

Fermentation of tomato and apple pomaces with *Aspergillus niger* improves organic acid profile and broiler chicken performance

Running title: Fermented pomace additives in broiler diets

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Fermentation of tomato and apple pomaces with *Aspergillus niger* improves organic acid profile and broiler chicken performance

Abstract

This study aimed to produce two functional feed additives via solid-state fermentation and evaluate their effects on performance, meat quality, and cecal microbiota in broiler chickens. A total of 288 mixed-sex Ross 308 broiler chicks were randomly assigned to nine dietary treatments (four replicates of eight chicks each). The control group received a basal diet, while treatment groups received the basal diet supplemented with increasing doses (300–2 400 mg/kg) of either fermented tomato pomace plus oat or fermented apple pomace plus rye. The trial lasted 49 days. Supplementation with the lowest and highest doses of fermented apple pomace plus rye significantly increased body weight and body weight gain ($P < 0.05$). The fermented tomato pomace plus oat increased breast meat lightness and yellowness at higher doses, whereas the fermented apple pomace plus rye decreased both color parameters ($P < 0.05$). The cecal lactic acid bacteria population increased with both additives at 300 mg/kg ($P < 0.05$). Both fermentation products yielded functional additives that improved body weight and weight gain during the starter period. The fermented apple pomace plus rye at 300 mg/kg showed the most consistent positive effects and can be recommended as a potential alternative feed additive for broiler chickens.

Keywords: Broiler chicken; Feed additive; Fruit pomace; Microbial fermentation; Organic acids.

Study contribution

Antibiotics have been used as feed additives in poultry nutrition for many years. However, due to the cross-resistance of bacteria and residues in animal products, the use of antibiotics was banned in 2006. Following this development, alternative feed additives are being investigated due to the continuity of production and possible stress factors in animals. In this study, the production of two different feed additives with high organic acid content and enzyme activity by the solid -state fermentation method and their effects on performance, meat quality, and cecal microbiota in broiler chickens were investigated. In conclusion, both products were converted into functional feed additives which increased body weight and body weight gain and improved feed utilization in the starter period of broiler chicks, and especially the 300 mg/kg dose of fermented apple pomace plus rye could be used as a feed additive in broiler chickens.

Introduction

The global population is increasing at a rapid rate, and it is expected to reach 9.7 billion by the year 2050.⁽¹⁾ The increase in the world population reveals the importance of white meat in meeting people's animal protein needs. Poultry meat plays a critical role in human nutrition due to its affordability compared to red meat, shorter production period, and greater accessibility. The productivity of animal products in intensive broiler chicken production is steadily rising in terms of quantity, capacity, and yield per unit area. This growth can be managed by increasing the production of white meat. Many advances have been made to enhance the productivity of animal products per unit of land while also reducing the time required for production. These include the development of hybrid breeds, the increase in

animal density per unit area, the precise determination of broiler chicken requirements, and the adjustment of feeding accordingly.⁽²⁾

These developments have led to the introduction of feed additives (FA) to improve feed nutrient utilization by animals. The FA sector had a market volume of 35.01 billion USD in 2019; it is projected to reach 56.22 billion USD by 2027.⁽²⁾ In this context, antibiotics have historically been the most widely used FA. However, long-term use of antibiotics can have some negative effects, such as microorganisms developing cross-resistance to antibiotics, leaving residues in animal products, and preventing the development of both beneficial and pathogenic microorganisms in the intestinal microflora. The European Commission banned its use as a growth factor in animal nutrition in 2006 for this reason.⁽³⁾ Following this decision, the search for natural, safe, and residue-free FA alternatives to antibiotics has gained prominence to mitigate the negative effects that could compromise economic sustainability and production continuity, such as health issues and decreased productivity.

As alternatives to antibiotics, researchers use organic acids, essential oils, plant extracts, enzymes, probiotics, prebiotics, and eubiotics using various production methods.⁽⁴⁻⁶⁾ The solid-state fermentation (SSF) method is the most widely used among these production methods. The general principle of SSF involves microorganism growth, proliferation, and biochemical reactions in a solid material. This process produces versatile FA that contains enzymes, antioxidants, organic acids, probiotics, prebiotics, and bioactive components. SSF was used to improve the nutritional quality of low-quality feeds.^(7, 8) Recently, the discovery of probiotics, enzymes, and organic acids in fermented end products has resulted in a growing use of this technique to produce feed additives. Studies indicate that fermented FA has the

potential to serve as a substitute for antibiotics, as suggested by Peng et al.⁽⁸⁾ and Yasar and Yegen.⁽⁹⁾

Research on fermented FA has demonstrated improvements in various parameters for broiler chickens, including body weight, body weight gain (BWG), feed consumption, feed utilization, carcass quality, and meat quality. Additionally, it has been effective in suppressing and reducing the population of pathogenic microorganisms in the small intestine and cecum.^(7, 8, 10) The market of FA produced through the SSF method has also been gradually expanding. For instance, Alltech⁽¹¹⁾ reported the development and marketing of an FA that features the active ingredient “Synergen” produced using the SSF method and *Aspergillus niger*.

Examining the literature on fermented feed additive production reveals that most studies focus on a single substrate, with limited research on mixtures of multiple substrates. This study produced a feed additive rich in organic acids by blending tomato pomace and oats, as well as apple pomace and rye, in suitable proportions, and subjecting them to solid-phase fermentation with *Aspergillus niger* under optimal fermentation conditions. Thus, fruit wastes were utilized to produce biologically active molecules blended in organic compounds and introduced as feed additives. The main purpose of this study was to produce a final product rich in organic acids, enzyme activity, and antioxidant capacity. Final products were then tested in broiler chickens.

Materials and methods

Ethical statement

All animal procedures were performed strictly in accordance with the guidelines and approved by the local Ethical Committee of Kafkas University (protocol number:2020-184).

Production of fermented feed additive

The pomaces and cereals used for fermentation were passed through a 3 mm sieve and homogenously mixed according to the proportions specified in **Table 1**. The variation in the cereal proportions across the mixes given in **Table 1** is due to the fact that apple pomace has a greater amount of sugar compared to tomato pomace. Consequently, we reduced the oat ratio and increased the molasses ratio in the mixture of tomato pomace and oat, aiming to balance the sugar levels of the substrates. Therefore, in the mixtures given in **Table 1**, the oat ratio was 40.714 % and the molasses ratio was 6 % in fermentation-1, and the rye ratio was 44.714 % and the molasses ratio was 2 % in fermentation-2.

The mixtures were added into 5-liter jars and closed with lids. They were then sterilized by autoclaving at a temperature of 121°C for a duration of 15 min. In the sterilization process, *Aspergillus niger* and sterile distilled water were added under aseptic conditions and thoroughly mixed until a uniform mixture was obtained at room temperature. Subsequently, we allowed it to undergo fermentation for a duration of 72 h, adhering to the specified parameters listed in **Table 1**. The sterilization of other glassware was performed at a temperature of 175°C for a duration of an hour.

Table 1. Fermentation conditions

Parameter	Fermentation-1	Fermentation-2
Substrate	Tomato pomace + oats	Apple pomace + rye
Mixing ratio (%)	50 % + 40.71	50 % + 44.71
Chemicals	1 % (NH ₄) ₂ SO ₄	1 % (NH ₄) ₂ SO ₄
	1 % (NH ₄) ₂ HPO ₄	1 % (NH ₄) ₂ HPO ₄
	0.1 % FeSO ₄ ·7H ₂ O	0.1 % FeSO ₄ ·7H ₂ O
	0.2 % KH ₂ PO ₄	0.2 % KH ₂ PO ₄
	0.05 % MgSO ₄ ·7H ₂ O	0.05 % MgSO ₄ ·7H ₂ O
	0.025 % CaCl ₂ ·2H ₂ O	0.025 % CaCl ₂ ·2H ₂ O
	0.01 % NaCl	0.01 % NaCl
	1 % CH ₄ N ₂ O	1 % CH ₄ N ₂ O
	6 % molasses	2 % molasses
Temperature	26–28°C	
Incubation period	72 h	
Sampling intervals	0 and 72 h	
Inoculant level	10 ⁶ cfu g ⁻¹	
pH	5–5.5	
Moisture (%)	65–70	

At the end of the fermentation periods, three replicate microbiological analyses were performed on wet samples taken. The counting of *Aspergillus niger* in fermented end products was conducted using the Thoma Chamber method. The dry matter,⁽¹²⁾ titratable acidity,⁽¹³⁾ and pH were determined in wet fermented products. The remaining fermented products were placed on drying stands and dried at room temperature. The dried fermented products were milled and stored at a temperature of +4°C until they were used for *in vitro* analyses and *in vivo* studies.

