*Original Paper***Dietary Nucleoforce® supplementation improves growth performance, feed efficiency and enhances intestinal morphology in broiler chickens**

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ABSTRACT

This study was carried out to estimate the effect of nucleoforce® on growth performance, feed efficiency and intestinal morphometry in broiler chickens. Two hundred forty, one-day old Ross 308 broiler chicks were randomly allocated into four equal groups (60birds for each group) with 3 replicates (20 birds for each replicate). Birds in the first group fed diet with no supplementation and considered as a control group (C), whereas birds of the second (200N), third (350N), and fourth (500N) groups were fed diet supplemented with nucleoforce® at concentrations of (200, 350 and 500 g/ton, respectively) from zero day till the end of the experiment. Birds were weighed weekly to record their body weight then calculate their weight gain and relative growth rate. Feed consumption was daily estimated and feed conversion rate was calculated. At days 21 and 49, intestinal samples (jejunum) were subjected to quantitative morphometrical analysis. The obtained results revealed improvement in growth parameters and enhancement of histomorphology of jejunum of small intestine in broiler chickens after dietary nucleoforce® supplementation. It could be concluded that dietary nucleoforce® as a commercial source of nucleotides can be considered as a promising feed additive in broiler chickens.

1. INTRODUCTION

Poultry production, as a food producing industry, represents one of the most rapidly developing animal industries all over the world. However, nutrition is the most critical aspect influences the productivity and profitability of this industry (Nguyen et al., 2021). In the commercial broiler industry, the most important challenge is to maximize the growth and quality of birds besides maintaining an optimal level of their health. Nucleotides are considered one of the best functional nutrients which have substantial roles in enhancing broiler chicken productivity (Salah et al., 2019). Nucleotides are phosphoric nucleoside esters consisting of a nitrogen base, a pentose sugar and one or more phosphate groups representing the basic units of the nucleic acids (Carver and Walker, 1995; Devresse, 2000). For maintaining the cellular nucleotide pools, three potential sources of nucleotides are available: de novo nucleotide synthesis, salvage pathway and dietary nucleotides supplementation (Hess and Greenberg, 2012). De novo nucleotides synthesis is a metabolically expensive process requires large amounts of energy in the form of ATP (Carver and Walker, 1995). The salvage pathway recycles about 90% of purine bases and is thought to depend on the availability of free bases (Cosgrove, 1998). It requires less energy than that is needed for the de novo synthesis. Exogenously supplied nucleotides are required for tissues possessing a limited capacity for the de novo synthesis (Gil and Uauy, 1995). These tissues include hematopoietic cells of the bone marrow, erythrocytes, leucocytes, lymphocytes and intestinal mucosa (Sanderson and He, 1994).

The small intestine, the major site for digestion and absorption of nutrients, is a vital organ that is related to growth performance (Brudnicki et al., 2017). Depending on the fact that part of the digestive and whole the absorptive capacity of the small intestine take place around and near the intestinal villi and crypts, it is recognized that the change in the villi length and the crypt depth are considered an index for gut health (Pluske 1996; Nguyen, et al., 2021). Therefore, this work was carried out to estimate the effect of different nucleotides concentrations on growth performance, feed efficiency and intestinal morphometry of broiler chickens.

2. MATERIAL AND METHODS*2.1. Nucleotides (Nucleoforce®)*

Nucleoforce® is a balanced concentrate of free nucleotides and active precursors obtained from dried yeast with a minimum concentration of 80%. It was obtained from Bioibérica, S.A., Spain in a powder form composed of 20.34% crude protein, 3.25% protein nitrogen, 12.09% non-protein nitrogen, 0.1% crude fiber and 3.38% Ash.

Birds, housing, and management

A total number of 240 one-day old broiler chicks (Ross 308) of both sexes with average weight of 43g obtained from a commercial hatchery (El-Desoky Company) were randomly allocated into four equal groups (60 birds/group) with 3 replicates (20 birds/replicate).

