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ORIGINAL PAPER

Supplementation of liquid organic acid blend as a protector against heat stress in broilers: Growth performance, intestinal morphology, blood biochemistry and ascites syndrome susceptibility

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ABSTRACT. This study investigated the effects of supplementation with a liquid organic acid blend (OAB) on the growth performance, intestinal morphology, blood biochemistry and susceptibility to ascites in Cobb 500 broilers exposed to heat stress. A completely randomised design was applied with three treatments: no OAB supplementation (control, CONT), OAB supplementation (0.2%) during the entire period (FP) (days 1–42, OAB/FP) and OAB supplementation (0.2%) during the late period only (LP) (days 21–42, OAB/LP). To induce heat stress, room temperature was maintained at 28–30 °C. At 42 days of age, the final body weight of broilers in OAB/FP and OAB/LP groups was higher (2632.6 and 2569.4 g, respectively), than in controls (2383.2 g, respectively, $P < 0.01$). Improved feed conversion ratio values at 42 days of age were also observed in the OAB/FP (1.71) and OAB/LP (1.75) groups compared to the CONT group (1.92, $P < 0.01$). OAB supplementation stimulated villus growth in the small intestine ($P < 0.01$). Mortality associated with ascites was highest in the CONT group (11.3%), followed by the OAB/FP (3.8%) and OAB/LP (1.3%) groups ($P < 0.05$). The supplementation with OAB increased immunoglobulin concentrations and decreased the heterophil-to-lymphocyte (H/L) ratio, a stress indicator ($P < 0.05$). In conclusion, organic acid blend supplementation improved productive performance, villus growth, and immune status, and reduced both ascites-related mortality and stress level during broiler exposure to high temperatures. Supplementation throughout the entire rearing period (days 1–42) appeared to be more effective as protection against heat stress.

Introduction

The ban on antibiotic use in animal production led to increasing interest in alternative approaches, including feed additives such as organic acids, prebiotics, probiotics, enzymes, essential oils and phytogenic feed supplements (Nguyen et al., 2020). Among these alternatives, organic acids and their salts, used either individually or as blended mixtures have increasingly gained importance and attention as potential agents supporting antibiotic-free poultry production worldwide. The use of organic acids and their salts as non-antibiotic feed additives in animal production is generally recognised as safe and has been authorised by the European Union (Zeeshan et al., 2022).

The term 'organic acids' also referred to as acidifiers, encompasses a broad range of chemical compounds naturally occurring as components of plants, animal tissues, and microorganisms, which are involved in important physiological and metabolic processes. In animal nutrition, organic acids such as acetic, formic, and butyric acids are used because of their nutritional value, antibacterial properties, and ability to stimulate energy metabolism. Moreover, organic acids reduce the abundance of pathogenic microbiota and lower gut pH, thereby suppressing the proliferation of pathogenic bacteria (Ao et al., 2009), which contributes to improved gut health and nutrient utilisation.

Due to the increasing occurrence of environmental stressors, e.g. global warming and elevated ambient temperatures, research has focused on minimising the adverse effects of stress through the use of feed additives (Mangan and Siwek, 2024). Heat stress is well known to negatively affect bird welfare and productivity, resulting in significant economic losses (Khan and Iqhbal, 2016). Therefore, additives, such as organic acids have been applied to alleviate the negative effects of heat stress and improve performance by regulating acid-base balance (Olanrewaju et al., 2007).

In poultry nutrition, feed consumption could be negatively affected by various factors, particularly heat stress (Oluwagbenga and Fraley, 2023). Therefore, supplementing drinking water with additives may be more beneficial than feed supplementation because water passes through the digestive tract more rapidly than solids (Olnood et al., 2015). Moreover, the use of liquid additives may be more practical under commercial production conditions. Consequently, water acidification is expected to be more effective than lowering pH through the diet, especially during heat stress.

In the present study, growth performance, villus development and acidity in the small intestine, blood biochemistry and ascites susceptibility were evaluated in broilers receiving organic acid blend (OAB) supplementation in drinking water during two different periods of the rearing cycle (days 1–42 and 21–42) and compared with a control group. The study aimed to determine whether supplementing of drinking water with OAB could improve broiler tolerance to heat stress and support the productivity and health status of the birds.

Material and methods

Birds, diets, housing, and experimental design

The care and use of animals for research purposes were approved by the Animal Use and Ethics Committee of Bursa Uludağ University in accordance with Turkish laws and regulations (Approval Number 2018-05/11).

A total of 240 one-day old Cobb 500 broiler chicks were randomly allocated to 12 floor pens, with 4 replicate pens per treatment and 20 chicks per pen (10 males and 10 females). The chicks were weighed using a balance with a precision of ± 0.1 g and assigned to three treatment groups: a control group without OAB supplementation (CONT), a group receiving OAB supplementation from days 1 to 42 (OAB/FP; full rearing period), and a group receiving OAB supplementation from days 21 to 42 (OAB/LP; late rearing period). The composition of OAB provided by the manufacturer is presented in Table 1. The supplementation level was 0.2% in drinking water (2 ml/l) according to the manufacturer's recommendation (R2 Agro A/S, Hedensted, Denmark). Drinking water samples were collected before and after OAB supplementation, and pH was measured using a digital pH meter (Mettler Toledo, SevenCompactTM pH/Ion S220, Greifensee, Switzerland). The pH values of water with and without OAB supplementation were 3.790 and 7.735, respectively.

