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Effect of dietary nucleotide supplementation on broiler intestinal villi length

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ABSTRACT

The present experiment was performed to investigate the influence of two different products of nucleotides as a feed additive for broiler chicks in terms of improvement of intestinal villi length. A total number of two hundred and seven-one-day-old broiler chicks (Ross 308) were randomly distributed into three different treatment groups (A, B, and C), of three replicates (23 chicks / replicate). The chicks in control group (A) were fed negative control diet, group B were fed diet containing (Nucleoforce®) (0.025%) while group C were fed diet containing (Immune force®) (0.1%). Results showed that, groups fed diet supplemented with nucleotides had expressed significant increase in length of intestinal villi when compared with the control one, although group B that was fed Nucleoforce® showed highest values among the three different groups. It was concluded that, inclusion of nucleotides (Nucleoforce® and Immune-force®) in broiler diets had a positive role in improvement of intestinal villi length.

1. INTRODUCTION

Healthy gut helps birds to gain optimum growth performance because gastrointestinal tract is responsible for digestion, absorption of nutrients and immune response.
Bacterial infection is the most critical factor that impairs both poultry digestive and immune systems. So, antibiotics are used at sub-therapeutic doses in poultry diets to enhance the performance of the birds (Chattopadhyay et al., 2014).
The usage of Antibiotic growth promoters (AGP) in animal diets has been prevented in many countries due to the dangerous effect of these residues on public health which concern the antibiotic-resistant bacteria in animals and human being (Ronquillo and Hernandez., 2017).
It has been necessary to find other substances, which can be used as alternatives to (AGP) in the diets. Those alternatives are supplemented in the diets in order to promote the performance of the animal while conserving environment and animal health (Mehdi et al., 2018). Nucleotides are among alternates to antibiotic-based drugs.
Nucleotides are a series of bioactive agents which have characteristics of low-molecular-weight and also, they are considered as intracellular compounds, which have a serious function in a number of physiological activities of animals (Superchi et al., 2012).
Nucleotide supplementation improves the body weight gain, feed consumption and feed conversion ratio (Rafique et al., 2020). Also, addition of nucleotide increases the absorption of nutrients from the small intestine as a result of increased height of the villi, as well as, the high levels of nucleotide addition are functional in reducing the counts of harmful bacteria in excreta (*Escherichia coli* and *Clostridia perfringens*) (Abd El-Wahab et al., 2019).
Nucleotides supplementation stimulates a faster and stronger immune response to routine vaccines in chickens (Wu et al., 2018). Where, involvement of nucleotide in diets of broiler chickens has achieved more economic profit per kg live weight (Khedr et al., 2020).
The objective of this study was to evaluate the influence of dietary supplementation of nucleotides on the length of intestinal villi.

2. MATERIAL AND METHODS

2.1. Birds, housing and management
The present study was carried out by using 207 one-day old chick (Ross 308) broiler chicks. The chicks reared at suitable environment. The chicks were randomly allocated into 3 groups, each group contain 3 replicates of 23 chicks were housed in clean well-ventilated cages. All groups were maintained under good ventilation and intermittent lightening program (23 hours light: 1 hour dark). The environmental temperature was adjusted according to the age of chicks. Feed and water were offered ad-libitum. All birds were routinely vaccinated against infectious bronchitis (IB), Newcastle and according to the vaccination program following (Younes, 2016) (Table 6). The other prophylactic measures were applied.

2.2. Diets
The chicks were distributed into 3 treatment groups (A, B and C). Group A was considered as negative control group and fed basal diet, group B were fed diet including Nucleoforce® at concentration 0.025% (table, 1) whereas, group C were fed diet containing (Immune-force®) at concentration 0.1% (table, 2) and were formulated to meet the requirements according National Research Council NRC, (1994), as shown in tables (3, 4 and 5)
2.3. Experimental procedure
Chicks had free choice access of feed and water.. Feed consumption was weekly calculated for each treatment. Live

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body weight was measured in grams for all birds at the start of the experiment and weekly.

