

# Effect of Using *Moringa oleifera* and *Spirulina platensis* as Feed Additives on Performance, Meat Composition and Oxidative Stability and Fatty Acid Profiles in Broiler Chicken

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## ABSTRACT

A study was conducted to determine mainly the n 3 fatty acids enrichment and decreased oxidation of broiler meat using the plant *Moringaoleifera* and a blue green algae *Spirulina*platensis. The effects of the dietary treatments were evaluated in terms of growth performance, carcass and meat yields, oxidative stability and fatty acids modulation. Two hundred and forty (240) one day old Cobb 500 broiler chicks were assigned to 5 dietary treatments for 5 weeks, with 4 replications having 12 chicks per replication. The diets of the treatments were formulated from the basal feed as follows: control (T<sub>1</sub>), including 2 different levels of *M. oleifera* leaf meal 1% (T<sub>2</sub>) and *M. oleifera* leaf meal 1.5%, (T<sub>3</sub>) and 2 different levels of *S. platensis* 1% (T<sub>4</sub>) and *S. platensis* 1.5% (T<sub>5</sub>). The birds were randomly distributed in each pen, and the data were analyzed using the SPSS statistical package. The final body weight (BW) gain was significantly higher in the T<sub>2</sub> and T<sub>5</sub> groups, and the feed conversion ratio improved in the T<sub>2</sub> group (1.68). The lowest (p ≤ 0.05) thiobarbituric acid-reactive substances values (TBARS) of breast and thigh were obtained in T<sub>2</sub> groups compared to T<sub>3</sub>-T<sub>5</sub> group after the second week of preservation. Regarding fatty acid profile of breast and thigh meat, the omega-3 fatty acid levels, such as those of linolenic and docosahexaenoic acid (DHA), were increased in the additives groups. The results of the present study elucidated that dietary inclusion of the 2 medicinal plants in the T<sub>2</sub> and T<sub>5</sub> groups could be promising functional ingredients to produce value-added broiler meat in terms of oxidative stability and omega-3 fatty acids enhancement.

**Keywords:** Broiler chicken; *Moringa oleifera*; *Spirulina platensis*; Oxidation; Fatty acid

## INTRODUCTION

At present, consumer demand is increasing for natural, safe, and eco-friendly products that can improve well-being, limit the risk of some chronic diseases, and promote health benefits beyond their nutritional value. These foods are called functional foods or nutraceuticals, a concept that was born in Japan early in 1980 [1,2] This consumer attitude applies to poultry products as it does to other value-added products. The advancements in poultry research have pooled knowledge of the biochemical and physiological mechanisms that increase the efficiency of feed utilization and desired carcass attributes through dietary manipulation [3]. Another reason for the broiler meat industry's success has been the consumer perception of a healthy product that contains less fat (< 5%), less cholesterol (<50 mg/100 g), and most predominantly unsaturated fatty acids as compared to beef or pork products

[4]. However, Sirri et al. [5] reported that bird genotype might strongly influence meat functional properties as well as nutritional characteristics. The feeding and rearing conditions under which the broilers are produced and slaughtered may also influence the meat's oxidative stability [6]. Lipid oxidation is a primary cause of quality deterioration in meat products through adverse changes in undesirable odors and flavors, which lowers the functional, sensory, and nutritive values of meat products [7].

To date, various plants and algae have been researched, and many are reported to contain functional properties that have an impact on the meat quality of poultry [8]. *M. oleifera*, a plant from the family Moringaceae is a major crop in Asia and Africa. For centuries, people in many countries have used moringa leaves as traditional medicine for common ailments. The most used parts of the plant are the leaves, which are rich in vitamins, carotenoids, polyphenols,

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phenolic acids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins [9]. These bioactive compounds might explain the pharmacological properties with having antioxidant, antiseptic [10], antimicrobials properties and can reduce microbial growth on the food and food products [11,12]. On the other hand, among edible algae, *Spirulina platensis*, blue-green microalgae, has recently served as an important source of valuable bioactive compounds. Dried spirulina is a good nutritional source with high protein and significant polyunsaturated fatty acids content, such as oleic, linoleic, gamma-linolenic, and docosahexaenoic (DHA) acid [12]. In addition, *M. oleifera* leaves and *S. platensis* act as good natural antioxidant sources due to the presence of various types of antioxidant compounds, such as ascorbic acid, flavonoids, phenolics, and carotenoids [13,14]. Antioxidants have the potential to maintain meat quality and protect body cells against the damaging effects of reactive oxygen species [15]. According to World Health Organization (WHO) developing countries populations, about 80% depends on the medicinal plants for their health care due to their beneficial properties [2,3]. However, information is limited concerning the oxidative stability and fatty acid profiles of broiler meat using *M. oleifera* and *S. platensis* as a natural functional ingredient in Bangladesh. This is an ongoing search for natural feed additives with excellent physiological activity for broiler meat. Therefore, the objectives of the present study were to evaluate the feeding effect of *M. oleifera* leaf meal and *S. platensis* on their performance, oxidative stability and fatty acid profiles of broiler meat.

## MATERIALS AND METHODS

### Preparation of *M. oleifera* leaf and *S. platensis* meal

Fresh *M. oleifera* leaves were collected and air-dried during the daytime. After 4-5 d of drying, the leaves were grinded to a fine powder to pass through a 0.15 mm sieve. The leaf meal was tightly packaged in polythene plastic bags and kept at room temperature until required. The blue-green alga *S. platensis* was collected from the Bangladesh Council of Scientific and Industrial Research (BCSIR) in Dhaka, Bangladesh. *M. oleifera* and *S. platensis* were analyzed in triplicate for Crude Protein (CP), Ether Extract (EE), moisture and ash as described by the Association of Official Analytical Chemists [16].