Table 1. The composition of organic acid blend (OAB) used in the experiment

Substances	Content (%)*
Ammonium formate	25–40
Formic acid	15–25
Propionic acid	10–15
Acetic acid	5–10
Lactic acid	2.5–5
Sodium propionate	1–2.5
Benzoic acid	0.25–1
Potassium chloride	0.25–1
Copper sulphate	0.25–1
Zinc acetate dehydrate	0.1–0.25

* the content of the OAB is given by the producer company

A maize-soybean mealbased diet formulated according to NRC (1994) recommendations for starter and grower broilers was used in the experiment. Chicks were fed a standard crumbled broiler starter diet from days 1 to 14 and a grower diet from days 15 to 42. The composition and proximate analysis of the basal diets are presented in Table 2. Water and feed were offered *ad libitum* throughout the experiment.

Stocking density was maintained at 27–30 kg live weight/m^2 based on final weight, and a standard lighting programme was applied according to the Cobb (2012) management guidelines. To induce heat stress, room temperature was maintained at 28–30°C during the experiment. Room temperature (T_R, %) and relative humidity (RH, %) were

Table 2. The composition and nutrient level of broiler diets

Dietary item	Starter (1–14 days)	Grower (15–42 days)
Composition, %		
maize	53.40	56.85
soybean meal (CP 48%)	38.50	34.00
vegetable oil	3.50	5.00
dicalcium phosphate	2.00	2.00
limestone	1.50	1.25
salt	0.30	0.30
L-lysine-HCl (99%)	0.10	0.10
DL-methionine (98%)	0.30	0.25
premix[1]	0.40	0.25
Calculated chemical composition		
ME, MJ/kg	12.88	13.53
Analyzed nutrient level, %		
crude protein	22.3	20.4
lysine	1.36	1.23
met + cyst	1.07	0.92
Ca	1.17	1.06
total P	0.45	0.44
crude fiber	3.5	5

[1] Premix provided per kg of diet: IU: vit. A 4000000, vit. D_3 800000; mg: vit. E 20000, vit. K_3 1200, vit. B_1 800, vit. B_2 2400, vit. B_6 2000, vit. B_{12} 6, vit. C 18000, niacin 8000, biotin 40, folic acid 400, choline chlorid 80000, manganese 32000, iron 24000, zinc 24000 , copper 2000, iodine 400, cobalt 80, selenium 60

monitored twice daily using a digital thermometer and hygrometer. Based on these measurements, the temperature–humidity index (THI) was calculated using the formula proposed by Thom (1959) and modified by Buffington et al. (1982):

$$THI = 0.8 \times T_R + RH \times \left(\frac{(T_R\ -\ 14.3)}{100}\right) + 46.3.$$

The mean values of T_R, RH and THI for each week are given in Figure 1. During the experimental period, the mean THI value was 84.3% which can be considered indicative of severe heat stress discomfort in birds (Timmons and Gates, 1988; Medeiros, 2001).

Growth performance parameters

Body weight and feed consumption of broilers were recorded weekly on a pen basis throughout the 6-week experimental period. Body weight gain and feed intake were determined weekly, and feed conversion ratio (FCR) was calculated based on weekly weight gains and feed intake values. FCR was adjusted for mortality within each pen. In addition, water intake was measured weekly on a pen basis. Cumulative body weight gain and feed intake were calculated for the period from days 1 to 42.

According to the productive performance data obtained at 42 days of age, the European Production Efficiency Factor (EPEF) was calculated using the formula provided by Zarghi et al. (2020):

$$EPEF = 100 \times [(\mathit{final\ body\ weight}\ (kg)] / \mathit{viability}\ (\%))] / [age\ (day) \times FCR].$$

Mortality was monitored daily on a pen basis during the experiment. Broilers that died after 14 days of age were subjected to gross necropsy examination for hydro-pericardium, right ventricular hypertrophy, and abdominal fluid accumulation to determine ascites-related mortality (Shinder et al., 2009).

Intestinal histomorphological traits

To evaluate intestinal morphology under heat stress conditions, samples of the small intestine were collected at 21 and 42 days of age for intesti-

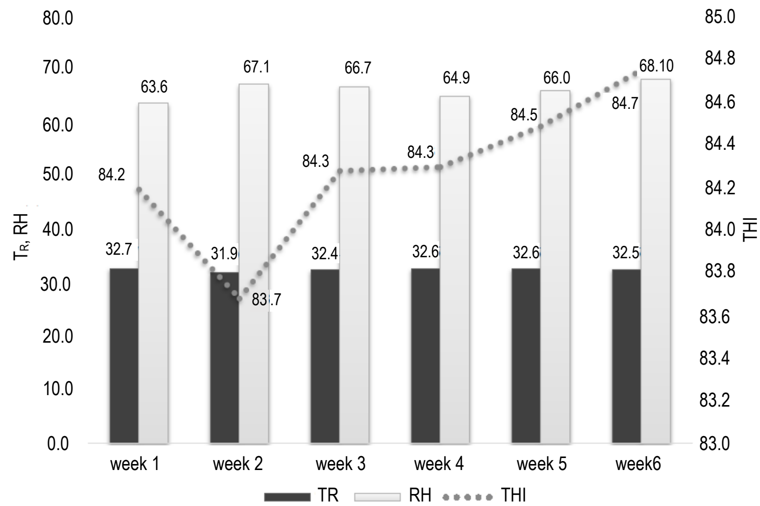

Figure 1. The mean value of room temperature (TR, °C), relative humidity (RH, %) and thermal heat index (THI, %) of each week during growing period