We obtained the targeted final product by adding thyme leaves to fermented products stored at +4°C just before *in vivo* and *in vitro* analyses. The dry matter (DM), crude ash, crude protein (CP), and crude fat (CF) contents of the samples were analyzed before and after fermentation using the methods described by AOAC.⁽¹²⁾ The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined using the methods reported by Van Soest et al.⁽¹⁴⁾ The samples were analyzed for organic acid content, including oxalic, citric, lactic, malic, acetic, propionic, and pyruvic acid, using HPLC with an Agilent Hi-Plex H column (300 × 7.7 mm). The analysis was conducted using the method described by Bevilacqua and Califano.⁽¹⁵⁾

The activities of xylanase, cellulase, and β-glucanase enzymes were measured by spectrophotometric methods as described by König et al.⁽¹⁶⁾ The activity of the amylase enzyme was determined using the method developed by Kumar and Duhan.⁽¹⁷⁾ Phytase enzyme activity was determined in accordance with the TS EN ISO 30024:2009⁽¹⁸⁾ standard. Protease activity was carried out by the method described by Yasuda et al.⁽¹⁹⁾ The samples were determined for antioxidant capacity. Free radical-scavenging activity was determined

using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method, as described by Martins et al.,⁽²⁰⁾ using a PerkinElmer spectrophotometer.

Birds, diets, and experimental design

A 288 Ross-308 day-old broiler chicks were randomly distributed into 36 floor pens with statistically similar body weights. The experimental design included 9 different diets, each with 4 pen replicates, and each pen contained 4 male and 4 female chicks. The pen had a dimension of 100 × 120 × 100 cm (width, length, and height). The mixed feeds utilized in this research were provided in powder form by a commercial company operating in Ağrı. We used starter and finisher feeds. The diet formulation was presented in **Table 2** and their chemical analysis in **Table 3**.

The starter and grower diets were supplemented with 300, 600, 1 200, and 2 400 mg/kg of fermented tomato pomace + oat mixture (FTPO) and 300, 600, 1 200, and 2 400 mg/kg of fermented apple pomace + rye mixture (FAPR). A control diet was also formulated without additives. The inclusion levels were arranged in a two-fold geometric progression to establish a biological dose–response relationship and to identify both effective and potentially inhibitory concentrations, as recommended for feed additive efficacy evaluation.^(4, 5, 8–10) During the study, feed and water were given *ad libitum* and 24-hour lighting was applied. In this study, the target body weight for the broiler chicken trial was aimed at being over 2 kg, and the trial period lasted for 7 weeks, considering the targeted body weight at the end of the trial. The room temperature was set at 33°C and gradually decreased according to the age of the broilers until reaching 23°C at 21 d.

Table 2. Chemical compositions of broiler diets

Composition	Starter periods (1–21 d)	Grower and finisher periods (21–49 d)
Crude protein (%)	24	20
Crude fiber (%)	2.5	3.2
Crude ash (%)	5	5
Crude fat (%)	4.5	7.7
Ca (%)	0.7	0.7
P (%)	0.6	0.6
Na (%)	0.2	0.2
Lysine (%)	1.4	1.2
Methionine (%)	0.5	0.6
Vitamin A (IU/kg)	13 400	10 000
Vitamin D ₃ (IU/kg)	3 200	2 750
Vitamin E (mg/kg)	79.6	50
MnO ₂ (mg/kg)	100	100
ZnO (mg/kg)	100	100
Na ₂ SeO ₃ (mg/kg)	0.2	0.2
CuSO ₄ (mg/kg)	9	9
FeSO ₄ (mg/kg)	45	15
Ca(IO ₃) ₂ (mg/kg)	1	1
Anticoccidial (mg/kg)	100	100

Table 3. Chemical composition of experimental feeds (analyzed values)

Nutrients	Starter periods (1–21 d)	Grower and finisher periods (21–49 d)
Crude protein (%)	23.48 ±0.48	20.78 ±0.75
Crude fiber (%)	3.10 ±0.52	2.64 ±0.69
Crude ash (%)	5.13 ±0.25	5.10 ±0.15
Crude fat (%)	3.56 ±0.84	6.81 ±0.94
Metabolic energy (kcal/kg)*	3 000	3 200

* Metabolic energy was calculated according to NRC (1994).

Growth performance

The body weights of the chicks were measured on a weekly basis using individual weighing. Feed intake (FI) was determined daily for each pen. The mortality rate was calculated based on the total count of deceased animals. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated on a weekly basis using weekly feed consumption and BWG per pen.

Determination of the characteristics of carcass and digestive system

To assess carcass features, the feeders were taken away from the animals 8 h prior to slaughter, and the animals were only given access to water. To determine the effect of treatments on carcass features, a total of 8 broilers (4 males and 4 females) were selected from each group based on their average body weight and were then sent to slaughter. Following slaughter, the internal organs were removed, the carcass was carefully cleaned, allowed to rest briefly, and subsequently weighed to ascertain its mass. Once carcasses were weighed, they were stored at a temperature of +4°C for one day. Afterward, they were weighed again to determine the weight of the cold carcass. The internal organs were

removed, and the liver, gizzard, heart, and digestive system were measured to determine the weight of each one.

Meat quality analysis

Breast meat pH values were measured 24 h after slaughter at three points with a digital pH meter (Hanna, USA) with a meat probe. After the electrode was immersed in the meat, the sample was kept in the meat until the value on the display screen was stable, and the stable value was recorded. The skin on the breast meat area of the carcass was removed, and then the values of three basic color parameters, L* (brightness), a* (redness), and b* (yellowness), were determined from three different points with a portable color scanner (PCE-CSM 4). The device was calibrated before measuring breast meat color values.

The breast meat peroxide value was determined according to the titrimetric method reported by NizamLIOđlu.⁽²¹⁾ Lipid oxidation of breast meat was determined by the spectrophotometric (PerkinElmer) method reported by Koniecko,⁽²²⁾ through the determination of thio barbituric acid reactive substances and malondialdehyde. The free radical scavenging activity of breast meat was assessed by the spectrophotometric method following the procedure described by Martins et al.⁽²⁰⁾ with 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Cecum microbiota

The cecum flora of broiler chickens was analyzed using the spread plate method. Following the cutting process, the contents of the cecum were transferred to sterile tubes. Subsequently, a 1 g sample was collected and put into 9 mL of sterile physiological water, resulting in a dilution ratio of 10^{-1} . Additional dilutions (10^{-4} – 10^{-8}) were then made, and the following selective media were prepared: De Man Rogosa Sharpe Agar (MRSA) (Merck, Germany),

MacConkey Agar (MCA) (Merck, Germany), Baird-Parker Agar (BPA) (Merck, Germany), and Eosin Methylene-Blue Lactose Sucrose Agar. The spread plate method was used to cultivate the samples under sterile conditions, and they were then incubated. Lactic acid bacteria (LAB) were cultured in MRSA medium at 37°C under anaerobic conditions for 48 hours. Coliform bacteria were cultured in MCA medium under aerobic conditions at 37°C for 24 hours.

Staphylococcus aureus was cultured in BPA medium under aerobic conditions at 37°C for 24 hours. *Enterobacteria* were cultured in Eosin Methylene Blue Agar medium at 37°C for 24 hours under aerobic conditions.⁽²³⁾ After the incubation period, the microbial counts in the cecum were determined using the most probable counting method. This process was performed in triplicate for each sample. Statistical analysis was conducted on the cecum microbiota data after it was transformed into logarithmic values using a base of 10.

