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EFFECTS OF DIETARY NUCLEOTIDES SUPPLEMENTATION ON THE PERFORMANCE OF BROILER CHICKS

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Abstract: This study examined the effects of nucleotide supplementation on broiler chick growth performance, immunological response, carcass traits, meat quality, serum biochemical parameters, total antioxidant capacity, intestinal morphology, mortality rate, and economic efficiency measures of broiler chicks. A total of 180 one-day-old chicks (Ross 308) were distributed into 6 groups, each of which consisted of 30 chicks, and each group was divided into 3 replicates so that each replicate contained 10 chicks fed on six experimental diets as follows: basal diet without oil or nucleotide (T1), basal diet with 1% soybean oil and without nucleotide(T2), 50 grams nucleotide per 100-kilogram diet with different fat sources (no oil(T3), 1% soybean oil(T4), 1% linseed oil(T5) &1% fish oil(T6) respectively during the experimental period (5 weeks). Growth performance parameters were detected per stage period. Four chicks from each replicate were used at the termination of the experiment for analysis of the above mentioned measurements. Results revealed that supplementation of nucleotide in diets of broiler chicks improved feed conversion ratio, carcass traits, intestinal morphology, serum biochemical parameters, immunological response, bursa of Fabricius weight, and the best ratios were observed in groups fed nucleotide in combination with fish and linseed oil. Also, there was an increased economic efficiency in the SBO fed group(T2) then group fed nucleotides in mix with linseed oil (T5) and control (T1). In comparison to the control groups (T1 & T2), groups fed nucleotides and PUFA oil sources had significantly lower n-6: n-3 ratio in breast muscle, and mortality rate.

Key words: broiler; nucleotide; PUFA oil sources; growth performance; immunity; gut morphology and economic effi-ciency

Introduction

Production of poultry meat has improved frequently over the years and is expected to remain. Alternatively, advancement in genetic characters of poultry strains and more understanding of nutrition help chickens to achieve the market weight of 2 kg at 35 days old, and the efficiency of converting feed into poultry products moreover continues to progress (1). Most vegetable oils have a high omega-6 to omega-3 fatty acid ratio. Soybean oil is in an intermediate omega-6 to an omega-3 proportion (2). The very long chain polyunsaturated fatty acids (PUFAs) (C18–C22) and n-3 Omega PUFAs are evidently generally acknowledged as a piece of current nutrition as a result of their valuable impacts on metabolism (3). Also, dietary nucleotides are emerging as one of the potential feed additives because of their ability to enhance the villous growth of the intestine and production performance (4). In addition, nucleotide supplementation has numerous significant physiological, gastrointestinal, and immunological capabilities in the body through times of fast growth and development, disease tasks, injury or conditions of stress like high stocking density or dirty litters in addition to saving energy (5).

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Nucleotides of yeast source which supplemented in bird feed led to higher body weight, daily body weight gains, and better feed conversion ratios (5) but, they didn't have any effect on the feed intake of broiler chicken (6). Improvement in weight gain which particularly noted when nucleotides nourished at the first three weeks of life signifying ideal early bird development and will support performance later because the accessibility of nucleotides might be rate-limiting in rapidly dividing tissues, like in young chickens which have juvenile digestive system (7). The positive effect of a nucleotide preparation in broiler chickens with a dose of 500 mg/kg diet was demonstrated by Esteve-Garcia et al. (8). Exogenous nucleotides lower the animal's energy needs because de novo synthesis of nucleotides requires a lot of energy (5).

Improvements in villus height, crypt depth, and villus height to crypt depth are signs of a better gut in chickens fed nucleotides (5) & (9). Furthermore, Wu et al. (9) found that supplementing diets with nucleotides improved the gut flora, as seen by higher levels of lactic acid bacteria and a more varied intestinal microbiota. The immune system after dietary nucleotide supplementation has been boosted (10). Additionally, chickens have a quicker and more potent antibody response to standard immunizations (9).

Nucleotide-fed birds had higher body weights and, as a result, delivered more carcasses, including heavier drumsticks, thighs, wings, and breasts (7). Additionally, their meat had greater nutritional qualities, was redder in color and tender, and had more lipids with higher unsaturation degrees, all of which are good for humans' health and are aesthetically acceptable to consumers (11).

New researches are required to determine the impact of nucleotides added to broiler diets under conditions typical of commercial farms Pelcia et al. (12). As a result, the current study's goal was to examine the impact of dietary nucleotide supplementation when combined with various fat sources (soybean oil, linseed oil, and fish oil) on broiler performance, carcass traits, immune response, serum biochemical markers, intestinal morphological characteristics, mortalities, economic effectiveness, and their impact on breast lipid unsaturation levels.

