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RESEARCH ARTICLE

Impact of Moringa oleifera and Nigella sativa Aqueous Extracts on Growth, Digestibility, Oxidative Stress, Gut Histology, and Cecal Microbiota in Broilers Under Heat Stress

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ABSTRACT

This study evaluated whether aqueous extracts of *Moringa oleifera* (MO) leaves and *Nigella sativa* (NS) seeds could mitigate the negative effects of heat stress in broiler chickens. A total of 240 day-old Hubbard chicks were randomly allocated to six treatments: a thermoneutral control (NC), a heat-stressed control (PC), three MO–NS combinations at the rate of 110mg/kg (MO40NS110), 75mg/kg (MO75NS75), and 40mg/kg (MO110NS40), and a heat-stressed group supplemented with vitamin C (250 mg/kg) as a reference antioxidant. Birds in the heat-stress treatments were exposed to 34 ± 1°C for 8 hours daily from day 22 to day 35, and all herbal preparations were administered through drinking water. Performance parameters—including body-weight gain, feed intake, and feed conversion ratio—along with nutrient digestibility and intestinal morphology were assessed. Broilers receiving the MO75NS75 mixture or vitamin C exhibited significant improvements ($P<0.05$) in growth and feed efficiency compared with the PC group. The MO75NS75 treatment yielded one of the highest final body weights, an improved FCR, and better crude-protein digestibility, values comparable to those of thermoneutral birds. Histological evaluations showed increased villus height and surface area in birds supplemented with MO and NS, with the greatest enhancements observed in the MO75NS75 group. Additionally, serum malondialdehyde levels—an indicator of oxidative stress—were markedly lower in both the MO75NS75 and vitamin C groups relative to the PC group. Overall, the findings suggest that combining *Moringa oleifera* and *Nigella sativa* extracts exerts a synergistic protective effect against heat stress by improving growth performance, antioxidant status, nutrient utilization, and intestinal health in broilers.

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INTRODUCTION

Poultry production has been frequently challenged with heat stress in tropical and subtropical zones of the world affecting growth, health and overall wellbeing in broilers (Abudabos *et al*., 2013; Rahman *et al*., 2017; Hafeez *et al*., 2021). Further, thermal stress leads to reduced feed consumption, nutrients absorption and increased oxidative stress resulting in economic losses in broilers (Zia ur Rehman *et al*., 2018; Khan *et al*., 2023). These situation demands for effective strategies to mitigate heat stress in modern poultry production.

Phytogenic feed additives, among the available mitigation strategies have received increased attention as safe and natural approach to synthetic antioxidants (Hafeez *et al*., 2020). *Moringa oleifera* and *Nigella sativa* are the two important botanicals in poultry production, which are rich in bioactive compounds and have well documented anti-inflammatory, antimicrobial and antioxidant effects (Haq *et al*., 2020; Mehwish *et al*., 2022; Khan *et al*., 2022; Imtiaz *et al*., 2023). Previous studies have reported that *M. oleifera* supplementation in broilers diets improved feed efficiency, reduced oxidative stress and maintained gut integrity (Ullah *et al*., 2022). On the other hand, *N. sativa* supplementation enhanced growth efficiency, heightened immune response and reduced lipid peroxidation during heat stress (Talebi *et al*., 2021).

Despite the well-documented individual benefits of both plants, limited work has examined their combined use under heat stress conditions. In particular, the potential synergistic effects of *N. sativa* and *M. oleifera* on intestinal morphology, growth performance, nutrient utilization, oxidative status, and microbial balance remain poorly explored. Therefore, the present study investigated how different concentrations of *N. sativa* seed extract and *M. oleifera* leaf extract, administered through drinking water, affect broilers subjected to cyclic heat stress. The study evaluated intestinal histopathology, growth performance (body weight gain, feed intake, feed conversion ratio), nutrient digestibility, serum MDA levels, and cecal microbiota.

