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Effects of Fermented Navel Orange Pulp on Growth Perfor-Mance, Carcass Characteristics, Meat Quality, Meat Nutrition, and Serum Biochemical Indicators of Finishing Tibetan Pigs

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Abstract: In order to cope with the limited supply of feed for global animal production, there is a pressing need to explore alternative feed resources. Orange pulp, a by-product of agriculture and industry, has shown potential to positively or neutrally impact pig productive performance when included in their diet. However, there is a lack of research on the effects of fermented navel orange pulp (FNOP) on pig growth and productive performance. This study aimed to investigate the effects of FNOP as a dry matter substitute on pig's growth performance, carcass characteristics, meat quality, meat nutrition, and serum biochemical indicators. The experiment involved 128 finishing Tibetan pigs, divided into four feed treatment groups, with varying levels (0%, 5%, 10% and 15%) of FNOP replacing dry matter in the basal diet. The results indicated that substituting 5% to 15% FNOP had no adverse effects on pig growth performance. However, at a 15% substitution rate, there was a decrease in serum growth hormone and IGF-1 levels, along with an increase in feed-to-gain ratio. A 10% FNOP replacement notably increased the eye muscle area of pigs. Additionally, 5% and 10% FNOP substitutions reduced the drip loss of pork. The study also found that substituting 5% to 15% FNOP increased unsaturated fatty acids and umami nucleotide contents in pork, as well as raised serum total protein and uric acid (nucleotide metabolism related-product) levels. These findings suggest that moderate FNOP substitution might enhance meat quality and maintain growth and productive performance in Tibetan pigs by improving protein synthesis and nucleotide metabolism, while also reducing feed costs. The optimal substitution ratio identified was 10%.

Keywords: fermented navel orange pulp; growth performance; carcass characteristics; meat quality; meat nutrition; serum biochemical indicators; finishing Tibetan pigs

1. Introduction

The global supply of feedstock for animal production is a pressing issue. The increasing demand for animal products worldwide is met with limited feed-crop availability due to climate change and competition with human food sources [1]. Farmers also face rising production costs and challenges due to industrial competition and limited land resources [2]. To address this, exploring unconventional feed resources is crucial. Utilizing agricultural and industrial by-products not only promotes circularity in the food system but also reduces the environmental impact of pork production by recycling non-edible plant materials. According to the Food and Agriculture

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Organization of the United Nations, there are at least 1.6 billion tons of agricultural by-products globally each year, and their utilization can significantly reduce carbon dioxide emissions, lessening the negative environmental impacts and economic losses [3,4].

Citrus fruits are a major global fruit, with an annual production exceeding 140 million tons [5]. Approximately 30% of citrus fruits, mainly oranges, are processed into juice, resulting in a substantial amount of citrus pulp that can make up 49-69% of the processed fresh fruit's weight [6]. Several studies have reviewed the chemical composition of citrus pulp [7,8], showing its nutritional value is comparable to conventional animal feed and holds promise for the animal feed industry [9,10]. Dehydrated citrus pulp has been successfully used in various livestock and ruminant feeds, including those for pigs [11–13]. However, almost two-thirds of fresh citrus pulp is discarded due to its high moisture content and perishability [14], with only a small portion being utilized by producers near processing facilities. In intensive pig farming, the high-moisture citrus pulp poses challenges for mechanized feed production, leading to additional energy costs for drying and pelleting.

In recent years, the popularity of liquid feed feeding models and advancements in biological fermentation technology have significantly broadened the potential applications of fresh citrus pulp in pig farming. Microbial fermentation not only prolongs the shelf life of high-moisture feed ingredients but also effectively breaks down anti-nutritional factors and promotes the production of probiotics and their beneficial metabolites [15,16]. Research indicates that incorporating fermented feed into diets can improve animal growth performance and meat quality [17–20]. Despite this, there is currently limited research on the utilization of fermented citrus pulp in pig feed.

The nutritional value of citrus pulp is influenced by various factors, such as the type of fruit, variety, season, ripeness, juicing method, and post-processing treatments like dehydration, ensilage, and fermentation [7]. This study specifically examines fresh by-products, focusing on navel orange pulp from 'Gannan Navel Orange', a local citrus resource in China. It investigates the effects of fermented navel orange pulp (FNOP) in the diet of Tibetan pigs, a native breed in China. The research evaluates how substituting FNOP for different proportions of the basal diet dry matter (DM) impacts the growth performance, carcass characteristics, meat quality, meat nutrition, and blood biochemical indicators of finishing Tibetan pigs. The results suggest that moderate FNOP usage as a substitute in feed does not negatively affect the growth performance or carcass characteristics of Tibetan pigs. Furthermore, it can improve the nutritional value, flavor, and taste of the pork, showing promise in enhancing pork quality and reducing feed expenses.

