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Fatty acid profile and storage stability of loin muscle in response to dietary supplementation of *Spirulina platensis* in finishing crossbred pigs

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ABSTRACT

The study was conducted to study whether feeding Spirulina platensis has beneficial effect on enhancing the fatty acid profile and storage stability of the loin muscle of pigs. A total of 24 male finishing pigs (*Large White Yorkshire X Desi*) were housed individually and distributed randomly into four equal groups with six animals in each group. Four isonitrogenous experimental diets (T1 to T4) were formulated by including dry Spirulina platensis at 0, 0.5, 1.0 and 1.5 %, respectively and fed during finishing phase (50 to 75 kg) live weight. Dietary treatments had no significant (P>0.05) effect on the total lipid and total cholesterol content of the loin muscle of pigs. The level of polyunsaturated fatty acids (PUFA) was higher (P<0.05) in Spirulina supplemented groups (T2, T3, and T4) compared to T1 leading to a higher (P<0.05) ratio of PUFA to saturated fatty acid (SFA) with increasing levels of Spirulina platensis inclusion in the diet. Whereas, the sum of SFA and monounsaturated fatty acids (MUFAs) was significantly (P<0.05) decreased in Spirulina supplemented groups compared to T1. The Ratio of n-6 to n-3 of muscle was decreased significantly (P<0.05) with increasing level of Spirulina and the values were 21.5, 20.2, 20.1, and 19.8 in T1 to T4, respectively. The other fatty acids namely gamma-linolenic acid, alpha-linolenic acid and eicosapentanoic acid were not statistically significant (P>0.05) among the treatment groups. Supplementation of Spirulina significantly (P<0.05) decreased the pH and 2-TBARS value of loin muscle during the refrigerated (4±1°C) storage at 0, 3, 6, and 9 days. Further, inclusion of Spirulina showed significant (P<0.05) improvement in the sensory attributes (flavour, tenderness, and juiciness) of the loin muscle of pigs. The results indicated that inclusion of dried Spirulina platensis powder up to 1.5 % in finishing pig diets was beneficial to achieve PUFA enriched pork and overall acceptability of the pork during refrigerated storage for a period of 9 days.

Keywords: *Loin muscle, Spirulina, Fatty acid profile, Storage stability, Sensory evaluation*

INTRODUCTION

The demand for high quality meat is increasing progressively to meet the protein requirements of a growing population. Pork is characterized by lower SFA, higher PUFA content, and n-6 to n-3 fatty acids ratio in comparison to poultry meat and beef (Blicharski et al. 2013). One of the major factors influencing the composition of pork and lard is the diet of the pig. It is well established that the diet provides a significant and effective approach for altering the fatty acid profile of pork, thereby modifying the impact of human dietary fat intake from pork. Therefore, researchers are interested in developing and validating new feeding strategies that increase animal performance, improve meat quality and its fatty acid composition (Bhaskar Reddy et al. 2023). One such strategy that has recently shown promising effects is dietary supplementation with microalgae. Blue-green algae, *Spirulina platensis* is attributed to the group of phytobiotics. These are grouped into diatoms, green algae, golden algae and blue-green algae cyanobacteria. It has been used as a nutritional supplement for humans and animals as it is rich in proteins, lipids, carbohydrates, sterols, and some vital elements such as zinc, magnesium, and selenium (Estrada et al. 2001; Babadzhanov et al. 2004). *Spirulina* is a rich source of gamma-linoleic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) which promote health (AlFadhly et al. 2022; Cohen et al. 1987). The high poly nutritive value and phyto pigments of *Spirulina* made it important in various applications for the production of safe and healthy food products, animal feed and many other diagnostic and therapeutic practices (Vonshak and Tomaselli, 2000). *Spirulina* has hepato-protective, neuro-protective and nephro-protective potential through normalizing the level of antioxidant and glutathione metabolizing enzymes, and managing hypercholesterolemia by lowering the contents of total lipid and triacylglycerol and lowering blood glucose level. The dietary inclusion of microalgae improved meat quality without negatively affecting the growth rate in poultry and pigs (Martins et al. 2021). Improved carcass qualities, slaughter output and quality of meat in fattening pigs with the addition of *Spirulina platensis* in rations (Simkus et al. 2013) were reported. Knowledge of the effects of microalgae supplementation on carcass quality of fattening pigs is relatively limited in India. Therefore, the effect of inclusion of *Spirulina platensis* in fattening pig diets on the meat quality, fatty acid profile and its stability during storage was studied in the present study.