Statistical analysis

Fermentation parameters (pH, fungal growth, nutrients, organic acids, enzyme activity, and antioxidant content) before and after fermentation were compared using a paired t-test. Differences were considered significant at $P < 0.05$. The data collected from the animal trial were analyzed using the General Linear Model procedure of SPSS (Version 17.0, SPSS Inc., Chicago, IL, USA). The statistical model used for the analysis was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observed parameter, μ is the overall mean, T_i is the fixed effect of treatment (supplementation level), and e_{ij} is the residual error.

The assumptions of normality and homogeneity of variances were checked using the Shapiro-Wilk and Levene's tests prior to analysis. When a significant treatment effect was

detected, differences among means were determined using Duncan's multiple range test at $P < 0.05$. Mortality data were analyzed using the chi-square test.

Results

Effect of fungal fermentations on the chemical composition of the substrate

The effects of both fermentations on pH, fungal growth, and chemical composition were found to be significant ($P < 0.05$) (**Table 4**). In FTPO samples, the pH level reduced from 5.13 to 4.60. Similarly, the pH level of FAPR samples declined from 5.20 to 4.59. It was determined that there was a 2-log fungal growth in both fermentations. The DM content in FTPO increased from 29.82 % to 37 %. In contrast, the DM content of FAPR decreased from 29.81 % to 25.84 %. The CP content of FTPO samples had a significant decrease, from 22.89 % to 20.94 %. FAPR samples showed no changes in CP and CF contents ($P > 0.05$), but the crude ash content increased from 2.16 % to 2.56 % ($P < 0.05$). The ADF content in FAPR samples reduced from 23.32 % to 21.20 %, and the NDF level decreased from 36.88 % to 31.83 % ($P < 0.05$).

Table 4. Effect of fermentation on pH, fungal growth, dry matter, and titratable acidity

	FTPO			FAPR		
	0. h	72. h	P value	0. h	72. h	P value
pH	5.13 ±0.01	4.60 ±0.04	0	5.20 ±0.03	4.59 ±0.02	0
FG (log ₁₀ cfu/g)	6.00 ±0.01	8.57 ±0.01	0	6.00 ±0.01	8.85 ±0.03	0
DM (%)	29.82 ±0.19	37.00 ±0.40	0	29.81 ±0.05	25.84 ±0.12	0
Ash (%)	3.25 ±0.01	3.26 ±0.01	0.753	2.16 ±0.01	2.56 ±0.01	0
CP (%)	22.89 ±0.29	20.94 ±0.27	08	13.16 ±0.25	13.11 ±0.18	0.880
CF (%)	2.25 ±0.11	2.55 ±0.23	0.313	5.66 ±0.10	5.43 ±0.64	0.745
ADF (%)	24.64 ±0.14	25.22 ±0.28	0.143	23.32 ±0.13	21.20 ±0.08	0
NDF (%)	35.56 ±0.43	36.36 ±0.91	0.468	36.88 ±0.68	31.83 ±0.61	05

Data are mean ± standard error. FTPO: tomato pomace + oat fermentation; FAPR: apple pomace + rye fermentation; cfu: colony-forming unit; FG: fungal growth; DM: dry matter; CP: crude protein; CF: crude fat; ADF: acid detergent fiber; NDF: neutral detergent fiber.

Effect of fungal fermentations on the organic acid content of the substrate

As a result of a 72-h fermentation with *A. niger*, significant changes were detected in organic acid contents ($P < 0.05$). As shown in **Table 5**, neither acetic acid (AA) nor oxalic acid (OA) content was detected in the fermentations conducted with *A. niger*. In both fermentations with *A. niger*, lactic acid (LA), citric acid (CitA), malic acid (MA), total organic acid, and titratable acidity increased significantly ($P < 0.05$). Conversely, propionic acid levels increased in FTPO but decreased in FAPR. Also, FTPO fermentation had no effect on the pyruvic acid (PIRA) level, whereas FAPR dramatically increased the PIRA level.

Table 5. Effect of fermentations on organic acids content

Organic acids		FTPO			FAPR		
		0 h	72 h	P-value	0 h	72 h	P-value
AA	g/100 mL	nd	nd	—	nd	nd	—
PROA	g/100 mL	0 ±0	1.21 ±0.05	0	0.82 ±0.05	0.01 ±0	0
LA	g/100 mL	0 ±0	0.92 ±0.03	0	0 ±0	1.02 ±0.07	0
CitA	g/100 mL	0.01 ±0	1.34 ±0.04	0	0 ±0	2.40 ±0.05	0
MA	g/100 mL	0.01 ±0	1.08 ±0.03	0	0.66 ±0.05	3.00 ±0.03	0
OA	g/100 mL	nd	nd	—	nd	nd	—
PIRA	g/100 mL	0.13 ±0.02	0.15 ±0.01	0.196	0 ±0	0.38 ±0.05	0
TOA*	g/100 mL	0.15 ±0.07	4.70 ±0.10	0	1.48 ±0.09	6.82 ±0.07	0
TA (%)		0.42 ±0.09	1.77 ±0.11	0	0.38 ±0.10	2.57 ±0.13	0

Data are mean ± standard error. FTPO: tomato pomace + oat fermentation; FAPR: apple pomace + rye fermentation. nd: not detected; AA: acetic acid; PROA: propionic acid; LA: lactic acid; CitA: citric acid; MA: malic acid; OA: oxalic acid; PIRA: pyruvic acid; TOA: total organic acid; TA: titratable acidity; * detected by calculation.

Effect of fungal fermentations on the enzyme activity and antioxidant capacity of the substrate

Fermentations of FTPO and FAPR with *Aspergillus niger* resulted in significant changes in enzyme activities and antioxidant capacity ($P < 0.05$) (**Table 6**). Alkaline protease activity increased by 82 % in FTPO and 42 % in FAPR ($P < 0.05$). Cellulase activity increased significantly by 87 % in FTPO and 44 % in FAPR ($P < 0.05$). In FTPO, β -glucanase, amylase, and phytase activity were not significant ($P > 0.05$). Antioxidant capacity (DPPH assay) increased by 87 % in FTPO and 14 % in FAPR ($P < 0.05$).

Table 6. Effect of fermentations on enzyme activity and antioxidant capacity

Enzyme	FTPO			FAPR		
	0 h	72 h	P-value	0 h	72 h	P-value
Alkaline protease (IU/g)	115.17 ±1.11	209.94 ±1.49	0	44.97 ±1.96	63.79 ±1.90	02
Cellulase (IU/g)	27.24 ±0.75	51.00 ±1.34	0	55.20 ±1.02	79.54 ±0.94	0
β-glucanase (IU/g)	33.80 ±0.40	33.05 ±0.97	0.514	27.30 ±1.15	33.10 ±0.56	0.011
Xylanase (IU/g)	13.07 ±0.41	12.66 ±0.38	0.501	28.57 ±0.44	27.64 ±0.42	0.202
Amylase (IU/g)	4.69 ±0.10	4.51 ±0.07	0.224	9.05 ±0.55	10.85 ±0.2	0.042
Phytase (IU/g)	1.03 ±0.04	1.01 ±0.04	0.646	0.82 ±0.02	1.11 ±0.02	01
DPPH (%)	12.03 ±0.58	22.45 ±1.20	01	16.57 ±0.30	18.86 ±0.28	05

Data are mean ± standard error. FTPO: tomato pomace + oat fermentation; FAPR: apple pomace + rye fermentation.