2. Materials and Methods

2.1. Animal Ethics Statement

All the procedures were approved by the Institutional Animal Care and Use Commit-tee at Jiangxi Academy of Agricultural Sciences.

2.2. Experimental Design and Diets

A total of 128 finishing Tibetan pigs (body weight = 30.78 ± 1.04 kg) were randomly assigned to 4 dietary treatments with 4 replications each (8 pigs per replication). The dietary treatments consisted of the basal diet (CON) and experimental diets where FNOP replaced 5% (5% FNOP), 10% (10% FNOP), and 15% (15% FNOP) of the basal diet DM, respectively. Chemical analysis of FNOP was carried out following the AOAC International guidelines (2005), and the results can be found in Table S1 of the supplementary material. The basal diet formulation comprehensively referred to the Chinese National Feed Standard for swine (lean-fat type pig) and the recommended nutritional requirements for finishing Tibetan pigs [21,22], as outlined in Table 1. Pigs were weighed after 3 days of pre-feeding and the experiment lasted 49 days. All pigs had ad libitum access to water, while feed intake was controlled. Initial feed intake for experimental pigs was calculated by multiplying the average body weight by a coefficient of 0.04, with subsequent weekly increases of 10% based on the previous week's intake. Pigs were fed 3 times daily at 07:30, 13:30, and 17:30.

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Table 1. Ingredients and nutrient levels of experimental diets (%, as-fed basis).

Item	CON	5% FNOP	10% FNOP	15% FNOP
Ingredient，%				
Corn	35.55	33.77	32.00	30.22
Soybean meal	6.20	5.89	5.58	5.27
Rice bran meal	45.00	42.75	40.50	38.25
Wheat bran	8.34	7.92	7.51	7.09
FNOP	0.00	5.00	10.00	15.00
Calcium hydrogen phosphate	0.87	0.83	0.78	0.74
Calcium carbonate	1.09	1.04	0.98	0.93
Salt	0.35	0.33	0.32	0.30
Baking soda	0.20	0.19	0.18	0.17
L-lysine sulfate (70%)	0.15	0.14	0.14	0.13
L-threonine (98.5%)	0.15	0.14	0.14	0.13
Choline chloride	0.05	0.05	0.05	0.04
Bentonite	1.00	0.95	0.90	0.85
Mildewcide[1]	0.05	0.05	0.05	0.04
Premix[2]	1.00	0.95	0.90	0.85
Calculated composition[3]				
DE, Mcal/kg[4]	2.70	2.66	2.62	2.58
CP,%	13.69	13.56	13.43	13.30
NDF,%	19.73	21.47	23.21	24.95
ADF,%	7.83	8.34	8.85	9.35
SID Lys,%	0.53	0.52	0.52	0.51
Ca, %	0.67	0.68	0.69	0.70
STTD phosphorus, %	0.38	0.37	0.35	0.34
Analysed composition				
CP,%	13.58	13.28	13.21	13.18

Note: [1] Mildewcide, ammonium propionate; [2] Supplied per kilogram of the diet: 60 mg Fe, 8 mg Cu, 20 mg Mn, 80 mg Zn, 0.35 mg I, 0.2 mg Se, 5,250 IU vitamin A, 1,125 IU vitamin D3, 75 mg vitamin E, 2.25 mg vitamin B1, 7.5 mg vitamin B2, 3 mg vitamin B6, 0.03 mg vitamin B12, 24 mg pantothenic acid, 0.9 mg folic acid, 30 mg niacin, 0.15 mg biotin, 12 mg antioxidant.[3] Calculated values based on the Tables of Feed Composition and Nutritional Values in China (2022) [23]. [4] DE was calculated as described by Noblet and Perez (1993) [24]. CON = basal diet; FNOP = fermented navel orange pulp; ME = metabolizable energy; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; SID Lys = standardized ileal digestible lysine; STTD phosphorus = standardized total tract digestibility of phosphorus.

2.3. Growth Performance Measurements

Throughout the experiment, the pigs' feed intake was meticulously recorded on a per replicate basis, allowing for the calculation of the average daily feed intake (ADFI) per pig. On the 49th day of the experiment, the pigs were weighed after an overnight fast, and the average body weight, average daily gain (ADG), and feed conversion ratio (F:G) of each pig were calculated.