The birds were housed in clean disinfected well-ventilated rooms bedded with a layer of fresh clean 4 cm deep wood shaving. The floor was divided into 12 separate pens of equal size by using wire net and bamboo materials. Intermittent lightening program (23 hours lighting: 1 hour darkness) was

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used. The temperature surrounding birds was adjusted with their age. Feed and fresh water were provided ad libitum.

2.1. Diets

Birds were randomly categorized into four equal groups: group1, birds were fed basal diet without supplementation

and considered as control group; group2, 3 and 4, birds were fed basal diet supplemented with 200,350 and 500 g nucleoforce@/ton during the whole period of the experiment (7weeks). The basal diet was formulated to provide the nutritional requirements of birds during different phases of age according to National Research Council (1994).The composition of the ration was shown in table 1.

Table 1. The chemical composition of the basal diet during different phases of growth

Feed Ingredients	Composition (%) mixed feed Broiler ration		
	Starter (0 to 17d)	Grower (17 to 36d)	Finisher (36d till slaughter)
Yellow corn (crushed)	53.03	55.51	60.68
Soya bean meal (CP 46 %)	35.00	33.70	27.50
Corn gluten meal	4.70	3.00	3.50
Soya bean oil	2.40	3.40	4.30
Di calcium phosphate	1.60	1.33	1.23
Limestone	1.50	1.40	1.25
L_Lysine	0.39	0.31	0.29
Sodium chloride	0.33	0.31	0.31
Vitamin and mineral premix	0.30	0.30	0.30
DL_methionine	0.33	0.30	0.26
Sodium bicarbonate	00.13	00.13	00.13
Anticoccedia	0.05	0.05	0.05
Antimycotoxin	0.05	0.05	0.05
L_Threonine	00.10	00.10	00.10
Anticolesteridia	0.03	0.03	0.03
Energy enzyme	0.03	0.05	0.05
Lysomax	0.10	0.10	0.10
Phytase enzyme	0.01	0.01	0.01
Protease B	0.01	0.01	0.01
Emulsifier	0.01	0.01	0.01
Total	100	100	100
Calculated composition			
Metabolizable energy ME (kcal/kg)	3001.88	3101.78	3226.25
CP	23.02	21.54	19.51
CF	3.56	3.50	3.17
Crude fat	5.03	6.02	7.02
Lysine	1.35	1.25	1.09
Lysine dig	1.26	1.16	1.01
Methionine	0.67	0.62	0.55
Methionine dig	0.63	0.58	0.52
Methionine+ cysteine	1.02	0.95	0.86
Methionine+ cysteine dig	0.92	0.85	0.77
Threonine	0.92	0.87	0.73
Threonine dig	0.79	0.75	0.62
Calcium	1.05	0.95	0.85
Available phosphorus	0.50	0.45	0.42
Sodium	0.18	0.17	0.17
Chloride	0.23	0.22	0.22
Potassium	0.88	0.85	0.75
Pellet quality factor	3.28	2.88	2.55
Acid base balance (me/kg)	223.67	217.11	188.50

Vitamin and mineral premix was composed of (Content per: 3.0 kg): vitamin A 12000000 IU; vitamin D 200000 IU; vitamin E 10000 mg; vitamin K3 2000 mg; vitamin B1 1000 mg; vitamin B2 5000 mg; vitamin B6 1500 mg; Biotin 50 mg; Niacin 30000 mg; Folic acid 1000 mg; D-Calpan 10000 mg; vitamin B12 10 mg; Iron carbonate 3000 mg; Cobalt Carbonate 100 mg; Manganese oxide 60000 mg; Calcium Iodate 1000 mg; Copper sulphate 4000 mg; Selenium Sodium 100 mg; Zinc (global) 50000 mg and carrier (CaCo3) Up to 3.0 kg. Vitamin and mineral premix produced by MULTI-VITA 6 of October city, Egypt.