### Experimental design, dietary treatments and bird management

A total of 240 one day old Cobb 500 broiler chicks were purchased from Nourish Poultry and Hatchery Ltd., a commercial hatchery. The chicks were randomly weighed and allocated to 20 floor pens (100 cm long × 90 cm wide) in shed containing fresh wood shavings at a depth of 5 cm. The internal temperature of the broiler house was set and maintained at 34°C for the first week, after which it was gradually reduced to 23°C at 3°C per week, and then maintained at this temperature until the end of the total experimental period. The experiment was divided into 5 dietary treatments, with 4 replications having 48 chicks in each group. Each pen served as an experimental unit. The chicks were vaccinated against commercial Newcastle disease virus (NDV) through eye drops and drinking water at days 4 and 18 during the experiment period, respectively. The chicks were inspected daily, and dead birds were removed following mortality recording (pen, date, and body weight [BW]).

Feed and fresh water were offered *ad libitum* feed intake and free access to water throughout the period (35 d rearing). The birds' care and basal diet was formulated to meet the Nutrient Requirements of Poultry [17] and applied for a total of 5 weeks in two stages: starter (0-3 weeks) and finisher (4-5 weeks), the diet and chemical composition of additives are shown in (supplementary information, Tables 1 and 2). All diets were in mashed form. Five dietary treatment groups were produced from the basal feed as follows: control ( $T_1$ ), including 2 different levels of *M. oleifera* leaf meal 1% ( $T_2$ ) and *M. oleifera* leaf meal 1.5%, ( $T_3$ ) and 2 different levels of *S. platensis* 1% ( $T_4$ ) and *S. platensis* 1.5% ( $T_5$ ). A proximate analysis for moisture, crude protein, ash, and ether extract was performed on the experimental diets (supplementary information (Tables 1 and 2) according to the Association of Official Analytical Chemists' (AOAC, 2000) methods. The neutral detergent fiber (NDF) concentration was analyzed according to the methods described by Van Soest et al. [18]. The diets' metabolizable energy (ME) contents were calculated on the basis of the determined nutrients in the proximate analyses. The BW per pen were recorded at placement, as well as at weekly intervals (i.e., at 7, 14, 21, 28, and 35 d of age, respectively). The BW (g) was calculated as final body weight ( $\Delta$ BW) minus initial BW; and average daily gains (ADG, g) were calculated accordingly. The feed intake (FI, g) was calculated as feed allocated minus feed refused; whereas, the feed conversion ratio (FCR) was calculated as the FI (g) per  $\Delta$ BW (g) on a pen weight basis. Experimental birds were reared in the Bangladesh Livestock Research Institute Savar, in research shed. The experiment was also subjected to an assessment of broiler chicken health status for its ethical acceptability and was approved by the Ethics Committees of Bangladesh Livestock Research Institute in Savar, Dhaka.

### Slaughter procedure

Eight birds were randomly selected per treatment, 2 per replicate pen, and were individually weighed at the age of 35 d. Birds were then fasted for 8 h with water offered *ad libitum* consumption and were reweighed before they were slaughtered. After bleeding, scalding, plucking, and washing, the feet, head, neck and skin were removed. Then, the carcasses were manually eviscerated and cut into breast, drumsticks (legs), wings, and thighs. The visceral organs and cuts were then weighed individually, and the yields and carcass dressing percentage (CW/BW) were calculated. The breast muscles and thigh muscles were separated from the bones and skin, and was trimmed of external/adjacent fat and connective tissue. The breast and thigh meat samples from each bird were ground separately using a meat grinder. The samples were subsequently divided into three parts, one for the oxidative stability analysis and another two for the proximate and fatty acid composition analysis. Finally, the samples were kept into zipper bag, after which those for oxidative rancidity analysis were refrigerated at 4°C and samples for other analyses were stored at -20°C.

### Determination of oxidative stability and fatty acids profile

The lipid oxidation of the broiler meat was determined according to the method described by Sarker et al. [19], with slight modification. For this analysis, 4 g of the thigh and breast meat samples were blended at full speed for 1.5 min in a chilled stainless watering blender cup with 10 mL of extracting solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid. The resulting sediment was quantitatively transferred to a 50 mL conical tube with 10 mL of distilled water, homogenized

and diluted by shaking. After, the aliquot was filtered through Whatman No.6 filter paper, then 5 mL of filtrate was transferred to a test tube, and 5 mL of 2-thiobarbituric acid (0.005 M in DW) was added. The solution was then subsequently shaken in a water bath at 80°C (HB-205 SW Hanbaek Scientific Co., Korea) for 30 min. After cooling, the color development was measured at 530 nm in a Jenway 6305 spectrophotometer (Bibby Scientific Ltd., Staffordshire, United Kingdom). TBARS values were expressed as micromoles of malondialdehyde (MDA) per 100 g of meat sample. The fatty acids compositions of breast and thigh meat were conducted at the BCSIR's Institute of Food Science and Technology in Dhaka, Bangladesh. The Fatty Acid Methyl Ester (FAME) was determined using a gas chromatograph (Shimadzu 14b, JAPAN) equipped with a flame ionization detector and identified by matching their retention times with those of their relative standards (Polyunsaturated Fatty Acid-2, Animal Source, SUPELCO, Bellefonte, PA, USA) as well as with the Food Composition Table (NRLSI, 2002).

### Statistical analysis

The analytical measurements were done in triplicates, and the results were presented as the average of 3 analyses  $\pm$  the standard deviation (SD). The statistical analysis was done using the SPSS statistical package (IBM Corp., IBM SPSS Statistics for Windows, Version 16.0, Armonk, NY, USA) with a one-way ANOVA. A p value of <0.05 was taken as statistically significant based on Tukey's tests.