nal villus micrometry analysis (n = 8 broilers/treatment group). The small intestine was divided into three segments: duodenum, jejunum and ileum. Samples were collected from the midpoint between the end of the stomach and bile duct entry (duodenum), between the bile duct entry and Meckel's diverticulum (jejunum), and between Meckel's diverticulum and the ileocecal junction (ileum) (Mahmoud and Edens, 2012). The samples were cut into 2-cm fragments and rinsed with 0.9% NaCl to remove intestinal contents. Subsequently, the tissues were fixed in 10% buffered formaldehyde and dehydrated in a graded ethanol series (30, 50, 70, 80, 95, and 100%) over 24 h. The samples were then embedded in paraffin and 5-µm sections were cut from the paraffin blocks using a microtome (Leica RM2155 Germany). The sections were mounted on glass slides and stained with haematoxylin and eosin for microscopic evaluation (Gridley, 1960).

For morphological analyses, intestinal morphometric parameters including villus height, villus width, crypt depth, thickness of the *tunica muscularis*, and apparent villus surface area were measured using a light microscope (Leica, DM-500, Switzerland) coupled with a computerised image system (Leica Application Suite, LAS Version 3.7.0, Leica Microsystems, Switzerland). For each bird, 15–20 villi, crypts, and *tunica muscularis* measurements were analysed.

Measurements of small intestinal pH

Small intestine samples (duodenum, jejunum and ileum) were collected for pH determination at 21 and 42 days of age. The pH of the samples was measured using a digital pH meter (Mettler Toledo, SevenCompactTM pH/Ion S220, Greifensee, Switzerland).

Serum biochemical parameters

At 42 days of age, 8 broilers from each treatment group were randomly selected and euthanised by decapitation. Approximately 1 ml of blood was collected for the determination of blood parameters, including haemoglobin concentration and haematocrit value, using standard methods described by Schalm et al. (1975). In addition, 4 ml of blood samples were collected into nonheparinised tubes for serum separation. Serum samples were analysed for activity of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) using an automatic analyser. Plasma levels of immunoglobulins (IgA, IgG, and IgM), triiodothyronine (T_3) and thyroxine (T_4) were determined using commercial kits and a Roche auto-analyser (Cobas 6000 series C501 module, Roche Diagnostic, Indianapolis, IN). Furthermore, 1 ml of EDTA blood samples were collected, and 2 blood smears per sample were prepared directly after drawing blood (n = 8 broilers/treatment group). After air drying, the slides were stained with May Grünwald and Giemsa stain (Clark et al., 2009). A total of 100 leukocytes including heterophils (H), eosinophils, basophils, monocytes, and lymphocytes (L) were counted once on each slide using a microscope at 100 × magnification with oil immersion. The H/L ratio was calculated as the number of heterophils divided by the number of lymphocytes.

Data analysis

The data were analysed using ANOVA procedures appropriate for a completely randomised experimental design with the general linear model (GLM) procedure of SAS Institute (version 9.4 2012, Cary NC, USA). The assumptions of normality and homogeneity (SAS, 2012) of variance were assessed using the ShapiroWilk and Levene tests, respectively. Treatment effects were evaluated using the least squares means test, and multiple comparisons were performed using Tukey's test. Performance parameters were analysed using a completely randomised repeated-measures design on a weekly basis, whereas intestinal morphology and small intestinal pH measurements were evaluated at 21 and 42 days of age. Repeated-measures ANOVA was used to evaluate performance and intestinal morphology traits over time. When significant differences were detected, Bonferroni-corrected comparisons were applied to identify the source of variation, and results were presented as day × treatment interaction effects. Only significant day × treatment interactions were reported in the Results section (body weight). Mortality data (dead/alive) were summarised for each experimental group and analysed using Fisher's exact test, which is suitable for categorical data with small sample sizes where the assumptions of the chi-square test are not met. Percentage data were analysed after arcsine square root transformation. Differences were considered significant at $P \leq 0.05$.