2.4. Histological observations

At 7, 14, 21, 28 and 35 days of age, two birds of each replicate were randomly dissected to collect samples from jejunum part of small intestine. All samples were fixed in 10% formalin. After fixation, the samples were dehydrated by increasing concentration of alcohol and embedded in paraffin. Four to eight micrometer thick transverse section were cut and stained with hematoxylin and eosin (H&E) (Drury and Wallington, 1967). Villus length was determined in cross section using upright light microscope by using image-analysis software.

2.5. Statistical analysis

Data obtained from the experiment were carried out by software SPSS program, Use one-way ANOVA variance analysis. Results for each group are expressed as Mean ± SEM. Differences between means were tested for significance by using Duncan's Range test (Duncan, SPSS Student Version 10.0.7, June

3. RESULTS

3.1. Length of villi

The effect of different dietary of nucleotides supplementation (Nucleoforce® and Immune force®) and control diets on intestinal villi length of broiler chicks was illustrated in table (7) and figures (1-15). During 1st ,2nd, 3rd ,4th and 5th weeks of the experiment the result showed a significant ($p \leq 0.05$) increase in intestinal villi length of nucleotides supplemented groups when compared with the control, although group B that fed Nucleoforce® showed highest values among the three different groups.

At 1s week, the intestine of the control group displayed normal intestinal villi lined by normal simple columnar epithelium (Fig. 1.1) with normal average length of intestinal villi by image J-analysis was (389 pixel).

Immune force® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.2) with increase length of intestinal villi (709 pixel). Nucleoforce® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.13) with increased length of intestinal villi (822 pixel).

The second week the , Control group intestine displays normal intestinal villi lined by normal simple columnar epithelium (Fig. 1.4) with normal average length of intestinal villi (586 pixel). Immune force® group: Intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.5) with increase length of intestinal villi with average length of villi (771.66 pixel). Nucleoforce® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.6) with increased length of intestinal villi with average length of intestinal villi (918.33 pixel).

Table 1 Chemical composition of Nucleoforce® as feed additive according to (Bioibérica, S.A Company)

Chemical composition	Amount
Crude Protein (CP)	20.34
Protein nitrogen	3.25
Non-Protein nitrogen (from nucleotides)	12.09
Crude fiber (CF)	0.1
Ash	3.38

At 3rd week, Control group displayed normal intestinal villi lined by normal simple columnar epithelium (Fig. 1.7) with normal average length of intestinal villi (750.66 pixel). Immune force® group displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.8) with increase length of intestinal villi with average length (849 pixel). Nucleoforce® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 1.9) with increased length of intestinal villi with average length (970.33 pixel).

Table 2 Chemical composition of Immune force® as feed additive according to(DAEHO CO., LTD Company)

Chemical composition	Amount
Lactobacillus plantrum	2 X 10^{10} CFU
Enterococcus faecium	2 X 10^{10} CFU
Saccharomyces cerevisiae	3 X 10^{10} CFU
Nucleotide (saccharomyces cerevisiae)	10 g
Carrier: Calcium carbonate up to	1 kg

Table 3 Physical and chemical composition (%) of the starter diets (0- 10 days) of the experimental groups

