

RESEARCH

Open Access



# Plasma activated water effects on behavior, performance, carcass quality, biochemical changes, and histopathological alterations in quail

Usama T. Mahmoud<sup>1\*</sup>, Ghada Abd El-Reda<sup>2,6</sup>, Fatma Abo Zakaib Ali<sup>3</sup>, Manal A.M. Mahmoud<sup>4</sup>, Sherief M.S Abd-Allah<sup>5</sup>, F. M. El-Hossary<sup>6</sup> and Nasser S. Abou Khalil<sup>7,8</sup>

## Abstract

**Background** Plasma-activated water (PAW) is an innovative promising technology which could be applied to improve poultry health. The current study investigated the effects of drinking water supply with PAW on quail behaviour, performance, biochemical parameters, carcass quality, intestinal microbial populations, and internal organs histopathology. A total of 54 twenty-one-day-old Japanese quail chicks were randomly allotted to three treatments provided with PAW at doses 0, 1 ml (PAW-1), and 2 ml (PAW-2) per one litter drinking water. Each treatment contained 6 replicates (3 birds/ cage; one male and two females).

**Results** The results clarified that there were no significant ( $P > 0.05$ ) changes in behaviour, and performance. For the biochemical indicators, the PAW-1 group showed significantly higher serum  $H_2O_2$ , total protein and globulin levels compared with the other groups ( $P = 0.015$ ,  $< 0.001$ , and  $0.019$ ; respectively). PAW groups had significantly lower serum creatinine and urea levels than the control ( $P = 0.003$ ). For the carcass quality, the internal organs relative weight between different treatments was not changed. In contrast, there was a significant increase in the meat colour, taste, and overall acceptance scores in PAW groups compared with the control one ( $P = 0.013$ ,  $0.001$ , and  $< 0.001$ ; respectively). For the intestinal microbial population, *lactobacilli* count was significantly higher in PAW-2 compared with the control group ( $P = 0.014$ ), while there were no changes in the total bacterial count between different treatment groups. Moreover, mild histological changes were recorded in the intestine, liver, and spleen of PAW groups especially PAW-2 compared with the control one.

**Conclusions** PAW offered benefits, such as reducing creatine and urea levels, improving meat characteristics, and increasing lactobacilli count, all of which are crucial for sustainable quail farming. Therefore, further research is needed.

**Keywords** Plasma activated water, Quail, Behaviour, Performance, Blood biochemical, Histopathology

\*Correspondence:  
Usama T. Mahmoud  
usama.mahmoud@aun.edu.eg

Full list of author information is available at the end of the article



## Background

Quail production is one of the poultry industry's expanding segments. Consumers greatly enjoy the diversity and good nutritional quality that quail meat offers to the human diet. Compared to other poultry species, quail offers several advantages, such as rapid growth, high productivity, early laying onset, high rates of reproduction, low feed intake, low investment, and disease resistance [1–4]. The significant increase in the rates of meat and egg production seen in quail farming can be attributed to advancements in genetics, better management practices, environment, and nutrition, as well as the growth of research employing various feed ingredients to satisfy production demands [5]. A societal issue with significant economic implications, antibiotic-resistant bacteria are becoming more prevalent in quail [6, 7]. The discovery of a naturally occurring chemical as an antibiotic substitute may contribute to better animal and human wellbeing. One of the established feeding strategies is the use of acidic compounds that have antibacterial activity to improve the performance and health status of quail and control the antibiotic resistance problem [8].

PAW is a relatively new and innovative technology that has gained attention in various industries, including the poultry industry. PAW is created by subjecting water to a plasma discharge, that generates chemically reactive species such as reactive oxygen species and reactive nitrogen species, which makes PAW acidic [9–12]. These species can have various antimicrobial and disinfectant properties, making PAW potentially useful in poultry farming and processing [13–15]. The PAW antibacterial modes of action were reviewed by Zhao et al. [16] and subdivided into 4 main modes of action as follow: First, primary and secondary reactive oxygen and nitrogen species (RONS) are generated in PAW, leading to reduced pH, increased ORP, and electrical conductivity, which cause physical stress on microbial cells. Second, these RONS induce oxidative stress, damaging the peptidoglycan structure of the cell wall and initiating lipid and protein peroxidation on the cell membrane. This results in compromised membrane integrity, shrinkage, distortion, and formation of cracks or pores, followed by depolarization of cell membrane potential. Third, RONS can be transported into the cell through these pores, leading to an accumulation of intracellular RONS and a reduction in intracellular pH due to proton influx. Fourth, the intracellular RONS oxidizes internal components such as DNA, proteins, lipids, and carbohydrates, resulting in DNA fragmentation and protein oxidation, with these oxidized components potentially exiting the cell through the membrane pores [16]. PAW has been shown to inactivate *Escherichia coli* and *Staphylococcus aureus* in chicken muscle, rough skin, and smooth skin [13]. It also effectively inactivates *Pseudomonas deceptionensis* CM2 on

the surface of raw chicken breasts [14]. Additionally, due to its antiviral and immunostimulant activities, PAW has been recommended as a promising strategy for the preparation of inactivated vaccines for Newcastle disease [15].

Several studies have shown that PAW is non-toxic and safe for use in animals including healthy CD1 mice [17], immuno-deficient nude mice [18], rabbits [19], and fish [20]. PAW is a promising technology for the poultry industry, but its safety has not been assessed before in different poultry species. Therefore, this pioneering study aimed to assess the impact of PAW oral administration on quail behavior, performance, biochemical parameters, carcass quality, intestinal microbial populations, and internal organ histopathology. We hypothesized that PAW's low pH and high content of RONS would enhance beneficial microbial populations and intestinal epithelium morphology, thereby improving nutrient absorption, performance, and meat quality. This study offers significant insights into PAW as a unique technique for increasing quail health and productivity. As an environmentally friendly solution, PAW has the potential to improve poultry management and advance sustainable quail farming techniques.

## Results

### The PAW effects on behaviour

Non-significant change in quail behavioral activities (feeding, drinking, standing, sitting, walking, preening, stretching, shaking, feather pecking, and wall pecking) were observed due to PAW treatment ( $P > 0.05$ ) (Table 1).

### The PAW effects on performance

The results indicate that there were no significant differences ( $P > 0.05$ ) in performance indicators including body weight, body weight gain, feed intake and feed conversion ratio among the different treatments (Table 2).

### The PAW effects on biochemical parameters

The results in Table 3 indicate that serum  $H_2O_2$ , total protein, and globulin levels were significantly higher in the PAW-1 group compared to the control group ( $P = 0.015$ ,  $< 0.001$ , and  $0.019$ ; respectively). The inclusion of PAW-1 and PAW-2 resulted in a significant reduction in serum creatinine and urea levels compared to the control group ( $P = 0.003$ ). However, levels of Total antioxidant capacity, albumin, albumin/ globulin ratio (A/G), alanine aminotransferase, and aspartate aminotransferase remained unchanged.

### The PAW effects on carcass quality

The outcomes presented in Table 4 demonstrate that the addition of PAW to drinking water had no significant impact ( $P > 0.05$ ) on the relative weights of the empty carcass, gizzard, spleen, heart, and liver. Carcass

**Table 1** The PAW effects on the behavioral activities of quails

Behavioral activities	Treatment groups			SEM	F	PValue
	Control	PAW-1	PAW-2			
Feeding%	4.86	3.59	5.67	0.86	0.899	0.438
Drinking%	5.86	4.86	5.58	0.83	0.295	0.751
Stand%	15.12	16.90	14.70	1.74	0.290	0.754
Sit%	50.23	48.15	46.98	2.72	0.269	0.770
Walk%	10.108	12.384	12.939	1.554	0.723	0.509
Preening%	9.182	10.880	9.580	0.991	0.602	0.566
Stretching%	1.08	0.93	1.52	0.23	1.029	0.392
Shaking%	0.39	0.23	0.15	0.13	0.641	0.547
Groom%	10.65	12.04	11.25	1.05	0.350	0.713
Feather pecking%	0.77	0.81	0.61	0.33	0.060	0.942
Wall pecking%	2.39	1.27	2.27	0.58	0.803	0.475

Behavioral activities of quails (Percentage of certain activity to the total number of activities/cage). ( $n=6$  replicates/group; 18 birds/ group)

characteristic measurements (Table 4) revealed that water-holding capacity, pH, juiciness, chewiness, and flavor were similar across all treatments. Values for color in the PAW-2 group were significantly higher than the control group ( $P=0.013$ ). Values of taste, and overall acceptability in birds provided with PAW-1 and PAW-2 were significantly ( $P=0.001$ , and  $<0.001$ ; respectively) higher than those in the control group.

#### The PAW effects on intestinal microbial populations

The data depicted in Fig. 1. revealed that the total bacterial count was not significantly different between treatments ( $P>0.05$ ). However, PAW-2 exhibited a significantly higher *Lactobacilli* count than the control group ( $P=0.014$ ).

#### The PAW effects on internal organs histopathology

The PAW effects on intestinal morphometry and histopathology were presented in Table 5; Figs. 2 and 3. indicate that compared to the control group, PAW-treated groups exhibited significantly higher ileum villus height ( $P=0.010$ ). However, no significant changes were observed in duodenum and jejunum villus height. PAW-2 showed the highest villus height for both duodenum and jejunum, while duodenum villus height in PAW-1 was significantly lower than in PAW-2. Crypt depth in the jejunum of PAW-1 was significantly reduced ( $P=0.001$ ) compared to both the control and PAW-2 groups. There were no significant changes ( $P>0.05$ ) in the villus: crypt ratio among the different treatment groups.

The impact of PAW on the histopathological changes in the liver tissue of quails is depicted in Fig. 4. The control groups exhibited an intact hepatic architecture characterized by normal hepatic lobules, central veins, and hepatocyte arrangement, with vesicular nuclei. The portal triad structure, consisting of the portal vein, hepatic artery, and bile duct, appeared normal (Fig. 4a and b). In PAW-1, no significant histological changes were observed in

the hepatic tissue, with hepatocellular structure appearing largely normal (Fig. 4c), except for a congested and dilated portal vein (Fig. 4d). However, PAW-2 exhibited various vascular alterations, including congested and dilated central veins and hepatic sinusoids between hepatic cords (Fig. 4e). Additionally, PAW-2 hepatocytes displayed signs of degeneration, and the portal vein appeared distended and congested with blood (Fig. 4f).