2.3. Experimental design

The initial body weights for all birds were recorded in grams at the beginning of the experiment and then birds were weighed weekly to record the live body weight. These data were used to calculate daily weight gain and relative growth rate. Body weight gain of chicks (expressed in grams) was calculated as difference between two successive weights.

Relative growth rate (%) was calculated according to Crampton and Liloyd (1959) using the following formula:
 $RGR = 100 (W_2 - W_1) / (W_2 + W_1)$

Where: W_1 = Body weight at the beginning of the week.

W_2 = Body weight at the end of the week.

The diet was provided regularly in the morning and the feed intake was calculated by difference between the weights of offered feed and remained portion. Feed conversion ratio was calculated according to Wagner et al. (1983) as follow:

$$FCR = \frac{\text{Feed intake (g) / bird/week}}{\text{Body weight gain (g) / bird/week}}$$

At days 21 and 49 of age, two birds from each replicate were slaughtered, and eviscerated to take specimens of jejunum for morphometrical examination (Rady et al., 2023). The specimens were fixed in 10% formalin and dehydrated by ascending concentrations of alcohol, cleared in xylol and then embedded in paraffin. Using Leica Rotary Microtome (RM 2145, Leica Microsystems, Wetzlar, Germany), serial 5- μ m sections were cut and routinely stained with hematoxylin and eosin according to Bancroft et al.2013. The histo-morphometric analysis of small intestine was carried out using Image J analysis software (National Institutes of Health, MD, USA). The number of goblet cells per unit of surface area (mm²) was also calculated.

2.4. Statistical analysis

It was performed by Graph Pad Prism software, 2007 version 5.04 (Graph Pad Prism, San Diego, CA, USA) for determining the significant difference between experimental groups using one way analysis of variance (ANOVA). Duncan's Multiple Range test using Costat Computer

Program (1986) was used to detect the significance of difference between each two groups. Data were represented as mean ± standard error. Significant differences between mean values were determined at P ≤ 0.05.

All procedures were authorized by the institutional review board for animal experiments of the Faculty of Veterinary Medicine, Benha University, Egypt with an ethical approval number (BUFVTM10-07-23)

3. RESULTS

Growth performance:

The effect of dietary nucleoforce® supplementation on the live body weight (g) of broiler chickens was shown in table 2. Dietary nucleoforce® significantly (P<0.05) increased the live body weight of broiler chickens in a concentration dependent manner during the whole experiment, where the highest concentration (500g/ton) resulted in the highest values.

Table 2: The effect of Nucleoforce® on the live body weight of broiler chickens

	C	200 N	350 N	500 N
IBW	43.69 ± 0.43 ^a	43.35 ± 0.43 ^a	42.28 ± 0.36 ^a	42.66 ± 0.44 ^a
1 st week	201.74 ± 1.0 ^d	209.74 ± 1.0 ^c	215.02 ± 0.92 ^b	218.00 ± 0.69 ^a
2 nd week	500.58 ± 1.9 ^d	528.66 ± 0.75 ^c	533.33 ± 0.59 ^b	537.35 ± 0.50 ^a
3 rd week	958.97 ± 0.71 ^d	986.79 ± 0.86 ^c	994.20 ± 0.80 ^b	1022.0 ± 2.30 ^a
4 th week	1535.00 ± 4.7 ^d	1577.23 ± 2.3 ^c	1590.13 ± 1.5 ^b	1605.86 ± 1.9 ^a
5 th week	2150.70 ± 1.6 ^c	2209.56 ± 2.2 ^b	2212.86 ± 2.6 ^b	2227.70 ± 1.9 ^a
6 th week	2781.93 ± 6.1 ^d	2843.93 ± 7.3 ^c	2891.53 ± 2.8 ^b	2927.6 ± 11.0 ^a
7 th week	3016.93 ± 4.2 ^c	3212.63 ± 4.4 ^b	3234.70 ± 3.2 ^a	3241.86 ± 3.3 ^a

Each value is expressed as mean ± standard error. Means with different letters in the same row are significantly different (p<0.05). IBW= initial body weight. C= control, 200 N= Nucleoforce® at a concentration of 200g/ton, 350 N= Nucleoforce® at a concentration of 350g/t, 500N= Nucleoforce® at a concentration of 500g/t.