MATERIALS AND METHODS

Rearing and Management Practices: A total of 240 day old Hubbard broilers were randomly allocated to six treatments and five replicates. Birds were housed in well-ventilated wire cages with standard feeding and watering equipment. From day 1-7, broilers were brooded at average 33°C and gradually reduced to 24°C. Sand dust litter was used and regularly replaced to maintain hygienic conditions. Birds had free access to water and feed. A lighting schedule of 23 h was used for first week followed by 20h for better growth performance. Mash diets were prepared, formulated according to the NRC (1994) recommendation as presented in Table 1.

Table 1: Broiler Diet Formulations and Nutrient Composition (NRC, 1994)

Ingredients (%)	Starter (1–21 d)	Finisher (22–35 d)
Maize	51.50	54.50
Soybean meal (44%)	38.50	34.50
Vegetable oil	2.70	3.10
Dicalcium phosphate	1.75	1.55
Limestone	1.05	1.00
DL-Methionine	0.27	0.22
L-Lysine HCl	0.22	0.16
Salt	0.30	0.30
Vitamin-mineral premix[1]	0.50	0.50
Total	100	100
Nutrient	Starter (1–21 d)	Finisher (22–35 d)
Metabolizable Energy (kcal/kg)	3020	3120
Crude Protein (%)	22.2	19.8
Calcium (%)	1.02	0.88
Available Phosphorus (%)	0.46	0.41
Lysine (%)	1.18	1.04
Methionine + Cystine (%)	0.88	0.79

[1] Vitamin-mineral premix provides per kg of diet: Vitamin A (11,500 IU), D3 (1,900 IU), E (12mg), K (2.8mg), B1 (1.8mg), B2 (5.5mg), B6 (2.8mg), B12 (0.018mg), niacin (38mg), folic acid (0.95mg), pantothenic acid (9.5mg), biotin (0.09mg), choline chloride (480mg), iron (58mg), zinc (58mg), manganese (58mg), copper (4.8mg), iodine (0.95mg), selenium (0.18mg).

Experimental Design and Feeding Regimen: From day 1 to day 14, all broilers were reared under standard husbandry conditions. Beginning on day 15 and continuing until day 35, the birds were subjected to cyclic heat stress and their designated experimental treatments. Six treatment groups were established as follows:
(1) **NC (Negative Control):** birds maintained under thermoneutral conditions and fed a conventional corn–soybean meal diet without additives; (2) **PC (Positive Control):** birds exposed to heat stress but receiving no *Moringa oleifera*, *Nigella sativa*, antibiotics, or antioxidants in drinking water; (3) **MO40NS110:** heat-stressed birds provided with drinking water containing 40mL/L *Moringa oleifera* leaf extract and 110mL/L *Nigella sativa* seed extract; (4) **MO75NS75:** heat-stressed birds receiving 75mL/L *Moringa oleifera* and 75mL/L *Nigella sativa* extracts; (5) **MO110NS40:** heat-stressed birds given 110mL/L *Moringa oleifera* and 40mL/L *Nigella sativa*; (6) **Antioxidant group:** heat-stressed birds supplemented with 250 mg/L vitamin C powder via drinking water.

Heat Stress Production and Management: After equal conditions of brooding period, birds were subjected to heat stress during the period of day 15 to 35 in all the experimental groups except NC. Birds were exposed to cyclic heat stress for a 34±1°C for 8 hours daily (09:00–17:00h) on daily basis. Temperature regulation was maintained using automated heating systems and controlled ventilation, and relative humidity was kept at 55–65%, reflecting typical summer housing conditions for broilers.

Plant Extracts Preparation: Aqueous extract of *M. oleifera* leaves and *N. sativa* seeds were prepared following a standard protocol. Both the contents were shade-dried and powdered. A preliminary antibacterial screening against *Escherichia coli* was conducted at 5-10% levels. Both the plants at 10% concentration exhibited the strongest antibacterial activity and were further used in this experiment. A stock solution was prepared by taking 50 g of powder in 500mL of distilled water after filtration. For the feeding trials, extract was supplemented in drinking water as mentioned above.

Growth performance: Feed intake, weight gain and feed conversion ratio (FCR) were calculated on weekly basis during the whole experimental period.