Ingredients	Groups		
	A	B	C
Yellow corn	53.03	53.005	52.93
Soybean meal (46)	35.00	35.00	35.00
Corn gluten meal	4.70	4.70	4.70
Soybean oil	2.40	2.40	2.40
Di calcium phosphate	1.60	1.60	1.60
Limesto	1.50	1.50	1.50
L –Lysine	0.39	0.39	0.39
DL –Methionine	0.33	0.33	0.33
Sodium chloride	0.33	0.33	0.33
Vit. &Min. mixture	0.30	0.30	0.30
Sodium bicarbonate	0.13	0.13	0.13
L –Threonine	0.10	0.10	0.10
Ant-coccidia	0.05	0.05	0.05
Ant-clostridia	0.03	0.03	0.03
Ant-mycotoxin	0.05	0.05	0.05
Energy enzymes	0.03	0.03	0.03
Nucleuforce®	_	0.025	_
Immune force®	_	-	0.10
Emulsifier	0.01	0.01	0.01
Phytase enzyme	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01
Total	100.0‘	100.0‘	100.0
Chemical composition			
ME (Kcal \ Kg diet)	3.001.88	3.001.88	3.001.88
CP	23.02	23.02	23.02
CF	3.56	3.56	3.56
Crude fat	5.03	5.03	5.03
Lysine	1.35	1.35	1.35
Methionine	0.67	0.67	0.67
Methionine dig	0.63	0.63	0.63
Methionine + cysteine	1.02	1.02	1.02
Methionine + cysteine dig	0.92	0.92	0.92
Threonine	0.92	0.92	0.92
Threonine dig	0.79	0.79	0.79
Calcium	1.05	1.05	1.05
Available phosphorus	0.50	0.50	0.50
Sodium	0.18	0.18	0.18
Chloride	0.23	0.23	0.23
Potassium	0.88	0.88	0.88
Pellet quality factor	3.28	3.28	3.28
Acid Base Balance (me/kg)	223.67	223.67	223.67

Table 4 Physical and chemical composition (%) of the grower diets (11 – 24 days) of the experimental groups

Ingredients	Groups		
	A	B	C
Yellow corn	55.51	55.485	55.41
Soybean meal (46)	33.70	33.70	33.70
Corn gluten meal	3.00	3.00	3.00
Soybean oil	3.40	3.40	3.40
Di calcium phosphate	1.33	1.33	1.33
Limestone	1.40	1.40	1.40
L –Lysine	0.31	0.31	0.31
DL –Methionine	0.30	0.30	0.30
Sodium chloride	0.31	0.31	0.31
Vit. &Min. mixture	0.30	0.30	0.30
Sodium bicarbonate	0.12	0.12	0.12
L –Threonine	0.1	0.1	0.1
Ant-coccidia	0.05	0.05	0.05
Ant-clostridia	0.03	0.03	0.03
Ant-mycotoxin	0.05	0.05	0.05
Energy enzymes	0.05	0.05	0.05
Nucleoforce®	_	0.025	_
Immune force®	_	_	0.1
Emulsifier	0.01	0.01	0.01
Phytase enzyme	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01
Total	100.00	100.00	100.00
Chemical composition			
ME (Kcal \ Kg diet)	3.101.78	3.101.78	3.101.78
CP	21.54	21.54	21.54
CF	3.50	3.50	3.50
Crude fat	6.02	6.02	6.02
Lysine	1.25	1.25	1.25
Lysine dig	1.16	1.16	1.16
Methionine	0.62	0.62	0.62
Methionine dig	0.58	0.58	0.58
Methionine + cysteine	0.95	0.95	0.95
Methionine + cysteine dig	0.85	0.85	0.85
Threonine	0.87	0.87	0.87
Threonine dig	0.75	0.75	0.75
Calcium	0.95	0.95	0.95
Available phosphorus	0.45	0.45	0.45
Sodium	0.17	0.17	0.17
Chloride	0.22	0.22	0.22
Potassium	0.85	0.85	0.85
Pellet quality factor	2.88	2.88	2.88
Acid Base Balance (me/kg)	217.11	217.11	217.11

At 4th week, Control group intestine showed normal intestinal villi lined by normal simple columnar epithelium (Fig. 2.10) with normal average length of intestinal villi (634.33 pixel). Immune force® group: Intestine displays normal intestinal villi with normal simple columnar epithelium (Fig. 2.11) with increase length of intestinal villi with average length (744.66 pixel).

Nucleoforce® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 2.12) with increased length of intestinal villi with average length (830 pixel).

At 5th WK, Control group intestine displayed normal intestinal villi lined by normal simple columnar epithelium (Fig. 2.13) with normal average length of intestinal villi (592 pixel). Immune force® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 2.14) with increase length of intestinal villi (717 pixel).

Nucleoforce® group intestine displayed normal intestinal villi with normal simple columnar epithelium (Fig. 2.15) with increased length of intestinal villi (785.66 pixel).