MATERIALS AND METHODS

Procurement of pigs, feed ingredients and *Spirulina platensis*

The study was carried out at the Department of Animal Nutrition and ICAR-All India Coordinated Research Project on Pigs (ICAR-AICRP), College of Veterinary Science, Sri Venkateswara Veterinary Science, Tirupati. A total of twenty four (*Large White Yorkshire X Desi*) crossbred finisher male pigs were weighed and randomly allocated into four treatment groups with six animals per each treatment. Four isonitrogenous experimental diets (T1 to T4) were formulated (NRC, 2012) by including *Spirulina* at 0, 0.5, 1.0, and 1.5 %, respectively (Table 1). The animals were housed in a pucca shed individually in well-ventilated pens with provision for individual feeding and watering. All four groups were offered respective experimental rations daily at 9:00 and 15:00 h during finisher phase (50 to 75 kg LW) and the animals were dewormed before the start of the trial.

Feed ingredients like maize, soybean meal, and deoiled rice bran (DORB) for the preparation of experimental diets were provided by ICAR-AICRP on pigs. *Spirulina* culture seed was procured from Arakonam, Tamil Nadu and was cultivated at the Department of Animal Nutrition, College of Veterinary Science, Tirupati. *Spirulina* was collected from the pond daily in the morning by straining it in a 50-micron nylon cloth. The collected *Spirulina* was washed once with tap water and placed in the form of threads on polythene cover and sun dried (Fig 1). The dried *Spirulina* was collected and ground into powder (Soni et al. 2017; Vardaka et al. 2016; and Zhang et al. 2015).

Table 1. Ingredient and chemical composition (% DM) of experimental diets.

Ingredient	T1	T2	T3	T4
Maize	71.0	70.7	71.0	71.2
Soya bean meal	19.75	18.5	17.7	17.0
De-oiled Rice bran	7.0	8.0	8.0	8.0
Spirulina platensis	-	0.5	1.0	1.5
Salt	0.5	0.5	0.5	0.5
Mineral mixture #	1.5	1.5	1.5	1.5
Lysine HCL	0.25	0.3	0.3	0.3
	100	**100**	**100**	**100**
Proximate composition (%)				
DM	91.03	90.96	90.55	91.25
CP	14.61	14.77	14.78	14.64
EE	2.22	2.39	2.20	2.09
CF	3.84	4.81	5.32	4.96
TA	7.37	8.32	9.51	8.69
AIA*	2.06	2.21	2.37	2.25
GE (kcal/kg)**	3883	3837	3845	4015

#per kg contained Ca, 25.5 %; P,12.75 %; S,0.72 %; Zn,9600mg;Mn,1500mg;Na,5.9 mg;Mg,6000mg; K,100mg; Fe,1500 mg; Iodine 325mg; Cu,12000 mg; Co,150mg.

DM-Dry matter; CP: Crude protein; EE: Ether extract; CF-Crude Protein; TA-Total ash; AIA- acid insoluble ash; GE-Gross Energy

Fig. 1. Cultivation and Production of *Spirulina platensis*

Ethical considerations, slaughtering of animals, and sampling

The permission to conduct the study was granted by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science, SVVU, Tirupati under the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India. At the end of the feeding trial, pigs were slaughtered by sticking, scalding, singeing, and evisceration. *Longissimus dorsi* muscle was dissected and collected from the carcass of each group. The muscle was cut into pieces and minced and the samples were immediately stored at - 20°C until further analysis for fatty acid profile, proximate composition and total cholesterol while the remaining meat sample was kept at 4±1°C for measuring meat quality attributes and sensory evaluation.

Proximate composition

The experimental diets, *Spirulina platensis* and stored meat samples were analyzed for proximate composition (AOAC, 2002) and mineral composition of *Spirulina platensis* was estimated by using AAS.

Total cholesterol estimation

Pipetted about 100 µl of lipid extract (prepared from 2 g of meat and lipid sample volume made to 5 ml with chloroform) and 50 µl of standard cholesterol separately in the test tube and evaporated to dryness in a hot air oven. Added 2 ml of chloroform, 1 ml of $ZnCl_2$ reagent, and 1 ml acetyl chloride. Heated in a water bath at 50°C for 10 min. For blank used 2 ml chloroform, 1 ml of $ZnCl_2$, and 1ml of acetyl chloride. Read optical density (pink red color) at 528 nm in a spectrophotometer (Hanel and Dam, 1955).