The impact of PAW on the histopathological changes in the spleen tissue of quails is illustrated in Fig. 5. Histological examination revealed the stroma and parenchyma of the spleen, characterized by a thin capsule and the absence of trabeculae. Distinct demarcation between white pulp and red pulp was not observed. Central arteries gave rise to penicilliform capillaries, encircled by perielipoidal lymphatic sheaths (PELS) (Fig. 5a). White pulp comprised periarterial lymphatic sheaths (PALS) and PELS, surrounded by arterioles and ellipsoids, respectively (Fig. 5b). Both PALS and PELS contained various white blood cells. Red pulp consisted of sinuses and cords (Fig. 5c) containing diverse cell types. In the PAW-1 treated group, mild vascular changes were observed, including dilatation and congestion in splenic sinuses, venules, and arterioles, with thickening and hyalinization of arterioles. Additionally, mild cellular changes such as lymphocytic depletion in PALS and hyperplasia in PELS were noted (Fig. 5d and e). The PAW-2 treated group exhibited mild thickening of the splenic capsule and dilated, congested subcapsular sinuses and venules. The lymphoid cellular density in the splenic parenchyma was normal, with mild hyperplasia observed in PELS (Fig. 5g-i).

#### Discussion

The drinking water pH is a critical factor for promoting overall bird health and productivity. Recently, several research studies have recommended adding acidifiers such as organic acids due to their low pH and antibacterial

**Table 2** The PAW effects on the quails' performance

Treatments	Body weight at age (day)/ g					Body weight gain/g			Feed intake/kg			Feed conversion ratio (g feed/g gain)				
	21	28	35	42		W1	W2	W3	Total	W1	W2	W3	Total	W1	W2	W3
Control	71.67	140.06	198.28	244.67	68.39	58.22	46.39	173.00	148.06	136.89	175.11	460.06	2.17	2.37	3.90	2.67
PAW-1	71.33	144.50	195.08	242.33	73.17	50.58	47.25	171.00	155.42	129.21	169.83	454.46	2.13	2.57	3.68	2.67
PAW-2	69.78	147.89	206.22	253.78	78.11	58.33	47.56	184.00	156.78	134.94	171.00	462.72	2.02	2.32	3.79	2.53
SEM	1.84	3.07	3.31	5.98	2.49	2.02	3.86	5.58	4.47	2.45	4.41	7.19	0.07	0.10	0.36	0.08
F	0.183	1.164	1.676	0.573	2.619	3.326	0.019	0.885	0.863	2.000	0.326	0.211	0.782	1.272	0.073	0.557
P value	0.836	0.351	0.236	0.581	0.122	0.078	0.982	0.443	0.451	0.186	0.729	0.814	0.484	0.322	0.930	0.590

n=6 replicates/group (18 birds/group; one male and 2 females per cage)

W1: First week of experiment; W2: Second week of experiment; W3: Third week of experiment

activities in poultry drinking water to improve the bird's health and productivity [21–23]. PAW has the similar advantages therefore the current study suggested it may be beneficial for improving the quail health. To our knowledge, this is the first study to evaluate the effects of PAW on one of the poultry species (quail). Several previous studies have predominantly examined the potential toxicity of plasma-activated liquid and PAW on various animal models. Rabbits subjected to plasma-activated liquid injection into bone marrow for 30 days displayed no adverse effects [19]. Likewise, mice administered PAW over 90 days exhibited no toxicity signs or behavioural abnormalities. Key indicators, such as skin colour, mucous membrane state, piloerection, diarrhoea, weight loss, food intake reduction, locomotor activity, and body posture, remained unaffected. This suggests the absence of adverse effects associated with PAW treatment in the tested mice [17]. However, CD1 female mice consuming PAW ad libitum for 180 days exhibited reduced appetite, vigour, and body weight [24]. Similarly, the current study recorded no changes in quail behaviour in PAW groups in compared with the control group. Quail behaviour can serve as an indicator of health status and well-being [25, 26]. Behavioural responses in quail can be used to evaluate chemical toxicity [24, 27]. Studies have shown that behavioural measures are often more sensitive and persistent than traditional physiological measures; and Japanese quail have been used as a model for avian toxicity tests due to their ease of husbandry and quick reproductive maturity [28].

Body weight and feed intake have significant effects on the health state of quail. High body weight resulted in better performance, including higher egg production and feed efficiency, compared to low body weight [29]. The composition of drinking water can profoundly influence body weight and feed intake in quails, as demonstrated by several studies on different additives such as common salt, moringa leaf solution, aqueous anise seed extract, and liquid probiotics [30–33]. However, there is no specific information available on the effect of PAW composition on quail body weight and feed intake. The current study reported that, quails supplemented with PAW-2 exhibited numerically higher total body weight and body weight gain compared to both the control group and PAW-1, although these differences were not statistically significant. The PAW-2 group exhibited the best feed conversion ratio (2.53), while the other groups recorded a ratio of 2.67.

Drinking water with low pH was found to be beneficial for quail and fast-growing poultry in terms of better performance and economic returns [34, 35]. Additionally, low pH levels (pH 5) in drinking water were found to improve the productive and physiological performance of Japanese quails, including increased egg production

**Table 3** The PAW effects on the levels of serum biochemical parameters in quails

Parameters	Treatment groups			SEM	F	P value
	Control	PAW-1	PAW-2			
H <sub>2</sub> O <sub>2</sub> (mM/L)	0.51 <sup>b</sup>	0.96 <sup>a</sup>	0.60 <sup>b</sup>	0.09	6.148	0.015
TAC (mM/L)	0.17	0.21	0.13	0.04	1.042	0.383
Albumin (g/dL)	1.93	2.60	2.45	0.19	3.606	0.059
Total protein (g/dL)	3.02 <sup>b</sup>	5.45 <sup>a</sup>	3.78 <sup>b</sup>	0.27	21.321	<0.001
Globulin (g/dL)	1.09 <sup>b</sup>	2.85 <sup>a</sup>	1.33 <sup>ab</sup>	0.42	5.194	0.019
A/G ratio	1.85	1.42	1.91	0.34	0.627	0.548
ALT (U/L)	9.17	7.17	8.17	1.67	0.356	0.707
AST (U/L)	10.00	10.17	11.33	1.86	0.152	0.861
Creatinine (mg/dL)	0.69 <sup>a</sup>	0.23 <sup>b</sup>	0.29 <sup>b</sup>	0.08	9.997	0.003
Urea (mg/dL)	22.55 <sup>a</sup>	12.43 <sup>b</sup>	14.07 <sup>b</sup>	1.71	10.048	0.003

*n* = 6 replicates/group (18 birds/ group; one male and 2 females per cage); for blood biochemical measures only 6 birds/ group (3males and 3 females) were used. TAC: Total antioxidant capacity; A/G: albumin/ globulin ratio; ALT: alanine aminotransferase; AST: aspartate aminotransferase

<sup>a-b</sup> Means with different superscripts in the same row indicate significant differences at  $P < 0.05$  (general linear model followed by Tukey post-hoc test)

**Table 4** The PAW effects on carcass quality of quails

Parameters	Treatment groups			SEM	F	P value
	Control	PAW-1	PAW-2			
Heart %	1.03	0.96	1.21	0.09	1.960	0.175
Liver %	1.98	2.20	1.90	0.31	0.258	0.776
Spleen%	0.05	0.06	0.06	0.01	0.784	0.475
Gizzard%	1.81	1.66	1.86	0.08	1.649	0.225
Empty carcass%	69.25	68.24	72.20	2.46	0.696	0.514
Water-holding capacity	62.01	63.19	63.20	1.61	0.181	0.836
PH	5.868	6.102	6.053	0.159	0.608	0.550
Color	6.50 <sup>b</sup>	7.20 <sup>ab</sup>	7.48 <sup>a</sup>	0.23	4.959	0.013
Taste	6.69 <sup>b</sup>	7.34 <sup>a</sup>	7.47 <sup>a</sup>	0.14	8.322	0.001
Juiciness	7.56	7.89	7.97	0.15	2.268	0.119
Chewiness	7.42	7.93	7.81	0.17	2.333	0.113
Flavor	7.00	7.39	7.39	0.17	1.699	0.199
Overall acceptance	7.03 <sup>b</sup>	7.55 <sup>a</sup>	7.62 <sup>a</sup>	0.09	11.521	<0.001

*n* = 6 replicates/group (18 birds/ group; one male and 2 females per cage); for carcass quality only 6 birds/ group (3males and 3 females) were used

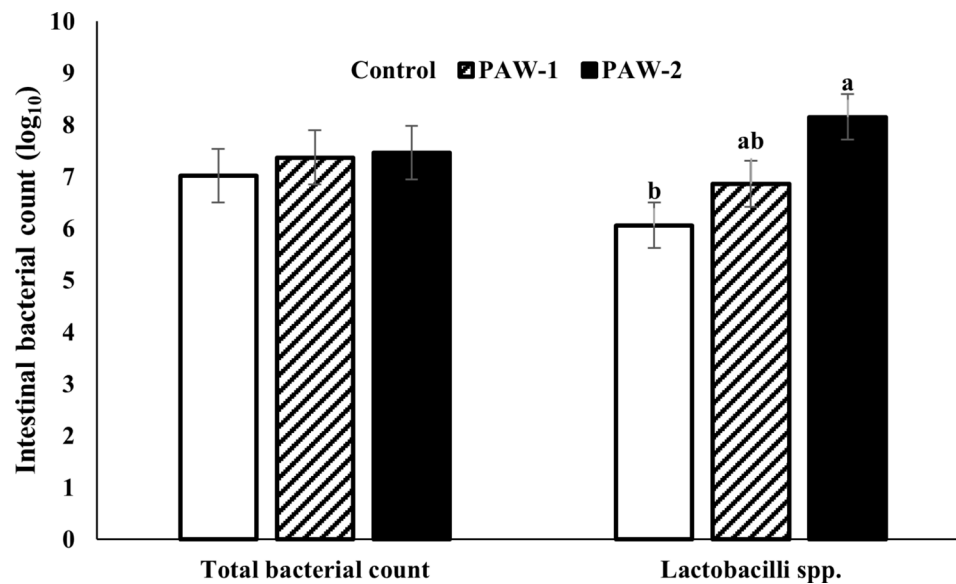
<sup>a-b</sup> Means with different superscripts in the same row indicate significant differences at  $P < 0.05$  (general linear model followed by Tukey post-hoc test)

and average egg weight [36]. The low pH of PAW is due to reactive species like reactive oxygen species and reactive nitrogen species formed when air is ionized in water, producing hydroxyls, nitrates, and nitrites, which generate hydrogen peroxide, nitric acid, and peroxy-nitrous acid, making PAW acidic [16, 37, 38]. Several previous studies reported that PAW, resulting from water exposure to air plasma for 15 min, had low pH values (2.11 [39], 3.65 [40], 3.66 [37]), like the one used in the current study. PAW doses evaluated in the current study were chosen based on previous studies examining drinking water commercial acidifying agents in quail and broilers, which showed effective results with a pH as low as 4 and a maximum dose of 2 ml per Liter [35, 41].

Non-thermal atmospheric plasma interacting with water results in the generation of PAW. This plasma treatment leads to the nonequilibrium dissociation of water molecules, producing short-lived species such as hydrated electrons and hydroxyl ions. The latter

intermediates rapidly react to form stable species, including ozone, superoxides, and hydrogen peroxide. Notably, hydroxyl radicals, with their high reactivity and short lifespan, interact with other liquid components, initiating further reactions in the system [42]. Thus, the marked elevation in serum hydrogen peroxide may be attributed to the saturation of PAW with a diverse range of reactive oxygen and nitrogen species. However, it appears that the production of reactive mediators did not surpass the capability of endogenous antioxidant defences in our experimental model, as indicated by the sustained levels of serum total antioxidant capacity. In a previous scholarly article, lipid peroxidation in chicken wings remained unaffected by the treatment with plasma-activated liquids [43].