## RESULTS

### Broilers' growth performance and meat composition

The effects of diets with added *M. oleifera* leaf meal and *S. platensis* on the FI,  $\Delta$ BW, and FCR are shown in Table 1. The dietary addition of *M. oleifera* leaf meal and *S. platensis* affected the broilers' growth performance. The final  $\Delta$ BW and BW gain were significantly higher ( $p < 0.05$ ) in the  $T_2$  and  $T_5$  groups compared to the  $T_3$  and  $T_4$  groups and the  $T_1$  control group. Similarly, on

d 35, birds fed diets with *M. oleifera* 1% and *S. platensis* 1.5%, in particular those in  $T_2$  and  $T_5$ , had the highest ADG, while those in  $T_1$  and  $T_3$  had the lowest ( $p < 0.05$ ).  $T_2$  group was observed decreased feed consumption ratio as compared to control ( $T_1$ ) group of broilers for the total period. No mortality was observed in the experimental period.

Addition of feed additives had an effect on the composition of breast and thigh meats (Table 2). Dietary addition of *M. oleifera* and *S. platensis* groups were increased ( $p < 0.05$ ) crude protein content in breast meat compared to the control group. In the breast meat crude fat content was decreased in  $T_2$ - $T_4$  group whereas increased in  $T_5$  group in compared to control group ( $P < 0.05$ ). Moisture content did not differ with control group; but there was found higher moisture content in the  $T_2$  group compared to other group ( $P < 0.05$ ). The crude ash content in thigh meat was lower in  $T_2$  group (0.65%) than the control group  $T_1$  (1.01%); however crude protein and crude fat content did not differ with the control group. In case of thigh and breast meat moisture content did not differ with the control group. Table 3 represents the data on internal organs of broiler chicks. Results showed that heart, liver, and small intestine weight were significantly different ( $p < 0.05$ ) among the treatments. However, other internal organs of birds there were no significant differences observed in this study. Abdominal fat pad was decreased in the additives group compared to control.

### Oxidative stability of broiler meat

The TBARS test, of broiler breast and thigh meats which determine the amount of malondialdehyde (MDA), a major secondary lipid oxidation byproduct was shown in Figures 1 and 2. Additives groups had lower ( $p < 0.05$ ) TBARS values after weeks 1 and 2 of preservation as well as 3<sup>rd</sup> weeks values in breast and thigh meat compare to control group,  $T_1$ . The lowest TBARS values obtained in breast and thigh meat were 9.95 and 12.88  $\mu$ mol MDA/100g, respectively, for  $T_2$  group in which other group's value were significantly higher in 2<sup>nd</sup> weeks. Similarly, the TBARS in preserved both thigh and breast meat were found to be significantly lowest for the  $T_3$ ,  $T_4$ , and  $T_5$  dietary groups, with values of 12.55, 13.47,

**Table 1:** Effect of dietary added *M. oleifera* leaf and *S. platensis* on the body weight, average daily gain, feed intake, and feed conversion ratio (Feed: Gain) of broiler chickens.

Experimental period (d) and parameters	Dietary treatments				
	$T_1$	$T_2$	$T_3$	$T_4$	$T_5$
<b>BW (g)</b>					
0 d	46.42 $\pm$ 0.34	46.38 $\pm$ 0.43	47.54 $\pm$ 0.88	47.06 $\pm$ 0.15	48.46 $\pm$ 1.4
21 d	779.50 $\pm$ 11.97 <sup>a</sup>	786.25 $\pm$ 5.06 <sup>a</sup>	751.75 $\pm$ 6.61 <sup>b</sup>	768.50 $\pm$ 11.25 <sup>b</sup>	778.75 $\pm$ 75 <sup>a</sup>
35 d	1512.65 $\pm$ 29.47 <sup>b</sup>	1632.28 $\pm$ 25.66 <sup>a</sup>	1521.71 $\pm$ 23.96 <sup>b</sup>	1518.31 $\pm$ 30.07 <sup>b</sup>	1629.47 $\pm$ 29.01 <sup>a</sup>
<b>ADG (g)</b>					
21 d	37.11	37.44	35.77	36.60	37.08
35 d	43.21 <sup>b</sup>	46.07 <sup>a</sup>	43.48 <sup>b</sup>	43.39 <sup>b</sup>	46.56 <sup>a</sup>
<b>FI (g/bird)</b>					
21 d	1004.12 $\pm$ 3.74	1003.75 $\pm$ 4.66	1008.10 $\pm$ 4.280	1000.66 $\pm$ 5.310	1007.50 $\pm$ 4.247
35 d	2714.37 $\pm$ 9.41 <sup>a</sup>	2716.54 $\pm$ 6.61 <sup>a</sup>	2661.14 $\pm$ 20.19 <sup>b</sup>	2694.08 $\pm$ 16.98 <sup>ab</sup>	2709.66 $\pm$ 7.49 <sup>a</sup>
<b>FCR (Feed: gain)</b>					
21 d	1.66 $\pm$ 0.09	1.62 $\pm$ 0.09	1.63 $\pm$ 0.07	1.61 $\pm$ 0.08	1.64 $\pm$ 0.09
35 d	1.79 $\pm$ 0.02 <sup>b</sup>	1.68 $\pm$ 0.03 <sup>a</sup>	1.75 $\pm$ 0.04 <sup>b</sup>	1.77 $\pm$ 0.02 <sup>b</sup>	1.72 $\pm$ 0.11 <sup>ab</sup>

<sup>a,b,c</sup>Means with different superscripts within same row are significantly different ( $p < 0.05$ ). BW - Body weight (g), ADG - Average Daily Gain, FI - Feed intake (g/bird), FCR - Feed Conversion Ratio;  $T_1$  - Control (basal diet);  $T_2$  - *M. oleifera* leaf meal 1%;  $T_3$  - *M. oleifera* leaf meal 1.5%;  $T_4$  - *S. platensis* 1%; and  $T_5$  - *S. platensis* 1.5%. The values are means  $\pm$  SD (n=4).

