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EFFECT OF DIETARY SUPPLEMENTATION OF ECHINACEA AND NUCLEOTIDES ON PRODUCTIVE PERFORMANCE, INTESTINAL HISTOMORPHOLOGY AND GENE EXPRESSION OF BROILER CHICKENS

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ABSTRACT

The current study was conducted to evaluate the effects of dietary *Echinacea purpurea* leaves powder, nucleotides single and in combination on growth performance, carcass traits, intestinal morphology, gene expression, biochemical parameters, immune and antioxidant status of broiler chickens. A total number of 108 one-day-old unsexed broiler chicks (Ross 308) were randomly distributed into 4 equal groups each of 27 chicks in 3 replicates (9 chicks per each). The first group (control) was fed a basal diet without additives, while the second group received a diet supplemented with 1.0% Echinacea. The third group fed on a diet containing 0.05% nucleotides and the fourth group received a diet supplemented with a combination of Echinacea 0.5% & nucleotides 0.05%. The results showed that birds in the second and third groups had significantly ($P<0.05$) higher weight gain (2012.60±75.82g & 2005.40±38.19g) and improved feed conversion ratios (1.44±0.05& 1.45±0.02). Dietary supplementation of Echinacea increased the relative weight of the bursa and thymus while decreased the relative weight of the spleen and liver. Addition of nucleotides to broiler diets significantly ($P<0.05$) increased the serum level of total protein, triglycerides and high density lipoproteins (HDL), while decreased cholesterol and low density lipoproteins (LDL). Echinacea addition significantly ($P<0.05$) increased the level of serum total protein and HDL, while decreased cholesterol and LDL. Group 3 showed improvement in the intestinal histomorphology, while group 4 showed degeneration in the jejunal epithelium. Nucleotides upregulated both (IL-10) and (IGF). It could be concluded that the dietary supplementation of Echinacea or nucleotides improved the growth performance, intestinal histomorphology, immunity and antioxidant status.

Keywords: Echinacea, nucleotides, broilers, growth performance.

INTRODUCTION

Poultry industry is exposed to many difficulties, the high prices of feed ingredients is the most important, therefore it is necessary not only to find a cheap source of protein or energy but also to find out additives high in medicinal value as an attempt to dismantle some of those difficulties especially in developing countries (Yadav *et al.*, 2016). Phytogenic compounds, organic acids, antimicrobial peptides, probiotics, and prebiotics widely

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acknowledged as viable alternatives for antibiotics in feed (Randrianarivelo *et al.*, 2010). *Echinacea purpurea* is one of the most used phytogenic compounds in poultry nutrition (Windisch and Kroismayr, 2006) due to its useful anti-inflammatory and immuno-stimulatory properties (Saeed *et al.*, 2018). In addition to its antioxidant, antibacterial and antiviral properties, Echinacea could be used as an alternative to antibiotics growth promoters in broilers feed (Manayi *et al.*, 2015). Echinacea contains a combination of active constituents like alkamides, phenolic complexes, caffeic acid derivatives, flavonoids, and glycoproteins (Erenler *et al.*, 2015). Dietary supplementation of Echinacea in broilers might be improved the feed conversion ratio, changed the number of blood cells and enhanced immunity response, changed the total and differential leucocytic count and antibody titers against avian influenza and Newcastle diseases (Dehkordi and Fallah, 2011).

Nucleic acid (DNA and RNA) building blocks are called nucleotides. They are created when a sugar (ribose or deoxyribose), a nitrogen base (purine or pyrimidine), and one or more phosphate groups are conjugated, (Voet and Voet, 2004). Nucleotides are indispensable for a variety of biological processes within the body; during normal conditions, endogenous sources of nucleotides are thought to be sufficient. In some other conditions such as reduced protein intake, intestinal lesions, rapid development and immune suppression, nucleotides supplementation is also necessary. Nucleotides sources include dietary source, de novo synthesis and the retrieval from salvage pathway (Hess and Greenberge, 2012).