2.4. Sample Collection and Carcass Characteristics Measurements

On the 49th day of the experiment, one pig of moderate size was randomly selected from each replicate of Tibetan pigs. Following a 12-hour fast, the live weight was measured, and subsequently, the pigs were stunned via electrocution and slaughtered. Throughout the slaughter process, the hot carcass weight of each pig was measured on-site to determine the dressing percentage. Additionally, a tape measure was utilized to record the straight length and chest circumference. The backfat thickness at the thickest part of the shoulder, thoracolumbar junction and lumbosacral junction were

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recorded and used to calculate the average backfat value. Caliper was used to measure the maximum length and height of the cross-section of the longissimus dorsi muscle (LDM) at the 6th and 7th ribs of thoracic vertebrae. The formula for calculating the eye muscle area is as follows:

$$\text{Eye muscle area } (\text{cm}^2) = \text{ Length (cm)} \times \text{ Width (cm)} \times 0.7$$

2.5. Meat Quality Measurements

The LDM samples stored at 4°C were used to analyze pH value, meat color, drip loss, shear force, and marbling score. The assessment of meat quality followed the methodology outlined in a previous study [25]. After slaughter, the pH of each LDM sample was measured at 45 minutes and 24 hours using a portable pH meter (pH-Star, Matthäaus GmbH, Päottmes, Germany). Meat color values, including lightness (L*), redness (a*), and yellowness (b*), were assessed at the same time points post-slaughter using a colorimeter (CR-10, Konica Minolta, Osaka, Japan). The color difference (ΔE*) between the two time points was calculated using a specific formula as follow:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Approximately 10 gram of each LDM sample was placed in a sealed plastic tube at 4°C, and after 24 hours, the surface moisture was removed before weighing to determine drip loss. Shear force was measured with a C-LM4 tenderness tester (Tenovo International Co., Limited, Beijing, China) following the manufacturer's instructions. The marbling score was determined 45 minutes post-slaughter based on the NPPC meat color chart (Nanjing Mingao Instrument Equipment Co., Ltd., Nanjing, China).

2.6. Meat Conventional Nutrition

The contents of moisture, crude protein (CP), ether extract (EE), cholesterol, inosine monophosphate (IMP) and adenosine monophosphate (AMP) of each LDM sample were determined using Association of Official Analytical Chemists (AOAC) methods (2005). The samples were cut into pieces, ground into a paste using a high-speed universal crusher (FW100, Taisite Ltd., Tianjin, China), and placed into sample cups. Moisture content (%) was calculated by measuring weight loss after oven drying samples (3 g) at 102 °C for 12 hours (until constant weight) in a Memmert laboratory dryer (UN 75, Schwabach, Germany). Crude protein content (%) was determined using the Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (SKD-200, Shanghai Peiou Analysis Instruments Co., Ltd., Shanghai, China). Fat content (%) was measured using the Soxhlet method with petroleum ether extraction in a Hanon Automatic Soxhlet Extractor (SZF-06A, Shanghai Lichen Instruments Technology Co., Ltd., Shanghai, China). The levels of IMP, AMP and cholesterol were determined using a high-performance liquid chromatography system (Agilen 1200, Agilen Technologies, CA, USA).

2.7. Amino Acid Composition

Approximately 0.1 g of each LDM sample was weighed and digested with 5 mL of 6 mol/L HCl solution at 105 °C in an oven for 24 hours. The volume was then adjusted to 50 mL in a volumetric flask, and the sample was filtered through a 0.22-mm water phase filter into a centrifuge tube. Subsequently, 2 mL of the filtrate was evaporated in an evaporating dish in a 60 °C water bath, followed by the addition of 4 mL of 0.02 mol/L HCl solution. Once dissolved, the sample was stored at 4 °C for detection using an ion-exchange AA analyzer (L8900, Hitachi, Tokyo, Japan).

2.8. Fatty Acid Profile

The fatty acid profile was analyzed using gas chromatography (GC) as outlined in previous studies (Hao et al., 2020). The LDM samples were first extracted with a mixture of chloroform and methanol (2:1; vol/vol). Approximately 20 g of each LDM sample was weighed and dried at 105 °C for 1 h, followed by weighing 1 g of the dried sample and leaching it with petroleum ether for 3 h. Subsequently, 60 mg of the extracted fat was dissolved in 4 mL of isooctane, with the addition of 200 mL of potassium hydroxide-methanol and 1 g of sodium bisulfate. After salt precipitation, the

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solution containing the methyl esters was separated into the upper layer and stored in a refrigerator at 4 °C. Prior to GC detection (Model 7890 A, Agilent Technologies, CA, USA), each sample was filtered through a 0.22-nm filter membrane. The fatty acid concentration was then determined using GC ChemStation software (Agilent Technologies, CA, USA).