Materials and methods

Place at which the study conducted

The study was conducted in the Department of Nutrition & Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental birds, accommodation and management

A commercial hatchery provided 180 unsexed one-day-old broiler chicks (Ross 308) in total. Chicks were reared in brooder battery cages. They were then divided into 6 equal groups, and weighed individually upon entry (as an initial average body weight 43.47, 43.47, 34.33, 34.47, 34.44, 34.58 grams for groups from T1 to T6 respectively) each of which consisted of 30 chicks, and each group was divided into 3 replicates, so that each replicate contained 10 chicks. The feeding trial persisted for 5 weeks. All chicks were fed crumble diet in the starter and grower period, then pellet diet until the termination of the experimental period, where feeding was ad libitum. Chicks were vaccinated as stated by vaccination programs against Newcastle disease through Eye drops (Hitchner and La Sota vaccine at 7 and 17 days of age respectively). Six experimental (isocaloric and isonitrogenous) diets divided into 3 phases were formulated in accordance with Aviagen (13) to satisfy the nutritional needs of broiler chicks as follows: control T1 basal diet without oil or nucleotide, control T2 basal diet with 1% soybean oil and without nucleotide T3 basal diet with nucleotide 50 Gm/100 kg diet without oil addition T4 basal diet with nucleotide 50 Gm/100 kg diet plus 1% soybean oil T5 basal diet with nucleotide 50 Gm/100 kg diet plus 1% linseed oil T6 basal diet with nucleotide 50 Gm/100 kg diet plus 1% fish oil and shown in Table 1. A representative sample of each feed component was examined using SupNIR (FPI SupNIR-2700 SERIES, Hangzhou, Zhejiang, China). Metabolic energy was calculated according to Janssen (14). The diets were kept in a cold dry place to avoid oxidative rancidity.

The growth performance was determined by weighing the daily feed consumption and the body weight for each cage after each period until

5 weeks. Total feed intake, weight gain, and feed to gain ratio were calculated at the conclusion of the feeding trial.

Tested feed additive: Nucleoforce Poultry is a concentrated form of unlimited nucleotides made from dry yeast extracts and suited for broilers and layers developed by Bioiberica. The product is a creamy-colored powder with a nucleotide content of 26.4%.

Sampling

at 5-wk-old, four birds from each replicate were sampled, weighed, slaughtered, and eviscerated without a feed withdrawal period according to Brake et al. (15). Eviscerated carcass, Liver, heart, gizzard, spleen, bursa, thymus gland, breast, thigh, abdominal fat yields and whole evacuated intestine were weighed for calculating dressing percent. Weights of the three lymphoid organs (thymus, spleen, and bursa of Fabricius) from the slaughtered birds in each group were recorded in order to determine the relative organ weight.

Humoral immune response for Newcastle virus vaccine antibodies was measured by hemagglutination Inhibition test according to Anon (16). Also, the total leukocyte count of non-coagulated blood samples during slaughter was measured at the termination of the trial using an automatic blood analyzer (Diagon ® Ltd D-Cell 60 auto hematology analyzer). According to Wahlefeld and Bergmeyer (17), triglyceride levels in the serum were examined, total cholesterol depending on Naito and Kaplan (18), serum low-density lipoprotein-cholesterol, serum very low-density lipoprotein (Triglyceride/5) based on Friedewald et al. (19), serum high-density lipoprotein-cholesterol on the basis of Burstein and Scholnick (20), total serum protein as stated by Grant et al. (21), albumin as stated by Doumas and Biggs (22) and globulin on the basis of Doumas and Biggs (23). Serum levels of total antioxidant capacity and glutathione peroxidase were tested in line with the methods approved by Koracevic et al. (24). Homogenized freeze-dried meat from the breast was evaluated for fatty acid content on the basis of the method described by Folch et al. (25), and fatty acid methyl esters were prepared as designated by Ichihara and Fukubayashi (26) through gas chromatography.

According to Drury et al. (27), representative samples from the jejunum were used in the histopathological examination of the intestine to measure villous height (at 100X), crypts depth (at 100X), villous height: crypt depth ratio (at 100X), intestinal wall thickness (at 40X), goblet cell proliferation, villus width (at 100X), and villus perimeter and surface area. The perimeter of the villus was computed as (2π × (average villi width/2) × villous height) and the surface area of villus was computed as (villus perimeter× villous height). Throughout the trial, mortality rates for each treatment were determined.

According to El-Kerdawy (28), an economic analysis was calculated using the following equation: Y= (A-B)/B x 100, where A is the selling cost of the acquired gain and B is the feeding cost of this gain.

Statistical analysis

The mean standard error and the coefficient of variation for the prior data were determined using the standard statistical formula which has been given by Snedecor and Cochran (29). The obtained data will be studied by ANOVA one-way classification via a totally randomized plan to detect the significance of the distinction between assorted treatment groups.

Our findings in table 2 demonstrated that the main impact of dietary nucleotides on broiler chick growth performance was that there was no significant difference in final BW, BWG, and FI between all groups.

All treatments containing nucleotides significantly reduced FCR compared to control treatments which did not have it, and the best FCR was recorded in dietary nucleotides in combination with fish and linseed oil treated groups. According to the results of the carcass traits in table 3, the dressing %, breast yield, bursa of Fabricius, thymus, and intestinal weight were considerably ($P<0.05$) higher in the nucleotide-fed

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Table 1: Composition of the experimental diets' ingredients (%)