Quail total protein primarily comprises albumin and globulins [44, 45]. Globulins are proteins that are mainly involved in the immune defence system. The elevated serum total protein and globulin levels observed in quails



**Fig. 1** The effects of PAW on intestinal microbial counts in quail. Data are expressed as means  $\pm$  standard errors. <sup>a-b</sup> Different superscripts above different columns for each parameter indicate significant differences ( $P < 0.05$ )

receiving PAW-1 supplementation, without affecting the serum albumin level, emphasize the potential of PAW as an immunostimulatory agent [46, 47]. However, this hypothesis was not evaluated in the current study. Supporting our hypothesis, a study by Ahmad et al. [43] mentioned that a higher level of globulin in quail serum is often used as an indicator for measuring immune response. They attributed the improvement in bird immunity to the inhibitory effects of organic acids on the gut system's microbial population, thereby enhancing non-specific immunity.

The reduction in serum urea and creatinine levels in PAW-treated groups is consistent with the findings observed in gamma-irradiated albino rats [48]. PAW-induced angiogenesis [49] may enhance the capillary filtration coefficient and increase the renal elimination of metabolic waste products. Providing quails with a high-protein diet may result in the development of hyperuricemia and gout [50–52]. Elevated serum uric acid levels associated with a protein-rich diet in poultry can adversely affect kidney function, increase the risk of gout, and negatively impact microbial communities and immunoregulation [53]. Histopathological deterioration in the liver and spleen, haematological abnormalities, and genotoxicity in erythrocytes in layer birds are linked to raised urea levels [54]. Hence, the potential of PAW to eliminate metabolic waste products of protein from circulating blood presents a promising approach to mitigate the inevitable side effects associated with feeding high-protein diets.

Muscle pH is an important post slaughter evaluating factor with effect on meat quality attributes such as meat color, Water holding capacity and the other muscle

characteristics [55]. Poultry meats with an ultimate pH between 5.7 and 6.1 are called normal and do not reveal any quality problems [56, 57]. The average pH determined in our study (5.8–6.1) was coherent with the other findings in the literature for quail meat [58, 59].

The colour of food is arguably the most crucial sensory characteristic, being the initial aspect noticed by consumers. It plays a vital role in shaping consumers' perceptions of a product's freshness, flavour, and overall quality [60]. The color attributes tend to be strongly associated with the pigment myoglobin, affected by myoglobin oxidation [61, 62]. Several studies have reported that the inclusion of low pH compounds in drinking water has the potential to improve poultry carcass and meat quality characteristics [21, 63, 64]. Similarly, the current study reported improvement in quail carcass quality parameters including increasing meat colour, taste, and overall acceptance score in PAW treated groups. Supporting these finding, Zhao et al. [65] reported that when fresh beef meat was directly treated with PAW, there was an enhancement in the redness index, with no significant alterations observed in the lightness or yellowness indexes. The meat's redness heightened either because of the oxidizing property of PAW [65] or due to its abundance in nitrite [66].

In our study, quails supplemented with PAW showed an improvement in the taste and overall acceptability of meat. High-voltage PAW has been shown to accelerate the flavour development of food products [67]. It is well known that taste perception is closely associated with textural characteristics experienced during food chewing [68]. The physical effects of PAW, including alterations in pH, oxidation-reduction potential, and the presence

**Table 5** The PAW effects on the intestinal morphometry of quails

Treatments	Duodenum			Jejunum			Ileum			Average		
	Villus height	Crypt depth	Villus: crypt ratio	Villus height	Crypt depth	Villus: crypt ratio	Villus height	Crypt depth	Villus: crypt ratio	Villus height	Crypt depth	Villus: crypt ratio
Control	708.94 <sup>ab</sup>	59.44	12.16	140.99	74.22 <sup>a</sup>	1.90	256.17 <sup>b</sup>	47.50	5.83	389.40	59.13 <sup>a</sup>	7.06
PAW-1	556.74 <sup>b</sup>	45.26	12.14	289.40	22.50 <sup>b</sup>	15.59	371.18 <sup>a</sup>	51.63	7.22	416.35	41.37 <sup>b</sup>	11.29
PAW-2	864.66 <sup>a</sup>	64.08	13.96	314.69	71.35 <sup>a</sup>	4.60	358.47 <sup>a</sup>	40.64	11.20	530.60	57.54 <sup>a</sup>	10.40
SEM	50.96	6.66	1.83	45.66	5.82	3.43	22.33	8.30	1.65	70.79	5.32	1.65
F	9.129	2.170	0.323	4.224	24.958	4.458	7.975	0.448	2.867	1.121	3.412	1.827
P Value	0.007	0.170	0.732	0.072	0.001	0.065	0.010	0.653	0.109	0.339	0.046	0.178

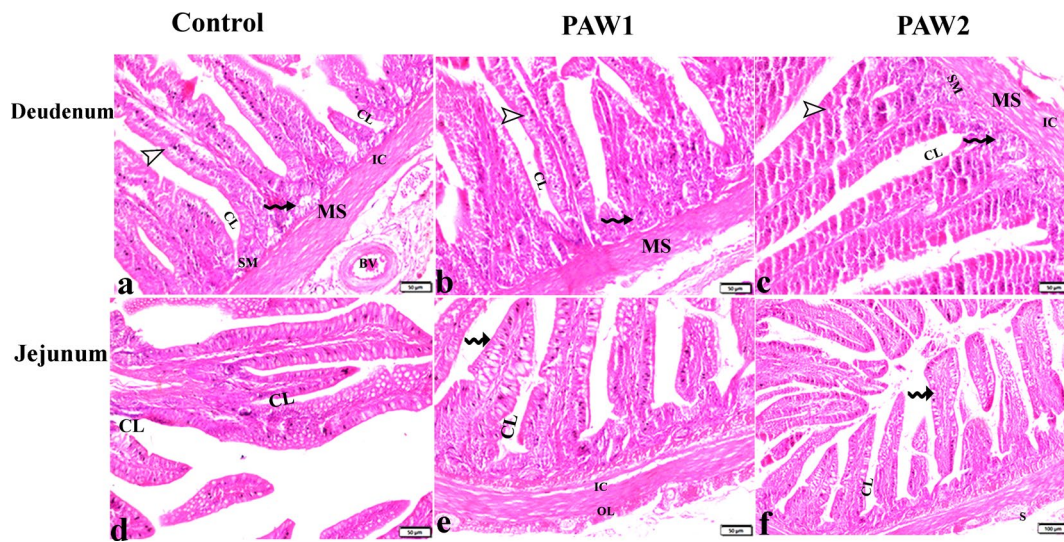
For intestinal morphometry only 6 birds/group (3 males and 3 females) were used. Different superscripts within the same column are significantly different at  $P < 0.05$  (general linear model followed by Tukey post-hoc test)

of reactive oxygen and nitrogen species, can induce significant changes to the food matrix. These modifications encompass enzyme inactivation, sugar breakdown, and lipid oxidation, thereby directly or indirectly influencing the textural properties of the food matrix [69]. Chicken breast treated with acidic electrolyzed water exhibited superior sensory scores in terms of texture and overall acceptability compared to the control [70]. The current study tracked enhancements in meat colour and taste present opportunities for increased consumer satisfaction and market appeal.

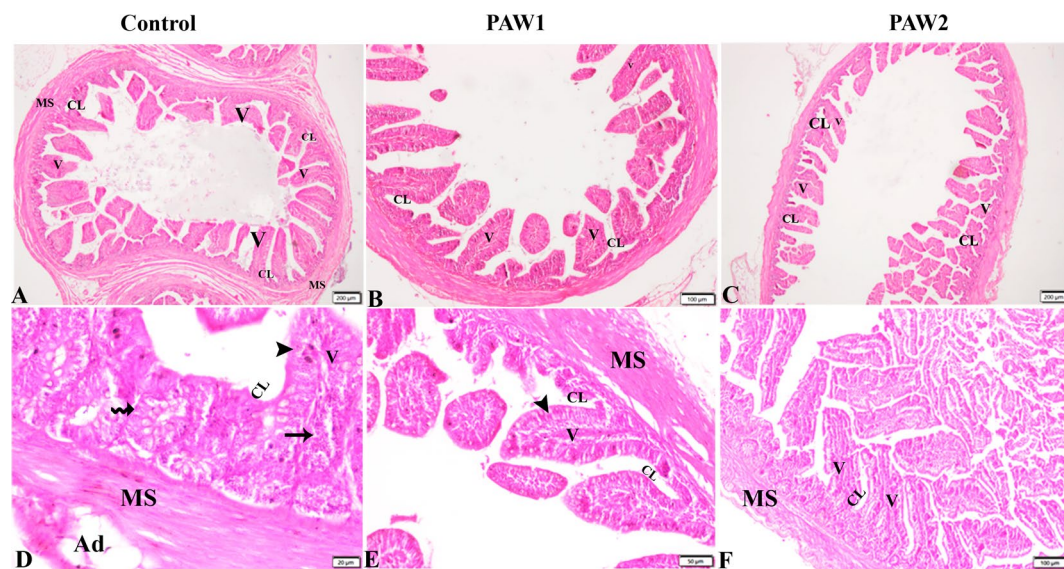
The current study recorded that PAW-2 exhibited a significantly higher *Lactobacilli* count than the control group. Supporting this finding, several studies reported that feed additives with acidic pH, such as organic acids, are utilized as alternatives to antibiotics because it promotes the growth of acid-tolerant beneficial bacteria while inhibiting the growth of acid-labile harmful pathogens [71, 72]. Lactic acid bacteria, like *Lactobacilli*, can flourish in lower pH environments [72, 73] because its high intracellular potassium concentration shields it from acid anions [74, 75]. The elevation of *Lactobacillus spp.* helps in inhibiting the colonization of pathogens such as *Salmonella spp.* in the gastrointestinal tract of broiler chickens [76], leading to improved BW and FCR [77–80]. The decrease in pH levels is associated with an elevated presence of *lactobacilli* populations, as observed in broiler chickens provided with acetic acid in their drinking water and exposed to *Salmonella enteritidis* [76]. Enhanced *Lactobacilli* colonization in the intestines exerts antimicrobial effects on pathogenic microbiota by creating a reduced pH environment in the gut [81]. Additionally, it produces antimicrobial compounds, including volatile fatty acids, organic acids, and bacteriocins [82]. *Lactobacilli* compete for nutrients, induce modifications in the structure and function of the intestinal epithelium, and flush out pathogens from the gastrointestinal tract, acting as receptor sites to prevent their adherence [83].