Determination of Apparent Nutrient Digestibility: On day 31, three birds per replicate, were randomly chosen and moved to individual metabolic cages. Titanium dioxide (TiO_2) was added to the diets at 0.5% to serve as an inert digestibility marker. At the conclusion of the collection period, birds were humanely euthanized, and ileal digesta were sampled from the region between Meckel's diverticulum and approximately 2 cm anterior to the ileocecal junction. Digesta from birds within each replicate were pooled, immediately frozen, and later freeze-dried for chemical analyses. Pooling produced one composite sample per replicate (n = 5 per treatment), making the pen replicate the experimental unit for statistical evaluation and reducing the degrees of freedom relative to using individual birds.

Feed and digesta samples were analyzed for crude protein (CP) using the Kjeldahl procedure, and ether extract (EE) using Soxhlet extraction. Gross energy was determined with an adiabatic bomb calorimeter. Titanium dioxide concentrations in both feed and digesta were measured using a UV–visible spectrophotometric method as described by Imtiaz *et al*. (2023).

Apparent ileal digestibility (AID) coefficients were calculated using the standard marker-to-nutrient ratio approach (formula in short form, with all concentrations expressed on a dry matter basis):

$$AID\ (\%) = 100 \times \left(1 - \frac{TiO_{2,feed} \times Nutrient_{digesta}}{TiO_{2,digesta} \times Nutrient_{feed}}\right)$$

Intestinal Histology: On day 35, tissue samples from the duodenum, jejunum, and ileum were collected to evaluate intestinal histomorphometry following the procedures of Chand *et al*. (2020). Two birds per replicate with body weights closest to the pen mean were randomly selected, yielding 10 birds per treatment (n = 60). Approximately 2-cm tissue segments were excised from standardized anatomical locations: (i) the duodenum, immediately distal to the gizzard; (ii) the jejunum, between the duodenal loop and Meckel's diverticulum; and (iii) the ileum, ~2 cm anterior to the ileocecal junction. Intestinal tissues were rinsed with 0.09% physiological buffer solution and fixed in 10% neutral-buffered formalin. Tissues were then dehydrated through ethanol solution, cleared in xylene, and finally embedded in paraffin wax. About 5 µm thickness sections of the tissues were obtained with the help of microtome, mounted on glass slides and stained with hematoxylin and eosin (H&E). Tissues were evaluated microscopically at 40x magnification and captured images using digital camera. Measurements were obtained with the help of ImageJ software.

Determination of MDA: On day 35 of the experiment, three birds from each replicate were humanely euthanized by cervical dislocation following a short period of feed withdrawal. Blood was collected from the jugular vein into plain tubes and allowed to clot at room temperature. The clotted blood was then centrifuged at 3,000 × g for 10 minutes at 4 °C to obtain serum. Serum malondialdehyde (MDA), an indicator of lipid peroxidation, was measured using the thiobarbituric acid reactive substances (TBARS) assay according to Ohkawa *et al*. (1979). In brief, serum samples were mixed with thiobarbituric acid under acidic conditions and heated at 95–100 °C for one hour. After cooling, the mixture was centrifuged to clarify the solution, and the absorbance of the resulting pink MDA–TBA_2 complex was read at 532 nm using a spectrophotometer. MDA concentrations were calculated from a standard curve prepared with 1,1,3,3-tetramethoxypropane (TMP), which hydrolyzes to form MDA. Results are reported in nmol/mL of serum, with a detection limit of approximately 0.1 nmol/mL.

Cecal microbiota enumeration: On day 35, three birds from each replicate pen (n = 5 replicates per treatment) were humanely euthanized by cervical dislocation following a brief feed withdrawal. The abdominal cavity was opened under aseptic conditions, and the ceca were carefully removed. Each cecum was tied at both ends, kept on ice, and transported immediately to the laboratory. Under sterile conditions, cecal contents from the three birds within each replicate were pooled to create a single composite sample per replicate (final n = 5 composite samples per treatment). Pooling at the replicate level ensured that the pen served as the experimental unit for microbiological analyses, which was accounted for in the statistical evaluation (see Statistical Analysis).