Results

Growth performance parameters

The effects of OAB supplementation on the growth curves of heat-challenged broilers are shown in Figure 2. Both treatment effects (OAB supplementation) and the day × treatment interaction were significant ($P = 0.023$ and $P < 0.001$, respectively). During the experimental period, the

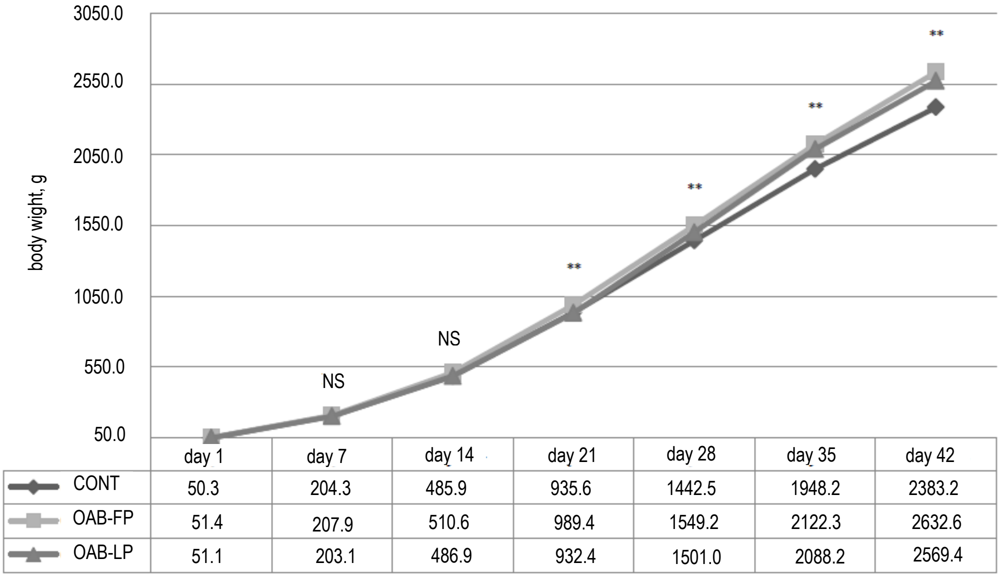

Figure 2. Effects of organic acid blend (OAB) supplementation on growth curves of broilers challenged with heat stress

CONT – control, no supplementation of OAB, OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OAB/FP – supplementation of OAB during 1–42 days, OAB/LP – supplementation of OAB during 21–42 days, n: 4 replicate pens/treatment group (20 broilers/pen), ** indicates significant differences among the experimental groups ($P < 0.05$), NS indicates any significant differences among the experimental groups ($P > 0.05$)

pattern of body weight changes differed between the treatment groups. The OAB-FP group showed the greatest increase in body weight compared to the other groups. Body weight on days 1 and 7 were similar among the experimental groups ($P > 0.05$), whereas broilers in OAB/FP were heavier at 14, 21 and 28 days than birds in the remaining groups ($P < 0.001$). Thereafter, supplementation of drinking water with OAB exerted a beneficial effect on body weight, and broilers in the OAB/FP and OAB/LP groups remained heavier than in the CONT group until the end of the growing period ($P < 0.001$). At 42 days of age, the final body weight of broilers in the OAB/FP and OAB/LP groups was higher (2632.6 and 2569.4 g, respectively) when compared to the control birds (2383.2 g, respectively, $P = 0.002$).

The effects of OAB supplementation on production parameters and water intake of broilers exposed to heat stress are presented in Table 3. Higher final body weight at 42 days of age, and improved FCR, were observed in the OAB/FP (2581.2 g and 1.71, respectively) and OAB/LP (2518.2 gand 1.75,

Table 3. Effects of organic acid blend (OAB) supplementation on productive parameters and water consumption of broilers challenged with heat stress

Parameters	Treatment groups			SEM	*P*-values
	CONT	OAB/FP	OAB/LP		
Productive performance					
BWG (g, days 1–42)	2332.9^b	2581.2^a	2518.2^a	70.3	0.002
CFC (g, days 1–42)	4563.9	4491.6	4502.2	88.7	0.735
FCR (day 42)	1.92^a	1.71^b	1.75^b	0.05	0.001
EPEF	240.0^b	343.5^a	336.3^a	19.5	<0.001
Water consumption (ml/bird)					
day 7	492.0^b	543.7^a	506.9^b	12.3	0.001
14	1049.0^{ab}	1014.6^b	1072.4^a	19.9	0.008
21	2061.3^a	1818.2^b	2036.6^a	49.6	<0.001
28	3258.4^a	2829.8^c	2998.9^b	54.1	<0.001
35	4808.0^a	4594.2^b	4510.9^b	101.7	0.007
42	6237.8^{ab}	6184.3^b	6336.5^a	69.2	0.035

N – 4 replicate pens/treatment group (20 broilers/pen); BWG – body weight gain, CFC – cumulative feed consumption, FCR – feed conversion rate, EPEF – European Production Efficiency Factor, CONT – control, no supplementation of OAB, OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OAB/FP – supplementation of OAB during 1–42 days, OAB/LP – supplementation of OAB during 21–42 days, SEM – standard error of the mean, ^{abc} – means within a columns with different superscripts are significantly different at $P < 0.05$

respectively) groups than the CONT group (2332.9 g and 1.92, respectively; P < 0.01). In contrast, the EPEF value was lowest in the CONT group (240.0), compared to the OAB/FP (343.5) and OAB/LP groups (336.3, P < 0.001). Water intake varied among the treatment groups during the experimental period; however, broilers in the CONT and OAB/LP groups tended to drink significantly more water than the broilers in the OAB/FP group (P < 0.05).