2.9. Serum Sample Collection and Serum Biochemical Measurements

On the 49th day, three pigs were randomly selected from each replicate. Fasting blood samples (5 to 10 mL) were collected from the anterior vena cava after a 12-hour fast. The blood was collected into sterile EP tubes without any anticoagulant. After centrifugation at 3000 rpm for 5 minutes, the supernatant was stored at -80°C for future use. Serum total protein (TP), blood urea nitrogen (BUN), and triglyceride levels were analyzed using a fully automatic biochemical analyzer (PBC22A Plus, LWPOCT, Shenzhen, China). Serum uric acid (UA) levels were determined using a colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels were measured using an enzyme-linked immunoassay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.10. Statistical Analyses

All data in the current study were analyzed by one-way analysis of variance (ANOVA) with the statistical software SPSS 20.0 (Chicago, IL, USA) followed by Duncan's multiple range analysis. $P < 0.05$ was considered to be statistically significant. All results are shown as the means ± standard deviation.

3. Results

3.1. Growth Performance

In this study, we investigated the effects of substituting FNOP for DM in the base diet on the growth performance of finishing Tibetan pigs by standardizing feed intake. The results presented in Table 2 show that there were no significant differences in ADFI across the experimental groups, indicating consistent control over feed consumption. Additionally, the ADG did not vary significantly among the experimental groups. It is noteworthy that the feed-to-gain ratio in the 15% FNOP group increased by 0.26 compared to the CON group, although this difference was not statistically significant. These findings suggest that replacing 5% to 15% of the base diet DM with FNOP during the finishing phase does not have a negative impact on the growth performance of Tibetan pigs. Moreover, a 15% replacement ratio may actually reduce the feed conversion rate.

Table 2. Effects of fermented navel orange pulp on the growth performance of finishing Tibetan pigs.

Item	CON	5% FNOP	10% FNOP	15% FNOP	*P*-value
ADFI, kg /d	1.73 ± 0.33	1.75 ± 0.33	1.77 ± 0.34	1.77 ± 0.31	0.635
Initial weight, kg	31.12 ± 0.58	30.39 ± 1.16	30.94 ± 1.03	30.67 ± 1.49	0.802
Final weight, kg	46.76 ± 0.84	46.10 ± 1.61	46.91 ± 1.22	45.89 ± 1.14	0.602
ADG, g/d	319.26 ± 10.52	320.63 ± 10.21	325.83 ± 13.39	310.75 ± 11.33	0.352
F:G	5.44 ± 0.18	5.47 ± 0.18	5.45 ± 0.20	5.70 ± 0.23	0.251

Note: Data are expressed as means ± standard deviation, n = 32. CON = basal diet; FNOP = fermented navel orange pulp; ADFI = average daily feed intake; ADG = average daily gain; F:G = feed-to-gain ratio.

3.2. Carcass Characteristics

The impact of FNOP on the carcass characteristics of finishing Tibetan pigs was further explored, with detailed results presented in Table 3. Live weight did not differ significantly among the three FNOP groups compared to the CON group, although the 15% FNOP group exhibited a lower live weight than the 5% and 10% FNOP groups ($P < 0.05$). In contrast to the CON group, the eye muscle

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area significantly increased in the 10% FNOP group ($P < 0.05$). No other carcass characteristic indicators showed significant differences across the experimental groups. These results suggest that substituting 5% to 15% of the base diet with FNOP does not negatively impact the productive performance of Tibetan pigs, and a 10% replacement may even improve lean meat production.

Table 3. Effects of fermented navel orange pulp on the carcass traits of finishing Tibetan pigs.

Item	CON	5% FNOP	10% FNOP	15% FNOP	*P*-value
Liver weight, kg	47.76 ± 5.01^{ab}	50.54 ± 3.29^{b}	49.79 ± 0.38^{b}	43.16 ± 1.51^{a}	0.023
Carcass weight, kg	30.41 ± 4.14	34.15 ± 2.77	32.10 ± 1.03	29.34 ± 1.97	0.121
Carcass yield, %	63.51 ± 2.42	67.57 ± 3.07	64.46 ± 1.72	67.97 ± 4.00	0.127
Body length, cm	77.00 ± 3.56	77.00 ± 7.70	75.50 ± 3.7	75.75 ± 4.99	0.963
Chest circumference, cm	83.00 ± 3.74	83.63 ± 7.65	83.70 ± 1.01	80.75 ± 1.71	0.756
BFT at thickest part of the shoulder, mm	36.24 ± 3.13	33.44 ± 5.52	34.38 ± 6.42	30.9 ± 3.41	0.688
BFT at thoracolumbar junction, mm	15.32 ± 1.73	15.99 ± 3.36	18.40 ± 6.79	14.64 ± 4.95	0.886
BFT at lumbar sacral junction, mm	20.86 ± 0.59	22.41 ± 5.79	22.73 ± 2.41	19.85 ± 3.70	0.864
BFT, mm	24.14 ± 1.49	23.94 ± 4.29	25.17 ± 4.34	21.80 ± 3.19	0.910
Eye muscle area, cm2	8.42 ± 0.72^{a}	9.20 ± 1.16^{ab}	12.23 ± 1.71^{b}	8.94 ± 1.17^{ab}	0.017

Note: Data are expressed as means ± standard deviation, n = 4. CON = basal diet; FNOP = fermented navel orange pulp; BFT = backfat thickness. ^{a,b} Within a row, values with different superscripts differ significantly at $P < 0.05$.