For each composite sample, approximately 1.0g of pooled cecal content was added to 9.0mL of sterile buffered peptone water (BPW) to prepare a 10^{-1} dilution and mixed thoroughly by vortexing for 60 seconds. Ten-fold serial dilutions were subsequently prepared in sterile peptone water. Appropriate dilutions were plated in duplicate on selective media as follows:

***Escherichia coli*:** Dilutions were plated on MacConkey agar (or TBX/MacConkey-based medium) and incubated aerobically at 37°C for 24 hours. Colonies showing typical lactose-fermenting morphology were counted, and representative colonies were confirmed using biochemical tests (IMViC or API 20E) when necessary. Results were expressed as colony-forming units per gram of wet cecal content (CFU/g).

***Lactobacillus* spp.:** Dilutions were plated on de Man, Rogosa, and Sharpe (MRS) agar and incubated anaerobically in an anaerobic jar with a gas pack at 37 °C for 48 hours. Colonies with characteristic morphology were counted, and representative colonies were Gram-stained and catalase-tested to confirm presumptive Lactobacillus.

***Salmonella* spp.:** Samples were processed according to standard screening protocols. Pre-enrichment was performed in BPW at 37°C for 18–24 hours, followed by selective enrichment in Rappaport–Vassiliadis and/or tetrathionate broths at 41.5 °C for 24 hours. Samples were then plated onto XLD (xylose-lysine-deoxycholate) agar following ISO 6579-1:2017 guidelines. Suspect colonies were confirmed using biochemical tests and serology. Salmonella counts were reported as CFU/g when colonies were detected. If direct plating yielded no colonies but enrichment was positive, results were recorded as presence/absence, and counts were noted as below the detection limit.

Colony counts were performed on plates containing 30–300 colonies and converted to CFU/g of the original cecal content by multiplying by the appropriate dilution factor. For statistical analysis, bacterial counts were $\log_{10}$-transformed to normalize the data distribution.

Statistical analysis: Data were analyzed using appropriate mixed-model statistical procedures. For growth performance parameters (body weight, feed intake, and feed conversion ratio), which were recorded weekly, a repeated-measures ANOVA was employed. In this model, time (week) was treated as the within-subject factor, dietary treatment as the between-subject factor, and pen (n = 5 per treatment) served as the experimental unit and random effect to account for correlations among repeated measurements within the same pen. For nutrient digestibility, blood biochemical indices, cecal microbiota, and histological traits, the individual bird was considered the experimental unit, and data were analyzed using a one-way ANOVA with treatment as the main effect. Prior to analysis, model assumptions for normality and homogeneity of variances were verified using the Shapiro–Wilk test and Levene's test, respectively. When a significant main effect of treatment was detected ($p < 0.05$), pairwise comparisons were performed using Tukey's post hoc test. All statistical analyses were conducted in SPSS (version 21). The sample size of five replicate pens per treatment was determined based on a power analysis (α = 0.05, power = 0.80), which indicated that this number of replicates was sufficient to detect biologically meaningful differences among treatments. Data are reported as mean ± standard error of the mean (SEM).

RESULTS

Table 2 summarizes weekly performance of heat-stressed broilers received different combination of *M. oleifera* and *N. sativa* seed extracts via drinking water. At the end of second week, no significance difference was observed among treatments in term of weekly feed intake, weight gain and FCR. However, broilers receiving MO75NS75 dose resulted in numerically higher weight gain and better FCR compared to the other treatments.

Table 2: Response of heat-stressed broilers in terms of weight gain, feed intake, and FCR offered with different levels of *Nigella sativa* seed and *Moringa oleifera* leaf extract in drinking water at different periods of production