Table 4. Effects of organic acid blend (OAB) supplementation on mortality of broilers challenged with heat stress

Treatment groups	Ascites induced mortality			Mortality (other causes)		
	Number	%	*P*- values	Number	%	*P-values*
CONT vs. OAB/FP	9/3	11.3/3.8	0.130	6/2	7.5/2.5	0.276
CONT vs. OAB/LP	9/1	11.3/1.3	0.017	6/2	7.5/2.5	0.276
OAB/FP vs. OAB/LP	3/1	3.8/1.3	0.620	2/2	2.5/2.5	1.000

CONT – control, no supplementation of OAB, OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OAB/FP – supplementation of OAB during 1–42 days, OAB/LP – supplementation of OAB during 21–42 days, P > 0.05 (no statistically significant)

The effects of OAB supplementation on mortality in broilers challenged with heat stress are presented in Table 4. Ascites-related mortality was highest in the CONT group (11.3%), followed by the OAB/FP (3.8%) and OAB/LP (1.3%) groups. Statistical analysis showed that only the difference between the CONT and OAB/LP groups was significant with respect to ascites-related mortality (P < 0.05), while deaths due to other causes were similar in all treatment groups and ranged from 2.5% to 7.5% (P > 0.05).

Intestinal histomorphological traits

The effects of OAB supplementation on intestinal morphology in broiler exposed to heat stress are presented in Table 5. OAB supplementation in drinking water improved villus growth and may have enhanced digestive and absorption processes. At 21 days of age, the highest villus height and apparent villus surface area were observed in the duodenum (449.5 µm and 22483 µm^2, respectively) and jejunum (400.0 µm and 24333 µm^2, respectively) of broilers in the OAB/FP group compared to the other groups. In the duodenum, the thickness of the *tunica muscularis* was lower in the OAB/FP (71.2 µm) and OAB/LP (77.7 µm) groups (P < 0.001), whereas crypt depth was the highest in the CONT group (49.9 µm; P < 0.01). Similarly, mean values for ileal crypt depth and *tunica muscularis* thickness were the highest in the CONT group (45.5 µm and 86.3 µm, respectively; P < 0.05).

At 42 days of age, OAB supplementation stimulated villus growth and development in all three sections of the small intestine compared to the CONT group. The highest villus height values in the duodenum (596.7 µm) and ileum (339.7 µm), as well as higher apparent villus surface area in the duodenum (29839 µm^2) and jejunum (32304 µm^2), suggested improved digestive and absorptive capacity of the intestinal tract. Lower crypt depth values in the duodenum (66.2 µm) and ileum (60.4 µm) were observed in the CONT group (P < 0.05). Lower thickness values of the *tunica muscularis* in the duodenum (94.9 µm), jejunum (88.2 µm) and ileum (86.5 µm) were recorded in the OAB/FP group. Moreover, reduced thickness of the *tunica muscularis* in the duodenum and jejunum was also observed in the OAB/LP group (103.6 µm and 90.0 µm, respectively; P < 0.01).

Small intestine pH

The effects of OAB supplementation on the pH of the small intestine in heat stresschallenged broilers are presented in Table 6. At 21 days of age, duodenal and jejunal pH values were lowest in the OAB/FP group (5.95 and 5.62, respectively) compared wih the CONT and OAB/LP groups (P < 0.05). Supplementation with OAB also caused a more acidic intestinal environment, reducing jejunal pH to 5.89 in the OAB/FP and 5.91 in the OAB/LP, when compared to the CONT group (6.10, P < 0.01). Ilea1,5 l pH was lowest in the OAB/FP group (5.30), followed by the OAB/LP (5.88) and CONT groups (6.54; P < 0.001).

Serum biochemical parameters

The effects of OAB supplementation on blood chemistry of broilers exposed to heat stress are presented in Table 7. Higher haemoglobin concentration and haematocrit values were observed in the CONT group (8.5 g/dl and 33.0%, respectively) compared to the OAB/FP (7.6 g/dl and 30.1 l/l) and OAB/LP groups (7.8 g/dl and 30.9 l/l, respectively). The highest T_3 concentration (1.22 ng/ml) was recorded in the CONT group, whereas T_4 concentration was highest in the OAB/FP group (1.30 µg/dl, P < 0.05). Lower AST, ALT and ALP

Table 5. Effects of organic acid blend (OAB) supplementation on intestinal morphology of broilers challenged with heat stress