4. DISCUSSION

The obtained results from our experiment was in agreement with Mohamed et al. (2020) who proved that nucleotides supplementation can positively influence the microflora within the alimentary canal due to the results of the improved intestinal morphological picture. Also, Abd El-Wahab et al. (2019) who found that supplementation of dietary yeast nucleotides has increased the absorption of nutrients from the small intestine as a result of increased villus height in Japanese quails. In addition, Wu et al. (2018) who mentioned that chickens fed the diets supplemented with 0.5% yeast nucleotides showed an increase in the height of the intestinal villi and the villus height to crypt depth ratio in the ileum.

Table 5 Physical and chemical composition (%) of the finisher diets (after 24 days) of the experimental groups

Ingredients	Groups		
	A	B	C
Yellow corn	60.68	60.655	60.58
Soybean meal (46)	27.50	27.50	27.50
Corn gluten meal	3.50	3.50	3.50
Soybean oil	4.30	4.30	4.30
Di calcium phosphate	1.23	1.23	1.23
Limestone	1.25	1.25	1.25
L –Lysine	0.29	0.29	0.29
DL –Methionine	0.26	0.26	0.26
Sodium chloride	0.31	0.31	0.31
Vit. &Min. mixture	0.30	0.30	0.30
Sodium bicarbonate	0.13	0.13	0.13
L –Threonine	0.04	0.04	0.04
Ant-coccidia	0.05	0.05	0.05
Ant-clostridia	0.03	0.03	0.03
Ant-mycotoxin	0.05	0.05	0.05
Energy enzymes	0.05	0.05	0.05
Nucleoforce®	-	.025	-
Immune force®	-	-	0.10
Emulsifier	0.01	0.01	0.01
Phytase enzyme	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01
Total	100.00	100.00	100.00
Chemical composition			
ME (Kcal \ Kg diet)	3226.25	3226.25	3226.25
CP	19.51	19.51	19.51
CF	3.17	3.17	3.17
Crude fat	7.02	7.02	7.02
Lysine	1.09	1.09	1.09
Lysine dig	1.01	1.01	1.01
Methionine	0.55	0.55	0.55
Methionine dig	0.52	0.52	0.52
Methionine + cysteine	0.86	0.86	0.86
Methionine + cysteine dig	0.77	0.77	0.77
Threonine	0.73	0.73	0.73
Threonine dig	0.62	0.62	0.62
Calcium	0.85	0.85	0.85
Available phosphorus	0.42	0.42	0.42
Sodium	0.17	0.17	0.17
Chloride	0.22	0.22	0.22
Potassium	0.75	0.75	0.75
Pellet quality factor	2.55	2.55	2.55
Acid Base Balance (me/kg)	188.5	188.5	188.5

pTable 6 Vaccination program of broiler chicks

Age (in days)	Name of vaccine	Type of vaccine	Route of vaccination
1	Hitchner IB	Living vaccine	Via coarse spray
12	Lasota	Living vaccine	Via drinking water
15	Gumboro	Living vaccine (mild strain)	Via drinking water
22	Lasota	Living vaccine	Via drinking water

Table 7 The effect of nucleotides (Nucleoforce®) and (Immune force®) on intestinal villi length

Period/weeks	Group fed Control diet	Group fed diet containing Nucleoforce®	Group fed diet containing Immune force®
1	389 ± 16.25 ^{c}	822 ± 13.31 ^{a}	709 ± 13.07 ^{b}
2	586 ± 19.07 ^{c}	918.33 ± 20.85 ^{a}	771.66 ± 17.62 ^{b}
3	750.66 ± 29.44 ^{c}	970.33 ± 13.98 ^{a}	849 ± 13.00 ^{b}
4	634.33 ± 25.18 ^{c}	830 ± 23.09 ^{a}	744.66 ± 17.52 ^{b}
5	592 ± 12.09 ^{c}	785.66 ± 13.59 ^{a}	717 ± 17.57 ^{b}

