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Effects of a Functional Mix Feed Additive on Growth Performance, Meat Quality and Vaccine Immunity in Broiler Chickens

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Abstract

In recent years, efforts to reduce antibiotic use in the poultry sector have generated increased interest in alternative feeding strategies. Natural ingredients such as propolis, omega-3 fatty acids, and Lactobacillus subtilis are increasingly being utilized as feed additives to increase production performance and bolster immunity in poultry. This study systematically investigated the effects of a feed additive comprising propolis, omega-3 fatty acids, and Lactobacillus subtilis on growth performance, meat quality, and Newcastle vaccine immunity in broiler chickens. The research involved 144 mixed-sex Ross 308 broiler chicks, aged one day, which were divided into four distinct groups: a control group (T1), a group receiving only the vaccine (T2), a group receiving both the vaccine and the feed additive (T3), and a group receiving only the feed additive (T4). The chicks were monitored over a period of 42 days to evaluate the outcomes.

The results indicated that chicks in group T4 achieved the greatest live weight gain, with an average of 2275 g, and exhibited a superior feed conversion rate of 1.85. Additionally, color changes were assessed separately for the skin-on and skin-off treatments among the study groups. The L color change values were significantly greater in the T1 and T2 groups than in the T3 and T4 groups ($p = 0.003$). Prior to vaccination, all groups displayed negative antibody levels, with no significant differences observed in the sample/positive (S/P) ratios among them. However, postvaccination evaluations revealed statistically significant differences in S/P ratios across the groups ($p < 0.001$), with the T1, T2, and T4 groups showing notably lower values. The vaccinated groups presented an increase in the immune response, which contributed to a more resilient profile against infectious diseases. These findings highlight the positive effects of the mixed feed additive on the efficacy of vaccination, suggesting that its inclusion not only enhances overall performance in broiler chickens but also strengthens their immune response to the Newcastle vaccine, thereby promoting a healthier and more resilient poultry production system.

Introduction

Newcastle disease is recognized as one of the most significant poultry diseases worldwide, representing an ongoing threat and economic burden to the agricultural production sector (Mayers et al., 2017). The symptoms and mortality rates associated with Newcastle disease vary and are influenced by factors such as the infective strain, viral dose, infection management practices, and the age and immunological status of the animals. Owing to the rapid transmissibility and increased morbidity and mortality observed in infected broilers, the disease can result in numerous respiratory, gastrointestinal, or neurological symptoms, at times leading to high mortality rates without evident clinical signs. To control Newcastle infections, numerous strategies, including the enhancement of immunity levels through vaccination and improved management in broiler farms, have been implemented; however, virulent viral strains persist in causing infections within endemic regions (Gharajalar et al., 2020). Vaccination, particularly against mildly virulent strains, remains the primary means of preventing Newcastle disease. However, the exclusive use of vaccines may compromise the immune response and result in adverse effects among

vaccinated individuals. Therefore, the selection and application of appropriate adjuvants are necessary to increase the immunogenicity and protective efficacy of Newcastle vaccines (Younas et al. 2025).

The incorporation of various hormones and antibiotics in broiler feeding practices to increase growth has raised concerns regarding the accumulation of these substances in animal tissues and organs. Moreover, the health risks associated with the consumption of products containing antibiotic residues, particularly antibiotic resistance, have prompted several countries to establish regulations that limit the use of antibiotics in poultry farming. These regulatory measures are subject to specific conditions (Beyene 2016; Önel and Aksu, 2020; Sarwar et al. 2025). As a result of these risks, probiotics have emerged as safer alternative feed additives, defined as "live microorganisms that confer health benefits to the host when consumed in adequate amounts as part of food" (Yan and Polk 2011; Sarwar et al. 2025). Probiotic strains such as Lactobacillus, Bifidobacterium, and Saccharomyces have been extensively studied and utilized in both human and animal health (Yan and Polk 2011). Commercial probiotics that contain Lactobacillus spp. are applied within the broiler industry to increase performance metrics, such as carcass weight and feed conversion efficiency, while also increasing resistance to infectious diseases (Nikpiran et al. 2013). Additionally, Lactobacillus and Bifidobacterium have the capacity to synthesize vitamins B9 (folate), A, C, and certain members of the B complex, which play critical roles in host metabolism (Peluzio et al. 2021). Lactobacilli exert their effects in the gastrointestinal tract by lowering the luminal pH, thus creating an environment detrimental to pathogenic bacteria (Axelsson, 2004; Menconi et al., 2011). They positively influence the composition of the gastrointestinal microbiota by increasing beneficial bacterial populations, such as Bifidobacterium spp., and reducing populations of potentially harmful bacteria, including Escherichia coli, Clostridia, and Staphylococci, thereby facilitating notable improvements in animal performance (Forte et al., 2016). Research has demonstrated the efficacy of these specific probiotics in enhancing antibody production against viral pathogens, including Newcastle disease (Forte et al., 2016).

In the poultry industry, infections resulting from bacteria, viruses, and fungi can lead to significant economic losses. To mitigate these infections, annual vaccination or biosecurity practices are essential (Asgharian et al. 2025). Probiotics may enhance the immune response against Newcastle disease virus by increasing the activity of macrophages and natural killer cells, regulating cytokine and immunoglobulin release, and contributing to the maintenance of intestinal epithelial barrier integrity (Shawky et al. 2026). The effective use of probiotics in alleviating heat stress has also been validated in studies with layer hens, where the administration of probiotics has shown positive effects on performance indicators such as average daily feed consumption and egg weight (Krysiak et al. 2021).