Addition of nucleotides to broilers diet improved weight gain, feed intake and FCR (Kruger & Werf, 2018; Salah *et al.,* 2019; Rafique *et al.,* 2020; Zahran *et al.,* 2020). Addition of nucleotides to the starter diet of broilers could be helpful to improve the economic value for broiler industry (Kocher *et al.*, 2010; Khedr and Ahmed, 2020). Dietary inclusion of nucleotides increased the absorption of nutrients from the small intestine as a result of the increased height of the villi, as well as, the high inclusion rates of nucleotides were functional in reducing the counts of harmful bacteria in excreta (*Escherichia coli* and *Clostridi aperfringens*) (Abd El-Wahab *et al.,* 2019). Nucleotides supplementation stimulated a faster and stronger immune response to routine vaccines in chickens (Wu *et al.,* 2018).

Consequently, the purpose of this study was to investigate the impact of dietary *Echinacea purpurea* leaves powder, nucleotides solo or in a combination on growth performance, serum biochemical markers, intestinal histomorphology, and antioxidant status as well as gene expression of broiler chickens.

MATERIALS AND METHODS

Animal ethics statement

For the care and use of animals, all applicable national and institutional guidelines were followed. All samples were from birds used in experiments approved by the Animal Ethics Committee of Assiut University's Faculty of Veterinary Medicine.

1- Experimental chicks and housing

The current work was carried out at the Applied Nutrition Research Unit (ANRU), Teaching Veterinary Hospital, Faculty of Veterinary Medicine, Assuit University. One-day-old, unsexed broiler chicks totaling 108 birds (Ross 308) were purchased from a local commercial source, weighed, and randomly assigned to 4 equal groups of 27 chicks each in three replicates(9chicks/each). The initial average weight of the experimental chicks was (39.87±1.25g). Birds in all groups were housed in floor pens and kept under the same management system and environmental conditions.

2-Experimental diets and feeding

Experimental mash diets (starter, grower and finisher) were formulated to sustain nutrient requirements of Ross following the nutrition specification of Ross broilers (2019) and illustrated in Table 1. All feed ingredients including *Echinacea purpurea* (SEKEM Company, Egypt) and formulated experimental diets were analyzed according to the Association of Official Analytical Chemists' procedures (AOAC, 2011). Metabolizable energy values were estimated using a bomb calorimeter. The three feeding phases program was followed when feeding birds: starter (0-14 days), grower (15- 28 days) and finisher (29-35 days).

The first group received a basal control diet (D1) with no additives (control group), while the second group was fed on a diet containing 1.0% *Echinacea purpurea* (D2). Chicks in the third group received basal control diet (D1) with 0.05% nucleotides (nucleoforce, yeast extract nucleotides were obtained from Ohly-GmbH Company), while birds in the fourth group fed the third diet (D3) containing a combination of Echinacea 0.5% and nucleotides 0.05%. Throughout the course of the experiment, birds were given unlimited access to fresh water and fed ad libitum on the appropriate diets.

Table 1: Composition and energy value of experimental diet

Item	Starter diets			Grower diets			Finisher diets		
	D1	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**
Physical composition (%)									
Yellow corn, ground	51.42	50.80	51.00	56.11	55.10	55.5	60.15	58.95	59.60
Soybean meal	40.88	40.52	40.79	35.95	35.70	35.8	30.90	30.80	30.85
Sunflower oil	3.82	3.80	3.83	4.36	4.62	4.62	5.65	5.67	5.75
Echinacea leaves powder	…..	1.00	0.50	…..	1.00	0.50	….	1.00	0.50
Nucleotides	…...	….	0.05	…..	….	0.05	….	….	0.05
Mono calcium phosphate	1.39	1.39	1.39	1.26	1.26	1.26	1.15	1.15	1.15
Limestone, ground	1.60	1.60	1.60	1.45	1.45	1.45	1.30	1.03	1.30
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.19	0.19	0.19	0.18	0.18	0.18	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.09	0.09	0.09	0.10	0.10	0.10
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chemical composition (%)									
Crude protein	23.02	23.05	23.01	21.06	21.02	21.02	19.0	19.01	19.02
Calcium	1.00	1.01	1.01	0.90	0.91	1.01	0.82	0.82	0.82
Available Phosphorus	0.47	0.48	0.48	0.41	0.41	0.48	0.37	0.37	0.37
Lysine	1.33	1.36	1.36	1.20	1.20	1.36	1.11	1.11	1.10
Methionine	0.54	0.55	0.55	0.50	0.50	0.55	0.46	0.46	0.45
Energy value:									
ME (kcal/kg diet)	3001.2	3000.6	3000.1	3080	3085	3088	3200	3201	3200