Effect of fermented feed additives on broiler chicken performance

The performance of broilers changed significantly when different doses of FTPO and FAPR were added to rations ($P < 0.05$). There was no significant difference in mortality rates between the groups during the trial period ($P > 0.05$). The effect of fermented feed additives on broiler chicken body weight (BW) was found to be significant ($P < 0.05$) (**Table 7**). Compared to the control group, the effect of additives on BW was found to be significant in the first week at doses other than 1 200 mg/kg in the FTPO group and at doses of 300 and 1 200 mg/kg in the FAPR group. In the later weeks of the experiment, although there was a difference in the FTPO 1 200 mg/kg dose on BW until the last week of the experiment, this difference disappeared in the last week. Broiler chickens fed with 300 and 2 400 mg/kg doses

of FAPR had higher BW compared to those receiving other doses and the control group throughout the trial.

The study determined that the addition of fermented feed additives to the broiler chickens did not have a significant effect on their weekly or total of FI ($P > 0.05$) [Table 8]. The inclusion of fermented additives in the broiler chicken diet had a significant effect on BWG ($P < 0.05$), as seen in Table 9. Although fermented additives influenced weekly BWG in the broiler chicken trial compared to the control group, the most significant effect was seen at the FAPR dose of 300 mg/kg by the end of the trial. The effect of the additives used in the experiment on FCR was found to be significant in some weeks (1, 2, and 7) ($P < 0.05$). Nevertheless, the total trial period was not significantly affected ($P > 0.05$) as shown in Table 10. FCR was found to be significant at the doses of FTPO 600 and 2 400 mg/kg and FAPR 300 mg/kg during the first week, at the dose of FAPR 2 400 mg/kg in the second week, and at the dose of FTPO 600 mg/kg in the final week of treatment.

Table 7. Weekly body weights of broiler chickens (g/animal)

Trial	Doses (mg/kg)	0 d	week 1	week 2	week 3	week 4	week 5	week 6	week 7
Control	0	41.32 ±0.37	97.05 ±1.05 ^c	179.56 ±3.16 ^d	355.58 ±8.48 ^e	744.45 ±14.58 ^{cd}	1 190.93 ±18.91 ^b	1 721.92 ±21.27 ^b	2 261.45 ±30.60 ^{bcd}
FTPO	300	41.18 ±0.47	101.35 ±1.40 ^{ab}	180 ±2.84 ^{cd}	375.76 ±6.98 ^{de}	701.81 ±13.57 ^d	1 178.29 ±19.22 ^b	1 728.81 ±25.52 ^b	2 289.06 ±34.01 ^{bcd}
	600	41.60 ±0.47	103.54 ±1.56 ^a	185.50 ±2.54 ^{cd}	387.73 ±5.92 ^{cd}	734.40 ±10.42 ^{cd}	1 182.98 ±18.09 ^b	1 739.39 ±26.51 ^b	2 349.32 ±34.14 ^{ab}
	1 200	41.32 ±0.57	99.47 ±1.12 ^{bc}	197.26 ±3.09 ^{ab}	408.61 ±8.60 ^{bc}	790.63 ±14.00 ^b	1 289.70 ±20.28 ^a	1 831.83 ±25.03 ^a	2 322.34 ±28.46 ^{abc}
	2 400	41.73 ±0.67	102.21 ±1.03 ^{ab}	183.09 ±2.71 ^{cd}	381.74 ±8.03 ^d	743.80 ±12.87 ^{cd}	1 189.78 ±16.10 ^b	1 703.66 ±23.68 ^b	2 204.70 ±30.01 ^d
FAPR	300	41.50 ±0.52	104.45 ±1.36 ^a	189.45 ±3.47 ^{bc}	414.36 ±9.47 ^{ab}	797.30 ±17.82 ^{ab}	1 285.73 ±26.50 ^a	1 825.52 ±37.69 ^a	2 414.14 ±41.75 ^a
	600	41.66 ±0.55	100.50 ±1.21 ^{abc}	188.67 ±2.23 ^{bcd}	385.28 ±5.60 ^{cd}	740 ±9.90 ^{cd}	1 187.72 ±15.20 ^b	1 742.20 ±22.47 ^b	2 223.57 ±25.53 ^{cd}
	1 200	41.55 ±0.55	101.59 ±1.15 ^{ab}	188.82 ±2.99 ^{bcd}	392.94 ±7.81 ^{bcd}	767.91 ±14.86 ^{bc}	1 229.71 ±25.22 ^b	1 785.14 ±29.82 ^{ab}	2 281.04 ±31.49 ^{bcd}
	2 400	41.82 ±0.72	99.04 ±1.44 ^{bc}	204.12 ±3.84 ^a	432.99 ±8.13 ^a	831.58 ±13.46 ^a	1 289.79 ±15.26 ^a	1 818.88 ±18.19 ^a	2 391.05 ±22.69 ^a
P-value	0.998	0.034	0	0	0	0	0	07	0

a, b, c, d, e Different superscript letters in the same column indicate significant differences ($P < 0.05$). Data are mean ± standard error. FTPO: mix of fermented tomato pomace and oat; FAPR: mix of fermented apple pomace and rye.

Table 8. Weekly feed consumption of broiler chickens

Trial	Doses(mg/kg)	week 1	week 2	week 3	week 4	week 5	week 6	week 7	0-49 d
Control	0	83.66 ±2.14	138.94 ±2.92	241.78 ±8.29	472.14 ±15.20	697.23 ±9.67	879.19 ±28.62	1 066.52 ±27.13	3 579.45 ±68.49
FTPO	300	86.75 ±1.94	142.63 ±1.42	251.72 ±12.46	454.50 ±25.11	679.69 ±4.01	903.80 ±10.93	1 098.90 ±19.05	3 617.98 ±24.30
	600	87.13 ±1.71	147.16 ±5.38	257.32 ±12.61	466.45 ±10.05	668.11 ±16.33	906.97 ±14.32	1 136.72 ±22.05	3 669.83 ±32.16
	1 200	90.75 ±1.83	148.00 ±5.12	275.03 ±9.99	495.94 ±27.02	704.88 ±20.93	909.88 ±23.50	1 053.88 ±23.13	3 678.35 ±101.87
	2 400	85.91 ±1.69	149.81 ±4.46	258.81 ±9.07	439.63 ±12.04	644.51 ±18.13	838.38 ±13.92	1 051.17 ±14.06	3 468.21 ±54.00
FAPR	300	88.99 ±2.89	153.25 ±8.26	282.42 ±16.07	503.15 ±16.95	705.40 ±25.07	929.08 ±6.94	1 139.48 ±21.77	3 801.77 ±85.90
	600	88.00 ±3.02	152.19 ±5.61	272.19 ±8.86	467.84 ±17.25	662.84 ±14.97	889.52 ±32.33	1 086.95 ±18.90	3 619.52 ±89.03
	1 200	89.60 ±4.24	155.25 ±6.13	261.00 ±21.18	481.06 ±27.63	657.94 ±41.06	888.19 ±32.20	1 051.78 ±7.35	3 584.82 ±135.31
	2 400	88.56 ±1.29	148.69 ±3.49	293.19 ±13.91	511.85 ±18.92	716.94 ±24.79	913.22 ±23.06	1 103.48 ±35.22	3 775.92 ±92.14
P value		0.931	0.737	0.294	0.151	0.093	0.239	0.206	0.096

The feed consumption was in g/animal. Data are mean ± standard error. FTPO: mix of fermented tomato pomace and oat; FAPR: mix of fermented apple pomace and rye.