3.3. Meat Quality

Table 4 illustrates the impact of FNOP on the meat quality of finishing Tibetan pigs. In comparison to the CON group, the 10% FNOP group displayed a tendency towards decreased pH_{24h} values in the meat ($P < 0.10$). The 5% FNOP group exhibited the highest L^*_{45min} value ($P < 0.05$). Moreover, the 15% FNOP group demonstrated the most significant color difference ($\Delta E^*_{(45min-24h)}$) ($P < 0.01$), correlating with the maximum pH fluctuation during the meat's acidification phase. Additionally, both the 5% and 10% FNOP groups experienced lower drip loss in the meat when compared to the CON group and the 15% FNOP group ($P < 0.05$). These findings indicate that substituting 5% and 10% FNOP improves meat quality, with the 10% replacement ratio showing superior acidification effects.

Table 4. Effects of fermented navel orange pulp on the meat quality of finishing Tibetan pigs.

Item	CON	5% FNOP	10% FNOP	15% FNOP	*P*-value
pH_{45min}	6.74 ± 0.16	6.55 ± 0.16	6.53 ± 0.09	6.62 ± 0.18	0.274
pH_{24h}	6.33 ± 0.21	5.82 ± 0.41	5.64 ± 0.24	6.14 ± 0.49	0.067
L^*_{45min}	34.95 ± 0.70	36.05 ± 0.52	35.06 ± 1.15	34.64 ± 1.44	0.286
a^*_{45min}	15.41 ± 0.71	16.76 ± 1.28	15.18 ± 0.47	16.25 ± 1.29	0.148
b^*_{45min}	0.38 ± 0.22^{a}	1.16 ± 0.52^{b}	0.30 ± 0.36^{a}	0.41 ± 0.41^{a}	0.032
L^*_{24h}	36.73 ± 3.29^{a}	38.51 ± 1.08^{a}	43.30 ± 2.94^{b}	37.17 ± 2.7^{a}	0.016
a^*_{24h}	15.40 ± 0.72	17.72 ± 1.11	16.62 ± 1.69	16.13 ± 0.78	0.079
b^*_{24h}	1.16 ± 0.53	1.45 ± 0.26	1.90 ± 0.17	1.30 ± 0.36	0.121
$\Delta E^*_{(45min-24h)}$	2.51 ± 2.08^{A}	2.98 ± 1.38^{A}	8.70 ± 2.18^{B}	2.98 ± 2.11^{A}	0.002
Drip loss, %	1.38 ± 0.29^{b}	0.86 ± 0.26^{a}	0.98 ± 0.31^{a}	1.26 ± 0.10^{b}	0.010
Shearing force, kgf	5.95 ± 1.25	5.66 ± 1.07	5.73 ± 0.84	6.34 ± 0.76	0.777
Marbling score	2.88 ± 1.03	2.5 ± 0.41	2.5 ± 0.71	2.88 ± 0.75	0.806

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Note: Data are expressed as means ± standard deviation, n = 4. CON = basal diet; FNOP = fermented navel orange pulp; $\Delta E^*_{(45min-24h)}$ = Color Difference of meat from 45 min to 24 h. [a,b] or [A,B] Within a row, values with different superscripts differ significantly at $P < 0.05$ and $P < 0.01$, respectively.

3.4. Meat Nutrition

The study further examined the nutritional composition of the meat and found no significant differences in moisture, CP, EE, ash, and cholesterol content among the meat from all experimental groups (Table S2). However, there was a notable increase in the content of IMP in the meat from the 5%, 10%, and 15% FNOP groups by 103.28%, 123.78%, and 85.97% respectively compared to the CON group ($P < 0.01$) (Table S2). Additionally, the content of AMP in the meat from the 5% and 10% FNOP groups showed a significant increase of 20.18% and 18.30% respectively ($P < 0.01$) (Table S2). Amino acid composition did not vary significantly across the experimental groups (Table S3). The fatty acid profile of pork is depicted in Tabe 5. The levels of C18:0 and SFA in the 5%, 10%, and 15% FNOP groups exhibited a significant linear decrease compared to the CON group ($P < 0.01$), while the levels of C18:1n9 and MUFA increased significantly ($P < 0.05$ and $P < 0.01$, respectively). Moreover, the levels of C18:2n6 and PUFA significantly increased in the 10% and 15% FNOP groups ($P < 0.01$ and $P < 0.05$, respectively). The UFA:SFA ratio in the meat from the 5%, 10%, and 15% FNOP groups displayed a linear significant increase compared to the CON group ($P < 0.01$). These findings suggest that FNOP substitution leads to higher levels of unsaturated fatty acids and umami nucleotides in the meat, enhancing its nutritional value and flavor, with the most pronounced effects observed at the 10% replacement level.