Day	Treatment groups	Villus height, µm	Villus width, µm	Villus apparent surface area, µm^2	Crypt depth, µm	*Tunica muscularis*, µm
Day 21	Duodenum					
	CONT	396.1^b	38.8	20076.0^b	49.9^a	94.1^a
	OAB/FP	449.5^a	42.5	22483^a	40.0^b	71.2^b
	OAB/LP	403.1^b	41.9	21235^b	41.8^b	77.7^b
	SEM	22.7	4.5	911.1	2.9	3.4
	P-values	0.038	0.102	0.046	0.015	<0.001
	Jejunum					
	CONT	348.6^b	46.7	21182^b	38.3	85.7
	OAB/FP	400.0^a	46.0	24333^a	43.5	84.7
	OAB/LP	357.9^b	48.0	21434^b	39.7	87.7
	SEM	21.0	2.5	961.9	2.2	7.8
	P-values	0.041	0.052	0.018	0.064	0.058
	Ileum					
	CONT	213.2	39.3	13590	45.5^a	86.3^a
	OAB/FP	256.0	46.9	14955	32.6^b	64.2^b
	OAB/LP	231.1	43.3	13928	35.1^b	65.8^b
	SEM	27.9	5.5	1445.6	4.4	6.6
	P-values	0.069	0.053	0.062	0.028	0.010
Day 42	Duodenum					
	CONT	515.0^b	45.3	26771^b	66.2^a	125.0^a
	OAB/FP	596.7^a	56.5	29839^a	53.3^b	94.9^b
	OAB/LP	563.7^{ab}	56.0	28837^{ab}	57.0^{ab}	103.6^b
	SEM	25.2	6.0	847.8	4.0	9.3
	P-values	0.021	0.111	0.012	0.020	<0.001
	Jejunum					
	CONT	466.7^b	49.1^b	28373^b	51.4	112.3^a
	OAB/FP	531.3^a	61.1^a	32304^a	57.7	88.2^b
	OAB/LP	545.3^a	64.6^a	31513^a	53.4	90.0^b
	SEM	31.5	2.6	737.3	2.6	4.2
	P- values	0.047	0.01	0.001	0.058	<0.001
	Ileum					
	CONT	286.7^b	52.8	15590	60.4^a	114.5^a
	OAB/FP	339.7^a	62.2	20295	43.9^b	86.5^b
	OAB/LP	311.3^{ab}	63.8	18795	56.8^{ab}	95.6^a
	SEM	18.9	4.8	1904.2	5.7	8.6
	P-values	0.038	0.062	0.057	0.027	0.010

For morphologic measurements, small intestine samples were collected from 8 broilers per experimental groups.

CONT – control, no supplementation of OAB; OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OAB/FP – supplementation of OAB during 1–42 days; OAB/lP – supplementation of OAB during 21–42 days; SEM – standard error of the mean; ^{ab} – means within a coloumns with different superscripts are significantly different at $P < 0.05$

Table 6. Effects of organic acid blend (OAB) supplementation on pH of small intestine of broilers challenged with heat stress

Day	Organs	Treatment groups			SEM	*P*-value
		CONT	OAB/FP	OAB/LP		
Day 21	Duodenum	6.04^a	5.95^b	6.05^a	0.02	<0.001
	Jejunum	6.18^a	5.62^b	6.09^a	0.04	<0.001
	Ileum	6.99	6.75	6.90	0.21	0.112
Day 42	Duodenum	6.10^a	5.89^b	5.91^b	0.09	0.009
	Jejunum	5.64	5.65	5.50	0.31	0.681
	Ileum	6.54^a	5.30^c	5.88^b	0.27	<0.001

For pH measurements, organ samples were collected from 8 broilers per experimental groups; CONT – control, no supplementation of OAB, OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OABOAB/FP – supplementation of OAB during 1–42 days, , OAB/LP – supplementation of OAB during 21–42 days, SEM – standard error of the mean; ^{ab} – means within a columns with different superscripts are significantly different at $P < 0.05$

Table 7. Effects of organic acid blend (OAB) supplementation on blood chemistry of broilers challenged with heat stress

Parameters	Treatment groups			SEM	*P*-value
	CONT	OAB/FP	OAB/LP		
Hematology					
hemoglobin, g/dl	8.5[a]	7.6[b]	7.8[b]	0.346	0.003
hematocrit, l/l	33.0[a]	30.1[b]	30.9[b]	1.029	0.002
Thyroid hormones					
T_3, ng/ml[1]	1.2[a]	0.85[b]	0.97[b]	0.126	0.048
T_4, µg/dl[2]	0.86[b]	1.30[a]	1.02[b]	0.108	0.039
Liver functions, IU/l					
AST[3]	57.3[a]	31.1[b]	47.0[a]	6.32	<0.001
ALT[4]	160.1[a]	130.6[c]	146.1[b]	9.36	0.048
ALP[5]	4889.5[a]	3287.0[b]	4559.0[a]	731.5	0.011
Immunoglobulins, mg/dl					
IgG[6]	131.2[b]	133.3[a]	131.3[b]	0.618	<0.001
IgA[7]	7.7[b]	9.0[a]	8.3[ab]	0.610	0.015
IgM[8]	4.6[b]	6.2[a]	6.2[a]	0.673	0.005
Stress indicator					
H/L[9]	0.76[a]	0.63[b]	0.62[b]	0.05	0.002

For these measurements, blood samples were collected from 8 broilers per experimental groups; CONT – control, no supplementation of OAB, OAB – organic acid blend, FP – full period of growing, LP – late period of growing, OAB/FP – supplementation of OAB during 1–42 days, OAB/LP – supplementation of OAB during 21–42 days; [1] T_3 – triiodothyronine, [2] T_4 – thyroxine, [3] AST – aspartate amino transferase, [4] ALT – alanine amino transferase, [5] ALP – alkaline phosphatase, [6] IgG – immunoglobulin-G, [7] IgA – immunoglobulin-A, [8] IgM – immunoglobulin-M, [9] H/L – heterophiles / lymphocytes; SEM – standard error of the mean; [abc] – means within a coloumns with different superscripts are significantly different at $P < 0.05$

activities were observed in OAB/FP group (31.1, 130.6 and 3287.0 IU/l respectively, $P < 0.05$) compared to the other groups. Supplementation of drinking water with OAB increased immunoglobulin titres. Broilers in the control group showed lower IgG, IgA and IgM levels than the OAB/FP and OAB/LP groups ($P < 0.05$). In addition, the highest H/L ratio was observed in the CONT group (0.76), followed by the OAB/LP (0.62) and OAB/FP groups (0.63, $P < 0.01$).