Fatty acid analysis of loin muscle

Samples for the analysis of fatty acids were prepared according to the method described by (Wang et al. 2000; Folch et al. 1957; Hubbard et al. 1977 and Guiheneuf et al. 2015) with slight modifications. Weighed exactly 4 g of loin sample and minced well with mortar and pestle. Homogenized the sample by adding 10-20 ml of Folch solution (Chloroform and Methanol in the ratio of 2:1) and allowed it to stand for 6-8 h. Filtered through Whatman No. 42 and rinsed the filter paper with 5 ml of Folch solution. Measured the volume and added approximately 25 ml of 0.88 % Sodiumchloride solution. Gently mixed it and left it for overnight. Discarded the top layer the next day and collected the chloroform layer. Evaporated the chloroform to dryness in a rotary evaporator (Make: Labocare and Model: 819-3001) at 45°C. Added 30 ml of alcoholic KOH (10 %) to this and allowed it to stay in the dark overnight. Then the saponified samples were refluxed for about one hour. Cooled the contents of the flask and then transferred into a separating funnel. Added 30 ml of distilled water and 50 ml of hexane. After thorough mixing, allowed the separation of the solution into two phases. Collected the lower phase in the same flask and discarded the upper phase. Added 15ml HCl and 50 ml hexane. Mixed and allowed to separate in a separating funnel. Collected the hexane (upper layer) in a separating flask. Again added 30 ml distilled water and 50 ml hexane. Finally, evaporated the hexane to dryness in a rotary evaporator. To the dried hexane layer, added 1 ml of methanol and 3 ml of acetyl chloride-methanol mixture in tubes fitted with air tight caps and heated at 85°C to 90°C in a water bath for 1 h. To this added 8 ml of 0.88 % NaCl and 3ml of n-hexane. Centrifuged at 2000 rpm for 10 min and collected the top hexane layer alone. Added a little sodium sulphate to remove any moisture and filtered through a 0.22 µm membrane filter. Fatty acids were determined by comparison with standard (37 Component FAME mix, Sigma- Aldrich, Supelco). Fatty acid methyl ester (FAME) was analyzed using GC (ACME 6100 - 6000 series) with a flame ionization detector (GC-FID). The GC was equipped with an SP GsBP capillary column (30 m x 0.32 mm x 0.25 µm). The oven temperature was set at 50° C for 1 min, and then raised to 200°C for 1min and held at 260°C. The temperatures of the injector and detector were 230° C and 220°C. The carrier gas flow rate was Nitrogen-4-5ml /min, Hydrogen- 30ml/ min, and Zero air- 300ml/ min with a split ratio of 25:1.

$$\text{Concentration of analyte in the sample (mg/g)} = \frac{\text{Area of analyte in sample} \times \text{concentration of analyte in standard (mg)}}{\text{Area of analyte in standard} \times \text{Sample weight}}$$

Meat quality attributes of loin muscle

pH

The pH of meat samples was determined (Bhaskar Reddy et al., 2013) by homogenizing 1 g of fresh meat sample using tissue homogenizer (Daihan Scientifics, Wise Mix, HG-15D, Korea) for 30 seconds. The pH of suspension was recorded with a digital pH meter (Systronics micro pH system 361, Model: 7856, Type 361) calibrated against a buffer of pH 4 and 7.

2-Thiobarbituric acid reactive substances (2-TBARS)

Thiobarbituric acid reactive substances(TBARS) value was determined as per the procedure of Witte et al. (1970).

Trichloroacetic acid (TCA) extract of the meat was prepared by homogenizing 4 g of sample with 20 ml of precooled 20% TCA solution for 2 min with mortar and pestle. The contents were allowed to extract for 10 minutes and then centrifuged at 3000 rpm (REMI R-8C, Serial no: JGLC-12753, Remi Elektrotechnik Limited, Vasai, India) for 10 min. Three ml of supernatant was mixed with an equal volume of 0.1% TBA reagent. The mixture was boiled in a water bath for 30 min, cooled and absorbance was measured at 532 nm using a UV-VIS spectrophotometer (Model: UV-1700 Pharma Spec, SHIMADZU, Japan), and the TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde / kg.

Sensory evaluation

The cooked pork samples were served to trained panelists and evaluated for color, flavor, tenderness, juiciness, and overall palatability using a 10-point descriptive scale (where, 10=extremely desirable, 1= extremely undesirable) as described by Keeton (1983) with slight modifications. Sensory evaluation was conducted between 3.30-4.00 PM and filtered potable water was provided to the panelists for rinsing their mouths in between evaluations of different samples.