Propolis is a viscous product composed of resinous, balsamic substances collected by bees from tree buds and plant exudates and subsequently digested with enzymes ingested orally (El Sherif et al. 2021; Irigoiti et al. 2021). It enhances the digestibility and absorption of nutrients in poultry by stimulating the activities of enzymes such as saccharase, amylase and phosphatase (El Sherif et al. 2021). The incorporation of propolis into diets significantly alters feed efficiency. This improvement can be attributed to the flavonoids present in propolis, leading to increased levels of beneficial gut microbiota, a

decrease in pathogenic bacteria, more stable gut flora, and thus increased digestibility (Abd El-Ghany 2024). Propolis is also a rich source of vitamins A, B1, B2, B3, C, and E, along with essential minerals and vitamins crucial for promoting growth (Abd El-Ghany 2024; Sorucu et al. 2025).

To achieve optimum production, broiler chicks must possess a healthy gut characterized by effective digestive function, adequate immune competence, and a balanced intestinal microbiota (Thanabalan 2021). Omega-3 and omega-6 fatty acids are particularly crucial during embryonic development, especially for the development of the immune and nervous systems in chickens. Notably, fatty acids exhibit visible differences in energy loads, which impact the overall efficiency and energy balance of the animals (Krysiak et al. 2021). Omega-3 fatty acids increase weight gain, immunity, and lipid parameters (Alagawany et al. 2020). Numerous investigations have been conducted to strengthen the immune system and enhance production in broiler nutrition through the addition of various immune-stimulating and immune-enhancing agents, with a particular focus on natural additives such as flaxseed oil and omega-3 fish oils (Gharajalar et al. 2020).

The hypothesis of this study is that a mixed feed additive comprising probiotics, propolis, and omega-3 fatty acids may yield positive results in terms of growth performance, meat quality, and immunomodulation in broiler chicks subjected to Newcastle vaccination at one day of age.

Materials and Methods

All procedures of this study were approved and monitored by the Animal Experiments Local Ethics Committee of Hatay Mustafa Kemal University, Hatay, Türkiye (Approval Number: 2021/05–11).

Experimental Design and Animals

A total of 144 one-day-old Ross 308 broiler chicks were utilized in this investigation. The experimental animals used in this study were purchased from a specialized company. The broiler chicks were obtained from Ross 308 broiler breeder hens that were 38 weeks old. Four groups were established, each comprising 36 chicks: control (T1), vaccine (T2), vaccine combined with feed additives (T3), and feed additive alone (T4) groups. The experimental design included a total of 12 groups, with 3 replicates of 12 chicks in each group. The T1 group did not receive the Newcastle vaccine and was fed exclusively a basal diet. In the experimental T4 group, the chicks were not vaccinated; they received a functional feed additive at a dose of 10 g/kg added to the basal diet. The T2 group was administered the vaccine without any additional feed additives, whereas the T3 group was given the Newcastle vaccine alongside the 10 g/kg functional feed additive incorporated into the basal diet. The formulation of the feeds used in the study is presented in Table 1, while the composition of the feed additives applied is detailed in Table 2. The functional feed additive was a commercial formulation containing vitamins (D3, C, E and beta-carotene), propolis, krill oil, Omega 3, and enzymes such as Endo-1,4-β-xylanase (1600 IU/kg), β-glucanase (480 IU/kg), and α-amylase (80 IU/kg), in addition to the probiotic Lactobacillus subtilis (1.6 × 1010 CFU/kg).

Assessment of Performance

The chicks were assigned to groups and given ad libitum access to feed. The research spanned a duration of 42 days. Live body weights and weight gains were recorded at the beginning of the trial and subsequently on days 7, 14, 21, 28, 35, and 42. The environmental parameters, including temperature, lighting, ventilation, and other conditions, were maintained according to the requirements of Ross-308 broiler chicks. Great care was taken to prevent any cross-contamination among the groups. The feed consumption and feed conversion rates were determined through weekly weight assessments.

Table 1
Nutritional Composition of the Feeds Used in the Experiment

Nutritional Values of Diets (%)				
Raw materials %	**Starter Broiler Chick Feed (0–13 days)**	**Broiler Chick Feed (14–28 days)**	**Broiler Finisher Feed (29–34 days)**	**Broiler Pre-Harvest Feed (35–42 days)**
Corn 6.2% CP	49.01	49.97	51.56	51.56
Soybean Meal 47% CP	37.81	32.24	27.07	27.07
Barley 12% CP	4.00	7.00	9.00	9.00
Soybean oil	3.17	4.23	5.28	5.28
Sunflower meal 33%CP	3.00	4.00	5.00	5.00
Limestone	0.75	0.65	0.55	0.55
Salt	0.27	0.27	0.26	0.26
Mineral Premix*	0.20	0.20	0.20	0.20
DL-Methionine	0.36	0.32	0.28	0.28
L-Lysine HCl	0.17	0.16	0.15	0.15
Dicalcium Phospahte	0.73	0.44	0.15	0.15
Sodium Bicarbonate	0.11	0.12	0.13	0.13
Phytase	0.12	0.12	0.12	0.12
L-Threonine	0.15	0.13	0.10	0.10
Vitamin Premix*	0.10	0.10	0.10	0.10
Choline Chloride —75%	0.05	0.05	0.05	0.05
Calculated nutrient compositions				
	(0–13 days)	(14–28 days)	(29–34 days)	(35–42 days)
ME (kcal/kg)	3100	3100	3100	3100
Crude Protein	24.11	22.83	20.5	20.5
Crude Fiber	3.65	3.34	3.16	3.16

Nutritional Values of Diets (%)				
Diethyl Ether Extract	5.98	6.44	7.1	7.1
Crude Ash	6.19	5.33	4.99	4.99
Calcium	0.98	0.89	0.81	0.81
Phosphorus	0.55	0.50	0.45	0.45
Sodium	0.17	0.18	0.19	0.18
Lysine	1.47	1.18	1.19	1.19
Methionine	0.67	0.61	0.55	0.55

*The vitamin and mineral premix contained the following per kg: 12000 IU vitamin A, 5,000 IU vitamin D3, 500 IU enzymes (protease, xylanase, phytase), 15 mg vitamin E, 4 mg vitamin B2, 10 mg vitamin B12, 2 mg vitamin K3, 60 mg manganese, 0.7 mg phosphorus, 0.30 mg sodium, and 1.5 calcium.