Table 2: Effect of *M. oleifera* and *S. platensis* on broiler meat composition.

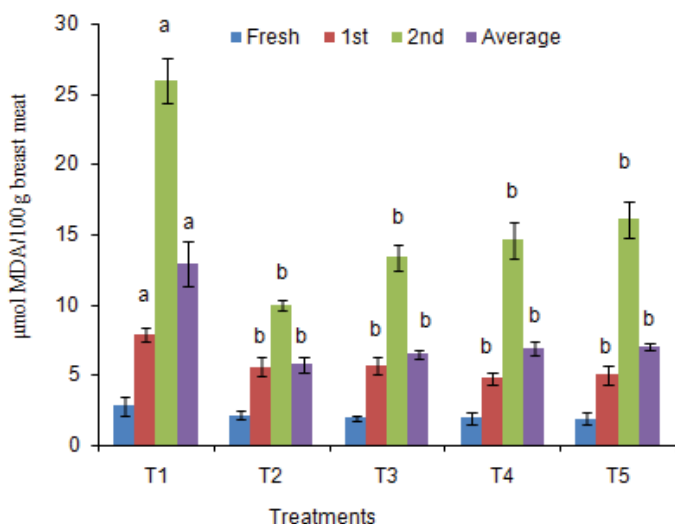
Parameters	Treatments				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
<b>Breast meat (%)</b>					
Moisture	74.75 ± 0.24	75.63 ± 0.09	74.62 ± 0.04	73.89 ± 0.25	73.93 ± 0.43
Crude ash	1.45 ± 0.12	0.90 ± 0.12	0.80 ± 0.43	1.50 ± 0.04	1.20 ± 0.22
Crude protein	22.36 ± 0.31	24.88 ± 0.06	23.99 ± 0.31	23.58 ± 0.24	23.01 ± 0.14
Crude fat	1.61 ± 0.12 <sup>bc</sup>	1.01 ± 0.13 <sup>b</sup>	1.20 ± 0.24 <sup>b</sup>	1.20 ± 0.41 <sup>b</sup>	2.42 ± 0.11 <sup>a</sup>
<b>Thigh meat (%)</b>					
Moisture	76.02 ± 0.42	76.02 ± 0.32	75.99 ± 0.22	75.21 ± 0.32	76.34 ± 0.32
Crude ash	1.01 ± 0.11	0.65 ± 0.43	0.85 ± 0.14	1.30 ± 0.41	0.85 ± 0.12
Crude protein	20.68 ± 0.33	20.25 ± 0.17	20.82 ± 0.32	20.28 ± 0.34	20.39 ± 0.42
Crude fat	2.80 ± 0.08	2.65 ± 0.21	2.60 ± 0.13	2.41 ± 0.15	2.53 ± 0.16

<sup>a,b,c</sup> Values with different superscripts in the same row differ significantly ( $p < 0.05$ ), T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1%; and T<sub>5</sub> - *S. platensis* 1.5%. The values are means ± SD (n=8).

Table 3: Effect of *M. oleifera* and *S. platensis* meal as feed additives on meat yield traits of broiler.

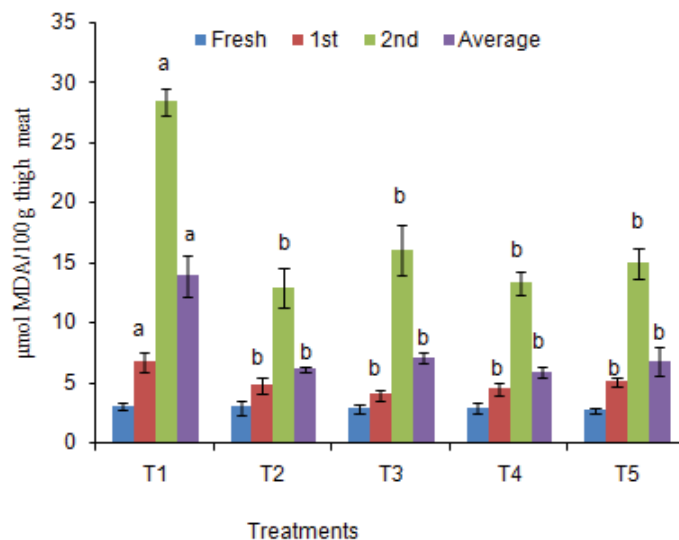
Parameters (g/bird)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	SEM
Head	38.30 ± 4.32 <sup>ab</sup>	41.50 ± 2.51 <sup>a</sup>	39.00 ± 3.46 <sup>ab</sup>	39.50 ± 3.78 <sup>ab</sup>	40.50 ± 3.41 <sup>a</sup>	0.75
Neck	44.52 ± 3.21 <sup>ab</sup>	46.31 ± 1.63 <sup>a</sup>	44.50 ± 3.46 <sup>b</sup>	44.43 ± 9.38 <sup>ab</sup>	44.51 ± 4.12 <sup>ab</sup>	1.08
Liver	37.50 ± 3.78 <sup>a</sup>	31.43 ± 3.46 <sup>b</sup>	32.50 ± 3.78 <sup>b</sup>	31.50 ± 1.00 <sup>b</sup>	31.00 ± 4.76 <sup>b</sup>	0.91
Gizzard	58.09 ± 1.02	57.32 ± 2.11	57.11 ± 3.10	58.01 ± 0.22	57.53 ± 2.41	0.87
Small intestine	73.21 ± 5.21 <sup>a</sup>	62.23 ± 11.43 <sup>b</sup>	66.50 ± 5.74 <sup>b</sup>	68.50 ± 5.03 <sup>ab</sup>	71.21 ± 4.76 <sup>b</sup>	1.64
Large intestine	9.37 ± 2.49	9.25 ± 0.95	10.75 ± 2.21	10.52 ± 1.29	11.02 ± 1.41	0.38
Abdominal fat	18.23 ± 2.82	17.02 ± 1.15	16.20 ± 3.26	15.51 ± 3.02	15.50 ± 2.51	0.57
Heart	6.11 ± 3.46	7.50 ± 1.31	7.50 ± 1.08	7.02 ± 1.15	6.50 ± 1.02	0.42