Statistical analysis

The obtained data were subjected to analysis through Statistical Package for the Social Sciences (version 20.0; SPSS, 2015, Chicago, IL, USA) by applying one way ANOVA and two way ANOVA for meat quality attributes (Snedecor and Cochran, 1995) and Duncan's multiple range test with significance at $P<0.05$ for comparing the means.

RESULTS AND DISCUSSION

The fresh *Spirulina platensis* contained 13% DM. It contained 88.5, 62.3, 1.8, 0.8, 11.5 and 1.8 % OM, CP, EE,CF,TA and AIA, respectively on DMB and its mineral composition (mg/100 g DM) was 888.64, 547.71, 238.52, 3.82, 928.85, 86.85 and 1.4 for Ca, Na, Mg, Zn, K, Fe and Cu, respectively.

Fatty acid profile of loin muscle

The fatty acid profile of *Spirulina* and experimental diets (Table 2) indicated the presence of higher levels of palmitic acid, palmitoleic acid, and gamma-linolenic acid in *Spirulina* whereas the experimental diets contained stearic acid, oleic acid and DHA. *Spirulina platensis* supplementation had no significant effect on the total lipids and total cholesterol content (Table 3) of loin muscle. Peiretti and Meineri, (2011) reported an increase in the lipid content of the *Longissimus* muscle of rabbits fed *Spirulina platensis*. On the other hand, Simkus et al. (2013) reported a significant decrease in the intra-muscular lipid content of *Longissimus dorsi* of pigs supplemented *Spirulina platensis* (2g/animal/d). The sum of saturated fatty acids (SFA) and mono unsaturated fatty acids (MUFAs) were significantly ($P<0.05$) lower in *spirulina* supplemented groups compared to the control whereas the sum of polyunsaturated fatty acids (PUFA) followed a reverse trend (Table 3). High intake of SFA leads to elevated serum cholesterol and increases the risk of cardiovascular diseases in humans. The SFA (Tetradecanoic acid C14:0) and MUFA (Oleic acid C18:1) were significantly lower ($P<0.05$) in experimental groups supplemented with *Spirulina* compared to the control group. The PUFA i.e., linoleic acid C18:2 and eicosadienoic acid C20:2 were significantly higher ($P<0.05$) in T4 fed pigs than in T1 and the values were 17.81, 20.61, 22.71, and 24.09 for C18:2; 0.54, 0.45, 0.45, and 0.6 for C20:2 in T1 to T4, respectively. The results of this study are in agreement with (Martins et al. 2021) that there was an increase in the levels of PUFA (C18:3 n-6) in the meat of *Spirulina* fed piglets. Peiretti and Meineri, (2011) reported similar trends of fatty acid in rabbits fed diets containing *Spirulina platensis*. Further, Dal Bosco et al. (2014) observed a significant increase in the levels of PUFAs (C18:2 n-6 and C18:3n-6) by supplementing *Spirulina* in rabbits. Palmegiano et al. (2008) also reported that the inclusion of *S. platensis* at higher levels elevated the levels of PUFAs and decreased MUFAs in Siberian sturgeon. The other fatty acids (Gamma-linolenic acid, Alpha-linolenic acid, and Eicosapentaenoic acid) were not statistically significant among treatments. There was a numerical increase in docosahexaenoic acid (DHA) content in the experimental groups with increasing *Spirulina* supplementation. The increment of PUFA, especially DHA (22:6 n-3) content, after feeding with *S. platensis* may be due to elongation and desaturation of linolenic acid. Increasing the levels of *S. platensis* may have elevated the conversion rate. The PUFA levels were significantly ($P<0.05$) higher in *Spirulina* supplemented groups (T2, T3, and T4) compared to T1, leading to a higher ratio of PUFA to SFA ($P<0.05$) with increasing levels of *Spirulina*. The Ratio of n-6 to n-3 of muscle was decreased significantly ($P<0.05$) with higher levels of *Spirulina platensis* in the diet.

Table 2: Fatty acid profile (% of total fatty acids) of *Spirulina platensis* and experimental diets.