Table 2
Composition of the active ingredients in the feed additives

Active Ingredient	Additive Name	Level in Premix	Unit
Vitamins and Similar Biological Effect Substances			
Vitamin D_3		200.000	IU/kg
Beta Carotene		900	mg/kg
Vitamin C		1.200	mg/kg
Vitamin E		2.000	mg/kg
Binders, Anti-Caking Agents, and Coagulants			
Sepiolit		100.000	mg/kg
Natural Substances			
Propolis		200	mg/kg
Krill Oil – Omega 3	EPA	1.560	mg/kg
	DHA	1.080	mg/kg
	Omega 3 Fatty Acids	3.200	mg/kg
Digestive Enhancers - Enzymes			
Endo – 1,4 – beta- xylanase		1.600	U/kg
Endo – 1,3 (4) beta- glucanase		480	U/kg
Fungal Endo – 1,4 – beta- glucanase		420	U/kg
Alpha-amylase		80	KNU/kg
Beta-glucanase		140	FBG/kg
Regulators and Probiotics			
Lactobacillus subtilis		1.6×10^{10}	CFU/kg

Fatty acid analysis

Fatty acid composition analysis was conducted on thigh meat samples obtained from broiler chickens following the fattening period. A total of 48 samples, comprising 4 samples from each replicate group, were analyzed. Body weight of each sampled bird was recorded, and following overt clinical evaluation, the birds were euthanized by cervical dislocation. After the complete removal of the skin from the thigh meat, approximately 10 grams of tissue samples were homogenized for fatty acid analysis. Following the methylation process, the analysis was performed via a modified three-stage method described by

Wang et al. (2024), which involved the addition of potassium hydroxide (10 M), methanol, and sulfuric acid (10 M).

The methylated fatty acids in n-hexane were transferred into vials sealed with PTE caps and analyzed via a gas chromatograph (GC, Thermo1300, Thermo Scientific, Waltham, MA, USA) equipped with flame-ionization detection (FID) and interconnected with an automatic sampler (Thermo AI 1310, Thermo Scientific, Waltham, MA, USA). The apparatus was fitted with a fatty acid methyl ester (FAME) column (length: 60 m, internal diameter: 0.25 mm, film thickness: 0.25 µm, maximum temperature: 250–260°C). The operational parameters included an injection split temperature of 255°C, a column temperature of 140°C, and a flow rate of 30 mL/min over a processing duration of 42 minutes. A standard FAME mixture (37°C) (CL.40.13093.0001, Chem-Lab, Zedelgem, Belgium) in dichloromethane was used for the identification of chromatographic peaks. The identification of fatty acids was accomplished by comparing the retention times of the chromatographic peaks with those of the standard (Kara, 2020).

Vaccination and serological analysis

Prevaccination maternal antibody tests were conducted for all groups, and the vaccination cohorts consisted of chicks that tested negative for maternal antibodies. Vaccination was administered via a commercial live vaccine containing the live La Sota strain of Newcastle disease virus (Hipraviar S, HIPRA Laboratorios, Amer, Spain), which was delivered via the ocular—nasal route at a dosage of 30 µL as a single dose.

Euthanasia was not performed on the animals. Blood samples of 0.5-1 ml were taken from the left cutaneous ulnaris vein (v. cutanea ulnaris) of each animal and collected in CaEDTA tubes through a cannula. Twenty days post-vaccination, approximately 0.5–1 mL of blood was extracted from the wing vein, and the antibody levels were evaluated via a commercial ELISA kit (Newcastle Disease Antibody ELISA, IDEXX, DEXX, Liebefeld-Bern, Switzerland). Antibody detection was performed on sera diluted to a ratio of 1:500. Optical density (OD) values were measured at a wavelength of 650 nm to determine the cutoff values for detecting NDV-specific antibodies as sample-to-positive (S/P) ratios exceeding 0.2.

Statistical Analyses

Statistical analyses were performed via SPSS version 20.0 (IBM, Inc., Chicago, IL, USA). Descriptive statistics are expressed as the mean ± standard deviation (SD) for all measurements. Comparisons among study groups across various subgroups and time intervals were executed via repeated measures two-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. For virological comparisons, one-way ANOVA was employed, followed by Tukey's HSD post hoc test. Significant pairwise comparisons are noted alongside their corresponding p values in the tables provided below. For comparisons between two independent groups, Student's t test was utilized. A p value of less than 0.05 was regarded as statistically significant, indicating a 5% Type-I error rate.

Results and Discussion

The analysis focused on discerning differences not only among subgroups but also across broader study groups. Each study group was monitored over a continuous six-week period, during which changes in live weight measurements were systematically tracked. The mean values for all the measurements are delineated in Table 3. A statistically significant increase in live weight was evident across all study groups during the repeated measurements throughout the study period ($p < 0.001$). However, no significant differences were detected among the subgroups ($p = 0.165$). Furthermore, no significant differences were recorded among the groups during the first week ($p = 0.315$). Notably, significant differences emerged starting from the second week of measurement. At week 2, the T2 group weighed significantly less weight than did the other groups did ($p = 0.013$). At week 3, the average weights of the T2 and T4 groups were significantly greater than those of the T1 and T3 groups ($p = 0.001$). Compared with the other groups, only the T3 group presented significantly lower weight averages by the end of week 4 ($p = 0.026$). By the end of week 5, the weights of the animals in group T1 surpassed those in the other groups, with those in group T2 demonstrating significantly greater weights than those in groups T3 and T4 ($p = 0.001$). At week 6, despite group T2 showing a slightly lower mean, no significant differences were observed among the groups ($p = 0.225$). By week 7, the mean weight of the T1 group was greater than that of the other groups, while the weight values of the T2 and T3 groups were significantly lower than those of the T4 group ($p = 0.042$) (Table 3). The interaction effect between repeated weekly measurements and study groups was significant ($p = 0.013$) (Table 3).