Groups	WFI (g)	WWG (g)	FCR
Day7-14			
NC	295	252	1.17
PC	297	253	1.17
MO40NS110	286	246	1.16
MO75NS75	298	260	1.15
MO110NS40	293	252	1.16
Antioxidant	296	254	1.17
SEM	2.40	1.90	0.003
p. Value	0.827	0.541	0.108
Day 15-21			
NC	736	432	1.70^{a}
PC	721	433	1.69^{a}
MO40NS110	709	438	1.67^{ab}
MO75NS75	736	446	1.62^{c}
MO110NS40	717	434	1.65^{bc}
Antioxidant	710	434	1.64^{bc}
SEM	7.25	4.03	0.01
p. Value	0.85	0.95	0.002
7-21 day			
NC	1031	684	1.51^{a}
PC	1018	686	1.48^{ab}
MO40NS110	995	685	1.45^{c}
MO75NS75	1034	706	1.47^{bc}
MO110NS40	1009	686	1.47^{bc}
Antioxidant	1006	688	1.46^{bc}
SEM	9.12	5.59	0.005
p. Value	0.860	0.902	0.005
Day 22-28			
NC	836^{a}	490^{a}	1.71^{b}
PC	733^{b}	395^{c}	1.86^{a}
MO40NS110	778^{ab}	438^{bc}	1.78^{ab}
MO75NS75	822^{a}	474^{a}	1.73^{b}
MO110NS40	781^{b}	444^{ab}	1.76^{ab}
Antioxidant	838^{a}	478^{a}	1.75^{b}
SEM	11.8	8.46	0.01
p. Value	0.031	<0.001	0.006
Day 29-35			
NC	1185^{a}	563^{a}	2.10^{c}
PC	907^{c}	409^{d}	2.22^{a}
MO40NS110	1079^{b}	494^{c}	2.18^{ab}
MO75NS75	1078^{ab}	522^{b}	2.06^{c}
MO110NS40	1100^{b}	514^{bc}	2.14^{bc}
Antioxidant	1100^{ab}	523^{b}	2.11^{bc}
SEM	21.9	11.8	0.015
p. Value	<0.001	<0.001	0.008

Means in each column with the same superscript are not significantly different at 0.05. NC = Standard group fed corn soybean meal diet; PC = Heat stress challenged group without offering any *Moringa oleifera* leaf and *Nigella sativa* seed extract and any Antibiotic in drinking water; MO40NS110= Heat stress challenged group offered with 40ml *Moringa oleifera* leaf and 110ml *Nigella sativa* seed extract per liter of drinking water; MO75NS75= Heat stress challenged group offered with 75ml *Moringa oleifera* leaf and 75ml *Nigella sativa* seed extract per liter of drinking water; MO110NS40= Heat stress challenged group offered with 110ml *Moringa oleifera* leaf and 40ml *Nigella sativa* seed extract per liter of drinking water; Antioxidant= *Heat* challenged group supplemented with 250 mg/kg vitamin C; WFI= Weekly feed intake: WWG= Weekly weight gain; FCR= Feed conversion ratio.

Table 3: Response of heat stress challenged broilers in terms of weekly water intake during various weeks offered with different levels of *Nigella sativa* seed and *Moringa oleifera* leaf extract in drinking water during the whole (d7-35) of the experiment

Groups	WI-I (mL)	WI-II (mL)	WI-III (mL)	WI-IV (mL)
NC	494	1234	1403	1988
PC	502	1221	1473	1822
MO40NS110	485	1200	1487	2057
MO75NS75	506	1249	1510	1985
MO110NS40	506	1238	1441	1996
Antioxidant	503	1192	1500	1985
SEM	4.96	14.1	17.7	28.4
p. Value	0.87	0.56	0.28	0.80

Means in each column with the same superscript are not significantly different at 0.05. NC = Standard group fed corn soybean meal diet; PC = Heat stress challenged group without offering any *Moringa oleifera* leaf and *Nigella sativa* seed extract and any Antibiotic in drinking water; MO40NS110= Heat stress challenged group offered with 40mL *Moringa oleifera* leaf and 110mL *Nigella sativa* seed extract per liter of drinking water; MO75NS75= Heat stress challenged group offered with 75mL *Moringa oleifera* leaf and 75mL *Nigella sativa* seed extract per liter of drinking water; MO110NS40= Heat stress challenged group offered with 110mL *Moringa oleifera* leaf and 40ml *Nigella sativa* seed extract per liter of drinking water; Antioxidant control group= Heat stress challenged group offered with SB Asper-C antioxidant in drinking water; WI-I= Water intake during first week of experiment; WI-II= Water intake during second week of experiment; WI-III= Water intake during third week of experiment; WI-IV= Water intake during fourth week of experiment.