Means with different letters at the same raw differ significantly at P ≤ 0.05. Values are means ± standard errors

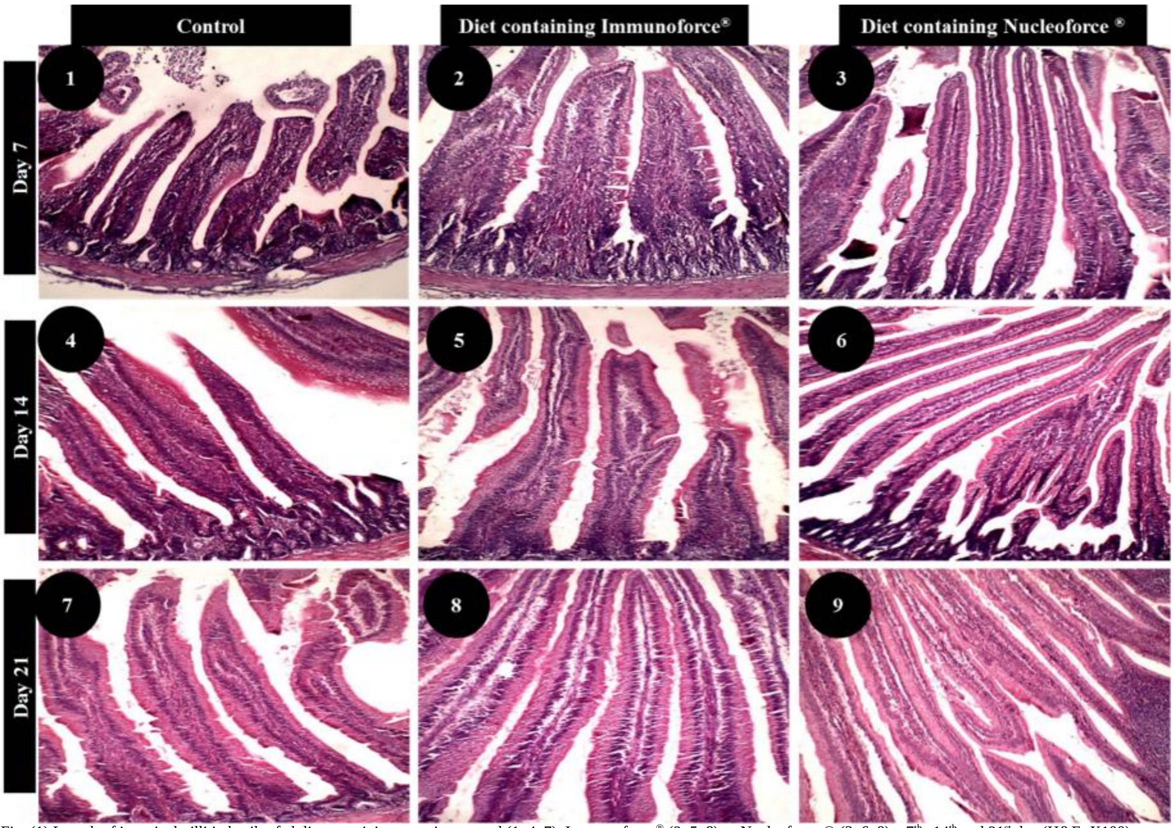

Fig. (1) Length of intestinal villi in broiler fed diet containing negative control (1, 4, 7), Immune force® (2, 5, 8) or Nucleoforce ® (3, 6, 9) at7th , 14th and 21st day (H&E, X100).