<sup>a,b,c</sup> Mean with different superscripts within same row are significantly different ( $p < 0.05$ ); T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1%; and T<sub>5</sub> - *S. platensis* 1.5%. The values are means ± SD (n=8)



**Figure 1:** Effect of *M. oleifera* and *S. platensis* on the thiobarbituric acid value (TBARS) in the broiler breast meat (n=8 for each treatment). TBARS values are expressed as micromoles of malondialdehyde (MDA) per 100 g of meat ( $\mu\text{mol MDA}/100\text{ g}$ ). Data are presented as the mean ± SD. Significant differences ( $p < 0.05$ ) are represented by different letters over the column. T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1% and T<sub>5</sub> - *S. platensis* 1.5%.

and 14.67  $\mu\text{mol MDA}/100\text{ g}$ , for thigh and 14.21, 12.27 and 13.47  $\mu\text{mol MDA}/100\text{ g}$  for breast meat compared to the T<sub>1</sub> (25.09 and 27.19  $\mu\text{mol MDA}/100\text{ g}$ ) group.



**Figure 2:** Effect of *M. oleifera* and *S. platensis* on the thiobarbituric acid value (TBARS) in the broiler thigh meat (n=8 for each treatment). TBARS values are expressed as micromoles of malondialdehyde (MDA) per 100 g of meat ( $\mu\text{mol MDA}/100\text{ g}$ ). Data are presented as the mean ± SD. Significant differences ( $p < 0.05$ ) are represented by different letters over the column. T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1%; and T<sub>5</sub> - *S. platensis* 1.5%.

### Fatty acid profile in broiler meat

The dietary treatments effect on the composition of saturated

fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFAs) in the meat is shown in Tables 4 and 5. A tendency of breast meat SFA (38.47 and 37.99 for T<sub>2</sub>

and T<sub>3</sub>, respectively) to be higher from *M. oleifera* added birds was found, whereas *S. platensis* added birds had a lower SFA (37.22 and 35.46 for T<sub>4</sub> and T<sub>5</sub>, respectively) composition. Eicosatrienoic

**Table 4:** Effect of *M. oleifera* and *S. platensis* as natural feed additives on fatty acid profile of broiler breast meat (g/100 g fatty acids).

Parameters (g/100 g)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
Myristic acid	0.43 ± 0.12	0.50 ± 0.31	0.58 ± 0.10	0.56 ± 0.55	0.65 ± 0.76
Palmitic acid	25.75 ± 1.55	25.24 ± 1.64	29.45 ± 1.48	24.33 ± 1.04	27.44 ± 1.35
Stearic acid	11.29 ± 1.69	12.58 ± 0.95	7.87 ± 1.01	12.24 ± 1.83	7.05 ± 0.88
Arachidic acid	0.03 ± 0.11	0.16 ± 0.56	0.09 ± 0.02	0.09 ± 0.12	0.31 ± 0.28
Myristoleic acid	0.03 ± 0.20	0.11 ± 0.56	0.03 ± 0.21	0.18 ± 0.21	0.16 ± 0.34
Palmitoleic acid	5.23 ± 0.37	3.61 ± 0.57	4.84 ± 0.17	3.14 ± 0.47	4.59 ± 0.24
Oleic acid	36.87 ± 0.91	33.64 ± 0.46	39.39 ± 0.43	36.30 ± 0.23	40.08 ± 0.74
Eicosenoic acid	0.09 ± 0.17	0.19 ± 0.22	0.31 ± 0.35	0.10 ± 0.11	0.25 ± 0.22
Eicosadienoic acid	0.03 ± 0.06	0.33 ± 0.69	0.10 ± 0.05	0.32 ± 0.45	1.65 ± 0.31
Eicosatrienoic acid	0.36 ± 0.03	0.50 ± 0.11	0.42 ± 0.03	0.40 ± 0.08	0.52 ± 0.04
Linolenic acid	0.43 ± 0.21	0.52 ± 0.13	0.29 ± 0.41	0.43 ± 0.14	0.64 ± 0.22
Eicosapentaenoic acid	ND	0.04 ± 0.34	0.02 ± 0.24	0.03 ± 0.41	0.05 ± 0.22
Docosahexaenoic acid	ND	0.06 ± 0.18	0.04 ± 0.11	0.07 ± 0.14	0.09 ± 0.14
Linoleic acid	18.06 ± 0.56	20.52 ± 0.51	15.91 ± 0.21	18.94 ± 0.09	16.41 ± 0.41
Arachidonic acid	1.19 ± 0.34	1.50 ± 0.15	2.02 ± 0.07	1.75 ± 0.23	1.81 ± 0.14
ΣSFA	37.50 ± 0.05	38.47 ± 0.33	37.99 ± 0.41	37.22 ± 0.36	35.46 ± 0.33
ΣMUFA	42.22 ± 0.33	37.54 ± 0.44	44.57 ± 0.21	39.73 ± 0.39	45.08 ± 0.57
ΣPUFA	20.99 ± 0.04	23.33 ± 0.24	18.21 ± 0.36	21.91 ± 0.28	21.17 ± 0.25
Σn - 6	19.25 ± 0.32	22.02 ± 0.18	17.92 ± 0.26	20.69 ± 0.15	18.22 ± 0.09

Saturated Fatty Acids (SFA); Mono Unsaturated Fatty Acids (MUFA); Poly Unsaturated Fatty Acids (PUFA) ); T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1%; and T<sub>5</sub> - *S. platensis* 1.5%. The values are means ± SEM (n=4).