Weight gain observations during the initial two weeks did not reveal significant differences across the groups. However, from day 15 onward, significant differences were detected among the study groups. During weeks 3 and 4, group T1 presented increased weight gain, whereas group T4 presented reduced weights. By the end of week 5, group T3 presented greater weight gain than the other groups did, but by the time of the study's completion, group T3 presented lower weight gain than did group T4, which maintained greater weight gain than the other groups did.

Table 3
Changes in weight among the study groups over the course of the study

Age, d	T1 (n = 36)	T2 (n = 36)	T3 (n = 36)	T4 (n = 36)	
	Mean ± SD				p_{groups}
	Live weight, g				
Initial	46,68 ± 3,79	45,79 ± 3,92	49,82 ± 1,68	49,68 ± 2,48	*0,315*
D 7	178,25 ± 17,47	171,63 ± 14,55	177,83 ± 21,76	191,42 ± 14,12	*0,013**
D 14	449,98 ± 50,46	489,64 ± 39,41	462,64 ± 40,88	493,03 ± 37,18	*0,001**
D 21	836,42 ± 101,66	868,33 ± 98,71	767,00 ± 84,20	855,33 ± 128,48	*0,026**
D 28	1335,58 ± 162,85	1365,33 ± 189,29	1281,92 ± 132,20	1219,50 ± 227,03	*0,001**
D 35	1725,42 ± 348,87	1582,75 ± 200,62	1662,17 ± 285,98	1752,25 ± 159,51	*0,225*
D 42	2157,92 ± 769,72	2032,58 ± 346,47	2010,00 ± 247,66	2275,00 ± 287,86	*0,142*
p_{days}	*< 0,001**	*< 0,001**	*< 0,001**	*< 0,001**	
				$p_{days*groups}$	*0,013**
		Weight gain, g			
D 1–7	132,26 ± 30,41	128,39 ± 29,52	125,52 ± 28,86	139,50 ± 32,08	*0,302*
D 7–14	260,13 ± 59,82	292,29 ± 67,92	281,40 ± 61,74	310,27 ± 73,08	*0,104*
D 14–21	374,37 ± 81,12	342,92 ± 78,93	343,62 ± 79,08	301,06 ± 71,24	*0,002**
D 21–28	514,94 ± 115,43	475,35 ± 109,33	442,19 ± 101,71	297,75 ± 68,48	*< 0,001**
D 28–35	413,74 ± 95,16	369,56 ± 85,99	428,14 ± 98,47	313,91 ± 71,94	*0,019**
D 35–42	518,88 ± 111,32	456,08 ± 103,84	365,11 ± 83,94	524,01 ± 118,42	*< 0,001**
p_{days}	*< 0,001**	*< 0,001**	*< 0,001**	*< 0,001**	
				$p_{days*groups}$	*0,041**
		Feed consumption, g			

*: significant at the 0.05 level according to two-way repeated-measures ANOVA

Age, d	T1 (n = 36)	T2 (n = 36)	T3 (n = 36)	T4 (n = 36)	
	Mean ± SD				p_{groups}
	Live weight, g				
D 1–7	121,17 ± 42,35	147,32 ± 21,04	117,54 ± 23,50	143,13 ± 28,48	*0,449*
D 7–14	348,32 ± 58,05	316,00 ± 39,78	369,81 ± 52,42	367,97 ± 52,25	*0,127*
D 14–21	566,38 ± 70,79	593,94 ± 98,59	628,24 ± 78,53	604,69 ± 85,86	*0,235*
D 21–28	703,35 ± 99,87	603,56 ± 100,19	606,13 ± 100,54	686,45 ± 97,47	*0,038**
D 28–35	950,58 ± 134,98	809,72 ± 101,21	857,16 ± 107,14	864,85 ± 122,80	*0,032**
D 35–42	1102,94 ± 156,61	1044,51 ± 148,32	933,54 ± 164,30	984,28 ± 149,61	*0,027**
p_{days}	*< 0,001**	*< 0,001**	*< 0,001**	*< 0,001**	
				$p_{days*groups}$	*0,035**
		Feed Conversion Ratio, g/g			
D 1–7	0,91 ± 0,22	1,10 ± 0,22	0,93 ± 0,23	1,02 ± 0,20	*0,267*
D 7–14	1,33 ± 0,21	1,08 ± 0,21	1,31 ± 0,20	1,18 ± 0,23	*0,185*
D 14–21	1,51 ± 0,24	1,73 ± 0,27	1,82 ± 0,36	2,00 ± 0,52	*0,041**
D 21–28	1,36 ± 0,27	1,26 ± 0,31	1,37 ± 0,27	2,30 ± 0,57	*0,038**
D 28–35	2,29 ± 0,57	2,19 ± 0,35	2,00 ± 0,50	2,75 ± 0,44	*0,031**
D 35–42	2,12 ± 0,42	2,29 ± 0,57	2,55 ± 0,58	1,87 ± 0,46	*0,028**
p_{days}	*< 0,001**	*< 0,001**	*< 0,001**	*< 0,001**	
				$p_{days*groups}$	*0,036**
*: significant at the 0.05 level according to two-way repeated-measures ANOVA					

Feed consumption and feed conversion ratios were systematically monitored over a six-week period across the various study groups. A significant increase in average weekly feed consumption was observed in all groups ($p < 0.001$). However, no significant differences in feed consumption were

detected among the animals subdivided into subgroups (p = 0.762). Throughout the initial three weeks, the study groups exhibited no significant differences in terms of feed consumption. In contrast, following the fourth week, an increase in feed consumption was recorded in group T1, whereas the other groups demonstrated reduced feed consumption. During the fifth week, the lowest feed consumption was observed in group T2, and in the final week, decreased feed consumption was noted in both groups 1 and 2. The feed conversion ratios significantly increased over time across all the study groups (p < 0.001). Despite this overall increase, no significant differences were found among the subgroups. In the first two weeks, there were no significant differences in the feed conversion ratios between the study groups. However, beginning in the third week, significant increases were documented in groups T3 and T4 relative to groups T1 and T2. Investigations into changes in antibody levels among the study groups were conducted. Prior to vaccination, all groups tested negative for antibodies, and no significant differences were identified in the S/P ratios among the groups. Postvaccination assessments revealed significant differences in both the S/P ratio and titer across the groups (p < 0.001). Notably, the serological values were significantly lower in all three groups, with no significant differences detected between groups T2 and T4 (see Table 4, Fig. 1).