Results of daily weight gain (g) were tabulated in table 3. Dietary nucleoforce® supplementation with its three different concentrations significantly (P≤ 0.05) increased the daily weight gain of broiler chickens during the 1st, 2nd and 7th weeks of the experiment as compared to the control

group. The difference among nucleoforce® supplemented groups varied in significance during different weeks of the experiment. The final weight gain was significantly higher in 500 and 350g /ton groups if compared to 200g /ton group while the control group had the lowest weight gain.

Table 3: The effect of Nucleoforce® on the daily weight gain of broiler chickens

	C	200 N	350 N	500 N
1 st week	22.57 ± 0.16 ^c	23.76 ± 0.13 ^b	24.67 ± 0.14 ^a	25.04 ± 0.12 ^a
2 nd week	42.69 ± 0.34 ^b	45.55 ± 0.18 ^a	45.47 ± 0.15 ^a	45.62 ± 0.10 ^a
3 rd week	65.48 ± 0.27 ^b	65.44 ± 0.18 ^b	65.44 ± 0.18 ^b	69.23 ± 0.35 ^a
4 th week	82.45 ± 0.68 ^c	84.29 ± 0.38 ^{ab}	85.12 ± 0.26 ^a	83.65 ± 0.48 ^{bc}
5 th week	87.95 ± 0.75 ^b	90.33 ± 0.40 ^a	88.96 ± 0.38 ^{ab}	88.83 ± 0.34 ^b
6 th week	90.17 ± 0.90 ^b	90.62 ± 1.2 ^b	96.95 ± 0.56 ^a	99.98 ± 1.60 ^a
7 th week	33.57 ± 1.1 ^d	52.67 ± 0.95 ^a	49.02 ± 0.60 ^b	44.89 ± 1.50 ^c
Final	2973.83 ± 4.2 ^c	3169.36 ± 4.3 ^b	3192.53 ± 3.2 ^a	3198.83 ± 3.2 ^a

Each value is expressed as mean ± standard error. Means with different letters in the same row are significantly different (p<0.05).

The effect of dietary nucleoforce® supplementation on the relative growth rate (%) of broiler chickens was shown in table 4. The values of relative growth rate differed among the groups and the different weeks of the experiment. The

final relative growth rate was significantly (P≤ 0.05) higher in nucleoforce® supplemented groups than in the control group.

Table 4: The effect of Nucleoforce® on the relative growth rate of broiler chickens

	C	200 N	350 N	500 N
1 st week	128.79 ± 0.70 ^c	131.49 ± 0.52 ^b	134.26 ± 0.52 ^a	134.54 ± 0.59 ^a
2 nd week	85.08 ± 0.58 ^b	86.39 ± 0.40 ^a	85.08 ± 0.36 ^b	84.56 ± 0.25 ^b
3 rd week	62.82 ± 0.34 ^a	60.46 ± 0.17 ^c	60.34 ± 0.12 ^c	62.14 ± 0.24 ^b
4 th week	46.37 ± 0.24 ^a	46.13 ± 0.15 ^a	46.03 ± 0.10 ^a	44.46 ± 0.23 ^b
5 th week	33.42 ± 0.32 ^a	33.39 ± 0.15 ^a	32.74 ± 0.13 ^b	32.44 ± 0.13 ^b
6 th week	25.58 ± 0.23 ^b	25.09 ± 0.29 ^b	26.59 ± 0.15 ^a	27.13 ± 0.37 ^a
7 th week	8.10 ± 0.26 ^d	12.18 ± 0.23 ^a	11.20 ± 0.14 ^b	10.20 ± 0.36 ^c
Final	194.37 ± 0.06 ^b	194.68 ± 0.06 ^a	194.85 ± 0.05 ^a	194.79 ± 0.06 ^a

Each value is expressed as mean ± standard error. Means with different letters in the same row are significantly different (p<0.05).