**Table 5:** Effect of *M. oleifera* and *S. platensis* as natural feed additives on fatty acid profile of broiler thigh meat (g/100 g fatty acids).

Parameters (g/100 g)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
Myristic acid	0.46 ± 0.67	0.71 ± 0.31	0.56 ± 0.10	0.60 ± 0.55	0.52 ± 0.76
Palmitic acid	23.05 ± 1.55	26.97 ± 1.64	28.03 ± 1.48	25.61 ± 1.04	23.86 ± 1.35
Stearic acid	11.56 ± 1.69	14.53 ± 0.95	13.71 ± 1.01	11.53 ± 1.83	13.02 ± 0.88
Arachidic acid	0.05 ± 0.20	0.32 ± 0.56	0.29 ± 0.17	0.26 ± 0.12	0.32 ± 0.28
Myristoleic acid	0.06 ± 0.37	0.10 ± 0.56	0.18 ± 0.43	0.11 ± 0.21	0.10 ± 0.34
Palmitoleic acid	7.00 ± 0.91	8.59 ± 0.57	8.15 ± 0.35	7.52 ± 0.47	6.42 ± 0.24
Oleic acid	35.10 ± 0.81	35.95 ± 0.46	36.96 ± 0.41	36.38 ± 0.23	35.66 ± 0.74
Eicosenoic acid	0.03 ± 0.11	0.12 ± 0.22	0.06 ± 0.31	0.04 ± 0.11	0.05 ± 0.22
Eicosadienoic acid	ND	0.05 ± 0.69	0.15 ± 0.12	0.04 ± 0.11	0.12 ± 0.31
Eicosatrienoic acid	0.15 ± 0.17	0.48 ± 0.11	0.47 ± 0.46	0.34 ± 0.45	0.39 ± 0.22
Linolenic acid	0.37 ± 0.64	0.53 ± 0.13	0.38 ± 0.21	0.46 ± 0.05	0.70 ± 0.41
Eicosapentaenoic acid	ND	0.05 ± 0.34	0.03 ± 0.11	0.05 ± 0.08	0.04 ± 0.25
Docosahexaenoic acid	0.02 ± 0.14	0.07 ± 0.18	0.03 ± 0.14	0.03 ± 0.11	0.08 ± 0.16
Linoleic acid	14.68 ± 0.03	18.91 ± 0.51	16.97 ± 0.31	17.19 ± 0.15	18.74 ± 0.11
Arachidonic acid	0.05 ± 0.23	0.08 ± 0.14	0.06 ± 0.24	0.09 ± 0.24	0.11 ± 0.31
ΣSFA	32.74 ± 0.06	42.52 ± 0.15	35.30 ± 0.21	37.99 ± 0.14	37.76 ± 0.09
ΣMUFA	42.25 ± 0.21	44.72 ± 0.33	45.35 ± 0.07	44.02 ± 0.09	42.22 ± 0.57
ΣPUFA	15.28 ± 0.34	20.08 ± 0.44	18.00 ± 0.41	18.20 ± 0.23	20.20 ± 0.33
Σn - 6	14.73 ± 0.05	18.99 ± 0.18	16.97 ± 0.36	17.28 ± 0.39	18.85 ± 0.19

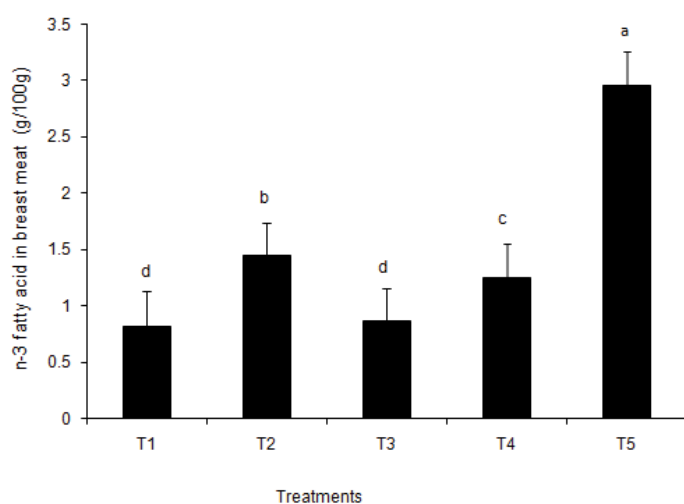
Saturated Fatty Acids (SFA); Mono Unsaturated Fatty Acids (MUFA); Poly Unsaturated Fatty Acids (PUFA) ); T<sub>1</sub> - Control (basal diet); T<sub>2</sub> - *M. oleifera* leaf meal 1%; T<sub>3</sub> - *M. oleifera* leaf meal 1.5%; T<sub>4</sub> - *S. platensis* 1% and T<sub>5</sub> - *S. platensis* 1.5%. The values are means ± SEM (n=4).

acid with values of  $0.50 \pm 0.11$ ,  $0.42 \pm 0.03$ ,  $0.40 \pm 0.08$  and  $0.52 \pm 0.04$  were found higher in the additives groups' meats than the  $T_1$  control group's  $0.36 \pm 0.03$ . Similar trends were observed in case of both EPA and DHA. Linolenic acid was found higher in  $T_2$  group both breast and thigh meat. The sum of omega-3 fatty acids content in breast meat was found higher in all additives groups except  $T_3$  and control ( $T_1$ ) (Figure 3). In Table 5, for the thigh meat, it was observed that sum of SFA differ in  $T_2$ - $T_5$  groups whereas sum of MUFA slightly differ in comparison to the control,  $T_1$ . In thigh meat,  $T_4$  group also decreased stearic acid content whereas arachidic and oleic acid contents were increased in  $T_2$  groups, compared to that of the  $T_3$  group. The sums of n-3 fatty acid in broiler thigh meat were observed significantly higher in all dietary additive groups (Figure 4) compared to control.