*Each 3kg contains: Vit. A, 12000000 IU; Vit. D3, 4000000 IU; Vit. E, 50000 mg; Vit. k3, 4000 mg; Vit B1, 5000 mg; Vit.B2, 8000mg; Vit. B6, 5000 mg; Vit. B12, 35 mg; Vit. B3, 70000 mg; Selenium, 250 mg; Pantothenic acid , 20000 mg; Folic acid 1000 mg; Biotin, 250 mg ;Manganse 100000 mg; Copper, 15000 mg; Iron, 50000 mg; Zinc 50000 mg; Cobalt,250mg ; Idoine,1500mg.(Universel Animal Care Company).

3- Performance parameters

Weekly records of performance parameters such as feed intake and live body weight were kept, and during the entire trial period, feed conversion ratios and body weight gain were computed.

4- Carcass Traits and intestinal histomorphology

At the end of the experimental period, three birds were randomly taken from each group weighed and slaughtered to complete bleeding after a night fasting. The dressed carcass weight, which is the total weight of the slaughtered birds after the removal of their feathers, heads, feet, and viscera but before any edible offal, was recorded. Some internal organs (liver, gizzard, and heart) and immunological organs (bursa, spleen, and thymus) had their absolute and relative weights measured and estimated. For the light microscopically examination, small specimens from the jejunum of the slaughtered birds of different experimental groups were taken and fixed in 10 % neutral buffered formalin. The fixed specimens were dehydrated in ascending grades of alcohol, cleared by methyl benzoate, embedded in paraffin wax and sectioned at 3-5 µm thick. The prepared sections were stained with the Harris Hematoxylin and Eosin stain (Bancroft *et al.,* 2013).

5- Blood sampling and serum parameters estimation

At the end of the experimental period, three randomly chosen birds from each group (one from each replicate) were selected for blood samples collection. Wing vein blood samples were collected in non-heparinized tubes. Centrifugation at 3000 rpm for 10 minutes was used to separate the serum, which was then kept at -18°C until further testing. Using a spectrophotometer and commercial test kits, serum samples were examined for the estimate of total protein and its fractions (albumin and globulin), triglycerides, cholesterol, LDL, HDL, malondialdehyde (MDA), glutathione peroxidase (GPX), and superoxide dismutase (SOD) (Spectrum, Cairo, Egypt).

6- Assessment of gene expression

Liver and spleen of three slaughtered broilers were taken for growth (IGF) and immune (IL-10) related gene expression. 200 mg of the pool chickens' preserved organs (liver and spleen) of the different experimental groups were used for total RNA extraction using the RNA mini extraction kit according to the applied biotechnology, following the manufacturer`s protocol. The RNA concentration and purity were determined by optical density at 260 nm and ratio optical density at 260/280 nm respectively using a nanophotometer and RNA samples were stored at -80°C until used.

RNA sample (1 µg) was reverse transcribed using the H-minus cDNA synthesis kit according to the manufacturer’s instructions. The complementary DNA was synthesized in 20 µl reaction end volume by using H minus mmlv (200unit/ 0.5 µl), 5x RT buffer (4 µl), dNTPs (2 µl) and gene-specific primer (2 µl) then the mixture was completed to 20 µl using diethylepyrocarbonate treated water. For random hexamer primed synthesis, the mixture was incubated for 5 min at 25°C, then for 60 min at 42°C, and finally for 5 min at 70°C to stop the process. The cDNA produced by the reverse transcription procedure is kept at -20°C for later use. Using Gene bank database sequences from the National Center for Biotechnology Information (Bethesda, MD), Primers sequences for β-actin (housekeeping gene), interleukin-10 (IL-10) and insulin-like growth factor (IGF) were designed, corresponding to each quantified gene Quantitative real-time (qRT) PCR was performed using the Step One Thermo Cycler on 48-well plate with 10 µL of total reaction volume as described by (Pfaffl and Hageleit, 2001).

7- Statistical analysis

Using SPSS 16 statistical software (SPSS, 2001, Inc., Chicago, IL, USA), all data have been analyzed using one-way analysis of variances (ANOVA) followed by Duncan'stest, www.spss.com.

Table ٢: Primers used in the current study.