	Starter						grower						finisher					
Feeding period (days)	0-10 days						11-24 days						25-35 days					
Groups	T 1	T 2	T 3	T 4	T 5	T 6	T 1	T 2	T 3	T 4	T 5	T 6	T 1	T 2	T 3	T 4	T 5	T 6
Yellow corn	59.71	57.10	59.69	56.74	56.64	56.64	65.00	62.74	65.00	62.68	62.71	62.71	71.42	69.11	71.40	69.08	69.08	69.08
Corn gluten meal 60%	12.2	9.66	12.25	9.91	9.90	9.90	15.55	12.73	15.50	12.75	12.73	12.73	17.85	15.10	17.90	15.10	15.10	15.10
Soybean meal 46%	23.32	27.60	23.23	27.70	27.80	27.80	14.93	19.12	14.93	19.11	19.15	19.15	6.45	10.62	6.37	10.60	10.60	10.60
Oil	0.00	1.00[1]	0.00	1.00[1]	1.00[2]	1.00[3]	0.00	1.00[1]	0.00	1.00[1]	1.00[2]	1.00[3]	0.00	1.00[1]	0.00	1.00[1]	1.00[2]	1.00[3]
Ground limestone	1.87	1.85	1.87	1.86	1.86	1.86	1.70	1.65	1.70	1.65	1.65	1.65	1.59	1.59	1.59	1.59	1.59	1.59
mono-ca. phosphate	1.29	1.25	1.29	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.10	1.05	1.10	1.05	1.05	1.05
L-lysine	0.41	0.33	0.41	0.32	0.32	0.32	0.46	0.38	0.46	0.38	0.38	0.38	0.53	0.44	0.53	0.44	0.44	0.44
Dl- methionine	0.19	0.20	0.19	0.20	0.19	0.19	0.11	0.13	0.11	0.13	0.13	0.13	0.07	0.09	0.07	0.09	0.09	0.09
L-threonine	0.07	0.06	0.07	0.05	0.05	0.05	0.04	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.03
Nucleoforce ***	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05
Vitamin mineral premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Common salts	0.33	0.35	0.33	0.33	0.33	0.33	0.33	0.35	0.33	0.33	0.33	0.33	0.35	0.35	0.35	0.35	0.35	0.35
Sod. bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Antimycotoxins	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritive value																		
CP	23.00	23.16	23.00	23.31	23.35	23.35	21.52	21.50	21.50	21.52	21.52	21.52	19.52	19.53	19.52	19.52	19.52	19.53
EE	2.69	3.56	2.69	3.58	3.55	3.55	3.74	3.74	3.74	3.74	3.74	3.74	3.94	3.94	3.94	3.94	3.94	3.94
ME	3000.16	3000.38	3000.18	3000.24	3000.09	3000.09	3100.91	3100.32	3100.25	3100.05	3100.74	3100.74	3200.36	3200.30	3200.36	3200.36	3200.36	3200.84
Calcium	0.96	0.96	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79	0.79	0.79
Av. Phosphorous	0.45	0.45	0.45	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40	0.40
N-3: N-6 ratio**	1:31.81	1:14.96	1:31.85	1:14.94	1:2.33	1:3.55	1:37.65	1:16.31	1:37.65	1:16.31	1:2.47	1:3.86	1:45.06	1:17.79	1:45.06	1:17.79	1:2.68	1:4.18

* Premix contain vitamins and minerals according to requirement for broiler chicks as recommended in Aviagen (13) and was Produced by Multivita Company. ** According to Gunstone's (30) calculations, various n-3 to n-6 fatty acid ratios. *** Tested feed additives: Nucleoforce Poultry which is a concentrated version of unrestricted nucleotides derived from dried yeast extracts and intended for broilers and layers obtained from dried yeasts extracts were produced by company called Bioiberica. The product is creamy colored powder contain 26.4% nucleotides. [1]=soybean oil, [2]=linseed oil, [3]= fish oil

Results

Table 2: Effect of dietary nucleotides supplementation on overall growth performance of broiler chicks

	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	**T2 0% nucleotides with 1% SBO**	**T3 0.05% Nucleotides without oil**	**T4 0.05% nucleotides with 1% SBO**	**T5 0.05 % nucleotides with 1% LO**	**T6 0.05 % nucleotides with 1% FO**
BW, g/bird	2253.15 ± 88.10	2355.89 ± 63.32	2241.42 ± 51.50	2286.63 ± 80.13	2365.00 ± 57.50	2412.08 ± 56.81
BWG, g/bird	2209.68 ± 88.02	2312.42 ± 63.33	2198.08 ± 51.58	2243.15 ± 80.06	2321.56 ± 57.62	2368.50 ± 56.95
FI, g/bird	3247.15 ± 143.90	3341.94 ± 88.50	3112.29 ± 59.69	3202.43 ± 65.12	3152.63 ± 64.53	3212.95 ± 103.36
FCR	1.47^{a} ± 0.03	1.45^{ab} ± 0.00	1.42^{abc} ± 0.04	1.43^{abc} ± 0.03	1.36^{bc} ± 0.02	1.36^{c} ± 0.01

$^{a, b, c}$ means ± standard Error values within the same row with various superscripts differ significantly (P<0.05).

BW, BWG, FI and FCR, stand for body weight, body weight gain, feed intake and feed conversion ratio, respectively.

Table 3: Effect of nucleotides supplementation in chicks' diets on carcass traits