The results of dietary nucleoforce® supplementation on the daily feed intake (g) were shown in table 5. There was non-significant difference in the daily feed intake of broiler chickens among the different groups and weeks of the experiment except at the 1st week where the control group

showed a significant (P≤ 0.05) decrease than 350 and 500g/ton groups and during the 6th week where the 500 g/ton resulted in a significant decrease than the other groups.

Table 5: The effect of Nucleoforce® on the daily feed intake of broiler chickens

	C	200 N	350 N	500 N
1 st week	29.41 ± 0.21 ^b	29.80 ± 0.98 ^{ab}	29.90 ± 0.09 ^a	30.24 ± 0.13 ^a
2 nd week	59.41 ± 0.40 ^a	59.61 ± 0.27 ^a	59.56 ± 0.26 ^a	59.81 ± 0.23 ^a
3 rd week	101.84 ± 0.40 ^a	102.14 ± 0.34 ^a	102.29 ± 0.35 ^a	102.29 ± 0.43 ^a
4 th week	144.37 ± 0.09 ^a	144.66 ± 0.31 ^a	144.91 ± 0.21 ^a	144.37 ± 0.23 ^a
5 th week	181.64 ± 0.92 ^a	181.89 ± 0.32 ^a	181.84 ± 0.30 ^a	182.62 ± 0.35 ^a
6 th week	216.61 ± 0.64 ^a	216.21 ± 0.34 ^a	216.21 ± 0.40 ^a	214.40 ± 0.27 ^b
7 th week	131.95 ± 0.18 ^a	132.34 ± 0.30 ^a	132.73 ± 0.15 ^a	132.83 ± 0.35 ^a
Final	6056.88 ± 1.8	6066.84 ± 2.1	6072.34 ± 0.91	6059.28 ± 7.3

Each value is expressed as mean ± standard error. Means with different letters in the same row are significantly different (p<0.05).

The results of feed conversion rate (%) were tabulated in table 6. The control group showed a significantly ($P \leq 0.05$) higher feed conversion rate during the whole experiment. The results of nucleoforce® supplemented groups varied in

significance among the different weeks of age. Finally, at the end of the experiment dietary nucleoforce® supplementation at a concentration of 500 g /ton had the lowest significant (best) feed conversion rate.

Table 6: The effect of Nucleoforce® on the feed conversion rate of broiler chickens

	C	200 N	350 N	500 N
1 st week	1.30 ± 0.009 ^a	1.26 ± 0.004 ^b	1.21 ± 0.003 ^c	1.21 ± 0.005 ^a
2 nd week	1.39 ± 0.009 ^a	1.31 ± 0.006 ^b	1.31 ± 0.006 ^b	1.31 ± 0.005 ^b
3 rd week	1.55 ± 0.006 ^a	1.56 ± 0.005 ^a	1.55 ± 0.005 ^a	1.47 ± 0.006 ^b
4 th week	1.74 ± 0.001 ^a	1.71 ± 0.004 ^c	1.70 ± 0.003 ^d	1.73 ± 0.003 ^b
5 th week	2.06 ± 0.010 ^a	2.01 ± 0.004 ^c	2.04 ± 0.003 ^b	2.05 ± 0.004 ^{ab}
6 th week	2.40 ± 0.007 ^a	2.38 ± 0.004 ^b	2.23 ± 0.004 ^c	2.22 ± 0.003 ^c
7 th week	3.93 ± 0.005 ^a	2.51 ± 0.006 ^d	2.70 ± 0.003 ^c	2.96 ± 0.008 ^b
Final	2.03 ± 0.001 ^a	1.91 ± 0.001 ^b	1.90 ± 0.001 ^c	1.89 ± 0.002 ^d

Each value is expressed as mean ± standard error. Means with different letters in the same row are significantly different ($p \leq 0.05$).