Table 9. Weekly and total body weight gain of broiler chickens

Trial	Doses (mg/kg)	week 1	week 2	week 3	week 4	week 5	week 6	week 7	0-49 d
Control	0	55.74 ±0.36 ^d	82.51 ±1.51 ^{bc}	176.02 ±3.50 ^c	388.87 ±18.71 ^{ab}	446.48 ±17.06	531.00 ±23.93	539.53 ±33.40 ^{abcd}	2 220.14 ±22.24 ^{cd}
FTPO	300	60.16 ±1.42 ^{abc}	78.65 ±1.07 ^c	195.76 ±5.97 ^b	326.06 ±8.97 ^d	476.48 ±11.62	550.52 ±39.45	560.25 ±15.62 ^{abc}	2 247.88 ±28.27 ^{cd}
	600	61.94 ±1.58 ^{ab}	81.96 ±1.37 ^{bc}	202.24 ±3.58 ^b	346.67 ±5.78 ^{cd}	448.57 ±11.08	556.41 ±21.46	609.93 ±30.37 ^a	2 307.72 ±27.94 ^{abc}
	1 200	58.15 ±1.53 ^{cd}	97.80 ±3.11 ^a	211.35 ±6.51 ^{ab}	382.03 ±6.17 ^{ab}	499.07 ±8.09	542.13 ±9.14	490.52 ±5.80 ^{cd}	2 281.02 ±27.19 ^{bc}
	2 400	60.48 ±1.17 ^{abc}	80.88 ±2.17 ^{bc}	198.65 ±7.27 ^b	362.05 ±8.91 ^{bc}	445.99 ±4.30	513.89 ±5.16	501.04 ±8.46 ^{bcd}	2 162.97 ±14.51 ^d
FAPR	300	62.95 ±0.34 ^a	85.01 ±1.10 ^{bc}	224.91 ±9.43 ^a	382.94 ±4.86 ^{ab}	488.43 ±8.32	539.79 ±23.72	588.62 ±34.45 ^a	2 372.64 ±41.86 ^a
	600	58.84 ±0.98 ^{bcd}	88.17 ±3.20 ^b	196.61 ±3.82 ^b	354.72 ±4.66 ^{bcd}	447.73 ±15.65	554.48 ±21.68	481.38 ±6.88 ^d	2 181.92 ±22.76 ^d
	1 200	60.05 ±1.17 ^{abc}	87.23 ±4.13 ^{bc}	204.11 ±7.96 ^b	374.97 ±9.33 ^{abc}	461.81 ±13.41	555.43 ±12.32	495.90 ±15.18 ^{cd}	2 239.50 ±36.65 ^{cd}
	2 400	57.23 ±1.31 ^{cd}	105.09 ±5.18 ^a	228.87 ±8.71 ^a	398.59 ±11.72 ^a	458.21 ±6.62	529.09 ±5.99	572.18 ±16.40 ^{ab}	2 349.24 ±25.14 ^{ab}
P-value		0.032	0	07	0.033	0.256	0.969	02	0

The feed consumption was in g/animal.

Table 10. Weekly and total feed conversion ratio of broiler chickens

Trial	Doses (mg/kg)	week 1	week 2	week 3	week 4	week 5	week 6	week 7	0–49 d
Control	0	1.50 ±0.03 ^{abc}	1.68 ±0.05 ^{ab}	1.37 ±0.06	1.21 ±0.06	1.56 ±0.07	1.66 ±0.05	1.98 ±0.10 ^{bc}	1.61 ±0.02
FTPO	300	1.44 ±0.03 ^{bc}	1.82 ±0.03 ^a	1.29 ±0.03	1.39 ±0.04	1.43 ±0.06	1.64 ±0.10	1.96 ±0.05 ^{bc}	1.61 ±0.03
	600	1.41 ±0.01 ^c	1.80 ±0.08 ^a	1.27 ±0.05	1.35 ±0.02	1.49 ±0.04	1.63 ±0.05	1.86 ±0.06 ^c	1.59 ±0.01
	1 200	1.56 ±0.03 ^a	1.51 ±0.03 ^{bc}	1.30 ±0.03	1.30 ±0.05	1.41 ±0.05	1.68 ±0.05	2.15 ±0.02 ^{ab}	1.61 ±0.03
	2 400	1.42 ±0.04 ^c	1.85 ±0.05 ^a	1.30 ±0.04	1.21 ±0.04	1.45 ±0.05	1.63 ±0.02	2.10 ±0.05 ^{abc}	1.60 ±0.02
	300	1.41 ±0.04 ^c	1.80 ±0.10 ^a	1.26 ±0.06	1.31 ±0.05	1.44 ±0.04	1.72 ±0.08	1.94 ±0.13 ^{bc}	1.60 ±0.06
FAPR	600	1.50 ±0.04 ^{abc}	1.73 ±0.08 ^a	1.38 ±0.02	1.32 ±0.04	1.48 ±0.03	1.60 ±0.08	2.26 ±0.04 ^a	1.66 ±0.03
	1 200	1.49 ±0.05 ^{abc}	1.78 ±0.06 ^a	1.28 ±0.05	1.28 ±0.05	1.42 ±0.08	1.60 ±0.03	2.12 ±0.08 ^{abc}	1.60 ±0.04
	2 400	1.55 ±0.04 ^{ab}	1.41 ±0.08 ^c	1.28 ±0.03	1.28 ±0.02	1.56 ±0.05	1.73 ±0.04	1.93 ±0.08 ^{bc}	1.61 ±0.04
	P value	0.029	0	0.334	0.103	0.256	0.833	0.032	0.962

The feed conversion ratio was expressed in g feed consumption/g body weight gain.

^{a, b, c} Different superscript letters in the same column indicate significant differences ($P < 0.05$). Data are mean ± standard error. FTPO: mix of fermented tomato pomace and oat; FAPR: mix of fermented apple pomace and rye.

The effect of fermented feed additives on broiler carcass yield, digestive system length, and weight

This study determined that the addition of different dosages of FTPO and FAPR to broiler chicken diets did not have a significant effect on hot and cold carcass yield, total digestive system weight (TDSW), as well as the weights of the liver, heart, and gizzard ($P > 0.05$) [Table 11].

Table 11. Effect of additive on hot and cold carcass yield, total digestive system weight and internal organ relative weights

Trial	Doses, mg/kg	Hot carcass yield, %	Cold carcass yield, %	TDSW, g	Liver, g	Heart, g	Gizzard, g
Control	0	75.13 ±0.31	74.18 ±0.32	9.68 ±0.22	1.93 ±0.05	0.54 ±0.03	3.21 ±0.20
FTPO	300	74.68 ±0.98	73.91 ±0.90	9.13 ±0.30	1.87 ±0.10	0.56 ±0.03	3.12 ±0.17
	600	74.55 ±0.41	73.69 ±0.37	9.36 ±0.26	1.87 ±0.04	0.56 ±0.02	2.82 ±0.23
	1 200	74.76 ±0.67	73.84 ±0.67	9.37 ±0.43	1.81 ±0.04	0.52 ±0.02	3.19 ±0.22
	2 400	73.12 ±0.48	72.20 ±0.49	9.43 ±0.52	1.83 ±0.08	0.50 ±0.02	3.30 ±0.27
	300	73.23 ±0.29	72.32 ±0.28	9.57 ±0.35	1.98 ±0.05	0.53 ±0.03	3.52 ±0.24
FAPR	600	74.53 ±0.49	73.59 ±0.49	9.60 ±0.34	1.99 ±0.08	0.52 ±0.03	2.99 ±0.22
	1 200	74.20 ±0.53	73.24 ±0.53	9.25 ±0.34	1.79 ±0.07	0.54 ±0.03	3.34 ±0.16
	2 400	74.25 ±0.40	73.45 ±0.36	9.05 ±0.28	1.74 ±0.06	0.53 ±0.02	3.19 ±0.14
	P value	0.183	0.092	0.709	0.451	0.630	0.791

It was measured in g/100 g body weight. Data are mean ± standard error. FTPO: mix of fermented tomato pomace and oat; FAPR: mix of fermented apple pomace and rye. TDSW: total digestive system weight.

The effect of fermented feed additives on breast meat color, pH, thiobarbituric acid reactive substances, peroxide, and antioxidant

The impact of the dietary additives on the L* and b* values of broiler chicken breast meat was significant ($P < 0.05$), while their effect on the a* and pH values was not significant ($P > 0.05$) (**Table 12**). The L* value of breast meat increased in the group that received the FTPO additive at a dosage of 2 400 mg/kg compared to the control group. Other dosages did not show any effect. In contrast, the addition of FAPR at the same dose of 2 400 mg/kg significantly decreased the L* value of breast meat, with this dosage leading to the most pronounced decrease. The experiment also revealed that the FTPO additive raised the b* value at doses of 1 200 and 2 400 mg/kg, with the 1 200 mg/kg dosage showing the highest increase. At doses of 300 and 600 mg/kg, FTPO had no effect. Conversely, as the dose of the additive increased in the diet, the b* value of breast meat decreased with FAPR supplementation.