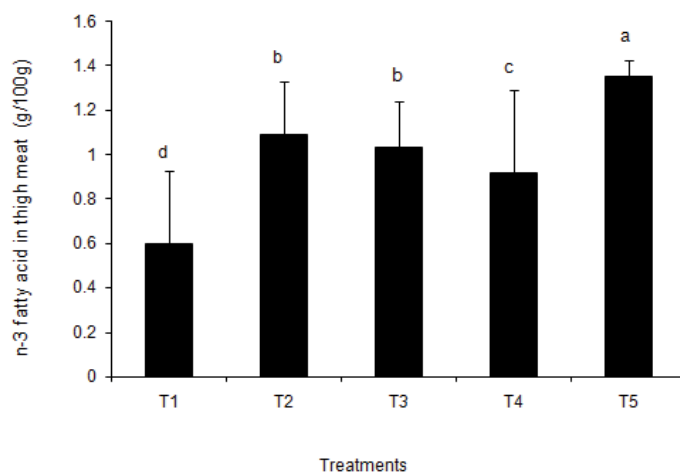
## DISCUSSION

Medicinal plants contain a wide range of bioactive components and can play important role in the biological reactions through their specific mode of action. *M. oleifera* and *S. platensis* has been recognized as containing a great number of bioactive compounds [3,4,20]. The dried leaves of *M. oleifera* are a great source of polyphenol compounds, such as flavonoids and phenolic acids. A number of animal studies documented the effects of *M. oleifera* leaves in protecting against cardiovascular disease, diabetes, hypertension and others, due the actions of the bioactive components in preventing lipid accumulation, reducing insulin resistance and inflammation [9,21]. Besides *M. oleifera*, the benefits of *S. platensis* has already been evaluated through a number of studies on the growth performances and carcass percentage of broiler chickens [20,22,23]. There is paucity of scientific reports on the antioxidant properties and fatty acid profile of broiler meat after addition of *M. oleifera* and *S. platensis* as feed additives.

Commonly, BW is used to monitor animals' nutritional status and growth [24]. Nkukwana et al. [25] described the fact that broiler  $\Delta$ BW and ADG were increased in birds fed diets with *M. oleifera*. It is possible that depending on the amount of *M. oleifera* addition, the concentration of anti-nutritional factors in these diets could have been relatively low to have a growth depression effect [25].



**Figure 3:** Omega 3 fatty acid in broiler breast meat after addition of *M. oleifera* and *S. platensis* on the diet. Data are presented as the mean  $\pm$  SD. Significant differences ( $p < 0.05$ ) are represented by different letters over the column.  $T_1$  - Control (basal diet);  $T_2$  - *M. oleifera* leaf meal 1%;  $T_3$  - *M. oleifera* leaf meal 1.5%;  $T_4$  - *S. platensis* 1%; and  $T_5$  - *S. platensis* 1.5%.



**Figure 4:** Omega 3 fatty acid in broiler thigh meat after addition of *M. oleifera* and *S. platensis* on the diet. Data are presented as the mean  $\pm$  SD. Significant differences ( $p < 0.05$ ) are represented by different letters over the column.  $T_1$  - Control (basal diet);  $T_2$  - *M. oleifera* leaf meal 1%;  $T_3$  - *M. oleifera* leaf meal 1.5%;  $T_4$  - *S. platensis* 1%; and  $T_5$  - *S. platensis* 1.5%.

In contrast, numerous studies have shown that *S. platensis* dietary addition can improve the growth performance of poultry [18,26]. In this present study, a 1.5% addition ( $T_5$ ) of *S. platensis* recorded a higher BW compared to the 1% addition ( $T_4$ ). It should be mentioned that a low FCR means chickens gain more weight when eating less feed. In our study significantly lowest FCR was observed in the  $T_2$  group at the finisher period in d 35 but, when considering the  $T_5$  group, there were no significant differences between the groups ( $p < 0.05$ ). The level of feed consumption is a basic and important factor that determines the rate of growth and body composition achieved by animals throughout their life cycles. The increase in FI as well as in weight gain could be due to the experimental diets' reduced palatability, following the  $T_2$  and  $T_5$  group. These results are in agreement with those of previous researchers, who recorded nonsignificant effects of dietary *S. platensis* addition on performance parameters. Furthermore, Bellof and Alarcon [27] reported that under organic farming, dietary *S. platensis* supplementation (5 g/kg or 10 g/kg) significantly improved broilers' growth and carcass performance parameters. Contradictory results are possibly due to the different inclusion levels and quality of *M. oleifera* and *S. platensis* in the present trials. In summary, it can be stated that natural additives have great potential, with the right combination and doses.

The crude protein content in feed additives group was increased and decreased crude fat content in thigh meat except *M. oleifera* 1.5% group in breast meat. This finding was consistent with the studies by Sarker et al. [19] and Hossain et al. [28], they reported crude protein content in breast meat was increased in the addition of natural herbs *Alisma canaliculatum* with probiotics. Abdominal fat were slightly decreased in feed additives groups compared to control. The health condition of experimental birds did not show any difference on addition of *M. oleifera* and *S. platensis* in diets. Du et al. [29] reported that dietary addition of *M. oleifera* and *S. platensis* might increase immunity of broilers.