Fatty acid	Chemical Name of FA	*Spirulina*	T1	T2	T3	T4
C4:0	Butanoic acid	Nd	0.13	0.21	0.24	0.12
C6:0	Hexanoic acid	Nd	0.37	0.25	0.58	0.24
C8:0	Octanoic acid	Nd	0.19	0.33	0.31	0.24
C10:0	Decanoic acid	0.08	0.26	0.39	0.53	0.22
C12:0	Dodecanoic acid	0.04	0.18	0.21	0.38	0.06
C14:0	Tetradecanoic acid	0.83	2.62	4.39	4.27	2.06
C16:0	Palmitic acid	49.28	24.42	22.71	20.98	25.15
C18:0	Stearic acid	2.04	5.48	4.88	6.28	6.26
C20:0	Arachidonic acid	0.39	0.55	0.10	0.38	0.40
C22:0	Docosanoate	Nd	1.83	Nd	Nd	Nd
C16:1	Palmitoleic acid	6.35	0.68	0.80	0.75	0.85
C18:1	Oleic acid (cis and trans)	6.31	27.75	28.69	28.26	25.62
C20:1	Methyl arachidate	0.21	0.75	0.17	0.78	0.47
C22:1 n-9	Cis-Docosanoate	Nd	Nd	1.05	1.41	0.93
C18:2 n-6	Linoleic acid	12.42	22.32	23.05	24.00	23.17
C18:3 n-6	Gamma- Linolenic acid	14.2	0.02	Nd	0.38	1.13
C18:3 n-3	Alpha- Linolenic acid	1.02	1.43	1.26	1.3	1.35
C20:4 n-6	Eicosatetraenoic acid	Nd	Nd	Nd	Nd	Nd
C20:5 n-3	Eicosapentaenoate	0	0.773	0.869	0.767	0.981
C22:6 n-3	Docosahexanoate	0.13	0.89	1.73	1.46	1.24
Other fatty acids		6.68	9.36	8.9	7.0	9.5
Σ SFAs		52.67	36.03	33.46	33.94	34.75
Σ MUFAs		12.87	29.18	30.71	31.20	27.87
ΣPUFAs		27.78	25.43	26.91	27.85	27.88
Σ n-3		1.16	3.10	3.87	3.52	3.57
Σ n-6		26.62	22.33	23.05	24.33	24.30
n-6/n-3		22.90	7.21	5.96	6.91	6.8
PUFA/SFA		0.53	0.71	0.80	0.82	0.8

ND: Not detected; SFA: saturated fatty acid; MUFA: mono unsaturated fatty acid;
PUFA: poly unsaturated fatty acid
Pork from pigs fed with experimental diets contains *Spirulina* at 0 (T1), 0.5 (T2), 1.0 (T3), and 1.5 % (T4) respectively.
Current nutritional recommendations are that the PUFA/SFA ratio in human diets should be above 0.45, and within PUFA, the n-6/n-3 ratio should not exceed 4.0 (Burghardt et al. 2010).

Table 3: Effect of experimental diets on fatty acid profile (%) of the loin muscle of crossbred (*LWY X Desi*) pigs fed experimental diets

Parameter	T1	T2	T3	T4	SEM	P-value
Total lipids (g/100g)	15.47	15.45	15.03	14.06	0.39	0.60
Total cholesterol (mg/100 g)	61.47	62.25	61.56	61.56	0.26	0.70
FA composition (% of total fatty acids)						
C10:0 (Decanoic acid)	0.19	0.13	0.06	0.11	0.02	0.10
C12:0 (Dodecanoic acid)	0.93	0.30	0.10	0.24	0.05	0.30
C14:0 (Tetradecanoic acid)	1.69^{b}	0.51^{a}	0.34^{a}	0.56^{a}	0.14	0.02
C16:0 (Palmitic acid)	25.59	24.69	25.11	23.67	0.28	0.13
C18:0 (Stearic acid)	8.07	9.25	8.27	7.86	0.23	0.17
C20:0 (Arachidonic acid)	0.74	0.66	0.57	1.56	0.14	0.07
C22:0 (Docosanoate)	Nd	Nd	Nd	Nd	-	-
C16:1 (Palmitoleic acid)	4.15	4.32	3.99	3.38	0.18	0.37
C18:1 (Oleic acid)	32.92^{c}	30.32^{b}	27.6^{ab}	29.1^{a}	0.56	0.01
C20:1 (Methyl arachidate)	1.04	0.50	0.43	0.43	0.11	0.16