The results of the quantitative morphometrical analysis of small intestine were illustrated in table 7 and figure 1. Nucleoforce® supplementation improved the jejunal morphology of broiler chickens in a dose and time dependent manner. There were substantial increases in the length of intestinal villi and the depth of crypt in nucleotide

supplemented groups. On the other hand, nucleotides supplementation was accompanied with significantly decreased inter villi spaces compared to control group. Moreover, nucleotide supplemented groups had significant increases in the numbers of goblet cells than the control group.

Table 7: The effect of Nucleoforce® on the quantitative morphometrical analysis of small intestine of broiler chickens

		Villi length (µm)	Base width (µm)	Crypt depth (µm)	Inter villi space (µm)	Goblet cells/mm ²
1 st slaughter	C	1015.04 ± 76.0 ^b	167.71 ± 17 ^a	163.93 ± 18.0 ^b	113.31 ± 6.5 ^a	135 ± 7.2 ^d
	200 N	1120.17 ± 25.0 ^b	159.64 ± 4.2 ^a	174.33 ± 7.7 ^b	96.51 ± 7.2 ^a	171 ± 8.4 ^c
	350 N	1320.17 ± 24.0 ^a	142.46 ± 5.0 ^a	227.35 ± 19.0 ^{ab}	70.78 ± 4.8 ^b	202 ± 5.5 ^b
	500 N	1429.19 ± 28.0 ^a	143.93 ± 8.8 ^a	285.73 ± 35.0 ^a	53.01 ± 8.0 ^b	231 ± 7.2 ^a
2 nd slaughter	C	1121.10 ± 9.3 ^c	194.85 ± 7.9 ^a	209.02 ± 7.9 ^c	97.04 ± 8.6 ^a	134.33 ± 11.0 ^c
	200 N	1231.59 ± 15.0 ^c	151.13 ± 17.0 ^{ab}	214.36 ± 13.0 ^c	74.51 ± 9.5 ^{ab}	207.33 ± 6.4 ^b
	350 N	1429.30 ± 25.0 ^b	122.70 ± 13.0 ^b	301.54 ± 20.0 ^b	53.98 ± 9.5 ^b	233 ± 6.6 ^{ab}
	500 N	1777.32 ± 21.0 ^a	126.56 ± 15.0 ^b	405.04 ± 32.0 ^a	46.06 ± 12.0 ^b	255.33 ± 7.2 ^a

Each value is expressed as mean ± standard error. Means with different letters in the same column are significantly different ($p \leq 0.05$).