Discussion

This study clearly demonstrated that OAB supplementation improved growth performance of broilers under heat stress conditions. The growth-stimulating effects of organic acids can be attributed to their ability to regulate gastrointestinal microbiota, improve gut microstructure, stimulate the secretion of digestive enzymes, and enhance the immune response in birds (Nguyen et al., 2018). Recently, Islam et al. (2024) investigated the effects of dietary supplementation with an organic acid mixture containing formic acid, propionic acid, ammonium formate, and ammonium propionate at different inclusion levels in broilers. The authors reported improved body weight gain and feed consumption in broilers receiving the organic acid blend at 200 mg/kg compared to the other treatment groups.

These effects may be attributed to improved nutrient utilisation associated with better development of the gastrointestinal tract and a barrier effect against pathogenic microorganisms (Khan and Iqhbal, 2016). In addition, organic acids play important roles in various physiological process in the gastrointestinal system, including the conversion of inactive pepsinogen into active pepsin, serving as an important energy source for intestinal tissues, and regulation of stomach-emptying rate (Chukwudi et al., 2024). These metabolic functions make organic acids valuable feed additives for stimulating broiler growth. However, the lower water intake observed in the control group may also have contributed to poorer growth performance compared with the OAB-supplemented groups. In a previous study, Bruno et al. (2011) reported that reduced water intake could decrease feed consumption and consequently impair broiler growth performance.

In the current study, mortality was classified into two categories: ascites-related and other causes, as ascites is considered one of the major factors contributing to death in broilers worldwide (Wideman and French, 2000). Dewil et al. (1996) reported that the incidence of ascites and associated mortality in fast-growing broilers tended to peak between 3 and 6 weeks of age due to an imbalance between oxygen demand and cardiopulmonary capacity resulting from rapid growth. In addition, environmental temperature and rapid growth rate during the rearing period are among the main factors contributing to ascites development by increasing metabolic rate and oxygen demand (Scheele et al., 1991). The present findings clearly demonstrate that OAB supplementation during both the 1–42-day and 21–42-day periods reduced ascites-related mortality under heat stress conditions compared to the control group. These results may provide useful information on the metabolic mechanisms associated with ascites development in broilers exposed to heat stress. The numerically lowest mortality value was observed in the OAB/LP group, which may suggest that acidifier supplementation has the potential to mitigate ascites under heat stress conditions.

Although studies focusing specifically on this topic are limited, Kopecký et al. (2012) reported that supplementation of acetic acid or citric acid in drinking water (at a concentration of 0.25%) reduced total mortality in broilers. The observed decrease in mortality due to ascites could be related with the regulatory effects of organic acids or their salts on acid-base homeostasis in birds, especially under stressful conditions. This protective effect of organic acids is further supported by the higher haemoglobin concentration observed in the control group compared with the OAB-supplemented birds.

Villus development may be considered an indicator of small intestinal function and is linked to nutrient absorption through increases in villus height, width and apparent surface. In addition, each segment of the small intestine performs specific functions in nutrient digestion and absorption. The jejunum is the main site of nutrient absorption, while the ileum plays an important role in nutrient assimilation (Islam et al., 2024). According to the present findings, OAB supplementation improved small intestinal morphology in broilers exposed to heat stress. Similarly, Pelicano et al. (2005) reported that organic acids suppressed the growth of pathogenic and non-pathogenic intestinal bacteria, thereby reducing intestinal colonisation and infectious processes. This may consequently reduce inflammatory reactions in the intestinal mucosa and improve villus development, as well as the secretory, digestive, and absorptive functions of the mucosa, which was also supported by the results of Islam et al. (2024).

Organic acid supplementation resulted in favourable changes in intestinal structure, as evidenced by increased villus height and reduced crypt depth in the OAB groups compared to the control group, reflecting improved absorptive capacity and reduced epithelial turnover. These morphological adaptations are indicative of a more functionally efficient intestinal environment and may be associated with improved nutrient utilisation (Celi et al., 2017). Moreover, reduced thickness of the *tunica muscularis* may indicate lower intestinal motility linked to enhanced digestibility and a reduced luminal load, as previously reported by Teirlynck et al. (2009). Overall, these findings demonstrate that organic acids positively affect intestinal structure and function in broilers. Similar results were also published by Stefanello et al. (2020), Ebeid et al. (2021) and Ko et al. (2025).