Enhanced intestinal colonization of *lactobacilli* played a significant role in augmenting histomorphometry parameters related to the intestinal absorptive surface, such as villus height [84] and mucosal layer height [81] crypt depth [83]. The observed increase in villus height implies the development of an expanded surface area capable of more efficient absorption of available nutrients [85]. Raised villous height and reduced crypt depth are associated with an enhanced epithelial turnover [86]. The heightened turnover of mucosal cells in the intestinal epithelium of birds plays a role in preserving mucosal integrity in the small intestine, acting as a preventive measure against the entry of pathogenic bacteria [83].

PAW contains a substantial amount of nitrate and nitrite, a powerful relaxant for vascular smooth muscle cells, leading to a reduction in vascular resistance [87].



**Fig. 2** The effects of PAW on duodenum and jejunum histopathological alterations in quail. (a-c): duodenum tissue sections from experimental groups. villi (V), mucosa (M), submucosa (SM), muscularis (MS), serosa (S) and blood vessel (Bv), inner circular (IC) and the outer longitudinal (OL) muscularis externa, simple columnar epithelial cells with goblet cells (arrowheads), crypts of Lieberkühn (CL), glands of Lieberkühn lined with Paneth cells (zigzag arrows). (d-f): jejunum tissue sections from experimental groups. villi (V), simple columnar epithelial cells with goblet cells (Zigzag arrows), crypts of Lieberkuhn (CL), glands of Lieberkuhn (GL), muscularis mucosa (MM), muscularis externa; inner circular muscle (IC), outer longitudinal muscle (OL), serosa (S). There is no structural difference between the groups



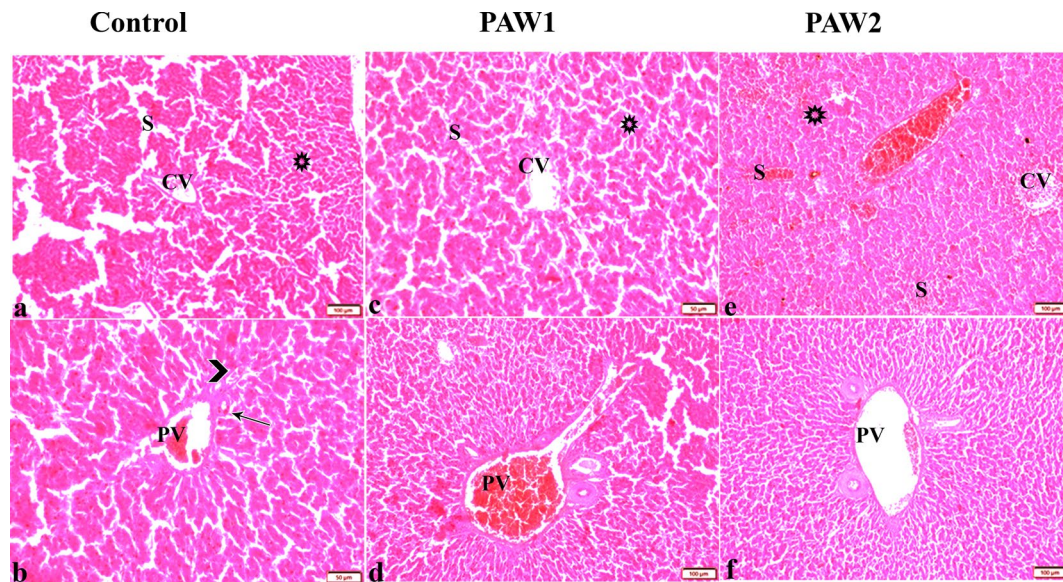
**Fig. 3** The effects of PAW on ileum histopathological alterations in quail. (a-f): ileum tissue sections from experimental groups. Control (A magnified in D), PAW1 (B magnified in E), PAW2 (C magnified in F): Villi(V), simple columnar epithelia with goblet cells (arrowheads), the crypts of Lieberkuhn (CL), Glands of Lieberkuhn (Zigzag arrow), muscularis externa (MS). Also shown lacteal (arrow) and adipose tissue (Ad). There is no structural difference between the groups except for a mild decrease in the thickness of mucosal villi from PAW2-treated groups in ileum sections

The acidification of the intestinal environment induced by *lactobacilli* results in the generation of nitric oxide (NO) through non-enzymatic nitrite reduction [88]. *Lactobacilli* provide additional source for conversion of nitrite to NO [89, 90]. NO acts as a crucial vasorelaxant by activating guanylyl cyclase [91]. All these vasodilator agents might be responsible for the congestion and dilation observed in the hepatic and splenic vasculature

especially in PAW-2 group. They also accelerate renal plasma flow and augment glomerular filtration rate [92] and subsequently increase urinary urea and creatinine clearance.

While our study presents promising results, it is important to acknowledge its limitations. This research is preliminary and exploratory, serving as the first work to discuss PAW effects on quail. Thus, we used the





**Fig. 4** The effects of PAW on liver histopathological alterations in quail. Photomicrograph of liver tissue sections from experimental group stained with HE stains showing: Control: Normal histological hepatic architectures compromising in (a&c): (a): normal central veins (CV), normal sinusoids (S), normal hepatocytes arrangement and structure with normal vesicular nucleus (star). (b): normal portal triad structure present in; normal portal vein (PV), hepatic artery (arrowhead) and bile duct (arrow). PAW1 (c&d): (c): normal central veins (CV), normal sinusoids (S), normal hepatocytes arrangement and structure with normal vesicular nucleus (star). (d): congested and dilated portal vein (PV). PAW (e&f): (e): congested central vein (CV), sinusoids (S), hepatocellular degeneration (Star). (f): dilated portal vein (PV)

minimum number of quail necessary, adhering to the 3R welfare standards (Replacement, Reduction, and Refinement), which emphasize the ethical use and care of animals in research. We were cautious about potential toxic effects. The lack of significant differences in performance could be attributed to the relatively short duration of the experiment (21–42 days) or the limited BW changes occurring during this period. We also investigated potential sex differences within each treatment and found no interaction between sex and treatment, this may be attributed to low number of each sex (3males vs. 3females). Current sample size may be on the lower side, However, the use of multiple replicates within each group helps to mitigate some of these limitations. The findings from our study provide valuable preliminary data. Future research should consider larger sample sizes and extended timelines is necessary to validate our findings and fully understand the mechanisms of PAW.

In conclusion, PAW treatments at doses of 1 ml or 2 ml per Liter of drinking water induced several biochemical, meat quality and histological changes in internal organs including higher *lactobacilli* count and lower serum creatinine and urea levels, as well as improving meat colour, taste, and overall acceptance score. These recorded changes are important for improving health and production in the sustainable quail farming industry. Therefore, further investigations are still needed to fine-tune the appropriate procedure and exact regimen for PAW treatment in quail and other poultry species.

## Materials and methods

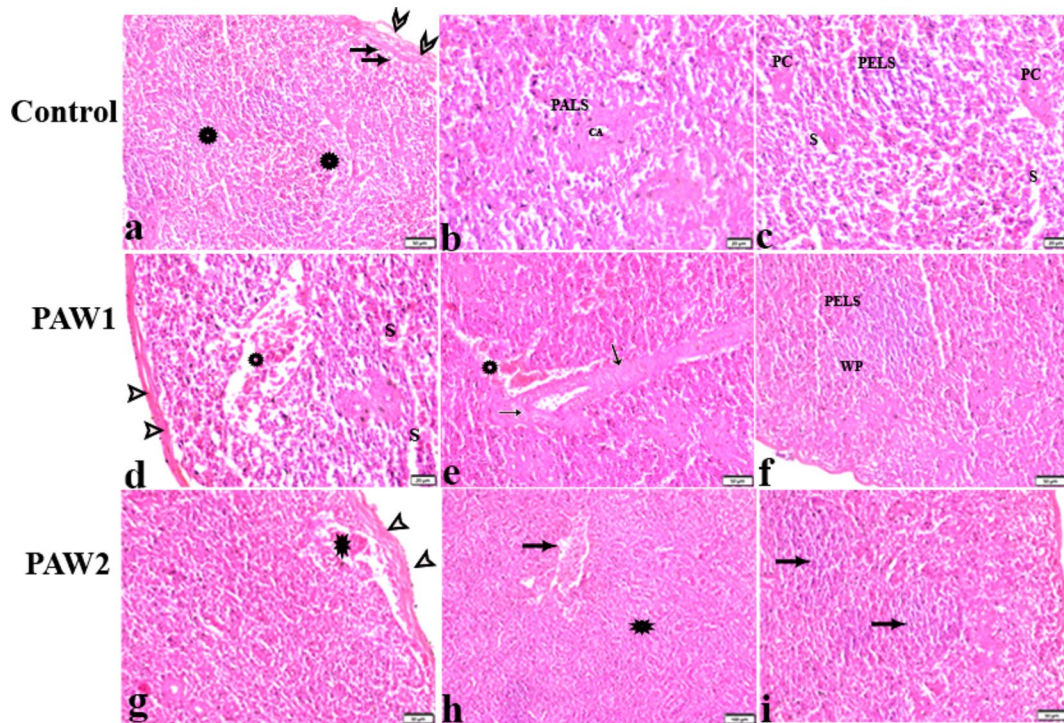
All study procedures were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt (Ethical Approval No. 06/2024/0182).

### Plasma activated water synthesis

PAW syntheses were done at Physics Department, Faculty of Science, Sohag University, (Sohag 82524, Egypt). Procedures details were formerly published by Abd El-Reda et al. [37]. Briefly, PAW was prepared by generating plasma on the distilled water surface using atmospheric air as the working supply gas at room temperature. 100 mL of distilled water was activated by plasma for 15 min, at 28 °C. The physicochemical measurements including pH, electrical conductivity, and hydrogen peroxide and Nitrites of the distilled water after the plasma activation were  $3.81 \pm 0.06$ ,  $150.54 \pm 5.97$  ( $\mu\text{S}/\text{m}$ ),  $0.38 \pm 0.07$  (mM/L) and  $5.35 \pm 2.27$  ( $\mu\text{M}/\text{L}$ ) respectively.