Table 12. Effect of fermented additives on brightness, redness, yellowness and pH values of breast meat

Trial	Dose (mg/kg)	L*	a*	b*	pH
Control	0	52.52 ±0.81 ^{ab}	1.14 ±0.17	1.00 ±0.11 ^{bc}	5.67 ±0.04
FTPO	300	52.39 ±0.57 ^{ab}	1.02 ±0.16	0.94 ±0.23 ^{bc}	5.74 ±0.05
	600	53.48 ±0.77 ^{ab}	0.98 ±0.14	1.07 ±0.20 ^{bc}	5.78 ±0.04
	1 200	53.09 ±1.03 ^{ab}	1.38 ±0.35	2.98 ±0.77 ^a	5.79 ±0.02
	2 400	53.99 ±1.15 ^a	0.79 ±0.17	1.12 ±0.57 ^b	5.80 ±0.02
FAPR	300	52.66 ±0.73 ^{ab}	1.51 ±0.25	0.60 ±0.41 ^{bcd}	5.84 ±0.01
	600	53.38 ±1.26 ^{ab}	1.06 ±0.10	0.45 ±0.24 ^{bcd}	5.94 ±0.02
	1 200	51.13 ±0.72 ^{bc}	0.72 ±0.30	-0.45 ±0.34 ^d	5.90 ±0.01
	2 400	48.80 ±0.53 ^c	0.50 ±0.16	-0.24 ±0.54 ^{cd}	5.93 ±0.01
P-value		0.011	0.090	01	0.106

^{a, b, c, d} Different superscript letters in the same column indicate significant differences ($P < 0.05$).

Data are mean ± standard error. FTPO: fermented tomato pomace + oat; FAPR: fermented apple pomace + rye. L*: brightness. a*: redness. b*: yellowness.

The study found that both fermented additives had no effect on the thio barbituric acid reactive substances (TBARS), peroxide, and DPPH values in breast meat ($P > 0.05$)

[Table 13].

Table 13. Effect of fermented additives on thiobarbituric acid reactive substances, peroxide and antioxidant values of breast meat

Trial	Dose (mg/kg)	TBARS	Peroxide	DPPH
Control	0	0.35 ±0.02	0.17 ±0.03	24.31 ±0.56
FTPO	300	0.31 ±0.04	0.16 ±0.02	23.42 ±0.10
	600	0.42 ±0.11	0.14 ±0.02	23.54 ±0.34
	1 200	0.38 ±0.05	0.12 ±0.02	23.45 ±0.43
	2 400	0.36 ±0.03	0.17 ±0.01	23.02 ±0.07
	300	0.38 ±0.07	0.21 ±0.02	23.20 ±0.37
FAPR	600	0.37 ±0.06	0.15 ±0.01	22.25 ±0.10
	1 200	0.39 ±0.06	0.21 ±0.04	25.08 ±2.13
	2 400	0.39 ±0.09	0.20 ±0.02	23.20 ±0.31
	P value	0.931	0.329	0.469

Data are mean ± SEM. FTPO: fermented tomato pomace + oat; FAPR: fermented apple pomace + rye.

Effect of fermented feed additives on broiler cecum microflora

Table 14 shows how adding FTPO and FAPR additives to the ration affects the population of some microorganisms in the broiler cecum microflora. We determined that the addition of FTPO to the broiler chicken diet did not significantly affect the cecum *Enterobacteriaceae* population compared to the control group. The addition of the FAPR supplement to the diet had a significant effect on the *Enterobacteriaceae* population. However, this effect varied significantly between doses. The addition of a 300 mg/kg dose reduced the *Enterobacteriaceae* population, while other doses had no effect. This study found that both fermented additives had an insignificant effect on the population of cecum *Staphylococcus aureus* and coliform microorganisms. This study found a significant effect of the fermented additives on the cecum LAB population. Compared to the control group, the LAB population increased in the FTPO group at doses of 300 and 1 200 mg/kg, and in the FAPR group at a dose of 300 mg/kg.

Table 14. Effect of fermented additives on the population of selected microorganisms in the cecum microflora

Trial	Doses, mg/kg	<i>Enterobacteriaceae</i>	<i>S. aureus</i>	Coliform	LAB
Control	0	6.06 ±0.03 ^a	6.16 ±0.03	5.81 ±0.19	7.14 ±0.07 ^{cd}
FTPO	300	6.08 ±0.01 ^a	6.14 ±0.01	5.60 ±0.26	8.10 ±0.11 ^b
	600	6.04 ±0.02 ^a	6.13 ±0.02	6.14 ±0.09	7.28 ±0.03 ^{cd}
	1 200	6.07 ±0.02 ^a	6.23 ±0.04	5.96 ±0.10	8.06 ±0.03 ^b
	2 400	6.14 ±0.07 ^a	6.27 ±0.15	6.05 ±0.10	7.36 ±0.08 ^c
FAPR	300	5.88 ±0.02 ^b	6.22 ±0.14	6.10 ±0.05	8.35 ±0.05 ^a
	600	6.17 ±0.10 ^a	6.25 ±0.05	5.90 ±0.06	7.25 ±0.06 ^{cd}
	1 200	6.05 ±0.03 ^a	5.86 ±0.26	6.13 ±0.02	7.32 ±0.10 ^{cd}
	2 400	6.03 ±0.11 ^a	5.92 ±0.26	6.11 ±0.03	7.09 ±0.08 ^d
P value		0.03	0.239	0.134	0

The cecum microflora was expressed by log₁₀ cfu/g. Different superscript letters in the same column indicate significant differences (P < 0.05). Data are mean ± standard error. FTPO: mix of fermented tomato pomace and oat; FAPR: mix of fermented apple pomace and rye. LAB: lactic acid bacteria.

Discussion

Passamani et al.⁽²⁴⁾ (2014) reported that the pH range in which *A. niger* operates optimally is between 4 and 6.5. This study found that the pH values in both FTPO and FAPR were at optimal levels. The reason for the decrease in pH in fermentations is the production of organic acid during fermentation. The production of organic acids was expected by the SSF method with *A. niger*.^(25, 26) The pH value of both fermentations were within the range specified in the literature, indicating that they were successful. In fact, a 2-log increase in fungal growth confirms the success of the fermentations. The fermentations had different effects on DM content, even though they used the same microorganism. While DM content increased in FTPO, it decreased in FAPR. This difference may indicate that the microorganism activity changes depending on the substrate composition.

The activities of the microorganisms in fermentations may have produced different components, leading to a potential decrease in the DM content in FAPR. According to Santos et al.,⁽²⁷⁾ the activities of microorganisms in fermentations form some volatile components, which in turn decrease the DM content of the substrate. In analyzing the impact of fermentations conducted with *A. niger* on crude ash content, it was found that there was no change in FTPO, but the crude ash content had an approximate 19 % increase in FAPR. The literature reports substantial variations in the effect of different substrates, including apple and tomato pomace, on the levels of crude ash in fermentation investigations using *A. niger*.^(28, 29)

Dei et al.⁽³⁰⁾ suggested that fungal microorganisms release the phytase enzyme through fermentation, thereby enhancing the concentration of inorganic substances by breaking phosphorus in its complex form. In FAPR, these results supported the opinion of Dei et al.⁽³⁰⁾ Villas-Boas et al.⁽³¹⁾ reported that the addition of nitrogen sources to SSF increased microbial

activity and microbial protein synthesis and consequently increased the CP content of the fermented end product. Although a nitrogen source was used in this study, it was determined that the CP content of FTPO decreased while FAPR did not change, and was not consistent with some studies in the literature.^(28, 31) On the other hand, there are some studies reporting that the CP content decreased as a result of the increase in protease activity in fermentation and the release of nitrogen gas during microorganism activities.^(32, 33)