Table 4
Serological results according to the experimental groups

Grups		**T1 (n = 36)**	**T2 (n = 36)**	**T4 (n = 36)**		
		Mean ± SD			***p***	***Tukey HSD***
	Pre S/P	0,06 ± 0,03	0,14 ± 0,08	0,11 ± 0,07	*0,126*	
	Post S/P	0,05 ± 0,05	3,20 ± 0,72	3,29 ± 0,74	*< 0,001**	*T1-T2* *T1-T4*
*: significant at the 0.05 level according to one-way ANOVA, Sample/Positive:S/P						

The water retention rates did not significantly differ among the study groups. Similarly, within each group, the water retention rates of groups D and E were comparable (62%). The ratios of butyrate fatty acids, as determined by measuring both saturated and unsaturated fatty acids, did not significantly differ across the study groups. Notably, the W9 fatty acid content was relatively lower in the T3 group than in the other groups, and the difference approached statistical significance (Table 5). Although there were no significant differences in butyrate fatty acids between subgroups D and E, in the T4 subgroup, the W9 acid level was significantly elevated in subgroup D (p = 0.047), oleic acid was significantly increased in subgroup D (p = 0.037), and pentadienoic acid was significantly greater in subgroup E (p = 0.037) (Table 6).

Table 5
Water retention rates and fatty acid contents of the thighs of the different experimental groups

Groups		**T1**	**T2**	**T3**	**T4**	
		(n = 36)	**(n = 36)**	**(n = 36)**	**(n = 36)**	
Measurements		Mean ± SD				*p*
WHC	%	63,71 ± 3,98	62,04 ± 2,06	62,28 ± 3,50	61,93 ± 4,43	*0,816*
SFA	%	34,65 ± 7,02	36,71 ± 6,72	35,61 ± 3,37	36,37 ± 3,06	*0,912*
UFA	%	65,34 ± 7,03	63,28 ± 6,72	64,18 ± 3,51	62,75 ± 3,44	*0,854*
MUFA	%	34,39 ± 3,27	34,53 ± 4,22	32,17 ± 2,43	30,58 ± 2,84	*0,143*
PUFA	%	30,94 ± 6,44	28,75 ± 3,07	32,00 ± 2,87	32,16 ± 3,98	*0,514*
W3	%	2,61 ± 0,78	2,71 ± 0,69	2,58 ± 0,89	2,88 ± 069	*0,907*
W6	%	28,33 ± 6,67	26,04 ± 2,94	29,42 ± 2,19	29,28 ± 3,39	*0,486*
W9	%	32,91 ± 4,12	32,83 ± 5,57	27,64 ± 1,73	28,91 ± 2,96	*0,059*
W3/W6	%	0,09 ± 0,04	0,10 ± 0,03	0,08 ± 0,02	0,09 ± 0,01	*0,777*
MCFA	*6–12 C*	0,18 ± 0,13	0,23 ± 0,18	0,23 ± 0,15	0,25 ± 0,11	*0,844*
LCFA	*14–20 C*	92,52 ± 9,36	92,86 ± 6,23	93,58 ± 6,18	87,66 ± 12,53	*0,658*
VLCFA	*> 20 C*	6,91 ± 9,36	6,28 ± 6,27	5,22 ± 6,26	10,70 ± 12,44	*0,738*
TRDCNC	%	0,37 ± 0,3	0,62 ± 0,23	0,75 ± 0,41	0,51 ± 0,2	*0,194*
PNTDCNC	%	0,59 ± 0,65	0,75 ± 0,31	0,74 ± 0,44	0,66 ± 0,29	*0,905*
PLMTC	%	21,07 ± 4,39	22,51 ± 4,09	20,93 ± 2,99	19,7 ± 5,2	*0,726*
STRC	%	7,16 ± 5,77	7,38 ± 8,24	9,05 ± 2,59	8,28 ± 2,69	*0,923*
OLEIC	%	30,69 ± 6,92	30,96 ± 6,08	25,86 ± 3,73	24,96 ± 7,66	*0,242*
LNLC	%	22,09 ± 9,59	19,43 ± 3,93	23,32 ± 4,11	20,63 ± 5,8	*0,728*
A-LNLC	%	1,52 ± 0,32	1,6 ± 0,77	1,55 ± 0,5	1,56 ± 0,54	*0,996*
ARCHDNC	%	1,87 ± 0,61	2,37 ± 1,07	3,19 ± 1,72	2,41 ± 0,79	*0,277*

TRDCNC; Total Relative Dry Compound Noncomponents, PNTDCNC; Plant Nontraditional Dry Compound Noncomponents, PLMTC; Polyunsaturated Long-chain Fatty Acids, STRC; Saturated Triacylglycerols and Related Compounds, OLEIC; Oleic Acid, LNLC: Linoleic Acid, A-LNLC; Alpha-Linolenic Acid, ARCHDNC; Arachidonic Acid Noncomponents.

Table 6
Values of water retention and thigh fatty acids between Groups D and E in the context of the study groups.