### Birds and housing

A total of 54 twenty-one-day-old Japanese quail chicks were purchased from a commercial hatchery. Chicks were kept at the same experimental room in multi-deck batteries, each with four decks and two cages per deck. Each cage, measuring 45×50×30 cm, accommodated one male and two females, ensuring standardized conditions for the study. A basal diet was formulated to meet the National Research Council (NRC, 1994)



**Fig. 5** The effects of PAW on spleen histopathological alterations in quail. Histology and general structure of the spleen of from experimental groups: (a-c): control normal quail. (a) Peritoneal layer covered the capsule (arrowheads) and subcapsular sinuses (arrows), unclear demarcation between white pulp and red pulp (stars). (B): PALS were surrounded central artery (CA) and consisted of various white blood cells including lymphocytes, macrophages, reticular cells, and plasma cells. (C): Penicilliform capillaries (PC) were encircled by PELS. PELS (lymphatic nodule) were formed mainly of lymphocytes of different sizes, macrophages, reticular cells, and heterophils. The Penicilliform (PC) continued with the blood sinuses (S). (d-f): PAW1 showing: (d): normal splenic capsule (arrowheads), dilated and congested sinuses (S), splenic venules dilatation and congestion (A&B stars). (d): thickening and hyalinization of splenic arterioles (arrows). (e): hyperplasia in PELS (WP). (g-i): PAW2 showing: (g): mild thickening in splenic capsule (arrowheads), dilated and congested sub capsular sinus (star). (h): splenic venules dilatated and congested (arrow). Normal lymphoid cellular density in splenic parenchyma (star). (i): lymphocytic hyperplasia in PELS (arrows)

**Table 6** Ration physical and chemical composition [93, 94]

	Ingredients	(%)	Chemical composition	(%)
1.	Yellow corn (ground)	49.50	Crude protein (%)	24.08
2.	Soya bean meal	45.00	Metabolizable energy (Mcal $kg^{-1}$ )	2.83
3.	Dried fat	2.20	Calcium (%)	0.87
4.	Ground limestone	1.00	Total phosphorus (%)	0.73
5.	Dicalcium phosphate	1.60	Crude fiber (%)	4.24
6.	Common salt	0.25		
7.	Mineral premix <sup>1</sup>	0.10		
8.	Vitamin premix <sup>2</sup>	0.12		
9.	Methionine	0.13		
10.	Lysine	0.10		
<b>Total</b>		<b>100%</b>		

<sup>1</sup>Supplied per kg of diet: Fe, 60 mg; Mn, 60 mg; Cu, 6 mg; I, 1 mg; Co, 1 mg; Se, 0.20 mg and Zn, 60 mg

<sup>2</sup>Supplied per kg of diet: Vitamin A, 12,000 IU; vitamin D3, 2200 IU; vitamin E, 26 IU; vitamin K3, 6.25 mg; vitamin B1, 3.75 mg; vitamin B2, 6.6 mg; vitamin B6, 1.5 g; pantothenic acid, 18.8 mg; vitamin B12, 0.31 mg; niacin, 30 mg; folic acid, 1.25 mg; biotin, 0.6 mg and choline chloride, 500 mg

recommendations (Table 6) and was offered ad libitum over the experimental period (21-42d). The chicks were fed a starter diet (CP: 24.08%; ME: 2830 kcal) and were kept under routine management practice and an optimum hygienic environment until the end of the experiment. On the first day of the experiment (21 day of age), chicks were randomly assigned to three PAW treatment groups: 0 (Control), 1 ml (PAW-1), and 2 ml (PAW-2) per liter of drinking water. Each treatment group consisted of six replicates (3 birds/cage). Water was provided ad libitum throughout the whole experimental period (21-42 days). The water was supplied via a single water container placed on the upper deck of each battery, connected with plastic tubes leading to nipple drinkers inside each cage. The water was replaced daily with freshly prepared PAW diluted water.

**Birds performance**

Live body weight (LBW), average live body weight gain (BWG), feed intake (FI), and feed conversion (FC) were recorded weekly throughout the experimental period (21-42 days).

### Behavioural observations

Direct live observations of quail behavior were conducted three times per week, once daily from 10:00 to 12:00 h, covering 18 cages (i.e., 6 cages per treatment). Instantaneous scan sampling at 10-minute intervals per hour was employed. A trained observer was present in the room during the observation periods but took precautions to minimize disturbance to the birds and maintain a consistent distance 1 m away from the battery cages. The observer was blinded to the treatment. The recorded activities, including drinking, feeding, standing, sitting, preening, stretching, feather pecking, and wall pecking (ethogram in Table 7), were reported for each cage. The data is expressed as the proportion of a certain activity to the total activities recorded/ cage.

### Blood collection and biochemical parameters

Blood samples were collected from six birds in each group (one bird per cage; 3 males and 3 females) by birds killing using the Islamic slaughter procedures, which involves severing the jugular vein, carotid artery, trachea, and esophagus with a sharp knife in a single swift motion to ensure loss of consciousness and minimize pain [98, 99]. The slaughter method adhered to the Halal protocol established by Malaysian institutes [100], where birds were slaughtered without anesthesia, and the major jugular veins were severed to facilitate effective bleeding [98] on the 42nd day of age. The collected serum samples were

stored at  $-20^{\circ}\text{C}$  until utilized for biochemical parameter analysis.

Total antioxidant capacity and hydrogen peroxide were measured using commercial colorimetric kits (catalog numbers: TA2513 and HP 25, respectively) according to the manufacturer's instructions (Biodiagnostic, Dokki, Giza, Egypt). Total protein, albumin, aspartate aminotransferase, alanine aminotransferase, urea, and creatinine were all assessed using commercial colorimetric kits (catalog numbers: 310001, 211001, 260001, 264001, 318001, and 234000, respectively) following the manufacturer's protocol (Egyptian Company for Biotechnology). The serum globulin level was determined by subtracting the serum albumin level from the serum total protein level Agina et al. [44].

### Carcass quality measurements

Following blood collection, the birds were subjected to scalding in a water tank at  $55\text{--}60^{\circ}\text{C}$  for 30 s, followed by plucking and evisceration, including the removal of the vent with intestines. The individual weights of the empty carcass, heart, liver, gizzard, and spleen were recorded and expressed as a percentage of body weight [101].

**Water-holding capacity:** The method described by [102] was employed to assess the water-holding capacity of post-rigor chilled quail breast and leg muscles. Approximately 300 mg of the sample was compressed between two filter papers (70mmu) and ceramic plates, applying 5 kg weight for 3 min. After pressing, the compressed sample was carefully separated from the moist filter paper, and its weight was immediately measured. The released water (RW) percentage was calculated using the formula:  $\text{RW}\% = (\text{W}_1 - \text{W}_2)/\text{W}_1 * 100$ , where  $\text{W}_1$  represents the weight of the meat sample, and  $\text{W}_2$  is the weight of the meat sample after pressing. Water-holding capacity was expressed as the percentage of water retained by the meat sample, calculated as:  $\text{Water-holding capacity (\% water retained)} = 100 - \text{RW}\%$ .

**Sensory assessment:** Consumer-based sensory panels were conducted to evaluate the acceptability of quail breast and thigh meat from quails subjected to different dietary treatments. Post-rigor frozen quail breasts and thighs were boiled until completely cooked ( $\sim 10\text{--}15$  min), cut into parts, and kept warm ( $60\text{--}70^{\circ}\text{C}$ ) in Petri dishes for panelist evaluation. Three members of the Food Hygiene Department at the Faculty of Veterinary Medicine, Assiut University, conducted blinded sensory evaluations, ensuring unbiased results free from prior knowledge influence. Sample order was randomized to mitigate sampling order bias. Panelists rated cooked quail breast and thigh samples for colour, taste, juiciness, chewiness, and flavour using a 9-point hedonic scale, where 1=dislike extremely, 5=neither like nor

**Table 7** Behavioural ethogram [95–97]

Behaviour	Definition
<b>Standing</b>	Posture where both feet are in contact with the floor, and no other body part is in contact with the ground.
<b>Sitting</b>	A sitting position is characterized by most of the ventral region of the bird's body being in contact with the cage floor, with no visible space between the floor and the bird.
<b>Walking</b>	Quail walking is observed when the bird is in the process of taking multiple steps, which includes more than two steps
<b>Feeding</b>	The quail's head is positioned inside the feeder, and it is actively pecking at the feed
<b>Drinking</b>	The quail's beak is in contact with the nipples of the drinker, and the bird is actively drinking water
<b>Grooming</b>	Preening (Gently pecking or scratching its own feathers), dust bathing, and leg or wing stretching
<b>Preening</b>	The bird uses its beak to clean its feathers while either standing or crouching.
<b>Stretching</b>	Quail stretching involves the extension of wings and legs, either unilaterally or bilaterally
<b>Shaking</b>	The behaviour described as shaking involves the bird moving its head and body while in a standing position
<b>Feather Peking</b>	Pecking actions directed towards the feathers of another bird.
<b>Wall pecking</b>	The behaviour of pecking at non-edible objects or the ground.

dislike, and 9=like extremely ([103]). Overall acceptability was determined as the mean of the evaluated traits.

**Determination of pH [104]:** Five grams of quail breast or thigh muscles were added to 45 ml of distilled water at 25 °C in a stomacher (Seward 400) bag and subjected to stomaching for 2 min. The mixture was then left for 10 min at room temperature. A pH meter (SD 50 pH meter, Germany), calibrated with buffers at pH 7 and 4, was immersed into the solution to determine the pH of the sample.

### Intestinal microbial analysis

The gastrointestinal tracts of euthanized quails were gathered for microbiological examination. Aseptically, 1 gram of intestinal contents was collected from each quail (one bird per cage) for the enumeration of total bacterial count and *Lactobacillus*. These samples were meticulously stored in cryovials at -80 °C until analysis. Microbial miniaturized plating was conducted following a previously published method [105]. Briefly, the intestinal contents (1 g) of each sample were thawed at room temperature for 15 min and were serially diluted (10-fold) with physiological saline in a 96-well plate. Ten microliters of each dilution were plated on Standard Plate Count Agar (Lab M Limited, Lancashire, UK) for the total bacterial count and MRS agar plates (Lab M Limited, Lancashire, UK) for the lactobacilli count. Total bacterial counts were incubated aerobically at 37 °C for 24 h, while lactobacilli counts were incubated anaerobically at 37 °C for 48 h using a Gas-Pak anaerobic system (Becton Dickinson Microbiology Systems). After incubation, colonies were counted and recorded as colony-forming units per gram (cfu/g) of the sample, expressed as log<sub>10</sub> cfu/g. The average of the counts from two plates was calculated.

### Histopathology

The intestine, liver, and spleen tissue samples were dissected and promptly fixed in 10% formalin. Following dehydration with increasing concentrations of ethyl alcohol (70–100%) and two passes in xylol, the samples were embedded in paraffin and sectioned at 5 µm using a rotary microtome. Subsequently, the histological slides underwent staining with hematoxylin and eosin (H&E) following the method described by [106].

Standard light microscopy was utilized for the evaluation of the histological sections. Quantitative histomorphometry measurements, specifically intestinal villus lengths and areas, were conducted using the NIH ImageJ software [107]. Measurements were taken from photomicrographs captured at 40× magnification. The lengths of both the villi and crypt regions were measured for a total of six selected individual and apparently complete, full-sized intestinal villi that exhibited no bending or mechanical damage in each sample. All measurements

were performed by a single individual who was blinded to the treatments to ensure unbiased data recording. Crypt to villus ratios were then calculated using the length data, as described by [108].

### Statistical analysis

The cage was designated as the experimental unit for analysis. Data were subjected to one-way analysis of variance using the General Linear Models (GLM) procedure in SPSS, version 16. Post hoc comparisons were conducted using Tukey's test upon detecting significant differences. Statistical significance was established at  $P < 0.05$ .