Parameter	T1	T2	T3	T4	SEM	P-value
C22:1 n-9 (Cis-Docosanoate)	Nd	Nd	Nd	Nd	-	-
C18:2 n-6 (Linoleic acid)	17.81[a]	20.61[b]	22.71[b]	24.09[c]	0.63	0.01
C18:3 n-6 (Gamma- Linolenic acid)	0.33	0.48	0.30	0.35	0.05	0.54
C18:3 n-3 (Alpha- Linolenic acid)	0.21	0.27	0.38	0.54	0.04	0.10
C20:2 n-6 (Eicosadienoic acid)	0.54[b]	0.45[b]	0.45[b]	0.62[a]	0.04	0.02
C20:4 n-6 (Eicosatetraenoic acid)	0.69	0.02	0.51	0.21	0.18	0.44
C20:5 n-3 (Eicosapentaenoate)	0.32	0.34	0.32	0.13	0.06	0.06
C22:6 n-3 (Docosahexanoate)	0.38	0.46	0.50	0.61	0.03	0.09
Others	4.40	6.70	8.37	6.53	-	-
Partial sum of FA						
∑SFA	37.22[b]	35.54[a]	34. 46[a]	34.1[a]	0.41	0.03
∑MUFA	38.11[b]	35.13[a]	32.03[a]	32.92[a]	0.40	0.02
∑PUFA	20.27[a]	22.63[b]	25.16[b]	26.55[c]	0.54	0.01
∑n-3 PUFA	0.90[a]	1.07[a]	1.19[b]	1.28[b]	0.05	0.01
∑n-6 PUFA	19.38[a]	21.56[b]	23.97[b]	25.27[c]	0.48	0.01
PUFA: SFA	0.54[a]	0.64[a]	0.73[b]	0.78[b]	0.01	0.03
n-6: n-3	21.46[b]	20.15[ab]	20.10[ab]	19.78[a]	0.46	0.03

[abc] values in a row differ significantly SEM: standard error of mean
ND: Not detected; SFA: saturated fatty acid; MUFA: mono unsaturated fatty acid;
PUFA: poly unsaturated fatty acid
Pork from pigs fed with experimental diets contains *Spirulina* at 0 (T1), 0.5 (T2), 1.0 (T3), and 1.5 % (T4) respectively.

In view of these figures, the n-6/n-3 ratio of *L. dorsi* muscle across dietary treatments is not in accordance with the recommended guidelines, in contrast to the PUFA/SFA ratio.

Storage stability of loin muscle pH

The pH of meat is an important measure to estimate relative alkalinity or acidity which might be an indication of the potential storage life of meat (Bhaskar Reddy et al. 2022). Live pig muscle is red and has a pH of about 7.0 and after slaughter, the fall in muscle pH is gradual reaching about 5.4 in about 24 h. If the fall in pH is rapid it may result in pale, soft, and exudative pork. Dietary *Spirulina platensis* and storage period had significantly (P<0.05) influenced the pH values (Table 4) during refrigerated storage (4±1°C). The loin muscle of pigs fed on control diet (T1) was spoiled after 6 days of refrigerated storage. There was a significant (P<0.05) decrease in pH in the experimental groups as the level of *Spirulina* increased in the diets. In the present study, the overall mean pH of muscle (Table 4) on different days of refrigerated storage was significantly lower (P<0.05) on the 3rd and 6th day than on the 0th and 9th day and the treatment effect was significant with 1.5% *Spirulina* (T4) in the diet. This might be due to the presence of conjugated polyphenols, sulfate groups, and phenolic compounds especially tannic acid, as it constitutes more than 63% of the phenols and also contains phycocyanin and beta-carotene which prevents the pork from lipid peroxidation. As the storage period progressed, there was a significant (p<0.01) decrease in the pHvalues of all the treatments. This might be due to differences in animal handling during the ante mortem period (Lawrie and Ledward, 2006) which causes the variations of ultimate pH formation but this relatively low pH did not affect the other meat quality characteristics. Furthermore, these variations in pH during storage could be due to protein denaturation and liberation of protein metabolites, mainly amines due to bacterial activity.

2-Thibarbituric acid reactive substance value (2-TBARS value)

TBA value is an empirical measure of the deterioration of fatty foods. It measures the aldehyde residues resulting from lipid peroxidation and deterioration in extractable and nonextractable lipids. In the present study, dietary *Spirulina* at 1.5% supplementation (T4) had significantly (P<0.05) lowered 2-TBARS values (Table 5) compared to all other treatment groups during refrigerated storage (4±1°C). This might be due to the presence of conjugated polyphenols, sulfate groups, and phenolic compounds especially tannic acid, as it constitutes more than 63% of the phenols that can fix roots by donating hydrogen atoms

Table 4. Effect of experimental diets on pH and 2-TBARS values of loin muscle during storage.