During the period of supplementation of day 15-21 days, better FCR was found in MO40NS110 group followed by antioxidant group. No significant difference was observed in weekly feed consumption and weight gain between the control and the treatment groups.

During the period of 22-28 days, birds received antioxidant and MO75NS75 supplemented groups achieved significantly ($P<0.01$) higher weight gain compared to PC group. Similarly, feed intake and FCR were significantly ($P<0.03$) higher in the NC, antioxidant and MO75NS75 groups. At the end of the study (28-35 days), treatments supplementation resulted in higher weight gain in the NC group, while the MO75NS75 and antioxidant group showed comparable results ($P<0.01$) compared to PC and other groups. In the same fashion, FCR was the best in the MO75NS75 group, while PC group showed the poorest FCR.

In the last week (day 28-35) of the experiment, feed intake and weight gain was significantly ($P<0.01$) higher in the NC group, while antioxidant and MO75NS75 groups achieved comparable results. Feed conversion ratio was poorest in the PC group and was most favorable in the MO75NS75 group.

As given in Table 4, crude protein digestibility was significantly ($P<0.01$) higher in the NC group followed by MO75NS75 and Antioxidant groups, exhibiting significantly greater values compared to PC MO75NS75 and antioxidant groups. Similar trend was also observed for AME values with the NC group showing the highest value followed by the other treatments while PC group exhibited the lowest AME. The serum MDA level was significantly ($P<0.01$) higher in the PC group compared to the NC group. In broilers, supplemented with MO75NS75 and Antioxidant supplemented groups exhibited MDA concentration comparable to the NC group.

As shown in Table 5, intestinal histology showed significant changes across the groups, exhibiting favorable dimension in the NC group. In contrast, the PC group showed marked deterioration in these parameters indicating compromised absorptive capacity under thermal stress. Significantly (P<0.01) higher duodenal morphological parameters were observed in MO75NS75 among the phytogenic-treated groups. These values were similar to those observed in the Antioxidant group.

Table 4: Effect of *Moringa oleifera* and *Nigella sativa* Extracts on Crude Protein Digestibility, Apparent Metabolizable Energy, and Malondialdehyde (MDA) in *Heat* Challenged Broilers

Groups	CP digestibility (%)	Apparent metabolizable Energy (Kcal/kg)	MDA (n Mol/mL)
NC	69.5^{a}	2970^{a}	6.17^{b}
PC	61.1^{c}	2588^{b}	7.82^{a}
MO40NS110	62.8^{c}	2657^{ab}	7.59^{a}
MO75NS75	67.8^{b}	2941^{a}	6.28^{ab}
MO110NS40	64.0^{c}	2706^{ab}	7.13^{a}
Antioxidant	67.5^{b}	2947^{a}	6.32^{ab}
SEM	6.71	23.45	1.42
p. Value	0.01	0.01	0.01

Means in each column with the same superscript are not significantly different (P<0.05). NC = Standard group fed corn soybean meal diet; PC = *Heat* challenged group without offering any *Moringa oleifera* leaf and *Nigella sativa* seed extract and any Antibiotic in drinking water; MO40NS110= *Heat* challenged group offered with 40mL *Moringa oleifera* leaf and 110mL *Nigella sativa* seed extract per liter of drinking water; MO75NS75= *Heat* challenged group offered with 75mL *Moringa oleifera* leaf and 75mL *Nigella sativa* seed extract per liter of drinking water; MO110NS40= *Heat* challenged group offered with 110mL *Moringa oleifera* leaf and 40mL *Nigella sativa* seed extract per liter of drinking water; Antioxidant= *Heat* challenged group offered with 250mg/kg vitamin C.