	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	**T2 0% nucleotides with 1% SBO**	**T3 0.05% Nucleotides without oil**	**T4 0.05% nucleotides with 1% SBO**	**T5 0.05% nucleotides with 1% LO**	**T6 0.05% nucleotides with 1% FO**
Body weight, g	2241.67^{b} ± 7.26	2358.33^{a} ± 4.41	2346.67^{ab} ± 52.07	2346.67^{ab} ± 42.06	2343.33^{ab} ± 18.56	2393.33^{a} ± 40.86
Carcass weight, g	1520.00^{d} ± 2.89	1614.33^{c} ± 2.33	1680.00^{bc} ± 33.29	1713.33^{ab} ± 36.09	1725.00^{ab} ± 10.41	1788.33^{a} ± 33.46
Carcass %	67.81^{d} ± 0.18	68.45^{d} ± 0.08	71.60^{c} ± 0.41	73.00^{b} ± 0.25	73.62^{b} ± 0.33	74.72^{a} ± 0.14
Liver%	2.06^{b} ± 0.05	2.36^{a} ± 0.04	2.15^{ab} ± 0.13	2.39^{a} ± 0.07	2.32^{a} ± 0.04	2.31^{a} ± 0.06
Heart%	0.44 ± 0.06	0.47 ± 0.03	0.57 ± 0.06	0.43 ± 0.03	0.44 ± 0.04	0.55 ± 0.03
Spleen %	0.14 ± 0.00	0.17 ± 0.06	0.11 ± 0.03	0.12 ± 0.01	0.10 ± 0.01	0.15 ± 0.00
Gizzard%	1.46 ± 0.10	1.15 ± 0.05	1.26 ± 0.12	1.27 ± 0.08	1.14 ± 0.17	1.30 ± 0.04
Total fat%	2.89 ± 0.12	2.80 ± 0.40	2.43 ± 0.36	2.89 ± 0.10	2.64 ± 0.20	2.17 ± 0.04
Bursa %	0.04^{b} ± 0.00	0.04^{b} ± 0.00	0.05^{ab} ± 0.01	0.05^{ab} ± 0.01	0.04^{ab} ± 0.00	0.06^{a} ± 0.00
Thymus %	0.26^{b} ± 0.04	0.28^{b} ± 0.01	0.42^{a} ± 0.07	0.34^{ab} ± 0.02	0.31^{ab} ± 0.02	0.37^{ab} ± 0.02
Intestine %	2.57^{b} ± 0.19	2.50^{b} ± 0.08	3.14^{a} ± 0.05	3.14^{a} ± 0.05	3.24^{a} ± 0.03	3.33^{a} ± 0.10
Pectoral muscle %	29.07^{b} ± 0.23	29.82^{b} ± 0.70	30.01^{b} ± 0.93	29.79^{b} ± 1.00	30.02^{b} ± 0.42	32.67^{a} ± 1.08
Thigh weight %	27.51^{ab} ± 0.12	27.91^{a} ± 0.35	26.33^{c} ± 0.45	26.53^{bc} ± 0.29	27.10^{abc} ± 0.30	27.26^{abc} ± 0.16

a, b, c, d Means ± standard Error values within the same row with various superscripts differ significantly (P <0.05).

Table 4: Effect of nucleotides supplementation in chicks' diets on Serum biochemical parameters and total Antioxidant Capacity

	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	**T2 0% nucleotides with 1% SBO**	**T3 0.05% nucleotides without oil**	**T4 0.05% nucleotides with 1% SBO**	**T5 0.05 % nucleotides with 1% LO**	**T6 0.05 % nucleotides with 1% FO**
Triglycerides (mg/dl)	$57.16^{a} \pm 1.96$	$48.74^{abc} \pm 1.40$	$53.41^{ab} \pm 2.76$	$46.82^{bc} \pm 5.19$	$41.78^{cd} \pm 1.93$	$37.61^{d} \pm 0.95$
Total cholesterol (mg/dl)	$141.71^{a} \pm 6.98$	$131.92^{ab} \pm 7.93$	$135.46^{ab} \pm 2.28$	$125.25^{b} \pm 0.08$	$105.63^{c} \pm 3.89$	$100.29^{c} \pm 0.63$
HDL (mg/dl)	56.26 ± 0.67	58.99 ± 0.75	53.58 ± 0.87	54.82 ± 2.38	56.28 ± 1.59	58.02 ± 3.34
LDL (mg/dl)	$71.45^{a} \pm 7.60$	$60.93^{a} \pm 7.24$	$68.61^{a} \pm 2.93$	$58.75^{a} \pm 2.77$	$39.10^{b} \pm 3.61$	$33.00^{b} \pm 3.69$
VLDL (mg/dl)	$11.43^{a} \pm 0.39$	$9.75^{abc} \pm 0.28$	$10.68^{ab} \pm 0.55$	$9.36^{bc} \pm 1.04$	$8.36^{cd} \pm 0.39$	$7.52^{d} \pm 0.19$
Cholesterol ester (mg/dl)	$2.57^{a} \pm 0.16$	$2.25^{a} \pm 0.12$	$2.58^{a} \pm 0.07$	$2.31^{a} \pm 0.11$	$1.89^{b} \pm 0.09$	$1.75^{b} \pm 0.11$
Total protein (mg/dl)	$3.24^{b} \pm 0.10$	$3.28^{b} \pm 0.09$	$3.51^{ab} \pm 0.12$	$3.47^{ab} \pm 0.06$	$3.72^{a} \pm 0.04$	$3.66^{a} \pm 0.11$
Albumin (g/dl)	1.89 ± 0.07	1.93 ± 0.04	1.83 ± 0.13	1.75 ± 0.01	1.72 ± 0.07	1.73 ± 0.08
Globulin (g/dl)	$1.35^{c} \pm 0.03$	$1.35^{c} \pm 0.09$	$1.68^{b} \pm 0.01$	$1.72^{b} \pm 0.07$	$2.00^{a} \pm 0.11$	$1.93^{ab} \pm 0.11$
TAC (mM/l)	$0.33^{a} \pm 0.01$	$0.29^{ab} \pm 0.04$	$0.27^{ab} \pm 0.03$	$0.24^{bc} \pm 0.01$	$0.18^{cd} \pm 0.02$	$0.14^{d} \pm 0.01$
GSH-Px (IU/mg)	$48.99^{c} \pm 1.89$	$50.98^{c} \pm 4.87$	$66.50^{b} \pm 3.62$	$68.50^{b} \pm 4.07$	$74.83^{ab} \pm 2.62$	$79.76^{a} \pm 2.80$

a, b, c, d Means ± standard error in the same row with different superscripts are significantly different (P<0.05).The abbreviations HDL, LDL, VLDL, TAC and GSH-Px stand for high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, total antioxidant capacity and glutathione peroxidase respectively.