Target gene		Oligonucleotide sequence (5' - 3')	Amplicon size (bp)	Accession number
IL-10	F	AGC AGA TCA AGG AGA CGT TC	103	AJ621614
	R	ATC AGC AGG TAC TCC TCG AT		
1GF-11	F	GGC GGC AGG CAC CAT CA	215	JN942579
	R	CCC GGC AAA AAG TTC AAG		
β-actin	F	CAA CAC AGT GCT GTC TGG TGG TA	205	X00182
	R	ATC GTA CTC CTG CTT GCT GAT CC		

RESULTS

1- Growth performance

Significantly (P<0.05) increased weight gain in *Echinacea purpurea* and nucleotides supplemented groups, while significantly decreased (P<0.05) in the fourth group supplemented with 0.5 % Echinacea and 0.05% nucleotides as shown in Table (3). The control group recorded the highest feed consumption (P<0.05) during different experimental period intervals. Dietary supplementation of Echinacea, nucleotides and their combination significantly improved (P<0.05) the feed conversion ratios (Table 3).

Table 3: Growth performance parameters of broilers during the whole experimental period.

Group / Item	G1 (control)	G2 Echinacea (1.0%)	G3 Nucleotides (0.05%)	G4 Echinacea (1.0%)+ Nucleotides (0.05%)
Initial body weight (g/chick)	39.70±*1.25	39.94±1.25	39.50±1.29	40.33±1.19
Live body weight (0-35 day)	1988.0^{b}±55.71	2052.5^{a}±76.00	2044.9^{a}±38.44	1795.7^{c}±52.51
Weight gain(0-35 day)	1948.2^{b}±55.47	2012.6^{a}±75.82	2005.4^{a}±38.19	1755.4^{c}±52.35
Feed consumption (0-35 day)	3163.0^{a}±55.48	2909.0^{b}±61.56	2912.2^{b}±67.55	2764.8^{c}±55.85
Feed conversion (0-35 day)	1.66^{a}±0.06	1.44^{c}±0.05	1.45^{c}±0.02	1.57^{b}±0.04

[*] Means in the same row having the same superscripts are not significantly different (P<0.05).

2- Carcass traits

Addition of *Echinacea purpurea* had no significant effect (P<0.05) on hot carcass%, eviscerated carcass% and dressed carcass%, while increased the relative weight of bursa and thymus. Supplementation of nucleotides did not have a significant (P<0.05) effect on carcass yield but increased the relative weight of bursa. The combination in group 4 did not have any significant effect (P<0.05) on hot carcass%, eviscerated carcass%, dressed carcass% and the relative weight of bursa (Table 4).

Table 4: Carcass traits and relative weight of immune organs of broilers fed different experimental diets

Item \ Group	G1 (control)	G2 Echinacea (1.0%)	G3 Nucleotides (0.05%)	G4 Echinacea (1.0%)+ Nucleotides (0.05%)
Pre-slaughter Wt. (g)	2035.0^{a}±*30.4	2063.3^{a}±39.3	2063.3^{a}±46.9	1813.3^{b}±24.0
Hot carcass%	86.8^{a}±0.50	87.8^{a}±0.35	87.4^{a}±0.33	85.9^{a}±0.56
Eviscerated carcass%	70.7^{a}±0.15	72.9^{a}±0.39	71.7^{a}±1.33	70.9^{a}±1.08
Dressed carcass%	75.8^{a}±0.66	77.1^{a}±0.34	76.0^{a}±1.17	75.9^{a}±1.1
Liver%	2.0^{a}±0.09	1.87^{ab}±0.11	1.94^{ab}±0.08	2.02^{a}±0.06
Heart%	0.45^{a}±0.03	0.43^{ab}±0.02	0. 46^{a}±0.04	0.43^{ab}±0.02
Gizzard%	1.37^{b}±0.04	1.38^{b}±0.01	1.37^{b}±0.02	1.42^{a}±0.10
Spleen %	0.12^{a}±0.05	0.11^{b}±0.01	0.10^{b}±0.02	0.11^{b}±0.04
Thymus%	0.43^{b}±0.03	0.48^{a}±0.02	0.45^{b}±0.05	0.38^{c}±0.03
Bursa%	0.11^{c}±0.01	0.15^{b}±0.01	0.17^{a}±0.02	0.12^{c}±0.02

*Means in the same row having the same superscripts are not significantly different (P<0.05).