Table 5: Intestinal histopathology of heat stress challenged broilers supplemented with different levels of *Nigella sativa* seed and *Moringa oleifera* leaf extract in drinking water during the experiment

Groups	DVL (µm)	DVW (µm)	DCD (µm)	DVSA (×10^{3} µm^{2})	VL/CD
Duodenum					
NC	1308^{a}	160^{a}	159^{d}	657^{a}	8.26^{a}
PC	1101^{d}	132^{c}	228^{a}	455^{d}	4.84^{d}
MO40NS110	1136^{cd}	135^{c}	212^{ab}	483^{cd}	5.36^{cd}
MO75NS75	1219^{b}	151^{ab}	178^{c}	578^{b}	6.86^{b}
MO110NS40	1157^{c}	141^{b}	198^{b}	512^{c}	5.86^{c}
Antioxidant	1254^{ab}	154^{a}	172^{cd}	609^{ab}	7.32^{b}
SEM	13.1	2.03	4.31	13.1	0.21
p. Value	<0.001	<0.001	<0.001	<0.001	<0.001
Jejunum					
NC	1222^{a}	147^{a}	196^{c}	563^{a}	6.27^{a}
PC	856^{d}	102^{d}	290^{a}	275^{d}	2.96^{d}
MO40NS110	957^{cd}	111^{cd}	261^{b}	335^{c}	3.67^{c}
MO75NS75	1087^{b}	134^{b}	214^{c}	456^{b}	5.11^{b}
MO110NS40	1001^{c}	121^{c}	243^{b}	381^{c}	4.14^{c}
Antioxidant	1128^{b}	138^{ab}	202^{c}	489^{b}	5.62^{ab}
SEM	21.0	2.81	6.07	16.9	0.20
p. Value	<0.001	<0.001	<0.001	<0.001	<0.001
Ileum					
NC	998^{a}	124^{a}	218^{c}	390^{a}	4.60^{a}
PC	735^{c}	85.8^{c}	368^{a}	198^{d}	2.00^{a}
MO40NS110	833^{b}	95.2^{bc}	293^{b}	249^{c}	2.88^{cd}
MO75NS75	950^{a}	115^{a}	245^{c}	342^{b}	3.88^{b}
MO110NS40	882^{b}	100^{b}	289^{b}	277^{c}	3.06^{c}
Antioxidant	964^{a}	114^{a}	233^{c}	346^{ab}	4.17^{ab}
SEM	16.2	2.46	9.01	11.7	0.15
p. Value	<0.001	<0.001	<0.001	<0.001	<0.001

Means in each column with the same superscript are not significantly different at 0.05. NC = Standard group fed corn soybean meal diet; PC = Heat stress challenged group without offering any *Moringa oleifera* leaf and *Nigella sativa* seed extract and any Antibiotic in drinking water; MO40NS110= Heat stress challenged group offered with 40mL *Moringa oleifera* leaf and 110mL *Nigella sativa* seed extract per liter of drinking water; MO75NS75= Heat stress challenged group offered with 75mL *Moringa oleifera* leaf and 75mL *Nigella sativa* seed extract per liter of drinking water; MO110NS40= Heat stress challenged group offered with 110mL *Moringa oleifera* leaf and 40mL *Nigella sativa* seed extract per liter of drinking water; Antioxidant= *Heat* challenged group offered with 250mg/kg vitamin C. DVL= Duodenal villus length; DVW= Duodenal villus width; DCD= Duodenal crypt depth; DVSA= Duodenal villi surface area; DVL/DCD= Duodenal villus length / Duodenal crypt depth.

Similarly, in the jejunum and ileum, reduced villus height and crypt depth were observed in the PC group with higher villus width (P<0.001). Broilers supplemented with MO75NS75 and the Antioxidant treatment exhibited significantly (P<0.01) improved villus dimensions compared to the NC group. In contrast, other phytogenic supplemented groups showed partial improvement but lesser in these histological investigations.

As shown in Table 6, different combination of *M. oleifera* an *N. sativa* did not impact the cecal population of *Escherichia coli*, *Lactobacillus* spp., or *Salmonella* among the experimental groups.

DISCUSSION

Recent studies have shown growing interest towards nutritional intervention that can support broiler productivity, health and meat quality (Chand *et al*., 2018). In the current study, the enhanced growth performance observed in heat-stressed broilers, receiving *M. oleifera* and *N. sativa* extract may be attributed to the presence of biologically active compounds, which can partially counteract the detrimental effects of heat stress. Heat stress commonly reduce feed consumption, compromise nutrients absorption, increases oxidative stress, leading to impaired growth and feed efficiency (Lara & Rostagno, 2013). Improved weight gain and FCR, particularly during the later growth period (day 22-35), a period associated with increased metabolic heat load. Although the MO75NS75 group showed better performance advantage, these results should be explained with caution, due to the absence of dose-response or factorial design.