### Acknowledgements

We would like to thank the graduate students and workers of Animal, Poultry and aquatic life Behavior and Management, Faculty of Veterinary Medicine, Assiut University for their help and support.

### Author contributions

U. M, F. A., F. H. and N. K. conceptualized and designed the study. G.R and F. H. were responsible for PAW synthesis and chemical analysis. U. M was responsible for animal housing, data collection of performance and behaviour. N. K. analysed blood parameters and interpreted the changes in blood measurements. F. A. collected intestinal, liver and spleen tissue, and interpreted histopathological lesions. S. A. analysed carcass quality data and interpreted its results. M. M. was responsible for intestinal bacterial count and interpreted its findings. All authors made significant contributions to statistical analysis and manuscript writing. U. M. and N. K. contributed to the drafting of the manuscript. The authors have contributed equally to this work. All authors have read and approved the final version of the manuscript.

### Funding

This study was not supported by any funding. Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Declarations

#### Ethics approval and consent to participate

The experiment adheres to the guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals. The authors confirm their compliance with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines [109]. All procedures in this study received approval from the Faculty of Veterinary Medicine, Assiut University, Egypt, under the OIE standards for the use of animals in research. The ethical approval number for this study is 06/2024/0182.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

#### Author details

<sup>1</sup>Department of Animal, poultry and aquatic life behavior and management, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

<sup>2</sup>Physics Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

<sup>3</sup>Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Sohag University, Sohag 82524, Egypt

<sup>4</sup>Department of Animal Hygiene and Environmental Pollution, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

<sup>5</sup>Department of Food Hygiene, Safety and Technology (Meat Hygiene, Safety and Technology), Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

<sup>6</sup>Physics Department, Faculty of Science, Sohag University, Sohag 82524, Egypt

<sup>7</sup>Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt

<sup>8</sup>Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine, Badr University, Assiut, Egypt

Received: 9 April 2024 / Accepted: 12 August 2024

Published online: 04 September 2024

## References

1. Minvielle F. The future of Japanese quail for research and production. *J World's Poultry Sci.* 2004;60(4):500–7. <https://doi.org/10.1079/WPS200433>.
2. Sabow AB. Carcass characteristics, physicochemical attributes, and fatty acid and amino acid compositions of meat obtained from different Japanese quail strains. *Trop Anim Health Prod.* 2020;52(1):131–40. <https://doi.org/10.1007/s11250-019-01991-2>.
3. Santos TC, Murakami AE, Fanhani JC, Oliveira CAL. Production and reproduction of egg- and meat type quails reared in different group sizes. *Braz J Poultry Sci.* 2011;13(1):09–14. <https://doi.org/10.1590/S1516-635X2011000100002>.
4. Quaresma MAG, Antunes IC, Ferreira BG, Parada A, Elias A, Barros M, et al. The composition of the lipid, protein and mineral fractions of quail breast meat obtained from wild and farmed specimens of common quail (*Coturnix coturnix*) and farmed Japanese quail (*Coturnix japonica* Domestica). *Poult Sci.* 2022;101(1):101505. <https://doi.org/10.1016/j.psj.2021.101505>.
5. Lima HJD, Morais MVM, Pereira IDB. Updates in research on quail nutrition and feeding: a review. *J World's Poultry Sci.* 2023;79(1):69–93. <https://doi.org/10.1080/00439339.2022.2150926>.
6. Silva V, Vieira-Pinto M, Saraiva C, Manageiro V, Reis L, Ferreira E, et al. Prevalence and characteristics of multidrug-resistant livestock-associated methicillin-resistant staphylococcus aureus (La-mrsa) cc398 isolated from quails (*Coturnix coturnix japonica*) slaughtered for human consumption. *Animals.* 2021;11(7):2038. <https://doi.org/10.3390/ani11072038>.
7. Farghaly EM, Samy A, Roshdy H. Wide prevalence of critically important antibiotic resistance in Egyptian quail farms with mixed infections. *Vet Sci Res Rev.* 2017;3(1):17–24. <https://doi.org/10.17582/journal.vsr.2017.3.1.17.24>.
8. Attia YA, Abd El-Hamid AE, Ellakany HF, Bovera F, Al-Harathi MA, Ghazaly SA. Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. *Ital J Anim Sci.* 2013;12(2):e37. <https://doi.org/10.4081/ijas.2013.e37>.
9. Bradu C, Kutasi K, Magureanu M, Puač N, Živković S. Reactive nitrogen species in plasma-activated water: generation, chemistry and application in agriculture. *J Phys D: Appl Phys.* 2020;53:223001. <https://doi.org/10.1088/1361-6463/ab795a>.
10. Liu K, Ren W, Ran C, Zhou R, Tang W, et al. Long-lived species in plasma-activated water generated by an AC multi-needle-to-water discharge: effects of gas flow on chemical reactions. *J Phys D Appl Phys.* 2021;54:065201. <https://doi.org/10.1088/1361-6463/abc211>.
11. Zhou R, Zhou R, Wang P, Xian Y, Mai-Prochnow A, et al. Plasma-activated water: Generation, origin of reactive species and biological applications. *J Phys D Appl Phys.* 2020;53:303001. <https://doi.org/10.1088/1361-6463/ab81cf>.
12. Mahdikia H, Saadati F, Freund E, Gaipal US, Majidzadeh-a K, Shokri B, et al. Gas plasma irradiation of breast cancers promotes immunogenicity, tumor reduction, and an abscopal effect in vivo. *Oncoimmunology.* 2021;10(1):1859731. <https://doi.org/10.1080/2162402X.2020.1859731>.
13. Royintarat T, Choi EH, Boonyawan D, Seesuriyachan P, Wattanutchariya W. Chemical-free and synergistic interaction of ultrasound combined with plasma-activated water (PAW) to enhance microbial inactivation in chicken meat and skin. *Sci Rep.* 2020;10:1559. <https://doi.org/10.1038/s41598-020-58199-w>.
14. Kang C, Xiang Q, Zhao D, Wang W, Niu L, Bai Y. Inactivation of *Pseudomonas deceptionensis* CM2 on chicken breasts using plasma-activated water. *J Food Sci Technol.* 2019;56(11):4938–45. <https://doi.org/10.1007/s13197-019-03964-7>.
15. Hongzhan Z, Ying T, Xia S, Jinsong G, Zhenhua Z, Beiyu J, et al. Preparation of the inactivated Newcastle disease vaccine by plasma activated water and evaluation of its protection efficacy. *Appl Microbiol Biotechnol.* 2020;104(1):107–17. <https://doi.org/10.1007/s00253-019-10106-8>.
16. Zhao YM, Patange A, Sun DW, Tiwari B. Plasma-activated water: physicochemical properties, microbial inactivation mechanisms, factors influencing antimicrobial effectiveness, and applications in the food industry. *Compr Rev Food Sci Food Saf.* 2020;19(6):3951–79. <https://doi.org/10.1111/1541-4337.12644>.
17. Nastasa V, Pasca AS, Malancus RN, Bostanaru AC, Ailincai LI, Ursu EL, et al. Toxicity assessment of long-term exposure to non-thermal plasma activated water in mice. *Int J Mol Sci.* 2021;22(21):11534. <https://doi.org/10.3390/ijms222111534>.
18. Xu D, Cui Q, Xu Y, Wang B, Tian M, Li Q, et al. Systemic study on the safety of immuno-deficient nude mice treated by atmospheric plasma-activated water. *PST.* 2018;20:044003. <https://doi.org/10.1088/2058-6272/aa9842>.
19. Xu Y, Peng S, Li B, Wang S, Zhang H, Li Q, et al. Systematic safety evaluation of Cold plasma-activated liquid in rabbits. *Front Phys.* 2021;9:659227. <https://doi.org/10.3389/fphy.2021.659227>.
20. Kim SW, Kim HJ, Kim SG, Kwon J, Lee S, Bin, Jung WJ, et al. Bactericidal efficacy of non-thermal plasma activation against *Aeromonas hydrophila* and immunological responses of Koi (*Cyprinus carpio* Haematopterus). *Fish Shellfish Immunol.* 2022;121:197–204. <https://doi.org/10.1016/j.fsi.2022.01.007>.
21. Eftekhari A, Rezaei pour V, Abdollahpour R. Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. *Livest Sci.* 2015;180:158–63. <https://doi.org/10.1016/j.livsci.2015.07.010>.
22. Açıkgoz Z, Bayraktar H, Altan Ö. Effects of formic acid administration in the drinking water on performance, intestinal microflora and carcass contamination in male broilers under high ambient temperature. *Asian-Australas J Anim Sci.* 2011;24(1):96–102. <https://doi.org/10.5713/ajas.2011.1.10195>.
23. Serrano-Gamboa MY, Arce-Menocal J, Ávila-González E, López-Coello C, Garibay-Torres L, Herrera-Camacho J. Organic acids for broilers: effects on intestinal morphology and growth performance. *Rev Colomb Cienc Pecu.* 2023;36(2):55–65. <https://doi.org/10.17533/udea.rccp.v36n2a1>.
24. Ottinger MA, Abdelnabi MA, Henry P, McGary S, Thompson N, Wu JM. Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail. *Horm Behav.* 2001;40(2):234–47. <https://doi.org/10.1006/hbeh.2001.1695>.
25. Yamazaki Y, Shinohara N, Watanabe S. Visual discrimination of normal and drag induced behavior in quails (*Coturnix coturnix japonica*). *Anim Cogn.* 2004;7(2):128–32. <https://doi.org/10.1007/s10071-003-0196-5>.
26. Pellegrini S, Orso GA, Marin RH, Guzman DA. The insemination status and social context influences quail laying and social behavior: a novel experimental set up. *Poult Sci.* 2023;102(2):102328. <https://doi.org/10.1016/j.psj.2022.102328>.
27. Kamata R, Takahashi S, Shimizu A, Morita M, Shiraishi F. In ovo exposure quail assay for risk assessment of endocrine disrupting chemicals. *Arch Toxicol.* 2006;80(12):857–67. <https://doi.org/10.1007/s00204-006-0113-1>.
28. Quinn M. Avian behavioral toxicity methods for use with the Japanese quail *Coturnix japonica* as a model species. *Avian Ecol Behav.* 2012;21:41–50.
29. Ratriyanto A, Widyan W, Prastowo S, Andrianto DF. 1st ICADA. Divergent selection towards body weight and betaine supplementation on quails performance in tropical environment. The First International Conference on Assessment and Development of Agricultural Innovation (2021). E3S Web of Conferences. 2021; 306: 05017. <https://doi.org/10.1051/e3sconf/202130605017>.
30. Susanti E, Tugiyanti E. Improving the Quality of Reproduction and Quail Production (*Coturnix coturnix japonica*) with Liquid Probiotics. *Anim Prod.* 2020;22(1):9–15. <https://doi.org/10.20884/1.jap.2020.22.1.42>.
31. Hussein Khalil al-Hilali A, Muhammad Najm Z. The Effect of Aqueous Extract of Anise seeds. *Pimpinella Anisum* L.Effect on Body Weight, Weight Gain, feed consumption and feed Conversion ratio of Japanese quail. *QJAS.* 2018;7(2):192–205. <https://doi.org/10.33794/qjas.Vol7.Iss2.40>.
32. Rohman F, Handarini R, Nur H. Performance of quails (*Coturnix coturnix japonica*) given drink containing moringa leaf solution in their growing period. *Jurnal Peternakan Nusantara.* 2019;4(2):75–82. <https://doi.org/10.30997/jpnu.v4i2.1538>.
33. MAHERİ SİS N, KHALILIPOUR G, TELİ AS. Effects of saline drinking water on growth performance and mortality rate of Japanese quails (*Coturnix coturnix Japonica*). *KSU J Agric Nat.* 2019;22(6):942–7. <https://doi.org/10.18016/ksu-tarimdogavi.553366>.