Table 5: Effect of nucleotides supplementation in chicks' diets on Newcastle vaccine antibody titter, total leucocyte count and mortality rate

	Dietary nucleotides supplementation%					
Exp. period (weeks)	**T1 0% nucleotides without oil**	**T2 0% nucleotides with 1% SBO**	**T3 0.05% nucleotides without oil**	**T4 0.05% nucleotides with 1% SBO**	**T5 0.05 % nucleotides with 1% LO**	**T6 0.05 % nucleotides with 1% FO**
Antibody titer1st day	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33
Antibody titer at 14-day age	3.33 ± 0.33	3.67 ± 0.67	4.33 ± 0.88	4.33 ± 1.20	5.67 ± 0.33	5.67 ± 0.33
Antibody titer at 26-day age	3.00 ± 0.00	3.33 ± 0.88	4.00 ± 1.00	4.33 ± 0.88	5.00 ± 0.00	5.00 ± 0.58
Antibody titer at 35-day age	2.33 ± 0.88	3.33 ± 0.33	2.67 ± 0.33	3.33 ± 0.33	4.00 ± 0.58	4.33 ± 0.88
Total leucocyte count	$166.07^{b} \pm 2.46$	$170.87^{b} \pm 3.01$	$188.83^{a} \pm 5.60$	$185.57^{a} \pm 6.37$	$193.17^{a} \pm 2.68$	$193.03^{a} \pm 3.33$
Mortality rate	13.87 ± 2.79	8.32 ± 4.81	11.11 ± 7.35	2.77 ± 2.77	2.77 ± 2.77	0.00 ± 0.00

a, b, means ± standard Error values within the same row with various superscripts differ significantly (P<0.05)

Table 6: Effect of nucleotides supplementation in chicks' diets on intestinal morphology

Intestinal morphology	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	T2 0% nucleotides with 1% SBO	T3 0.05% nucleotides without oil	T4 0.05% nucleotides with 1% SBO	T5 0.05 % Nucleotides with 1% LO	T6 0.05 % nucleotides with 1% FO
VH	$823.00^{f} \pm 7.51$	$896.00^{e} \pm 4.04$	$1091.33^{d} \pm 3.76$	$1112.33^{c} \pm 4.33$	$1178.00^{b} \pm 1.15$	$1222.00^{a} \pm 5.03$
CD	$84.40^{e} \pm 2.83$	$93.60^{d} \pm 1.85$	$114.10^{c} \pm 2.48$	$122.67^{b} \pm 2.62$	$133.73^{a} \pm 3.12$	$130.43^{ab} \pm 2.24$
VH/CD	$9.78^{a} \pm 0.42$	$9.58^{ab} \pm 0.15$	$9.57^{ab} \pm 0.17$	$9.07^{ab} \pm 0.16$	$8.82^{b} \pm 0.20$	$9.37^{ab} \pm 0.12$
Mucosa thickness	$418.50^{f} \pm 4.05$	$431.00^{e} \pm 2.16$	$724.13^{d} \pm 3.35$	$973.87^{b} \pm 3.10$	$1390.00^{a} \pm 2.89$	$846.77^{c} \pm 3.45$
wall thickness	$492.67^{e} \pm 2.90$	$472.47^{f} \pm 1.55$	$1037.83^{d} \pm 2.52$	$1200.13^{b} \pm 2.89$	$1516.20^{a} \pm 2.72$	$1083.90^{c} \pm 2.48$
VW	$47.83^{d} \pm 1.17$d	$73.28^{c} \pm 1.09$	$75.03^{bc} \pm 2.07$	$77.43^{bc} \pm 1.37$	$83.02^{a} \pm 2.13$	$79.73^{ab} \pm 1.54$
villus perimeter x 10^4	$12.36^{d} \pm 0.19$	$20.63^{c} \pm 0.23$	$25.73^{b} \pm 0.75$	$27.06^{b} \pm 0.57$	$30.73^{a} \pm 0.79$	$30.61^{a} \pm 0.53$
villus surface area x 10^6	$10.17^{a} \pm 0.08$	$18.48^{c} \pm 0.15$	$28.08^{b} \pm 0.88$	$30.11^{b} \pm 0.75$	$36.19^{a} \pm 0.94$	$37.40^{a} \pm 0.61$

a, b, c, d, e, f Means ± standard Error values within the same row with various superscripts differ significantly (P<0.05). The abbreviations VH, CD, VH/CD and VW stand for villus height, crypt depth, Villous height to Crypt depth and Villous width respectively.