Parameter	Treatment	Storage days				Mean
		0	3	6	9	
pH	T1	5.62	5.16	5.24	Spoiled	5.36^{ab}±0.021
	T2	5.48	5.31	5.22	5.82	5.46^{c}±0.018
	T3	5.48	5.27	5.27	5.55	5.39^{b}±0.018
	T4	5.48	5.22	5.24	5.33	5.32^{a}±0.018
	Mean	5.52^{B}±0.018	5.25^{A}±0.018	5.25^{A}±0.018	5.57^{B}±0.021	
2-TBARS	T1	0.046	0.211	0.636	Spoiled	0.29^{b}±0.29
	T2	0.028	0.169	0.518	0.803	0.37^{c}±0.01
	T3	0.022	0.123	0.358	0.685	0.29^{b}±0.01
	T4	0.016	0.080	0.340	0.592	0.25^{a}±0.01
	Mean	0.02^{A}±0.01	0.14^{B}±0.01	0.46^{C}±0.01	0.69^{D}±0.01	

^{ABCD}values in a row differ significantly* (P<0.05)
^{abc}values in a column differ significantly * (P<0.05)

or an electron (Zheng et al. 2012) and also containing phycocyanin and beta-carotene thus reducing the loin from lipid peroxidation. As the storage period progressed, there was a significant (P<0.05) increase in 2-TBARS values of all the treatments. This could be because as the storage period progressed the intensity of lipid oxidation enhanced and the production of more secondary products of lipid oxidation formed from the decomposition of oxidized lipids which yielded more 2-TBARS values. The results obtained in this study are in agreement with the observations of (Dal Bosco et al. 2014; Liu et al. 2016; Teimouri et al. 2019; and Zhao et al. 2019; Abbas et al. 2021) showed that a progressive increase in lipid oxidation with an increase in refrigerated storage and dietary *Spirulina* supplementation reported lower lipid oxidation compared to T1 fed animals.

Sensory evaluation of loin muscle

Appearance

Appearance score of muscle of pigs fed with 1.5 % *Spirulina* (T4) was significantly (P<0.05) higher compared to other treatments (Table 5). This might partly be due to the preventive nature of the myoglobin oxidation by *Spirulina*, which in turn delays the surface color deterioration during storage of pork. Martins et al. (2021) and Altmann et al. (2020) also reported that dietary inclusion of *Spirulina* in piglets showed significantly (P<0.05) higher appearance scores compared to pigs fed on the control diet. Pestana et al. (2020) observed more yellowness scores in *Spirulina* supplemented chicken than control group due to the accumulation of zeaxanthin within the muscle. Dietary *Spirulina* levels at 1 % of the total ration in the week before slaughter have been found to result in broiler muscle tissue pigmentation at levels best representing consumer preferences (Dismukes et al. 2008 and Khadeer et al. 2023). As the progression of the storage period, there was a significant (P<0.05) detrimental in appearance scores in all treatments. This might be due to nonenzymatic browning resulting from a reaction between lipid oxidation products and amino acids as reported by Che Man et al. (1995).

Flavour

Higher (P<0.05) flavour scores of loin muscle were reported in T3 and T4 fed pigs than in muscle of other treatments (Table 5) and this might be due to the anti-oxidative effect of active components in *Spirulina* that were effective in inhibition of lipid peroxidation and development of rancid flavour in stored meat. Increased rancidity in stored meat leads to the development of higher intensity of off-flavour during storage (Purrinos et al. 2011; Amaral et al. 2018). As the progression of the storage period, there was a significant (P<0.05) detrimental in flavour scores in all treatments. The decrease in flavour scores might be due to the cumulative effect of the oxidative process during the storage period and as the storage prolonged antioxidant constituents of *Spirulina* showed some positive effects in the supplemented groups. The results of this study are incongruent with (Luo et al. 2018; Altmann et al. 2020; Martins et al. 2021) that polysaccharides in *Spirulina platensis* improve anti-oxidant capacity of sausages. *Spirulina* at largest dosages improved sensory attributes (P<0.05) indicating that *Spirulina* incorporation had no negative effect on meat flavour.

Tenderness

Meat tenderness is influenced by the level of proteolytic degradation of muscles, connective tissues, and sarcomere length (Lavanya et al 2023) or might be due to the presence of antioxidant compounds present in *Spirulina*. The Tenderness score was significantly (P<0.05) higher in stored loin muscle of T3 and T4 fed pigs than in T1 fed pigs (Table 5). The results of this study are incongruent with (Luo et al. 2018 and Martins et al. 2021) who observed that polysaccharides in *Spirulina platensis* improved anti-oxi-

Table 5. Effect of experimental diets on sensory quality of loin muscle quality during storage.