The positive impact associated with supplementation of *M. oleifera* can likely be attributed to its richness in phenols, flavonoids and vitamins. These compounds are known to contribute to the body's antioxidant capacity. These compounds may also support digestive efficiency by enhancing the secretion of enzymes in the gastrointestinal tract (Siddhuraju & Becker, 2003; Anwar *et al*., 2007). In a similar manner, *Nigella sativa* seeds contain thymoquinone, a bioactive molecule with strong antioxidant, anti-inflammatory and immunomodulatory actions. Thymoquinone has been shown to improve mitochondrial performance and limit oxidative damage, thereby conserving energy that can be utilized for growth rather than physiological maintenance (Al-Zahrani *et al*., 2011; Darakhshan *et al*., 2015). Together, these effects may underline the improved feed efficiency observed in supplemented groups, even feed consumption remained largely unchanged.

The digestibility results provide additional support for this explanation. The enhanced crude protein digestibility and apparent metabolizable energy values reflect improved digestive and absorptive functions in birds supplemented with *N. sativa* and *M. oleifera.* Improved digestibility and nutrients absorption. Similar findings were reported in previous studies indicating that feed additives stimulate digestive enzymes and protect mucosal structure particularly under stress conditions (Khalid *et al*., 2020; Hassan *et al*., 2016; Mohamed & Hassan, 2023). In particular, flavonoids such as quercetin and kaempferol from *M. oleifera*, and thymoquinone from *N. sativa* are known to reduce intestinal inflammation and promote more effective nutrients uptake (Shaterzadeh-Yazdi *et al*., 2018).

Changes in serum MDA concentration provides additional evidence of the protective antioxidant role associated with dietary supplementation. As a reliable marker of oxidative stress, and is known to increase under heat stress (Hafeez *et al*., 2024). In the current study, birds supplemented with *M. oleifera* and *N. sativa* resulted in significantly lower serum MDA concertation compared with unsupplemented, heat-stressed control birds, aligning with reports evaluating antioxidant intervention studies in broilers Puthpongsiriporn *et al*., 2001; Surai *et al*., 2019). The decline in oxidative stress marker seems to be the results of combined effects *M. oleifera* flavonoids and thymoquinone, which are well studied for their neutralizing action against the free radicals and protective effect from oxidative injury (Majdalawieh & Fayyad, 2015).

Histological observations of intestinal tissue confirmed the improvements in digestive functions supportive the observed enhancements in nutrients absorption. In the current study, the phytogenic supplementation expanded the villus dimensions when compared with birds in the control group during heat stress. These morphological observations indicate a more developed structural enhancement and more functionally mature intestinal epithelium, both of which contribute to greater efficiency of nutritional uptake, digestibility and absorption (Awad *et al*., 2009; Montagne *et al*., 2003). Under the thermal stress, the ability to preserve the villus morphology may be explained by the antioxidant effects of the bioactive compounds present in *M. oleifera* and *N. sativa*, which are known for mitigating cellular damage from oxidative and inflammatory injury Shaterzadeh-Yazdi *et al*., 2018; Aydogan *et al*., 2020).

Conclusions: Dietary supplementation with equal concentration of *M. oleifera* and *N. sativa* extracts (MO75NS75) markedly reduced the negative impacts of heat stress, leading to enhanced growth performance, enhanced oxidative balance and intestinal morphology.

Conflict of interest: Authors declare no conflict of interest

Competing interest: None declared.

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Ethical statement: This study was approved by the ethical committee of, the Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan under notification No. L- 452/AH/UAP dated 16/11/2022.

Data availability statement: Data is available from the corresponding author upon reasonable request.

Authors contribution: Conceptualization; MT, Investigation; AA. Writing-original draft preparation; RUK. Writing-review and editing; RUK, Visualization; RH. supervision; NAK and NC project administration; MT. All authors have read and agreed to the published version of the manuscript.

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