Lipid oxidation causes the loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce shelf life. Enhancing the antioxidant capability in muscle tends to improve meat quality and extend shelf life [7]. TBARS values of the additives groups were

significantly different from the T<sub>1</sub> group. The result of the current study is consistent with those of Aksu et al. [30]. *M. oleifera* leaves and *S. platensis* were revealed to have significantly high oxidative stabilities, indicating the presence of natural antioxidants [31] and phytochemicals. Predominantly, *M. oleifera* leaves contain flavonols, quercetin, and kaempferol in their 3-O-glycoside forms, which are well-known compounds with radical scavenging properties [32]. Surprisingly, in the second week, breast and thigh meat from birds fed T<sub>1</sub> diets had found the highest TBARS value (p<0.05), about two fold higher than the first week's TBARS value. On the other hand, *S. platensis* has antioxidants compounds, such as tocopherol, butylated hydroxyanisole and carotene, which possess antioxidant properties beneficial in the prevention of cancer and cardiovascular diseases [6]. These antioxidant compounds also assist in the prevention of meat degradation by oxidation. Several researchers concluded that the percentage of lipid oxidation inhibition observed in the meat from *M. oleifera* and *S. platensis* supplemented animals stimulated the defense mechanism in the animals' systems to prevent the formation of excessive free radicals [6,33]. The results from the TBARS test provided evidence that the additives groups could improve the oxidative stability in broiler meat. These 2 substances, *M. oleifera* and *S. platensis*, may intercept and neutralize free radicals, preventing the oxidation process propagation.

Consumers are becoming more conscious of their health and are particularly interested in reducing the risk of cardiovascular and other diseases by consuming more PUFA, especially omega-3 fatty acids [34]. Due to enrichment of energy and fatty acid pattern of poultry, the composition of meat can be modified by dietary manipulation, in which fat and oil sources are important dietary ingredients [35]. Therefore, the fatty acid composition of meat products is an important parameter of meat quality. Noteworthy is the relatively high PUFA content observed in meat from birds fed *M. oleifera* and *S. platensis* added diets both breast and thigh meat. The most important omega-3 fatty acids in human nutrition are eicosapentaenoic acid (EPA) (20:5n-3), DHA (22:6n-3), and  $\alpha$ -linolenic acid (ALA; 18:3n3), which serve as precursors for EPA and DHA synthesis [36]. However, in the present study, EPA was found to be lower than DHA in all the dietary groups. Herein, DHA was found to be higher in the T<sub>2</sub> and T<sub>5</sub> groups of breast meat, with values of 0.06  $\pm$  0.18 and 0.09  $\pm$  0.14, respectively. The addition of *M. oleifera* and *S. platensis* may have the potential to improve the fatty acids due to the phenolic compounds and organic acids because they exert the antioxidant potential and consequently prevent PUFA oxidation [34]. The antihyperlipidemic activity due to the phenolic acids of *M. oleifera* and *S. platensis* as well as medicinal plants might be attributed in the improvement of the fatty acid content of broiler meat through the lipid homeostasis and fatty acid synthase enzyme [36].

The fatty acid profile of the thigh meat was enriched in PUFA, especially eicosapentaenoic acid and docosahexaenoic acid. The elevation of PUFA in the both breast and thigh meat of additives group mainly upgradation of linolenic and linoleic acid. However, in breast meat T<sub>3</sub> group the content of linoleic acid was found lower compared to control group. Reduction of linoleic acid content led to decreased levels of PUFA. Linoleic acid is an essential fatty acid that acts as the primary precursor of n6 PUFAs [37]. Linoleic acid in the diet can suppress lymphocyte proliferation in rats [38]; and linolenic acid can prevent cardiovascular disease [39], which

all could be beneficial for human health through consumption of broiler meat. A total of n3 fatty acid content in broiler breast meat was found to be higher than thigh meat. The fatty acid composition can be different between these different muscle tissues possibly due to their different phospholipid contents [40]. The n3 PUFA of fatty acids are important to human health since they are precursors for the biosynthesis of eicosanoids, which are considered as an important bio-regulator of many cellular metabolic processes, blood pressure and clotting, tissue growth and immune system modulation [41]. The enhancement of fatty acids especially omega 3 fatty acids in thigh meat after addition of feed additives might be due to the dietary active components. Although this trend was supportive in thigh meat perfectly as dose-response relationship, which was not followed for breast meat in T<sub>3</sub> i.e., *M. oleifera* 1.5% group. Actually, many factors are responsible for the mechanism of PUFA modulation and also lipid oxidation. These results provide valuable information on screening of two additives for enriching broiler meat with n3 fatty acids and reducing oxidation using *M. oleifera* and *S. platensis*.

## CONCLUSION

The dietary inclusion of *M. oleifera* leaf 1% and *S. platensis* 1.5% meal in the starter and finisher diets did not alter the diets' nutrient compositions. The growth performance and yield results indicate that T<sub>2</sub> group significantly improved the feed utilization efficiency in the broiler chickens used in this study. The current study results demonstrated that the addition of *M. oleifera* leaf meal and *S. platensis* at the levels of 1.5% of the birds' reduced TBARS value with influenced the fatty acid composition in broiler meat. The tendency to have lower TBARS in meats from the additives groups' birds was a clear indication that it is capable of reducing the lipid oxidation in broiler meat. Furthermore, additives groups increased the  $\omega$ 3 fatty acid in breast and thigh meat. Therefore, *M. oleifera* leaf meal and *S. platensis* could be promising functional ingredients in broiler chicken meat production.

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## CONFLICTS OF INTERESTS

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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