		T1 (n = 36)			T2 (n = 36)		
		D	**E**	***p***	**D**	**E**	***p***
Measurements		Mean ± SD				Mean ± SD	
WHC	%	62,47 ± 4,30	64,95 ± 4,07	*0,508*	62,16 ± 1,89	61,93 ± 2,64	*0,911*
SFA	%	38,75 ± 1,25	30,54 ± 8,45	*0,171*	40,36 ± 6,67	33,06 ± 5,34	*0,214*
UFA	%	61,23 ± 1,26	69,44 ± 8,45	*0,172*	59,64 ± 6,68	66,93 ± 5,34	*0,214*
MUFA	%	32,54 ± 2,23	36,24 ± 3,39	*0,190*	32,44 ± 4,94	36,61 ± 2,68	*0,269*
PUFA	%	28,69 ± 3,46	33,20 ± 8,74	*0,453*	27,19 ± 1,94	30,31 ± 3,54	*0,252*
W3	%	3,04 ± 0,98	2,19 ± 0,15	*0,215*	2,57 ± 0,78	2,85 ± 0,73	*0,676*
W6	%	25,65 ± 3,28	31,01 ± 8,88	*0,383*	24,62 ± 2,60	27,46 ± 2,98	*0,282*
W9	%	29,86 ± 1,44	35,95 ± 3,54	*0,051*	29,62 ± 6,30	36,04 ± 2,63	*0,179*
W3/W6		0,12 ± 0,04	0,07 ± 0,02	*0,184*	0,10 ± 0,04	0,10 ± 0,02	*0,918*
MCFA	*6–12 C*	0,26 ± 0,14	0,10 ± 0,02	*0,123*	0,29 ± 0,25	0,17 ± 0,08	*0,461*
LCFA	*14–20 C*	87,50 ± 11,97	97,54 ± 0,58	*0,220*	93,62 ± 4,04	92,10 ± 8,90	*0,801*
VLCFA	*> 20 C*	11,76 ± 12,17	2,06 ± 0,53	*0,241*	5,31 ± 3,95	7,25 ± 8,93	*0,748*
TRDCNC	%	0,46 ± 0,34	0,28 ± 0,3	*0,527*	0,77 ± 0,23	0,47 ± 0,09	*0,106*
PNTDCNC	%	0,89 ± 0,84	0,29 ± 0,27	*0,301*	0,94 ± 0,35	0,56 ± 0,04	*0,138*
PLMTC	%	19,14 ± 5,53	23 ± 2,54	*0,334*	22,44 ± 5,37	22,57 ± 3,61	*0,974*

*: significant at the 0.05 level according to Student's t test

		T1 (n = 36)			T2 (n = 36)		
		D	**E**	***p***	**D**	**E**	***p***
STRC	%	9,91 ± 1,49	4,4 ± 7,63	*0,287*	10,38 ± 9,47	4,37 ± 7,27	*0,433*
OLEIC	%	25,68 ± 5,61	35,69 ± 3,63	*0,060*	29,02 ± 6,67	32,9 ± 6,06	*0,497*
LNLC	%	16,06 ± 6,82	28,12 ± 8,63	*0,130*	17,3 ± 4,88	21,55 ± 1,11	*0,215*
A-LNLC	%	1,48 ± 0,5	1,56 ± 0,05	*0,796*	1,6 ± 1,07	1,59 ± 0,58	*0,989*
ARCHDNC	%	2,28 ± 0,45	1,47 ± 0,5	*0,102*	2,6 ± 0,94	2,15 ± 1,36	*0,664*
		T3 (n = 36)			T4 (n = 36)		
WHC	%	59,90 ± 1,87	64,66 ± 3,19	*0,090*	62,71 ± 6,73	61,16 ± 1,42	*0,715*
SFA	%	34,19 ± 4,21	37,02 ± 2,19	*0,361*	37,05 ± 4,68	35,70 ± 0,27	*0,645*
UFA	%	65,39 ± 4,65	62,97 ± 2,19	*0,462*	62,95 ± 4,69	62,56 ± 2,71	*0,906*
MUFA	%	32,84 ± 3,59	31,50 ± 0,75	*0,564*	32,65 ± 1,78	28,52 ± 2,06	*0,059*
PUFA	%	32,55 ± 3,43	31,46 ± 2,81	*0,694*	30,30 ± 5,35	34,03 ± 0,83	*0,299*
W3	%	2,32 ± 1,06	2,84 ± 0,82	*0,540*	2,43 ± 0,60	3,33 ± 0,46	*0,109*
W6	%	30,23 ± 2,37	28,62 ± 2,11	*0,432*	27,87 ± 40,75	30,70 ± 0,42	*0,363*
W9	%	27,90 ± 2,67	27,39 ± 0,38	*0,759*	31,12 ± 2,38	26,70 ± 1,26	*0,047**
W3/W6		0,07 ± 0,03	0,09 ± 0,02	*0,417*	0,08 ± 0,01	0,10 ± 0,01	*0,055*
MCFA	*6–12 C*	0,23 ± 0,12	0,23 ± 0,20	*0,964*	0,22 ± 0,12	0,29 ± 0,11	*0,506*
LCFA	*14–20 C*	93,32 ± 2,04	90,84 ± 8,29	*0,328*	96,64 ± 0,71	78,68 ± 12,27	*0,065*

*: significant at the 0.05 level according to Student's t test

		T1 (n = 36)			T2 (n = 36)		
		D	**E**	***p***	**D**	**E**	***p***
VLCFA	*> 20 C*	2,26 ± 1,51	8,19 ± 8,33	*0,292*	2,75 ± 0,60	18,65 ± 14,03	*0,122*
TRDCNC	%	0,76 ± 0,56	0,73 ± 0,33	*0,933*	0,39 ± 0,19	0,64 ± 0,16	*0,152*
PNTDCNC	%	0,76 ± 0,62	0,73 ± 0,30	*0,950*	0,43 ± 0,17	0,88 ± 0,18	*0,037**
PLMTC	%	21,94 ± 0,24	19,92 ± 4,39	*0,471*	23,54 ± 0,97	15,85 ± 4,74	*0,051*
STRC	%	8,61 ± 3,75	9,48 ± 1,44	*0,726*	9,22 ± 3,59	7,34 ± 1,61	*0,455*
OLEIC	%	27,48 ± 2,65	24,24 ± 4,47	*0,341*	30,83 ± 2,4	19,09 ± 6,13	*0,037**
LNLC	%	25,65 ± 4,45	20,99 ± 2,49	*0,189*	23,66 ± 6,02	17,6 ± 4,52	*0,235*
A-LNLC	%	1,72 ± 0,72	1,37 ± 0,13	*0,458*	1,71 ± 0,81	1,42 ± 0,11	*0,577*
ARCHDNC	%	3,35 ± 2,55	3,02 ± 0,88	*0,846*	2,03 ± 0,65	2,78 ± 0,83	*0,284*
*: significant at the 0.05 level according to Student’s t test							