Table 7: Effect of nucleotides supplementation in chicks' diets on breast muscle content of fatty acids (%)

fatty acids percent of breast muscle	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	T2 0% nucleotides with 1% SBO	T3 0.05% nucleotides without oil	T4 0.05% nucleotides with 1% SBO	T5 0.05 % nucleotides with 1% LO	T6 0.05 % nucleotides with 1% FO
SFA	$29.31^{e} \pm 0.14$	$54.23^{a} \pm 0.25$	$27.83^{f} \pm 0.13$	$51.36^{b} \pm 0.24$	$34.36^{c} \pm 0.10$	$32.06^{d} \pm 0.15$
USFA	$65.71^{b} \pm 0.31$	$45.47^{e} \pm 0.22$	$69.57^{a} \pm 0.33$	$47.82^{d} \pm 0.22$	$62.65^{c} \pm 0.30$	$66.43^{b} \pm 0.31$
Total n-3 fatty acids	$1.69^{e} \pm 0.14$	$2.19^{de} \pm 0.17$	$2.80^{c} \pm 0.23$	$2.59^{cd} \pm 0.21$	$9.18^{b} \pm 0.04$	$22.93^{a} \pm 0.11$
Total n-6 fatty acids	$36.55^{a} \pm 3.01$	$14.14^{b} \pm 1.09$	$38.67^{a} \pm 0.18$	$15.37^{b} \pm 1.27$	$18.24^{b} \pm 0.09$	$15.07^{b} \pm 0.07$
n-6:n-3 ratio	$22.94^{a} \pm 1.89$	$6.97^{c} \pm 0.53$	$15.38^{b} \pm 1.27$	$6.87^{c} \pm 0.52$	$2.09^{d} \pm 0.17$	$0.69^{d} \pm 0.06$

a, b, c, d, e, f Means ± standard Error values within the same row with various superscripts differ significantly (P<0.05). The abbreviations USFA = unsaturated fatty acid, SFA =saturated fatty acid; n-6=omega 6, n-3=omega 3

Table 8: Effect of nucleotides supplementation on the economic efficiency of the experimental diets

	Dietary nucleotides supplementation%					
	T1 0% nucleotides without oil	T2 0% nucleotides with 1% SBO	T3 0.05% nucleotides without oil	T4 0.05% Nucleotides with 1% SBO	T5 0.05 % Nucleotides with 1% LO	T6 0.05 % nucleotides with 1% FO
Feeding cost of the obtained gain (LE)	18.52	18.93	18.94	19.43	19.42	20.27
Selling price of the obtained gain (LE/kg live weight)	23	23	23	23	23	23
Selling cost of obtained gain (LE)	50.82	53.19	50.56	51.59	53.40	54.48
Economic efficiency (EE) %	174.41	180.92	166.90	165.55	174.94	168.73