34. Alam Khan A, Bandy MT, Shahnaz S, Tanveer S. Moderately lower pH of drinking water proves beneficial to poultry. *JPSST*. 2013;1(1):17–9.
35. Khan RU, Chand N, Ali A. Effect of organic acids on the performance of Japanese quails. *Pak J Zool*. 2016;48(6):1799–803.
36. Shihab IM, Ezzat HN, Hussein MA. Effect of using ionized water on some productive and physiological performance of Japanese quails. *Poult Sci*. 2019;98(10):5146–51. <https://doi.org/10.3382/ps/pey590>.
37. Abd El-Reda G, Mahmoud MAM, Khalaf M, Saber AA, El-Hossary FM. Impact of Cold Atmospheric plasma treatment duration on Distilled Water Physicochemical characteristics. *Sohag J Sci*. 2024;9(2):190–7. <https://doi.org/10.21608/sjsoci.2024.247829.1146>.
38. Ikawa S, Tani A, Nakashima Y, Kitano K. Physicochemical properties of bactericidal plasma-treated water. *J Phys D Appl Phys*. 2016;49:425401. <https://doi.org/10.1088/0022-3727/49/42/425401>.
39. Wang Q, Salvi D. Evaluation of plasma-activated water (PAW) as a novel disinfectant: effectiveness on *Escherichia coli* and *Listeria innocua*, physicochemical properties, and storage stability. *LWT*. 2021;149:111847. <https://doi.org/10.1016/j.lwt.2021.111847>.
40. Sivachandiran L, Khacef A. Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment. *RSC Adv*. 2017;7:1822–32. <https://doi.org/10.1039/C6RA24762H>.
41. van Bunnik BAD, Katsma WEA, Wagenaar JA, Jacobs-Reitsma WF, de Jong MCM. Acidification of drinking water inhibits indirect transmission, but not direct transmission of *Campylobacter* between broilers. *Prev Vet Med*. 2012;105(4):315–9. <https://doi.org/10.1016/j.prevetmed.2012.03.007>.
42. Rahman M, Hasan MS, Islam R, Rana R, Sayem ASM, Sad MAA, et al. Plasma-activated water for Food Safety and Quality: a review of recent developments. *Int J Environ Res Public Health*. 2022;19(11):6630. <https://doi.org/10.3390/ijerph19116630>.
43. Qian J, Wang C, Zhuang H, Zhang J, Yan W. Oxidative stress responses of pathogen bacteria in poultry to plasma-activated lactic acid solutions. *Food Control*. 2020;118:107355. <https://doi.org/10.1016/j.foodcont.2020.107355>.
44. Agina OA, Ezema WS, Iwuoha EM. The haematology and serum Biochemistry Profile of Adult Japanese Quail (*Coturnix coturnix japonica*). *Not Sci Biol*. 2017;9:67–72. <https://doi.org/10.15835/nsb919928>.
45. Ahmad EAM, Abdel-Kader IA, Abdel-Wahab AA, Abd A-W, Abdel-Wahab A. Organic acids as potential alternate for antibiotic as growth promoter in Japanese quail. *Egypt Poult Sci*. 2018;38(II):359–73.
46. Azzariti A, Iacobazzi RM, Di Fonte R, Porcellini L, Gristina R, Favia P, et al. Plasma-activated medium triggers cell death and the presentation of immune activating danger signals in melanoma and pancreatic cancer cells. *Sci Rep*. 2019;9:4099. <https://doi.org/10.1038/s41598-019-40637-z>.
47. Wang S, Wang P, Thompson R, Ostrikov K, Xiao Y, Zhou Y. Plasma-activated medium triggers immunomodulation and autophagic activity for periodontal regeneration. *Bioeng Transl Med*. 2023;8(4):e10528. <https://doi.org/10.1002/btm2.10528>.
48. Eldeighdy SM, Allam TM, Hassanin WF. The protective effect of non-thermal plasma against gamma irradiation in albino rats. *J Radiat Res Appl Sci*. 2021;14(1):467–76. <https://doi.org/10.1080/16878507.2021.1996844>.
49. Chen F, He H, Wang L, Yang X, Gao J, Wang J, et al. Toxicity study of rats treated by plasma-activated solution. *Heliyon*. 2023;9(12):e23116. <https://doi.org/10.1016/j.heliyon.2023.e23116>.
50. Wu H, Wang Y, Huang J, Li Y, Lin Z, Zhang B. Rutin ameliorates gout via reducing XOD activity, inhibiting ROS production and NLRP3 inflammasome activation in quail. *Biomed Pharmacother*. 2023;158:114175. <https://doi.org/10.1016/j.biopha.2022.114175>.
51. Chu MZ, Lin ZJ, Zhang B, Wang Y, Dong YZ, Wang X, et al. Induction and preliminary study of quail visceral gout model. *Chin Pharm Bull*. 2020;36(6):879–83.
52. Wu H, Wang Y, Ren Z, Li Y, Huang J, Lin Z, et al. Overnutrition-induced gout: an immune response to NLRP3 inflammasome dysregulation by XOD activity increased in quail. *Front Immunol*. 2022;13:1074867. <https://doi.org/10.3389/fimmu.2022.1074867>.
53. Xi Y, Huang Y, Li Y, Huang Y, Yan J, Shi Z. The effects of dietary protein and fiber levels on growth performance, gout occurrence, intestinal microbial communities, and immunoregulation in the gut-kidney axis of goslings. *Poult Sci*. 2022;101(5):101780. <https://doi.org/10.1016/j.psj.2022.101780>.
54. Ghaffar A, Hussain R, Abbas G, Ahmad MN, Abbas A, Rahim Y, et al. Sodium arsenate and/or urea differently affect clinical attributes, hemato-biochemistry and DNA damage in intoxicated commercial layer birds. *Toxin Rev*. 2018;37(3):206–15. <https://doi.org/10.1080/15569543.2017.1342096>.
55. Aberle E, Forrest J, Hedrick H, Merkel R. Principles of meat science. 1989.
56. Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, et al. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. *Poult Sci*. 2005;84(7):1015–21. <https://doi.org/10.1093/ps/84.7.1015>.
57. Barbut S. Problem of pale soft exudative meat in broiler chickens. *Br Poult Sci*. 1997;38(4):355–8. <https://doi.org/10.1080/00071669708418002>.
58. Gevrekçi Y, Oğuz I, Akşit M, Önenç A, Özdemir D, Altan Ö. Heritability and variance component estimates of meat quality in Japanese quail (*Coturnix coturnix japonica*). *Turk J Vet Anim Sci*. 2009;33(2). <https://doi.org/10.3906/vet-0603-30>.
59. Oğuz I, Akşit M, Önenç A, Gevrekçi Y, Özdemir D, Altan Ö. Genetic variability of meat quality characteristics in Japanese quail (*Coturnix coturnix japonica*). *Archiv für Geflügelkunde*. 2004;68(4):176–81.
60. Spence C. The Psychological Effects of Food Colors. *Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color*. 2016: 29–58. <https://doi.org/10.1016/B978-0-08-100371-8.00002-6>.
61. Hughes JM, Oiseth SK, Purslow PP, Warner RD. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci*. 2014;98(3):520–32. <https://doi.org/10.1016/j.meatsci.2014.05.022>.
62. Faustman C, Sun Q, Mancini R, Suman SP. Myoglobin and lipid oxidation interactions: mechanistic bases and control. *Meat Sci*. 2010;86(1):86–94. <https://doi.org/10.1016/j.meatsci.2010.04.025>.
63. Nosrati M, Javandel F, Camacho LM, Khuro A, Cipriano M, Seidavi A, et al. The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *JAPR*. 2017;26(2):295–306. <https://doi.org/10.3382/japr/pfw073>.
64. Ndelekwute EK, Uzegbu HO, Amaefule KU, Okereke CO, Umoh BI. Effect of organic acids fed through drinking water on carcass and internal organs of broiler chickens. *Nigerian J Anim Prod*. 2021;41(1):60–7. <https://doi.org/10.51791/njap.v41i1.2681>.
65. Zhao Y, Chen R, Tian E, Liu D, Niu J, Wang W, et al. Plasma-activated Water Treatment of Fresh Beef: bacterial inactivation and effects on Quality attributes. *IEEE Trans Radiat Plasma Med Sci*. 2020;4(1):113–20. <https://doi.org/10.1109/TRPMS.2018.2883789>.
66. Jung S, Kim HJ, Park S et al. In Yong H, Choe JH, Jeon HJ. The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage. *Meat Sci*. 2015;108:132–137. <https://doi.org/10.1016/j.meatsci.2015.06.009>.
67. Patra A, Prasath VA, Pandiselvam R, Sutar PP, Jeevarathinam G. Effect of plasma activated water (PAW) on physicochemical and functional properties of foods. *Food Control*. 2022;142:109268. <https://doi.org/10.1016/j.foodcont.2022.109268>.
68. Herianto S, Hou CY, Lin CM, Chen HL. Nonthermal plasma-activated water: a comprehensive review of this new tool for enhanced food safety and quality. *Compr Rev Food Sci Food Saf*. 2021;20(1):583–626. <https://doi.org/10.1111/1541-4337.12667>.
69. Shanker MA, Khanashyam AC, Pandiselvam R, Joshi TJ, Thomas PE, Zhang Y, et al. Implications of cold plasma and plasma activated water on food texture - a review. *Food Control*. 2023;151:109793. <https://doi.org/10.1016/j.foodcont.2023.109793>.
70. Rahman SME, Park J, Song K, Bin, Al-Harbi NA, Oh DH. Effects of slightly acidic low concentration electrolyzed water on microbiological, physicochemical, and sensory quality of fresh chicken breast meat. *J Food Sci*. 2012;77(1):M35–41. <https://doi.org/10.1111/j.1750-3841.2011.02454.x>.
71. Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, et al. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poult Sci*. 2022;101(4):101696. <https://doi.org/10.1016/j.psj.2022.101696>.
72. Damayanti E, Julendra H, Sofyan A, Hayati SN. Bile salt and acid tolerant of lactic acid bacteria isolated from proventriculus of broiler chicken. *Media Peternakan*. 2014;37(2):80–6. <https://doi.org/10.5398/medpet.2014.37.2.80>.
73. Hamida F, Wiryawan KG, Meryandini A. Selection of lactic acid bacteria as probiotic candidate for chicken. *Media Peternakan*. 2015;38(2):138–44. <https://doi.org/10.5398/medpet.2015.38.2.138>.
74. Russell JB, Diez-Gonzalez F. The effects of fermentation acids on bacterial growth. *Adv Microb Physiol*. 1998. [https://doi.org/10.1016/S0065-2911\(08\)60017-x](https://doi.org/10.1016/S0065-2911(08)60017-x). 39:205–34.
75. Hutkins RW, Nannen NL. pH homeostasis in lactic acid Bacteria. *J Dairy Sci*. 1993;76(8):2354–65. [https://doi.org/10.3168/jds.S0022-0302\(93\)77573-6](https://doi.org/10.3168/jds.S0022-0302(93)77573-6).