Color changes were analyzed separately for both skinned and unskinned individuals across the study groups as well as within each specific group. The L color change values were significantly greater in the T1 and T2 groups than in the T3 and T4 groups (p = 0.003). No significant differences were detected in the a and b color change values. In the T1 group, there were no significant differences in the L, a, or b color change values between the skinned and unskinned individuals. Conversely, in the T2, T3, and T4 groups, the L and b color changes were significantly greater in the skinned individuals, whereas the a color change was significantly greater in the unskinned individuals (Table 7).

Table 7
Color change values according to the experimental groups.

Measurements	**T1 (n = 36)**	**T2 (n = 36)**		**T3 (n = 36)**	**T4 (n = 36)**	
		Mean ± SD				***p***
L color	25,07 ± 2,31	26,51 ± 2,72		21,69 ± 3,86	24,74 ± 2,91	*0,003** *T1-T3* *T2-T3*
a color	70,61 ± 11,07	70,29 ± 6,59		75,15 ± 7,08	66,47 ± 5,80	*0,079*
b color	14,49 ± 2,33	14,37 ± 2,35		13,82 ± 2,43	16,29 ± 2,64	*0,090*
	T1 (n = 36)			T2 (n = 36)		
	Skinned	Skinless	*p*	Skinned	Skinless	*p*
Measurements	Mean ± SD					
L color	26,80 ± 1,85	23,34 ± 1,10	*0,055*	27,80 ± 2,68	25,22 ± 2,25	*0,036**
a color	71,53 ± 13,57	69,68 ± 9,13	*0,124*	67,40 ± 5,25	73,19 ± 6,93	*0,006**
b color	15,84 ± 2,39	13,15 ± 1,41	*0,087*	16,15 ± 1,74	12,60 ± 1,25	*0,017**
	T3 (n = 36)			T4 (n = 36)		
		Mean ± SD			Mean ± SD	
	Skinned	Skinless	p	Skinned	Skinless	p
L color	24,53 ± 2,08	18,86 ± 3,02	*0,004**	26,78 ± 2,14	22,70 ± 2,02	*0,006**
a color	71,38 ± 6,76	78,93 ± 5,52	*0,028**	63,46 ± 5,26	69,49 ± 4,94	*0,016**
b color	15,47 ± 1,67	12,16 ± 1,91	*0,034**	17,29 ± 2,78	15,28 ± 2,29	*0,037**
*: significant at the 0.05 level according to Student's t test						

Discussion and Conclusion

Newcastle disease continues to pose a significant economic burden on the poultry industry worldwide. In the absence of effective treatment options, the poultry sector relies primarily on stringent biosecurity measures and vaccination protocols to control this devastating disease. Currently, vaccines are the most effective means of mitigating the occurrence of Newcastle disease (Li et al., 2020). However, the continuous development of innovative vaccine technologies, combined with improved biosecurity

measures through training, may provide viable solutions for reducing the global threat of Newcastle disease within the poultry industry (Mayers et al., 2017). This study highlights the positive effects of a feed additive containing propolis, omega-3 fatty acids, and Lactobacillus subtilis on the growth performance, meat quality, and immunization of broiler chickens. These findings demonstrate that diets enriched with propolis and omega-3 promote weight gain and increase feed conversion ratios. These outcomes are linked to the beneficial impact of propolis on the intestinal microbiota, which in turn increases digestibility (Elsherif et al., 2021; Abd El-Ghany, 2024). This study suggests that the use of feed additives alongside vaccination can positively influence growth performance, digestive health, meat quality, and overall immunity in broiler chickens. Additionally, feed additives containing propolis, omega-3, and Lactobacillus subtilis have the potential to enhance vaccine efficacy. The application of lipopolysaccharides, vaccines, and propolis supplements at an optimal dosage of 5 mg/kg body weight throughout the rearing period may bolster the efficiency and immunity of broiler chickens. Consequently, it is recommended that low doses of LPS, vaccines, and propolis be administered to poultry to reinforce immune defense mechanisms within their bodies (Hammadi et al., 2020). Similarly, Shreif & El-Saadany (2017) reported that increasing propolis levels in the diets of bandarah chicks resulted in increased weight gain. These findings indicated that incorporating propolis (0.05 g/kg) into broiler diets achieved a performance that was comparable to that of a group fed an antibiotic-based diet and was vital for eliciting a robust immune response to the Newcastle vaccine. Moreover, an increase in the immune response was documented in the vaccinated groups. This finding aligns with the literature indicating that probiotics such as Lactobacillus subtilis enhance immunity, thereby increasing resistance to infectious diseases (Forte et al., 2016; Cai et al., 2024). Furthermore, propolis has been shown to support immunological responses and assist in infection control (Zulhendri et al., 2021). Water retention rates in broiler meat, alongside the fatty acid composition of the thigh, are critical parameters influencing the nutritional value and quality of meat. The results of this study revealed no significant differences in water retention rates across all the groups. Similar findings may be associated with the balanced utilization of various nutrients that affect meat quality (Kumar et al., 2018). Analyzing thigh fatty acids is essential for establishing the nutritional value and health effects of meat. On the basis of these findings, only the W9 fatty acid group was significantly lower in the T3 group. This suggests that the other groups may have exhibited a more balanced fatty acid profile, along with potential health benefits. However, the lack of significant differences among the other fatty acid groups indicates that the feed additive employed effectively prevented fatty acid imbalances (Alagawany et al., 2020). Following the vaccination protocols, significant increases in antibody levels were recorded across all study groups. Notably, the T2, T3, and T4 groups presented pronounced increases in antibody titers compared with the T1 group, confirming the efficacy of probiotics in immune stimulation. These findings support the potential of probiotics containing Lactobacillus subtilis to enhance immune responses (Forte et al., 2016). Moreover, the negative antibody levels in all groups prior to vaccination established a critical baseline for evaluating immunization effectiveness. The Newcastle vaccine is effective in promoting immune development in vaccinated animals, yielding an improved immune response upon exposure to the virus (Zohaib et al., 2025).