Table 5. Effects of fermented navel orange pulp on the fatty acid profile of the longissimus dorsi muscle in finishing Tibetan pigs (%).

Item	CON	5% FNOP	10% FNOP	15% FNOP	*P*-value
C10:0	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.277
C12:0	0.05 ± 0.01^{A}	0.09 ± 0.02^{B}	0.06 ± 0.01^{A}	0.05 ± 0.01^{A}	0.009
C14:0	1.26 ± 0.05	1.25 ± 0.08	1.14 ± 0.08	1.21 ± 0.05	0.085
C16:0	26.15 ± 0.21	25.73 ± 0.40	26.05 ± 0.07	25.85 ± 0.19	0.244
C17:0	0.20 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.18 ± 0.04	0.267
C18:0	12.97 ± 0.22^{C}	12.40 ± 0.12^{B}	11.78 ± 0.19^{A}	11.76 ± 0.27^{A}	<0.001
C20:0	0.42 ± 0.03	0.37 ± 0.03	0.40 ± 0.05	0.44 ± 0.02	0.053
C16:1	5.78 ± 0.12	6.08 ± 0.11	5.91 ± 0.38	5.95 ± 0.12	0.285
C17:1	0.23 ± 0.01	0.21 ± 0.04	0.23 ± 0.03	0.19 ± 0.03	0.227
C18:1n7	0.92 ± 0.04	1.00 ± 0.05	1.01 ± 0.10	1.01 ± 0.05	0.180
C18:1n9	38.00 ± 0.29^{a}	38.84 ± 0.45^{b}	38.80 ± 0.22^{b}	38.97 ± 0.49^{b}	0.014
C20:1	0.48 ± 0.03^{ab}	0.52 ± 0.03^{b}	0.46 ± 0.04^{a}	0.42 ± 0.04^{a}	0.010
C18:2n6	10.30 ± 0.21^{A}	10.06 ± 0.10^{A}	10.70 ± 0.23^{B}	10.80 ± 0.18^{B}	<0.001
C18:3n3	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.262
C18:3n6	0.27 ± 0.02	0.32 ± 0.02	0.30 ± 0.03	0.30 ± 0.03	0.071
C20:3	0.14 ± 0.02	0.15 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.355
C20:4	2.60 ± 0.09	2.58 ± 0.13	2.63 ± 0.11	2.46 ± 0.07	0.168
MUFA[1]	45.39 ± 0.29^{A}	46.65 ± 0.46^{B}	46.40 ± 0.18^{B}	46.53 ± 0.50^{B}	0.002
PUFA[2]	13.43 ± 0.27^{ab}	13.24 ± 0.19^{a}	13.90 ± 0.35^{c}	13.85 ± 0.26^{bc}	0.013
SFA[3]	41.18 ± 0.37^{B}	40.12 ± 0.30^{A}	39.70 ± 0.19^{A}	39.62 ± 0.38^{A}	<0.001
UFA:SFA[4]	1.43 ± 0.02^{A}	1.49 ± 0.02^{B}	1.52 ± 0.01^{BC}	1.52 ± 0.02^{C}	<0.001

Note: Data are expressed as means ± standard deviation, n = 4. [1] MUFA = C16:1 + C17:1 + C18:1n7 + C18:1n9 + C20:1; [2] PUFA = C18:2n6 + C18:3n3 + C18:3n6 + C20:3 + C20:4; [3] SFA = C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0; [4] UFA = MUFA + PUFA. CON = basal diet; FNOP = fermented navel orange pulp; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid. [a,b] or [A,B] Within a row, values with different superscripts differ significantly at $P < 0.05$ and $P < 0.01$, respectively.

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3.5. Serum Biochemical Indicators

Table 6 displays the serum biochemical indicators related to growth and metabolism. The serum TP levels in all FNOP experimental groups were significantly higher compared to the CON group ($P < 0.01$), while there was no significant difference in the levels of BUN, a product of protein metabolism. This suggests a potential promotion of protein synthesis metabolism in pigs. Moreover, the levels of UA, a purine metabolism product, increased notably in the 5%, 10%, and 15% FNOP groups compared to the CON group ($P < 0.01$). The 15% FNOP group also exhibited a significant rise in serum triglyceride levels compared to the CON group ($P < 0.05$). In terms of growth-related hormones, there was a linear and significant decrease in GH levels with increasing FNOP replacement ($P < 0.01$). Additionally, IGF-1 levels were notably reduced in the 15% FNOP group compared to the CON group ($P < 0.05$). These results suggest that FNOP replacement enhances protein synthesis metabolism and nucleotide metabolism in pigs, but exceeding 10% may lead to a reduction in growth-related hormone levels.