Parameter	Treatment	Storage days				Treatment Mean
		0	3	6	9	
Appearance	T1	7.66	6.66	5.33	Spoiled	6.55^{a}±0.118
	T2	8.33	7.66	7.0	5.5	7.12^{b}±0.102
	T3	8.16	8.0	7.83	5.66	7.24^{b}±0.102
	T4	8.50	8.0	7.16	5.66	7.33^{b}±0.102
	Mean	8.16^{D}±0.102	7.58^{C}±0.102	6.83^{B}±0.102	5.61^{A}±0.118	
Flavour	T1	7.83	7.33	6.50	Spoiled	7.22^{ab}±0.126
	T2	8.16	7.66	7.0	5.66	7.12^{a}±0.109
	T3	8.66	8.33	7.33	5.23	7.38^{b}±0.109
	T4	8.33	8.0	7.66	5.86	7.46^{b}±0.109
	Mean	8.25^{D}±0.109	7.83^{C}±0.109	7.12^{B}±0.109	5.58^{A}±0.126	
Tenderness	T1	7.50	7.66	5.66	Spoiled	6.94^{a}±0.131
	T2	8.33	7.5	6.83	6.0	7.16^{ab}±0.13
	T3	8.66	8.16	7.13	5.66	7.40^{b}±0.113
	T4	8.66	8.03	7.16	5.96	7.45^{b}±0.113
	Mean	8.29^{D}±0.113	7.83^{C}±0.113	6.69^{B}±0.113	5.87^{A}±0.131	
Juiciness	T1	8.0	7.5	6.3	Spoiled	7.27^{a}±0.118
	T2	8.33	8.0	6.8	5.5	7.16^{a}±0.096
	T3	9.0	8.33	7.5	5.66	7.62^{b}±0.096
	T4	9.0	8.66	7.33	5.93	7.73^{b}±0.098
	Mean	8.58^{D}±0.096	8.12^{C}±0.096	7.0^{B}±0.096	5.7^{A}±0.112	
Overall Palatability	T1	7.83	7.16	6.0	Spoiled	7.0^{a}±0.084
	T2	8.33	8.0	7.16	6.0	7.37^{b}±0.073
	T3	8.83	8.0	7.53	5.13	7.37^{b}±0.073
	T4	8.83	8.0	7.16	5.72	7.43^{b}±0.073
	Mean	8.45^{D}±0.073	7.79^{C}±0.073	6.96^{B}±0.073	5.61^{A}±0.084	

^{ABCD} values in a row differ significantly, ^{ab} values in a column differ significantly * (P<0.05)

dant capacity of sausages and improved sensory attributes (P<0.05). As the storage period progressed, there was a significant (P<0.05) detrimental effect on tenderness scores in all treatments. The decrease in tenderness scores might be due to the cumulative effect of the oxidative process on the storage period and as the storage prolonged antioxidant constituents of *Spirulina* showed some positive effect in the treated groups (T2 to T4) and (Purrinos et al. 2011 and Amaral et al. 2018) also reported a similar effect of *Spirulina* supplementation.

Juiciness

The juiciness of loin muscle obtained from pigs fed on T3 and T4 was significantly (P<0.05) higher than in T1 and T2 (Table 5) and *Spirulina* provided through diet might have helped to retain more water molecules and also subsequent delay in breakdown of proteins by active constituents of *Spirulina* leading to more retention of water. As the storage period progressed, there was a significant (P<0.05) detrimental effect on juiciness scores in all treatments. This could be due to the dehydration of the meat during the storage period.

Overall acceptability

The overall acceptability scores (Table 5) of loin muscle obtained from pigs fed *Spirulina* in diets (T2 to T4) were significantly (P<0.05) higher compared to pigs fed with control diet and this might be due to superior appearance, flavour, and juiciness scores awarded by panelists. As the storage period progressed, there was a significant (P<0.05) reduction in overall acceptability scores in all treatments and this might be due to reduced sensory scores and increased lipid oxidation, protein degradation, and development of bland flavour due to fat degradation (Bariya, 2016).

CONCLUSION

It was concluded that *Spirulina platensis* at 1.5 % of diet was beneficial to produce nutritionally healthier pork for human

consumption by enhanced PUFA content and reduced SFAs and MUFAs in loin muscle of crossbred pigs with improvement in storage stability and sensory attributes.

COMPETING INTERESTS

The authors do not have any competing interests among themselves or others related to this research work.

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