In conclusion, the incorporation of natural components such as propolis and omega-3 fatty acids, in conjunction with vaccination, has produced positive outcomes regarding both growth performance and the enhancement of the immune system in broiler chickens. These findings underscore the feasibility of implementing alternative and natural feeding strategies to reduce antibiotic usage in poultry farming.

Declarations

Ethics approval and consent to participate

All procedures of this study were approved and monitored by the Animal Experiments Local Ethics Committee of Hatay Mustafa Kemal University, Hatay, Türkiye (Approval Number: 2021/05–11).

Consent for publication:

Not applicable

Competing interests:

The authors declare that they have no competing interests

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Author Contribution

Suleyman Ercument ONEL was responsible for the care and nutrition of the animals as well as the writing of the manuscript. Veysel Soydal ATASEVEN conducted the virological analyses and provided interpretation of the results. Mikail BAYLAN contributed to the animal care and feeding aspects of the study. Kanber KARA performed laboratory analyses and interpretation of the data. Adnan KARAİBRAHİMOĞLU was responsible for processing and interpreting the statistical data related to the study. Mehmet Alpay DEMİRAL oversaw the laboratory procedures pertaining to virological analyses.

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Data Availability

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

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Figures

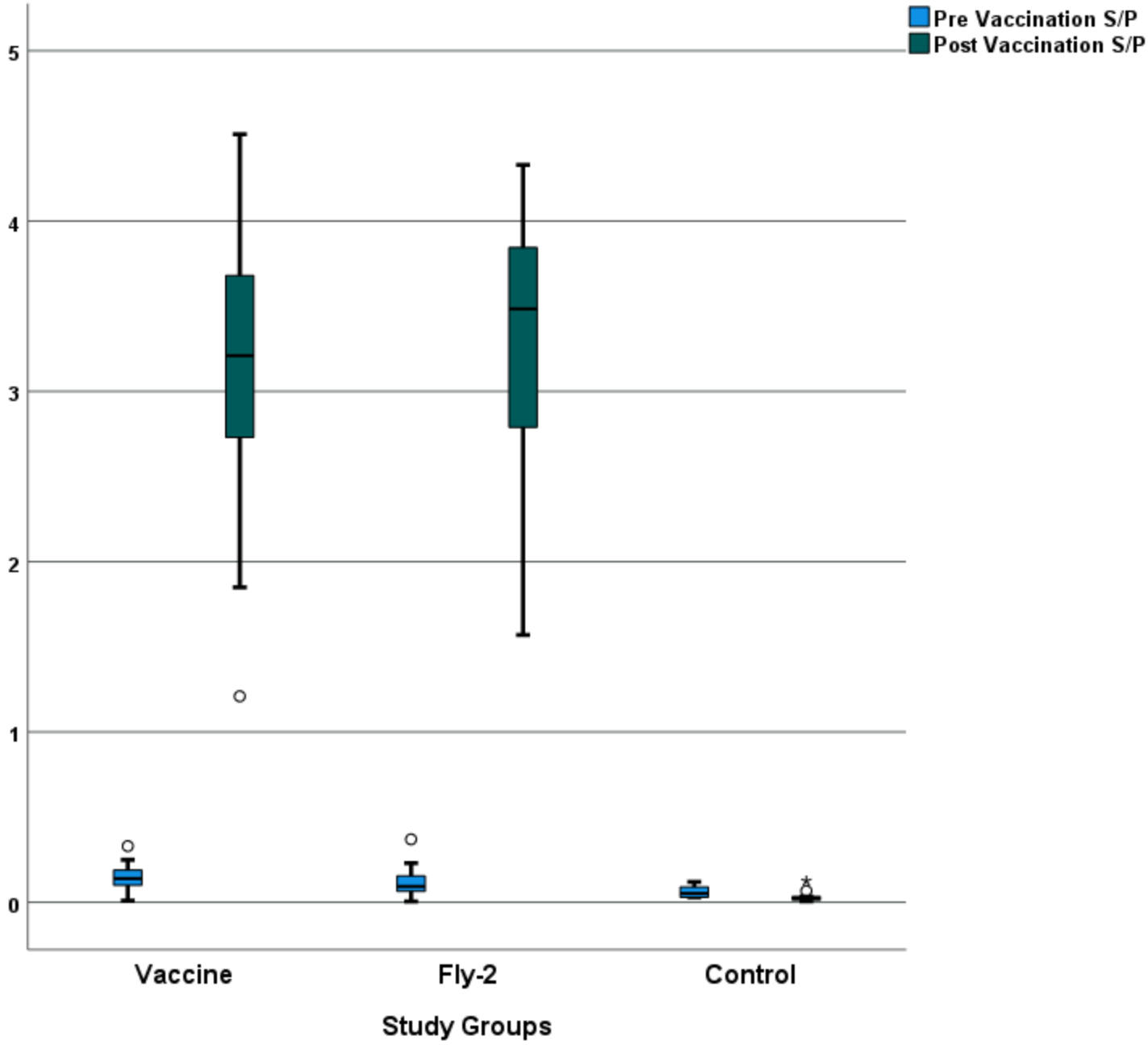


Figure 1

Serological results according to the experimental groups