Table 6. Effects of fermented navel orange pulp on the serum biochemical indexes of finishing Tibetan pigs.

Item	CON	5% FNOP	10% FNOP	15% FNOP	P-value
TP, ug/uL	73.46 ± 8.18^{A}	89.17 ± 9.36^{B}	82.33 ± 9.02^{B}	84.42 ± 9.59^{B}	0.001
BUN, mmol/L	7.59 ± 1.48	7.30 ± 1.71	7.59 ± 1.03	7.80 ± 1.35	0.865
UA, mmol/L	23.72 ± 12.47^{A}	42.24 ± 16.13^{B}	43.11 ± 10.35^{B}	49.20 ± 25.73^{B}	0.007
TG, mmol/L	0.59 ± 0.24^{a}	0.69 ± 0.29^{ab}	0.78 ± 0.13^{ab}	0.92 ± 0.37^{b}	0.036
GH, ug/L	11.85 ± 1.62^{C}	10.84 ± 2.69^{BC}	9.66 ± 1.58^{B}	7.48 ± 1.00^{A}	0.002
IGF-1, μg/L	9.33 ± 1.43^{b}	9.69 ± 1.35^{b}	9.06 ± 1.69^{ab}	7.82 ± 1.19^{a}	0.040

Note: Data are expressed as means ± standard deviation, n = 4. CON = basal diet; FNOP = fermented navel orange pulp; TP = total protein; BUN = blood urea nitrogen; UA = uric acid; TG = triglyceride; GH = growth hormone; IGF-1 = insulin-like growth factor-1. ^{a,b} or ^{A,B} Within a row, values with different superscripts differ significantly at $P < 0.05$ and $P < 0.01$, respectively.

4. Discussion

This study aimed to investigate the effects of replacing 5%, 10% and 15% of the basal diet DM with FNOP on the growth performance, carcass characteristics, meat quality, meat nutrition, and blood biochemical indicators of finishing Tibetan pigs. The results demonstrated that the moderate replacement of the basal diet DM with FNOP did not negatively impact the growth and productive performance of the pigs. Furthermore, FNOP also enhanced the metabolic processes of nucleotides and protein synthesis within the pigs, which ultimately improved the nutritional value and flavor of the pork.

In this study, FNOP was found to have higher levels of protein and neutral detergent fiber compared to unfermented citrus pulp reported in previous studies [8,13], while showing reduced levels of carbohydrates and energy. These changes indicate that the biofermentation process significantly altered the chemical composition of the navel orange pulp. The high level of neutral detergent fiber content can be attributed to the rich content of hemicellulose, which may make the fiber in FNOP more easily fermentable in the gut [26–28].

Previous studies have indicated that the addition of 15% citrus pulp to the diet of finishing pigs is appropriate [29,30]. In the present study, the replacement of 5%, 10%, and 15% of the basal diet DM with FNOP led to a slight decrease in the energy and protein content of the diet. However, under the condition of uniform feed intake, the growth performance of the pigs was not significantly adversely affected. However, it is important to note that when the replacement ratio reached 15%, there was a decrease in the feed conversion efficiency of the pigs, potentially linked to the reduced energy and protein density of the diet.

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In terms of carcass characteristics, compared with 5% and 10% replacement ratios, the live weight of slaughter pigs using 15% FNOP replacement ratio decreased, which is related to the random principle followed in the selection process. In this trial, FNOP substitution did not have a significant impact on most carcass characteristics, including carcass weight, carcass yield, body length, chest circumference and backfat thickness, which is different from the findings of Ferrer et al.'s study. Ferrer et al. found that as the proportion of dehydrated citrus pulp in the diet increased, carcass weight and backfat gain showed a linear downward trend [13]. These differences may be related to the different breeds of experimental pigs and the citrus pulp materials used.