76. Atabaigi Elmi V, Moradi S, Ghazi S, Rahimi M. Effects of *Lactobacillus acidophilus* and natural antibacterials on growth performance and *Salmonella* colonization in broiler chickens challenged with *Salmonella enteritidis*. *Livest Sci*. 2020;233:103948. <https://doi.org/10.1016/j.livsci.2020.103948>.
77. Kalavathy R, Abdullah N, Jalaludin S, Ho YW. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *Br Poult Sci*. 2003;44(1):139–44. <https://doi.org/10.1080/0007166031000085445>.
78. De Cesare A, Sirri F, Manfreda G, Moniaci P, Giardini A, Zampiga M, et al. Effect of dietary supplementation with *Lactobacillus acidophilus* D2/CSL (CECT 4529) on caecum microbiota and productive performance in broiler chickens. *PLoS ONE*. 2017;12(5):e0176309. <https://doi.org/10.1371/journal.pone.0176309>.
79. Wang L, Lin Z, Ali M, Zhu X, Zhang Y, Li S, et al. Effects of lactic acid bacteria isolated from tibetan chickens on the growth performance and gut microbiota of broiler. *Front Microbiol*. 2023;14:1171074. <https://doi.org/10.3389/fmicb.2023.1171074>.
80. Pradipta MSI, Sri-Harimurti. Widodo. Feed supplementation with encapsulated indigenous probiotic lactic acid Bacteria increased broiler chicken performance. *ASEAN J SCI TECHNOL DEV*. 2019;36(1):29–34. <https://doi.org/10.29037/ajstd.569>.
81. Forte C, Manuali E, Abbate Y, Papa P, Viecelli L, Tentellini M, et al. Dietary *Lactobacillus acidophilus* positively influences growth performance, gut morphology, and gut microbiology in rurally reared chickens. *Poult Sci*. 2018;97(3):930–6. <https://doi.org/10.3382/ps/pex396>.
82. Olhood CG, Beski SSM, Choct M, Jiji PA. Novel probiotics: their effects on growth performance, gut development, microbial community and activity of broiler chickens. *Anim Nutr*. 2015;1(3):184–91. <https://doi.org/10.1016/j.aninu.2015.07.003>.
83. Vineetha PG, Tomar S, Saxena VK, Kapgate M, Suvarna A, Adil K. Effect of laboratory-isolated *Lactobacillus plantarum* LGFCP4 from gastrointestinal tract of guinea fowl on growth performance, carcass traits, intestinal histomorphometry and gastrointestinal microflora population in broiler chicken. *J Anim Physiol Anim Nutr (Berl)*. 2017;101(5):e362–70. <https://doi.org/10.1111/jpn.12613>.
84. Meimandipour A, Hair-Bejo M, Shuhaimi M, Azhar K, Soleimani AF, Rasti B, et al. Gastrointestinal tract morphological alteration by unpleasant physical treatment and modulating role of *Lactobacillus* in broilers. *Br Poult Sci*. 2010;51(1):52–9. <https://doi.org/10.1080/00071660903394455>.
85. Chichlowski M, Croom WJ, Edens FW, McBride BW, Qiu R, Chiang CC, et al. Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, Primalac, and salinomycin. *Poult Sci*. 2007;86(6):1121–32. <https://doi.org/10.1093/ps/86.6.1121>.
86. Fan YK, Croom J, Christensen VL, Black BL, Bird AR, Daniel LR, et al. Jejunal Glucose Uptake and oxygen consumption in Turkey Poults selected for Rapid Growth. *Poult Sci*. 1997;76(12):1738–45. <https://doi.org/10.1093/ps/76.12.1738>.
87. DeMartino AW, Kim-Shapiro DB, Patel RP, Gladwin MT. Nitrite and nitrate chemical biology and signalling. *Br J Pharmacol*. 2019;176(2):228–45. <https://doi.org/10.1111/bph.14484>.
88. Tiso M, Schechter AN. Nitrate reduction to Nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. *PLoS ONE*. 2015;10(3):e0119712. <https://doi.org/10.1371/journal.pone.0119712>.
89. Sobko T, Huang L, Midtvedt T, Norin E, Gustafsson LE, Norman M, et al. Generation of NO by probiotic bacteria in the gastrointestinal tract. *Free Radic Biol Med*. 2006;41(6):985–91. <https://doi.org/10.1016/j.freeradbiomed.2006.06.020>.
90. Sobko T, Reinders CI, Jansson EÅ, Norin E, Midtvedt T, Lundberg JO. Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. *Nitric Oxide*. 2005;13(4):272–8. <https://doi.org/10.1016/j.niox.2005.08.002>.
91. Laurindo FRM, Liberman M, Fernandes DC, Leite PF. Endothelium-Dependent Vasodilation: Nitric Oxide and Other Mediators. *Endothelium and Cardiovascular Diseases: Vascular Biology and Clinical Syndromes*. 2018; 97–113. <https://doi.org/10.1016/B978-0-12-812348-5.00008-8>
92. Mangan C, Stott MC, Dhanda R. Renal physiology: blood flow, glomerular filtration and plasma clearance. *Anaesth Intensive Care Med*. 2018;19(5):254–7. <https://doi.org/10.1016/j.mpac.2018.02.013>.
93. Mahmoud UT, Abdel-rahman MA, Darwish MHA, Mosaad GM. The effect of heat stress on blood picture of Japanese quail. *J Adv Vet Res*. 2013;3:69–76. <https://www.advetresearch.com/index.php/AVR/article/view/118/117>.
94. Mahmoud UT, Abdel-rahman MAM, Darwish MHA, Mosaad GMM. The effect of separate-sex rearing of Slaughter Quail on serum corticosterone level. *J Adv Vet Res*. 2012;2:206–7. <https://advetresearch.com/index.php/AVR/article/view/182/179>.
95. Mossad GM, Abdel-Rahman MA, Darwish MH, Mahmoud UT. Performance, Behaviour, carcass traits and blood constituents of Japanese quails reared under heat stress. *Assiut Vet Med J* 2010;57(126). [https://journals.ekb.eg/article\\_173963\\_e6c9078c037d5aae1f6a39617887752c.pdf](https://journals.ekb.eg/article_173963_e6c9078c037d5aae1f6a39617887752c.pdf)
96. Mahmoud UT, Khalil NSA, Elsayed MSA. Behavioral and physiological effects of Mannan-oligosaccharide and  $\beta$ -glucan Prebiotic Combination on Heat stressed broiler chickens. *J Adv Vet Res*. 2017;7:81–6. <https://advetresearch.com/index.php/AVR/article/view/258/245>.
97. Mahmoud UT, Abdel-Rahman MAM, Darwish MHA, Applegate TJ, Cheng HW. Behavioral changes and feathering score in heat stressed broiler chickens fed diets containing different levels of propolis. *Appl Anim Behav Sci*. 2015;166:98–105. <https://doi.org/10.1016/j.applanim.2015.03.003>.
98. Elzahr HAA, Ibrahim ZA, Ahmed SA, Salah AS, Osman A, Swelum AA, et al. Growth, carcass criteria, and blood biochemical parameters of growing quails fed *Arthrospira platensis* as a feed additive. *Poult Sci*. 2023;102(12):103205. <https://doi.org/10.1016/j.psj.2023.103205>.
99. Nakyinsige K, Che Man YB, Aghwan ZA, Zulkifli I, Goh YM, Abu Bakar F, et al. Stunning and animal welfare from Islamic and scientific perspectives. *Meat Sci*. 2013;95(2):352–61. <https://doi.org/10.1016/j.meatsci.2013.04.006>.
100. JAKIM (Department of Islamic Development Malaysia). Malaysian protocol for the halal meat and poultry productions. 2011. <https://law.resource.org/pub/my/ibr/ms.halal.protocol.2011.pdf>
101. Fathi MM, Al-Homidan I, Ebeid TA, Galal A, Abou-Emera OK. Assessment of residual feed intake and its relevant measurements in two varieties of Japanese quails (*Coturnixcoturnix Japonica*) under high environmental temperature. *Animals*. 2019;9:299. <https://doi.org/10.3390/ani9060299>.
102. Honikel KO, Hamm R. Measurement of water-holding capacity and juiciness. In: Quality attributes and their measurement in meat, Poultry and Fish products. In: Pearson AM, Dutson TR, editors. *Quality attributes and their measurement in meat, Poultry and Fish products*. Advances in Meat Research. Volume 9. Boston, MA: Springer; 1994. [https://doi.org/10.1007/978-1-4615-2167-9\\_5](https://doi.org/10.1007/978-1-4615-2167-9_5).
103. Cville GV, Carr BT. Sensory evaluation techniques. 5th ed. CRC; 2015. <https://doi.org/10.1201/b19493>.
104. Konuk Takma D, Korel F. Active packaging films as a carrier of black cumin essential oil: development and effect on quality and shelf-life of chicken breast meat. *Food Packag Shelf Life*. 2019;19:210–7. <https://doi.org/10.1016/j.fpsl.2018.11.002>.
105. Sieuwerts S, de Bok FAM, Mols E, de Vos WM, van Hylckama Vlieg JET. A simple and fast method for determining colony forming units. *Lett Appl Microbiol*. 2008;47:275–8. <https://doi.org/10.1111/j.1472-765x.2008.02417.x>.
106. Bancroft JD, Gamble M. *Theory and practice of histological techniques*. Elsevier health sciences; 2008.
107. Ferreira T, Rasband W. ImageJ user guide user guide ImageJ. Image J user Guide. 2012;1:46r. <https://imagej.net/ij/docs/guide/>
108. Wilson FD, Cummings TS, Barbosa TM, Williams CJ, Gerard PD, Peebles ED. Comparison of two methods for determination of intestinal villus to crypt ratios and documentation of early age-associated ratio changes in broiler chickens1,2,3. *Poult Sci*. 2018;97(5):1757–61. <https://doi.org/10.3382/ps/pex349>.
109. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the arrive guidelines for reporting animal research. *PLoS Biol*. 2010;8(6):e1000412. <https://doi.org/10.1371/journal.pbio.1000412>.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.