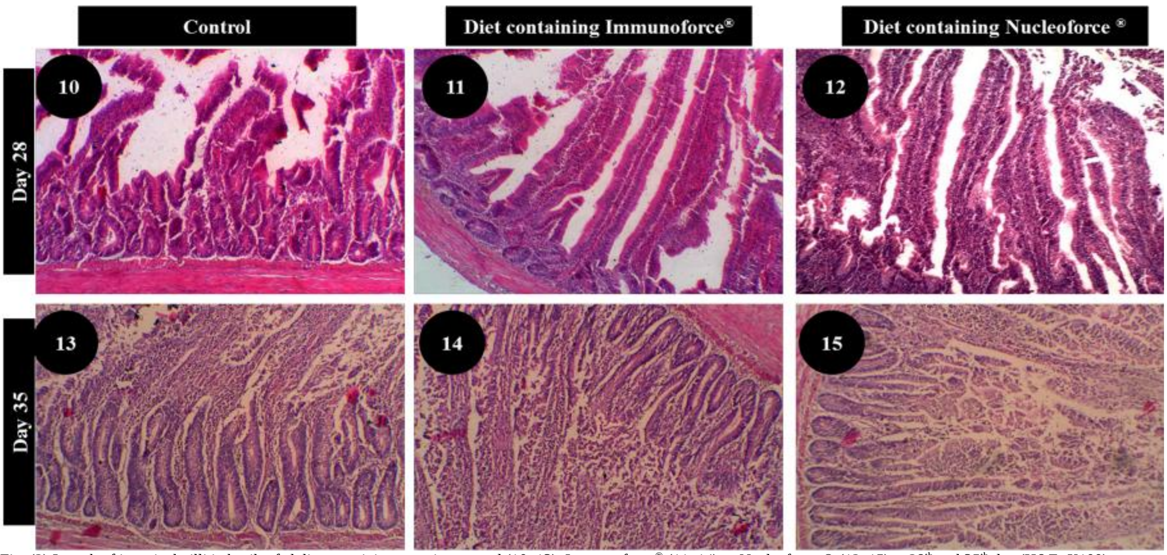

Fig. (2) Length of intestinal villi in broiler fed diet containing negative control (10, 13), Immune force® (11, 14) or Nucleoforce ® (12, 15) at 28th and 35th day (H&E, X100).

Furthermore, Brudnicki et al. (2017) reported that the addition of yeast nucleotides in diets has positively reflected on improving the structure of small intestinal which is important for digestive and absorptive function of small intestine and is exceedingly attached to the morphological changes of small intestinal villus length and crypt depth ratio. Moreover, Pourabedin et al. (2014) found that the addition of dietary yeast in diets of broilers significantly improves the villus height in the duodenum of the treated bird. Also, Luquetti *et al*. (2012) who mentioned that *S. cerevisiae* yeast cell wall (YCW)supplementation in diets of broiler chickens may be a helpful management tool to keep out the integrity of intestinal epithelium of broiler chickens vaccinated against coccidiosis. They added that the intestinal villi of birds supplemented with YCW at rate of (2 g/kg) are higher than control, irrespective of a vaccination against coccidiosis. Furthermore, Jung and Betal, (2012) demonstrated that when dietary nucleotides are supplemented to the feed of broiler chickens, these chickens express a healthier gut indicated by increased villus height and villus height to crypt depth in the intestinal villi. In addition, Swarnkar et al. (2011) reported that health status of gastrointestinal tract was improved as a result of addition of dietary nucleotides. This improvement of gut health was expressed in the form of increasing villous height and crypts‟s depth ratio. Also, Gao et al. (2008) found that addition of yeast nucleotides in diets with doses 2.5 g/kg and 7.5 g/kg has improved the length of the intestinal villi in the duodenum on day 21 and d 42 and so, resulted in a wider surface area of the intestine and higher activities of digestive enzymes, therefore, increased absorption of essential nutrients and improved digestibility. These results were confirmed by the findings of Uauy et al. (1990) who recorded that the involvement of yeast nucleotides in feed has resulted in an increase of the intestinal surface area, mucosal protein, villous height and brush border enzyme activities, consequently, expresses a better assimilation of nutrients and therefore lowers feed to gain ratio.

5. CONCULOSIONS

From obtained results, we can conclude that supplementation of nucleotides is beneficial, because the groups fed diets with Nucleoforce® and Immune force® showed improvement in their length of the intestinal villi when compared with the control one, but the group fed Nucleoforce® showed the best results because its values were higher than those of the group fed Immune force® as well as the negative control group.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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