When FNOP replaced 10% of the basal diet DM in the experiment, there was a significant increase in the eye muscle area of the longissimus dorsi muscle of pigs. This suggests that FNOP may have a positive effect on promoting protein deposition in pigs. The rise in TP levels in blood biochemical indicators further supports this hypothesis. Bakare et al. observed a similar trend, noting that the serum TP level of finishing pigs initially increased and then decreased with the addition of dehydrated orange pulp in the diet, peaking at a 24% addition level [30]. This aligns with the results of the current study. In the study by Bakare et al., it was observed that as the level of dehydrated citrus pulp added to the diet increases, the protein content decreases. However, at a 24% addition ratio, the crude protein level can reach 14.76%, which still exceeds the NRC (2012) recommendations for meeting the protein deposition needs of finishing pigs. This may be an important factor in increasing their serum TP levels. Belloumi et al. found that incorporating dehydrated citrus pulp into pig diets could potentially enhance nitrogen absorption and utilization in the intestines [31]. This was supported by a decrease in fecal metabolites linked to bacterial protein fermentation and an increase in serum metabolites associated with protein metabolism. Cui et al. suggested that citrus extracts may also boost nitrogen absorption and utilization in pigs by improving intestinal morphology and digestive enzyme activity [32]. Additionally, Noh et al. showed that a diet supplemented with a product fermented using Bacillus subtilis on a mixture of citrus pulp and fish by-product improved the digestibility of various nutrients in pigs [33]. The observed increase in eye muscle area and TP levels in our current experiment could be a result of enhanced nitrogen digestion and absorption in the pigs' intestines. However, it is important to note that a 15% FNOP substitution did not lead to an improvement in eye muscle area, possibly due to reduced levels of GH and IGF-1.

Meat quality traits are intricate quantitative features comprising various indicators like meat color, pH value, water-holding capacity, shear force, and marbling. Following slaughter, pork typically undergoes an aging process lasting 24 to 36 hours, during which rigor mortis occurs and resolves. A key biochemical change during this period is the conversion of glycogen to lactate through anaerobic glycolysis, resulting in a decrease in pH value. In our study, we observed that pork from the 5% and 10% FNOP groups exhibited appropriate pH_{24h} values, while the pH_{24h} values of the CON group and the 15% FNOP group remained above 6. Postmortem aging contributes to enhancing the meat's water-holding capacity, thereby improving the juiciness of pork [34], as evidenced by reduced drip loss in the 5% and 10% FNOP groups. A lower final pH value generally indicates a higher glycogen content in the muscle at slaughter, which is linked to the muscle's activity level and metabolic state, although this aspect was not further explored in our study. Concurrent with pH changes, alterations in meat color occur; notably, the 10% FNOP group showed the greatest color difference from 45 min to 24 h after slaughter. Throughout the aging process, the lightness value (L*) and yellowness value (b*) of pork from all experimental groups increased, consistent with previous research findings [35,36]. Particularly, the 24-hour lightness value of pork from the 10% FNOP group significantly improved, possibly due to enhanced protein denaturation and structural changes resulting from the pH decrease. The denaturation of sarcoplasmic proteins and increased transverse spacing of myofibrils contribute to heightened light scattering [37,38].

The nutritional quality and taste of meat play a crucial role in how consumers perceive it. The consumption of fatty acids is intricately linked to consumer health. Research suggests that consuming excessive saturated fatty acids may elevate the risk of developing Type 2 diabetes and heart disease [39], whereas unsaturated fatty acids have shown to have positive effects on health, such as reducing inflammation, regulating glycolipid metabolism, and supporting muscle growth. In this study,

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replacing 5%, 10%, and 15% of the basal diet DM with FNOP led to an increase in monounsaturated fatty acids and a decrease in saturated fatty acids in pork. Notably, substituting 10% of the basal diet DM with FNOP significantly raised the levels of polyunsaturated fatty acids. These results align with previous research and suggest that the changes of fatty acid composition in diet due to FNOP substitution could be responsible for these outcomes [13,18]

Nucleotides play a crucial role in the nutritional and flavor profile of pork products, particularly IMP which enhances the umami taste of meat [40]. This study observed that substituting 5%, 10%, and 15% FNOP increased IMP levels in pork, with 5% and 10% FNOP also boosting AMP levels. These findings align with prior research indicating that fermented feed can upregulate genes linked to IMP synthesis in muscle tissue, thereby enhancing IMP concentration [19]. This phenomenon is likely attributed to the heightened nucleotide production facilitated by microbial fermentation processes [41,42]. The higher serum UA levels across all FNOP groups, a byproduct of purine metabolism, indirectly suggest that fermented feed may stimulate nucleotide synthesis and accumulation.

5. Conclusions

The study found that substituting 5% to 15% of the basal diet DM with FNOP did not negatively impact the growth and productive performance of finishing Tibetan pigs. However, the substitution of 15% FNOP led to a decrease in the levels of growth-related hormones in the pigs' serum and also resulted in a reduced feed conversion efficiency, suggesting that 15% may be the maximum threshold for the FNOP substitution ratio. The most optimal substitution ratio of FNOP appears to be 10%, as it maximizes the nutritional value, flavor, and texture of the meat. This is supported by an increase in the eye muscle area of the pigs' longissimus dorsi muscle, a rise in unsaturated fatty acids and savory nucleotides in the pork, and a decrease in drip loss. While further research is needed to understand the specific mechanisms behind FNOP's enhancement of pork quality in finishing pigs, this study highlights the potential benefits of FNOP in improving pork quality and reducing feed costs.

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