(LE)= Egyptian pound date of experiment= 1/3//2019

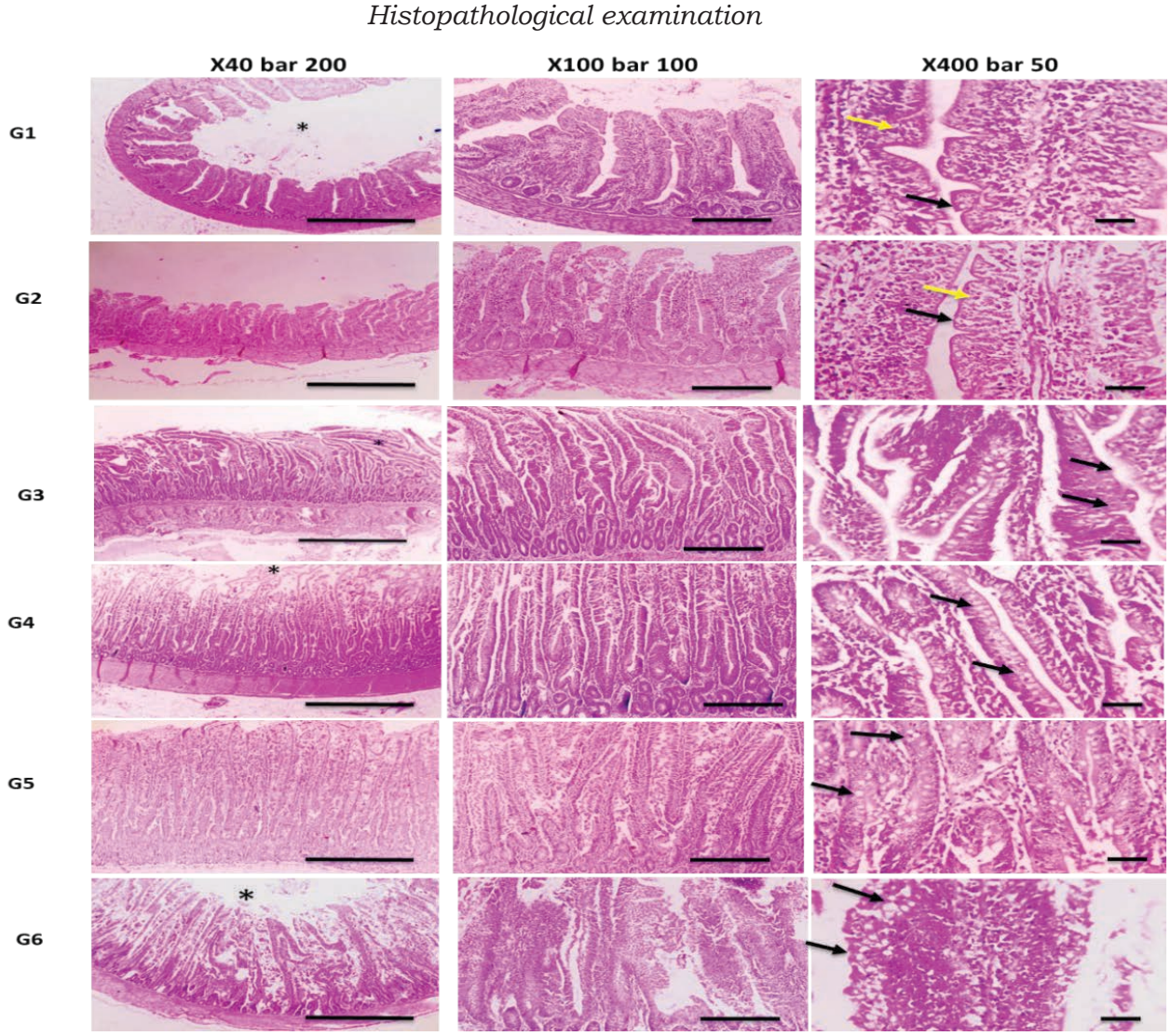

Figure 1: microscopic images of the jejunal sections stained with H&E

Microscopic images of H&E-stained jejunal slices from the control group show normal villi, lamina propria, and muscular coat. Higher magnification X: 400 show presence of few goblet cells (black arrow) along with epithelial vacuolization (yellow arrows). Meanwhile, groups supplemented with mixture of different oils and nucleotide showing gradual increase in villous height, numbers and size of goblet cells (black arrow) and decrease epithelial vacuolization (yellow arrows). (Asterisks point to lumen of jejunum).

table 3, the dressing %, breast yield, bursa of Fabricius, thymus, and intestinal weight were considerably ($P<0.05$) higher in the nucleotide-fed groups than in the control groups. The group administered nucleotides together with fish and linseed oil showed the best carcass characteristic, bursa of Fabricius, and intestinal weight.

Our findings in table 4 demonstrated that serum concentrations of triglycerides, cholesterol, LDL, VLDL, TAC, and albumin (A) were significantly ($P<0.05$) decreased with increased serum content of total protein (TP), globulin, and GSH-Px values with nucleotide supplementation, particularly in combination with PUFA oil sources.

According to the results in table 5, there was no significant difference in antibody titer against Newcastle virus vaccine between all groups but TLC significantly ($P<0.05$) increased with nucleotides fed than control groups and the best value was recorded in group fed nucleotides in combination with fish and linseed oil. Also, significant ($P<0.05$) decreases mortality ratio with nucleotides fed groups and the best value was observed in in group fed nucleotides in combination with fish oil.

As shown in table 6 significant ($P<0.05$) improvement in villus height, crypt depth, mucosal thickness, wall thickness, villus width, villus

perimeter, and villus surface area in nucleotide fed groups compared to control groups, especially when combined with PUFA oil sources (FO, LO, and then SBO).

As shown in table 7, there was a substantial difference between each group. With T1, T3, T5, and T6 compared to T2 and T4, there were significantly ($P<0.05$) higher levels of USFA and lower levels of SFA in the breast muscle. N-3 fatty acids are present in greater percentages in T3, T4, T5, and T6 than in T1 and T2. In comparison to other groups, T1 and T3 had significantly ($P<0.05$) greater levels of N-6 fatty acids. As a result, the n-6: n-3 ratio gradually dropped with the addition of nucleotides to the diet and USFA oil sources.

Our results showed in table 8 that the feeding cost of the obtained gain (LE) and the selling cost of the obtained gain (LE) had increased with nucleotide use and the highest economic efficiency was recorded in the control group (T2), which was supplemented with SBO then group fed nucleotides in combination with linseed oil and control group(T1) than other groups.

Discussion

Effect of nucleotides dietary supplement on growth performance

The chief conclusions of the positive effect of dietary nucleotides on the growth response of broiler chicks are presented in Table 2, where the best *growth performance* was recorded in dietary nucleotides in combination with fish and linseed oil treated groups. These findings were in line with Jung & Batal (5) and Salah et al. (6) who discovered that adding yeast nucleotides to bird diet increased body weight, daily weight gains, and feed conversion ratios without affecting feed consumption. However, Pelcia et al. (12) discovered that the 0.05, 0.06, and 0.07% additions of nucleotides to broiler feed had no effect on the broiler's performance. The encouraging outcomes could be attributed to the easier access to nucleotides for the growth of intestinal cells and subsequently improved activity of the digestive enzymes, which lead to improved digestion and nutritional absorption (6) Additionally, according to Jung and Batal (5) supplementing broiler feed with nucleotides increased villus height and villus height-to-crypt depth ratio while lowering the animal's energy consumption because de novo synthesis of nucleotides requires a lot of energy. Oil sources like fish and linseed oil, which are well known as important nutrients for health and are essential for many regular bodily activities as well as stimulating growth, also have a dual effect with omega-3 fatty acids (31). Additionally, the involvement of n-3 PUFA in the enhancement of bile production, which enhances fat digestion in the colon, enhances the effectiveness of feed digestion and absorption (32).

Effect of nucleotides dietary supplement on carcass composition

According to our findings in Table 3, nucleotide supplementation improved carcass characteristics and intestinal weight and decreased the fat content of the breast muscles. The group administered nucleotides together with fish and linseed oil showed the best carcass characteristics and intestinal weight. The liver, gizzard, and lean meat production showed the maximum performance in the group treated with 0.03% of nucleotides, according to Fonia et al (33) findings that dietary nucleotide supplementation improves the weight of different organ cut up sections. However, Pelcia et al (12) findings were different, showing that adding nucleotides to broiler feed in amounts of 0.05, 0.06, and 0.07% had no impact on carcass output. On the other hand, nutritional augmentation with omega-3 fatty acids enhances the carcass by lowering broiler abdominal fat distribution (34).