According to **Table 6**, it is thought that alkaline protease production (82 %) is high in FTPO, and as a result, CP content decreases. Studies indicate that apple pomace releases the cellulase enzyme when fermented with *A. niger*, resulting in the breakdown and decrease of structural carbohydrates such as ADF, NDF, and fiber.⁽³⁴⁾ In studying the effects of fermentation on ADF and NDF levels, this study observed an increase in cellulase activity in both cases. However, the amounts of ADF and NDF were unchanged in FTPO, whereas they decreased in FAPR. The fermentation method is widely used to produce organic acids using various microorganisms.⁽³⁵⁾ *A. niger* is one of these microorganisms, and it reportedly produces nearly all microbial CitA.⁽³⁶⁾

Copetti et al.⁽³⁷⁾ fermented cocoa beans with *A. carbonarius* and *A. niger* at different pH values and investigated organic acid production. According to the researchers, microorganisms produce the highest amounts of CitA and LA within a pH range of 4.2–5.8, while a high pH is optimal for AA production. The fermentation conditions in this study are thought to be ideal for LA and CitA production but not for AA production, as the initial pH of 5.10–5.20 dropped to 4.60. Indeed, the absence of AA production and the significant production of LA and CitA lend support to this theory. Zhang et al.⁽³⁸⁾ suggested adding 200 mg L⁻¹ (NH₄)₂HPO₄ and 0.3 mg L⁻¹ thiamine to the fermentation medium in order for the fermented end product to have low levels

of MA and PIRA and high levels of LA and succinic acid. $(\text{NH}_4)_2\text{HPO}_4$ was added to the medium for both of the fermentations in this study.

High levels of MA and LA were found in both fermentations, and PIRA production was found in the FAPR. The LA results obtained by Zhang et al.⁽³⁸⁾ are in agreement with this study. The MA and PIRA results are in numerical agreement with those obtained by Zhang et al.,⁽³⁸⁾ but it is thought that adding $(\text{NH}_4)_2\text{HPO}_4$ to the medium does not affect the production of these two organic acids. Despite the absence of thiamine in the FTPO substrate, no PIRA production occurred. However, PIRA production increased despite the presence of rye, which contains thiamine, in the substrate used for FAPR. This study found that fermentations increased titratable acidity in tandem with the effects of organic acids. Roja et al.⁽³⁹⁾ associated the increase in titratable acidity in fermentation with microorganism activity and concluded that the increase in microorganism activity in fermentation decreases the ambient pH, which increases titratable acidity. The titratable acidity results of this study are similar to fermentation studies in the literature.^(39, 40)

The results of this study on organic acids indicate that both fermented products are significant sources of organic acids, with mixed apple pomace and rye fermented products being particularly important as functional additives for LA, CitA, and MA. *A. niger* is one of the microorganisms used safely in enzyme production and is the most important microorganism used in microbial protease enzyme production.⁽⁴¹⁾ Paranthaman et al.⁽⁴²⁾ reported that *A. niger* fermented rice by-products to produce protease enzyme, with the optimal fermentation conditions being 35°C, 96 h, and pH 7.0. Muthulakshmi et al.⁽⁴³⁾ reported a 5.8-fold increase in protease activity when they fermented agricultural by-products with *Aspergillus flavus* at 30°C,

7 days, and pH 5.0. In this study, alkaline protease increased significantly, which is consistent with the studies in the literature.^(41, 42, 44)

When *A. niger* ferments agricultural by-products with high lignin and cellulose content, the addition of Fe, Zn, Cu, Mn, and Mg minerals to the medium triggers the microorganism to break down cellulolytic, hemicellulolytic, and lignocellulolytic structures. This, in turn, increases fungal growth, sporulation, and activity and increases the production of cellulase enzymes along with some secondary metabolites.^(45, 46) Moran-Aguilar et al.⁽⁴⁶⁾ reported fermenting sugarcane stalks and brewer's grains with different strains of *Aspergillus niger* to produce cellulase and xylanase enzymes. They also stated that the fermentation period was five days for maximum enzyme production from lignocellulolytic substrates. Despite the three-day fermentation period in this study, it produced a significant level of cellulase enzyme, which aligns with the findings of Moran-Aguilar et al.⁽⁴⁶⁾

Aspergillus terreus fermented rice straw to produce the β -glucanase enzyme, with 70–80 % moisture content and KH_2PO_4 addition serving as the ideal fermentation conditions for optimum production.⁽⁴⁷⁾ The study found that there was no increase in β -glucanase activity during FTPO, whereas there was a 21 % increase in FAPR. The β -glucanase results obtained from FAPR agree with the findings reported in the literature.^(47, 48) da Luz et al.⁽⁴⁹⁾ reported that the amount of moisture had a large effect on enzyme production. Researchers found that fermenting agricultural by-products with *A. niger* at 70 % moisture content increased α -amylase activity by 90 %.⁽⁴⁹⁾ Amylase activity increased similarly in other studies conducted with *A. niger*; the maximum increase was 176.30 U/g.^(50–52)

Reports also indicate that *A. niger* significantly produces phytase enzyme from agricultural by-products, similar to its amylase activity. For example, Mahmood et al.⁽⁵³⁾ stated

that phytase activity increased by 1.37 as a result of their study with *A. niger*. This study found a considerable increase in amylase and phytase activities in FAPR, which is consistent with previous studies.^(50–53) There are studies in the literature reporting that the antioxidant capacity of the substrate exposed to SSF increases.^(32, 54) Erskine et al.⁽⁵⁵⁾ stated that antioxidant effective compounds are synthesized through microbial hydrolysis occurring in the cell walls of microorganisms during fermentation and that the antioxidant capacity of the substrate increases as a result of the emergence of antioxidant compounds such as some phenolics and flavonoids. Apple pomace has flavonoids, phenolic compounds, vitamins C and E, and biological compounds with antioxidant effects like α -tocopherol, lycopene, phenolic acids, and flavonoids.^(56, 57)

Tomato pomace has the same biological compounds with antioxidant effects. In this study, antioxidant capacity was found to increase in both fermentations, and this increase is thought to be the result of microbial hydrolysis, as described by Erskine et al.⁽⁵⁵⁾ In the literature, there are numerous studies investigating the potential of fermenting agricultural by-products as additives to broiler chicken rations.^(5, 9, 58, 59) Yasar and Yegen⁽⁹⁾ fermented a mixture consisting of grain flour, tomato pomace, and citrus pomace with *Saccharomyces cerevisiae*, aiming to produce additives by adding rosemary leaves to the fermented end product. It was stated that when this product was added to broiler chicken rations, FCR was significantly improved. Studies indicated that a blend of corn, soybean meal, and wheat bran was fermented with *Lactobacillus casei* and added into the diets of broiler chickens.⁽⁸⁾

The FCR was significantly improved, while no beneficial effect on BW and FI was observed.⁽⁸⁾ Bostami et al.⁽⁵⁸⁾ found that adding fermented pomegranate pomace, fermented with a mixture of yeast and bacteria, to the broiler chicken ration increased BWG but had no

effect on FI and FCR. In another study, the addition of grape pomace fermented with *A. niger* to the broiler chicken ration increased BW but had no effect on FI and FCR.⁽⁶⁰⁾ According to Yasar and Yegen,⁽⁹⁾ it is normal for the addition of low doses of fermented additives to broiler chicken rations to act in the digestive system, affecting BW, BWG, and FCR rather than FI. In our study, there was no effect on FI, similar to the results of many studies in the literature.^(8, 9, 60)

On the other hand, in this study, significant effects were found on BW and BWG, and the notable improvements were at FAPR doses of 300 and 2 400 mg/kg. According to these results, mixed apple pomace and rye fermentation has a higher efficiency, and the doses determined in this study have no effect on BW or BWG. The FAPR product is believed to enhance the release of enzymes and fluids in the digestive system, leading to improvements in BW and BWG, particularly in the 300 and 2 400 mg/kg dose groups. **Tables 5** and **6** show that FAPR has significantly higher enzyme activity and organic acid content compared to FTPO. This difference in organic acid and enzyme activity is thought to be due to the fact that organic acids, especially lactic, citric, and malic acids, lower gastrointestinal pH and improve intestinal microbial balance in broilers.