Table 5: Serum biochemical parameters and antioxidant status of broilers fed different experimental diets

Item \ Group	G1 (control)	G2 Echinacea (1.0%)	G3 Nucleotides (0.05%)	G4 Echinacea (1.0%) + Nucleotides (0.05%)
Total protein (g/dl)	3.15^{c}±*0.03	3.27^{b}±0.07	3.50^{a}±0.02	3.10^{c}±0.02
Albumin (g/dl)	1.70^{b}±0.06	2.05^{a}±0.26	1.45^{c}±0.03	1.85^{ab}±0.03
Globulin (g/dl)	1.45^{b}±0.03	1.22^{c}±0.2	2.02^{a}±0.02	1.25^{c}±0.03
A/G	1.17^{b}±0.06	1.80^{a}±0.41	0.72^{c}±0.02	1.48^{ab}±0.06
Cholesterol (mg/dl)	152.63^{b}±11.75	150.05^{b}±18.00	124.60^{c}±2.19	151.35^{b}±11.05
Triglycerides (mg/dl)	92.10^{b}±1.96	93.30^{b}±0.35	96.45^{a}±0.09	89.15^{c}±0.82
LDL (mg/dl)	106.90^{a}±8.14	73.80^{c}±1.61	71.90^{c}±3.92	95.30^{b}±9.35
HDL (mg/dl)	27.40^{c}±3.29	34.55^{b}±3.15	33.40^{b}±6.12	38.20^{a}±1.33
MDA (Nmol/ml)	10.50^{a}±0.46	8.50^{b}±0.12	7.45^{b}±0.14	6.00^{c}±0.12
GPX (mU/ml)	393.90^{c}±8.42	396.30^{c}±8.60	466.90^{a}±16.86	451.50^{b}±20.09
SOD (U/ml)	18.55^{c}±1.53	20.90^{c}±1.44	26.50^{b}±0.75	39.70^{a}±0.29

*Means in the same row having the same superscripts are not significantly different (P<0.05).

3- Serum biochemical parameters and antioxidant status

Addition of nucleotides to broiler diets significantly increased (P<0.05) total protein, globulin, triglycerides and high density lipoproteins (HDL) level, and decreased serum cholesterol and low density lipoproteins (LDL) level. *Echinacea purpurea* supplementation significantly increased (P<0.05) total protein, albumin, A/G ratio and HDL level, while decreased serum cholesterol and LDL. However, a combination between Echinacea and nucleotides had no significant effects on total protein, albumin, A/G ratio and cholesterol level. Inclusion of Echinacea, nucleotides or their combination in broiler diets decreased significantly (P<0.05) MDA level. Addition of nucleotides significantly increased (P<0.05) GPX and SOD enzymes level, while *Echinacea purpurea* had no significant effect. Supplementation a mixture of Echinacea and nucleotides significantly (P<0.05) increased GPX and SOD level. (Table 5).

4- Growth and immune-related genes

Figure (1) showed the fold change of immune-related gene (interleukin-10) in different experimental groups. Addition of Echinacea or nucleotides to broiler diets in the second and third groups significantly (P<0.05) upregulated IL-10 expression in spleen tissue, while downregulated the gene expression in the fourth group. The fold change of insulin-like growth factor (IGF) in figure (2) showed that the addition of nucleotides significantly (P<0.05) upregulated (IGF) expression in liver tissue. *Echinacea purprea* did not have a significant effect on the growth gene expression, while the combination of Echinacea with nucleotides in the fourth group caused down-regulation (P<0.05) of the growth-related gene.

