Belal, D. R. Kang et al., "Influence of probiotic-friendly pigs production on met quality and physicochemical characteristic Korean," *Journal of Food Science of Animal Resources*, vol. 38, no. 2, pp. 403–416, 2018.

[29] E. R. Grela, M. Swiatkiewicz, M. Florek, M. Bakowski, and G. Skiba, "Effect of inulin source and a probiotic supplement in pig diets on carcass traits, meat quality and fatty acid composition in finishing pigs," *Animals*, vol. 11, no. 8, p. 2438, 2021.

[30] T. Zhimei, C. Yiyan, L. Huijie, W. Gang, and M. Xianyong, "Effect of long-term dietary probiotic *Lactobacillus reuteri 1* or antibiotics on meat quality, muscular amino acids and fatty acids in pigs," *Meat Science*, vol. 171, pp. 108–234, 2021.

[31] J. D. Wood, R. I. Richardson, G. R. Nute et al., "Effects of fatty acids on meat quality: a review," *Meat Science*, vol. 66, no. 1, pp. 21–32, 2004.

[32] K. Suzuki, M. Ishida, H. Kadowaki, T. Shibata, H. Uchida, and A. Nishida, "Genetic correlations among fatty acid compositions in different sites of fat tissues, meat production, and meat quality traits in Duroc pigs," *Journal of Animal Science*, vol. 84, no. 8, pp. 2026–2034, 2006.

[33] B. Lebret, P. Ecolan, N. Bonhomme, K. Méteau, and A. Prunier, "Influence of production system in local and conventional pig breeds on stress indicators at slaughter, muscle and meat traits and pork eating quality," *Animal*, vol. 9, no. 8, pp. 1404–1413, 2015.

[34] B. Chen, D. Li, D. Leng, H. Kui, X. Bai, and T. Wang, "Gut microbiota and meat quality," *Frontiers in Microbiology*, vol. 13, no. 13, Article ID 951726, 2022.

[35] L. E. T. Vissers, J. Rijksen, J. M. A. Boer, W. M. M. Verschuren, Y. T. van der Schouw, and I. Sluijs, "Fatty acids from dairy and meat and their association with risk of coronary heart disease," *European Journal of Nutrition*, vol. 58, no. 7, pp. 2639–2647, 2018.

[36] Q. Zhu, M. Song, M. A. K. Azad, C. Ma, Y. Yin, and X. Kong, "Probiotics and synbiotics addition to Bama mini-pigs' diet improve carcass traits and meat quality by altering plasma metabolites and related gene expression of offspring," *Frontiers in Veterinary Science*, vol. 9, Article ID 779745, 2022.

[37] A. Kasprzyk, M. Tyra, and M. Babicz, "Fatty acid profile of pork from a local and a commercial breed," *Archives of Animal Breeding*, vol. 58, no. 2, pp. 379–385, 2015.

[38] A. Rybarczyk, E. Bogusławska-Was, and B. Pilarczyk, "Carcass and pork quality and gut environment of pigs fed a diet supplemented with the bokashi probiotic," *Animals*, vol. 11, no. 12, p. 3590, 2021.

[39] National Research Council (Nrc), *Nutrient Requirements of Swine*, National Academies Press, Washington, DC, USA, 2021.