Effect of nucleotides dietary supplement on serum biochemical parameters

Our findings in table 4 demonstrated that nucleotide supplementation improved serum biochemical parameters, particularly in combination with PUFA oil sources. These outcomes were consistent with studies that observed decreased serum cholesterol and LDL cholesterol and increased HDL cholesterol with higher levels of nucleotide fed groups in addition to a significant improvement in total protein, albumin, and globulin, but A/G ratio showed no effect (35) and (36). A heightened immunological response and the proteinic character of the antibodies may be the cause of the increase in serum concentra-

tion of total protein with omega-3 fatty acid (37). However, adopting diets including fish oil as an omega-3 fatty acid source considerably increased blood HDL-c, total protein (TP), globulin (GL), and GSH-Px values while significantly lowering serum triglycerides, cholesterol, LDL, VLDL, albumin (A), and TAC concentrations (P<0.05) (38). Additionally, Qi et al. (39) and Ibrahim et al. (40) discovered that adding n-3 PUFA to the diets clearly improved antioxidative status.

The effect of nucleotides dietary supplement on avian immune function

According to our findings in Table 5, the groups fed nucleotides in conjunction with fish and linseed oil had the best outcomes, with antibody titers against the Newcastle disease vaccination, TLC and bursa of Fabricius weight. These findings support the work of Raheel et al. (10), who demonstrated that the immune system is strengthened by nucleotides when they are added to the diet. increased bird immunoglobulin production is another factor (41). in order to lessen the effects of pathogens (42). Likewise, adding nucleotides at a dosage of 0.5 g/kg raised bursa of Fabricius weight in comparison to the control group but had no impact on spleen weight (6). This outcome could have occurred because the percentage of cell turnover in tissues like the Fabricius bursa was increased during stresses. They need enough extra nucleotides to synthesize DNA and RNA for maintenance and growth in this way (41). The intestinal lumen is where foreign nucleotides are absorbed, and they subsequently go to immunological organs like the bursa (43). In addition, Hassanein et al. (38) discovered that TLC substantially (P< 0.05) provided the best ratio in fish oil-fed groups as opposed to control groups.

Effects of nucleotides dietary supplement on mortality ratio

According to our findings in Table 5, nucleotide supplementation decreased the mortality ratio, and the lowest value were observed when mixed with fish oil. This outcome was consistent with the findings of Daneshmand et al. (41), who discovered that broilers given nucleotides had a decreased mortality rate. It could be because, in addition to storing energy, nucleotides perform several vital physiological, gastrointestinal, and immune roles in the organism during rapid growth and development, disease problems, injury or stress situations like high stocking density or unclean litters (5). In addition, Hassanein et al. (38) shown that supplementing with fish oil considerably (P 0.05) reduces the mortality ratio.

Effects of nucleotides dietary supplement on intestinal morphology

By significantly, Table 6 demonstrates that intestinal morphology in nucleotide-fed groups is significantly improved when compared to control groups, especially when combined with PUFA oil sources (FO, LO, then SBO). These findings were in agreement with those of Khedr et al. (44), who demonstrated that, in the jejunum region of the small intestine, broiler groups fed nucleotides showed a substantial increase in intestinal villi length when compared to control groups. And yet, Hassanein et al. (38) found that fish oil-based diets significantly improved intestinal morphology compared to control groups in terms of villus height, crypt depth, mucosal thickness, wall thickness, villus width, villus perimeter, and villus surface area. However, Aziza et al. (45) found that when compared to fish oil and camelina meal holding diets, control and camelina meal diets as a source of n-3 PUFA increased villus height, VH: CD, and villus perimeter of the jejunum, and there was no significant difference in villus width, surface area, or muscularis thickness between different groups. These effects might be caused by fish oil, which enhances the intestine's absorption capacity. As a result, broilers fed these diets should thrive and have more robust immune systems (37).

Effect of nucleotides dietary supplement on meat quality

Significant changes were found between the groups, as shown in Shawn's table 7, where the addition of nucleotides and USFA oil sources to the diet gradually reduced the n-6: n-3 ratio in the breast muscle. These findings corroborated those of Chiofalo et al. (11), who concluded that meat had better nutritional qualities because it included more lipids with greater unsaturation degrees, which had positive impacts on human health when consumed with nucleotides.

The economic evaluation of nucleotides dietary supplement

According to our results in Table 8 nucleotide supplementation increased the feeding cost of obtained gain (LE) and selling cost of obtained gain and had the highest economic efficiency only when used in conjunction with linseed oil. But as demonstrated by Zahran et al. (46), Ahiwe et al. (47), and Fathi et al. (48), broiler groups given a diet containing nucleotides performed significantly better economically than the control group. However, Hassanein et al. (38) report that the group given 1% FO had the best economic efficiency, followed by 1% SBO, 2% FO, 3% FO, and 0% oil, in that order.

Conclusion

The performance of growth, dressing percentage, meat quality, serum biochemical parameters, immunological response, and intestinal morphology in chicks is considerably enhanced by the addition of nucleotides to their diets. It also lowers mortality, but it's only cost-effective when used in conjunction with linseed oil.

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