Previous studies have indicated that organic acids have such effects on poultry.^(4, 5, 7–10, 60–62) On the other hand, the high enzyme activity in FAPR is thought to reduce the viscosity of digestive fluids and increase the availability of metabolizable energy in broilers. Increased phytase activity may improve phosphorus bioavailability and indirectly support skeletal development and feed digestion.^(7–10, 23) The improvement in total BWG observed, particularly in the FAPR 300 mg/kg (2 372.64 g) and FAPR 2 400 mg/kg (2 349.24 g) groups, despite the lack of a significant difference in feed intake, indicates an increase in feed digestibility rather

than increased consumption. The effect of FAPR on BW and BWG is insignificant at different doses; it can be concluded that the doses have no effect on BW and BWG.

The lack of difference between the dose groups is believed to result from adequate levels of enzymes and organic acids, even at the lowest dose. The absence of these effects in FTPO fermentation may be attributed to insufficient levels of enzymes and organic acids to influence performance. Furthermore, despite the lack of statistical significance, it is apparent that FI has an important relationship with BWG. The statistical insignificance of the FI data is evident due to the significant variation in FI data across experimental groups. However, it is observed that the BWG reflects the numerical changes in the FI data, leading to an increase in BWG at the 300 and 2 400 mg/kg doses in the FAPR group. Also, in this study, the effect of fermented additives on FCR was significant in the first, second, and last weeks of the experiment.

Wang et al.⁽⁶¹⁾ stated that the digestive enzyme activities, especially lipase and trypsin, of broiler chickens reached their maximum level at 21 days. In this study, it is believed that the additives' effect diminishes as their digestive enzyme activities increase, reaching their maximum level within the first two weeks of improving FCR. The findings of Lin et al.⁽⁶²⁾ support this theory as well. Lin et al.⁽⁶²⁾ fermented wheat bran with *Trichoderma pseudokoningii*, extracted xylanase and cellulase enzymes from the fermented product, and added them to broiler chicken rations. They stated that enzyme addition significantly improved the BW, BWG, and FCR of broiler chickens in the initial period, but this effect disappeared afterwards.⁽⁶²⁾ This study observed the effect on FCR in the beginning period of broiler chickens, suggesting that adding fermented additives to the starter ration will improve FCR.

The color scoring of meat is an indicator of its sensory quality, which plays an important role in consumer preference. According to Zhou et al.,⁽⁶³⁾ the meat's sensory quality generally

improves with a higher a^* value and a lower b^* value. Previous studies have reported that some color pigments can improve the meat color score of broiler chickens.⁽⁶⁴⁻⁶⁶⁾ For instance, studies have reported that adding tomato or its pomace, which is rich in color pigments like lycopene and β -carotene, to the diet improves meat color.⁽⁶⁷⁾ Additionally, Liu et al.⁽⁶⁸⁾ stated that microorganisms produce some metabolites during fermentation and that these metabolites significantly affect meat quality.

In Güngör,⁽⁵⁹⁾ it was stated that the 1 % fermented pomace mixture added to broiler chicken rations increased the L^* value, and the highest increase was in the pomace mixture fermented with *A. niger*. The researcher stated that fermented pomace had no effect on breast meat a^* and pH values, while the b^* value decreased. The groups with the addition of a pomace mixture fermented with *A. niger* showed the highest decrease. The additives used in this study had different effects on the meat's color scoring. Namely, while the breast meat L^* value of broiler chickens fed with the FTPO additive showed a slight increase, the FAPR additive caused the L^* value to decrease. The effect of FTPO on breast meat L^* value was similar to the results obtained by Güngör, but the effect of FAPR on L^* value was not similar.

This study suggests that the varying effects of FTPO and FAPR on the L^* value of breast meat can be attributed to the additive content. Because tomato pomace is rich in color pigments such as lycopene and β -carotene, it may have increased the L^* value. Furthermore, despite using the same microorganism in this study's fermentations, it is possible that the different substrates altered the microorganism's activity and the metabolites it produced. In this study, it is believed that the variation in the L^* value of breast meat among the additives is attributed to the differing metabolites produced by microorganisms during fermentations and the varying

content of color pigments in the substrates. The effect of additives on breast meat b* value showed significant differences, as did the L* value.

The effect of additives on breast meat b* value generally had an increasing effect in the FTPO group, and the most significant increase was at the dose of 1 200 mg/kg. In the FAPR group, it had a reducing effect, and the most significant decrease was at the dose of 1 200 mg/kg. The observed differences in the b* value of the additives are believed to stem from variations in their raw material compositions. Studying the literature, it becomes evident that various factors, including the composition of the fermented additive, fermentation conditions, and microorganisms, affect the breast meat b* value. While some studies demonstrate an effect on the b* value, some do not.^(68, 69)

Tomato and apple pomace are known to contain phenols and flavonoids, which have antimicrobial and antioxidant properties.^(56, 57) An early study showed that tomato and apple pomace contain antibacterial and antioxidant features, which effectively inhibit and decrease the presence of pathogenic microorganisms in the intestines and cecum of broiler chicks.⁽⁷⁰⁾ There are also studies reporting that the addition of fermented feeds to broiler chicken rations supports the digestive system, increases the population of beneficial microorganisms, and, as a result, suppresses and reduces pathogenic microorganisms.^(60, 71, 72) The fermented additives FTPO and FAPR (except the 300 mg/kg dose of FAPR) in this study had no effect on the *Enterobacteriaceae* found in the cecum

The literature shows that adding additives to the broiler chicken ration has varying effects on the cecum pathogen microbiota.^(59, 70-74) While it has no effect in some studies, it appears to decrease in others.^(59, 70, 72) In this study, it is thought that either the additive dose was insufficient or *A. niger* used phenolic compounds with antioxidant and antimicrobial properties

as an energy source during fermentation, which is why the additives did not have an effect on caecal pathogens, as Altop et al.⁽⁶⁹⁾ stated that microorganisms use some phenolic compounds as energy sources during fermentation. In addition, Güngör⁽⁵⁹⁾ suggested in a study that if the additive dose is low in broiler chickens, the additive may lose its effect on the cecum microorganism population. There are studies in the literature stating that fermented additives have a positive effect on the cecum LAB population, and similarly, that fermented additives have an increasing effect on the small intestine and cecum LAB population.^(8, 70, 74)

Conclusions

The fermentation studies carried out in this work found that *A. niger* SSF had significant effects on the chemical composition of the substrates, resulting in increased organic acid production, enzyme activity, and antioxidant capacity. Particularly, when *A. niger* ferments apple pomace and rye mixtures, the amounts of ADF and NDF decrease while the amounts of organic acid and enzyme activities increase. This means that the fermentation is successful and its efficiency is higher. As a result of the fermentation studies, the mixture of apple pomace and rye turned into a functional product, considering the organic acid content, enzyme activity, and antioxidant contents of the end product of fermentation with *A. niger*. This shows that the solid-state fermentation method, carried out under suitable conditions using agricultural by-products as substrates, enables the production of functional feed additives.

In the broiler chicken trial, it was found that doses of FAPR at 300 and 2 400 mg/kg resulted in increased body weight and BWG compared to the control group. The first two weeks and the final week showed a generally positive effect on feed conversion value. While groups receiving the FTPO additive showed a tendency to increase breast meat L* and b* values, the

addition of the FAPR additive was associated with a decrease in these values. An increase in the presence of cecum LAB was observed at FTPO doses of 300 and 1200 mg/kg, as well as at the 300 mg/kg dose of FAPR. This study concluded that broiler chickens could benefit from a 300 mg/kg dose of FAPR to enhance their body weight, body weight gain, or feed conversion ratio during their early stages.

Data availability

All relevant data are inside the manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization, investigation, methodology, visualization, writing-original draft, and writing-review and editing: R Tosun.

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Supervision: S Yasar.

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