One of the main reasons for using organic acids in drinking water is their potential to lower intestinal pH and reduce the colonisation and growth of pathogenic bacteria, particularly during critical growth stages and stressful conditions (Kammon et al., 2019). However, conflicting results have been reported regarding the ability of organic acids supplied in drinking water to reduce the pH of small intestine, and gastrointestinal tract in general (Hamid et al., 2018). These discrepancies may be related to differences in the type and concentration of organic acids used in individual studies, buffering capacity of dietary ingredients, presence of other anti-microbial additives in the diet, variation in gut microbiota and health status, as well as environmental conditions and stressor (Rynsburger and Classen, 2007).

Dietary organic acids were found to generally reduce intestinal pH in broilers, with more pronounced effects observed in the distal segments, such as the ileum, compared to the duodenum and jejunum (Suiryanrayna and Ramana, 2015; Liu et al., 2018), although results published in the literature are not fully consistent. Similarly, Ndelekwute et al. (2019) observed that dietary supplementation with acetic and butyric acids (0.25%) reduced pH in the duodenum and caecum of broilers. On the other hand, Ma et al. (2021) detected a decrease in duodenal pH in broilers supplemented with a mixed organic acid preparation (formic acid, ammonium formate, propionate, acetate, and lactate), while no significant differences were observed in jejunum and ileum.

Based on current knowledge, the effects of organic acids on intestinal pH may vary depending on the method of supplementation (feed vs. water), the dose applied, and the intestinal segment considered. While dietary inclusion of organic acids provides a more consistent reduction in gastrointestinal pH, supplementation via drinking water leads to a rapid but transient acidification, mainly affecting the upper digestive tract (Dibner and Buttin, 2002; Van Immerseel et al., 2006).

Blood biochemical parameters are widely used as indicators of animal health status, providing information on infections, dehydration, haematological disorders, and nutritional imbalance (Kareem et al., 2024). This study clearly showed that OAB supplementation via drinking water affected blood biochemical parameters, as well as growth parameters, ascites-related mortality, intestinal morphology, and pH in broilers exposed to heat stress. A significant increase in haemoglobin and haematocrit values in control broilers was observed in the control group. Under stressful conditions, haemoglobin concentration may increase to improve oxygen transport in the blood (Olanrewaju et al., 2007). Additionally, the higher incidence of ascites in the control group was accompanied by elevated haemoglobin concentration and haematocrit values, which was consistent with the findings of Maxwell et al. (1986). Higher haemoglobin and haematocrit values in the control group may also have been related to lower water intake. Dehydration resulting from reduced water intake and heat stress may cause haemoconcentration in broilers, resulting in increased haematocrit and haemoglobin levels, as previously reported by Altan et al. (2000). These results indicate that the analysed parameters were interrelated.

Thyroid hormones regulate energy metabolism and accelerate both basal and oxidative metabolic rate. Rapid growth is known to increase T_3 level, which reflects elevated metabolic activity and oxygen demand (Luger et al., 2001). This may exceed cardiopulmonary capacity and predispose fast-growing broilers to hypoxia, pulmonary hypertension and subsequent ascites development (Ezzulddin, 2023; Hossain and Akter, 2022). In the present study, supplementation with organic acids reduced T_3 and increased T_4 concentrations in the OAB/FP group, while remaining within physiological ranges. The increase in T_4 concentration could be associated with reduced susceptibility to ascites under stress conditions, as lower thyroid hormone levels may impair tissue oxygen supply and contribute to hypoxia and heart failure (Hassanzadeh, 2009). In addition, hepatic enzyme activity is commonly used as an indicator of liver function and health status (Cheesborough, 1987). The higher AST, ALT and ALP activities observed in the control group could therefore indicate a greater susceptibility to ascites.

A previous study showed that organic acidifiers can stimulate the immune response in birds (Khan and Iqhbal, 2016). In this study, OAB supplementation increased immunoglobulin concentrations. The H/L ratio is employed to assess immune response of birds and is considered an indicator of physiological stress (Lentfer et al., 2015). The current findings clearly showed that the supplementation of OAB may reduce the effects of heat stress by lowering the H/L ratio and improving immune status, which is consistent with the results reported by Akaichi et al. (2022). The increased value of this parameter observed in the control group may have also been associated with lower water intake and its negative effects on physiological status. Based on these results, lower water consumption may increase the susceptibility of broilers to heat stress and consequently increase the H/L ratio. Similar observations were reported by Gil et al. (2023).

Conclusions

In summary, supplementation of drinking water with acidifiers may be used as a growth-promoting strategy, particularly under heat stress conditions. Organic acid blend (OAB) exerted a positive effect on the growth performance irrespective of the supplementation period (days 1–42 or 21–42). One limitation of the present study was the relatively small number of birds per treatment group, which may have reduced the ability to detect minor but biologically relevant differences between treatments. Nevertheless, the results provide preliminary evidence supporting the hypothesis of this study and indicate a consistent relationship between the evaluated parameters. Future studies with a greater number of animals are required to confirm these findings, improve statistical robustness, and strengthen the broader applicability of the conclusions.

This study provides important evidence supporting the potential of OAB as an effective alternative strategy for improving intestinal health, broiler productivity, and reducing susceptibility to ascites, particularly under stressful conditions. The adoption of OAB supplementation under different production conditions may contribute to more sustainable poultry production.

Conflict of interest

The Author declares that there is no conflict of interest.

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