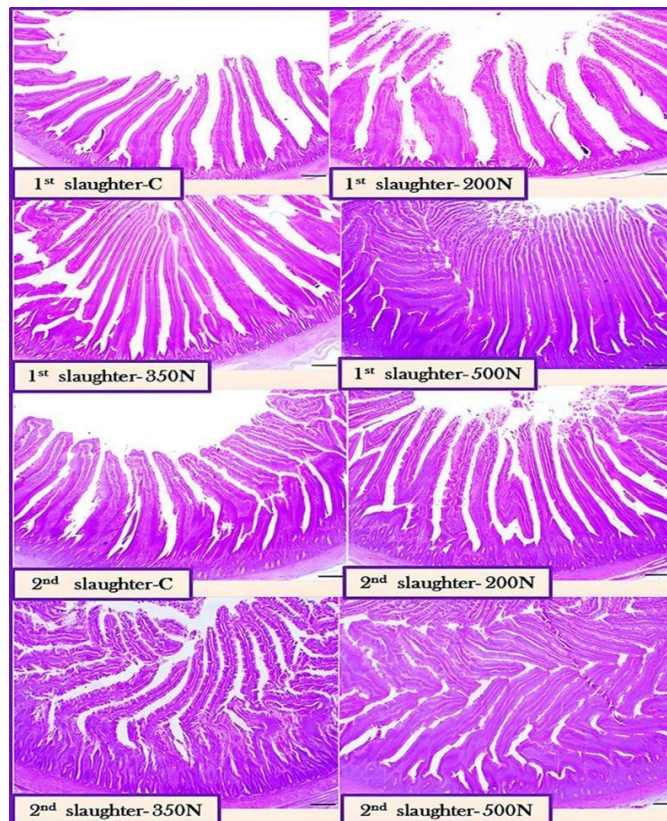


Figure 1: Photomicrograph showing the morphology of intestine (jejunum) of control, dietary nucleoforce® at 200, 350 and 500 gram/ton at the 1st and 2nd slaughters. H&E, X40, bar = 200 µm.

4. DISCUSSION

Nucleotides supplementation is thought to play a crucial role in broilers industry by growing them up much faster and enabling them to reach marketing weight at earlier age which in turn occurs due to early and rapid development of intestinal tract and mature villi (Kocher et al., 2010, Alizadeh et al., 2016, Leung, 2018 and Wu et al., 2018). In the present study, dietary nucleotides supplementation resulted in significant increases in broiler live body weights, daily weight gain, and final relative growth rates. These results came in agreement with Pelícia et al. (2010), Salah et al. (2019), Khedr et al. (2020a), Mohamed et al. (2020) and Rafique et al. (2020). Regarding the feed intake, this study revealed no significant difference between control and nucleotides supplemented groups which came in harmony with Deng et al. (2005), Zauk et al. (2006) and Salah et al. (2019) who found that nucleotide supplementation had no significant importance on the feed intake. Increasing growth performance without affecting the daily feed intake resulted in better feed conversion rates with nucleotides supplementation as compared to control group. Moreover, the current study also showed substantial increases in the length of intestinal villi and the depth of crypts in nucleotide supplemented groups. Increases in villi length indicate more cells for the absorption process, which improves nutrition absorption in the gut. The crypt is the villus factory, a big crypt suggested a quick tissue turnover and a high need for new tissue regenerating, as well as being responsible for the nutrition absorption process indicating positive effect of dietary supplementation of nucleotides. On the other hand, nucleotides supplementation was accompanied with smaller inter villi space compared to control group. The empty space between two villi appears to be affected by the number of villi. When number of villi increase, the inter-villi gap decrease. Similarly, nucleotide supplemented groups had higher numbers of goblet cells than the control group. These goblet cells secrete mucin, which dissolves in water to create mucus, which coats the gut wall and functions as a first line of defense against pathogen invasion, implying a beneficial effect of nucleotide supplementation on intestinal health status. These findings came in harmony with Khedr et al. (2020b), Kamel et al. (2021) and Rady et al. (2023) who returned the cause of improvement in growth performance after nucleotides inclusion to the increased villi length and increased number of intestinal glands which in turn led to increasing the surface area of absorption and consequently increased digestion and absorption of nutrient.

The positive results of nucleoforce® inclusion could be attributed to the pronounced effects of nucleotides on the growth of intestinal cells and increasing the activity of digestive enzymes, which in turn augments nutrients digestion and absorption (Salah et al., 2019). In addition, nucleotides supplemented exogenously lower the animal's energy requirements because de novo nucleotides synthesis inside the body requires a high cost of energy (Jung and Batal, 2012).

5. CONCLUSION

It could be concluded that dietary nucleoforce® supplementation from zero day till 7 weeks of age improved growth performance and enhanced intestinal histomorphology in broiler chickens. The superlative results were shown in the highest concentration (500 g/ton diet).

CONFLICT OF INTEREST STATEMENT

None of the paper authors has a financial or personal relationship with other people or organizations that could inappropriately influence the content of the current paper.

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