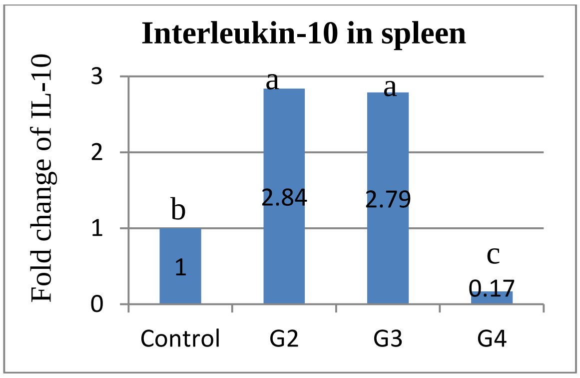

Figure 1: Fold change expression of interleukin-10 (IL-10) in broilers spleen

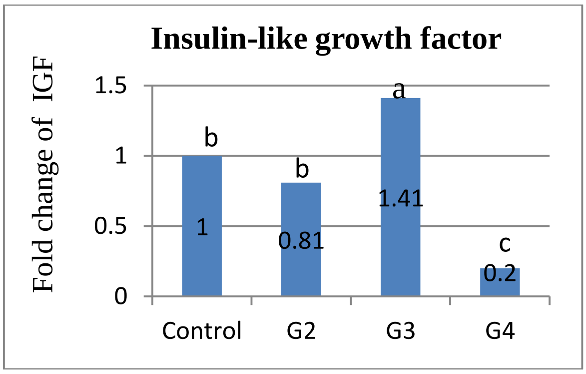

Figure 2: Fold change expression of insulin-like growth factor (IGF) in broiler's liver

5- Intestinal histomorphology

The control group showed normal jejunum histomorphology consisting of four layers namely; mucosa, submucosa, musculosa and serosa. The mucosa of the jejunum is thrown into a finger-like villi (V) that consisted of a lamina propria of loose connective tissue (CT) supporting the mucosal membrane (figure 3).

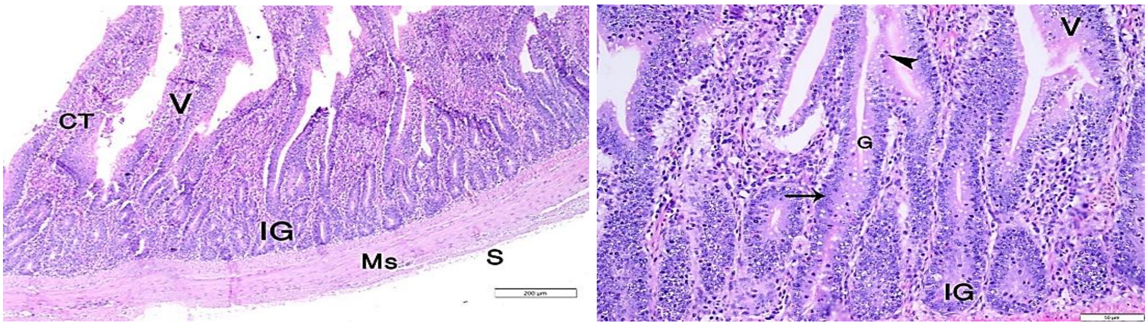

Fig.3: H&E stained sections showed the jejunal mucosaof broilers in first group (control).

Jejunum in *Echinacea purpurea* supplemented group did not show differences compared to the control one. However, it was noticeable that the number of goblet cells (G) and lymphocytes (Ly) increased in comparison to the control. In addition the intestinal gland (IG) became longer and more coiled. The thickness of muscular layer increased compared to the control groups (figure 4).

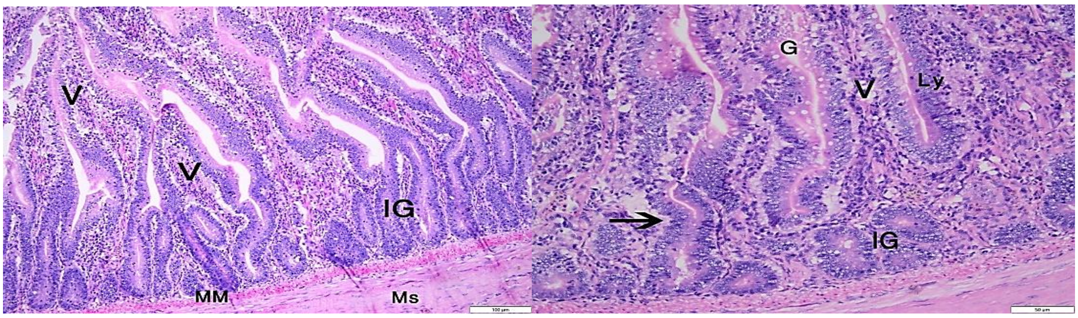

Fig.4: H&E sections showed the jejunal mucosa of broilers in the second group supplemented with *EP*.

Nucleotides supplemented group had the best histomorphological changes. The length of the intestinal villi (V) and the intestinal gland (IG) increased obviously compared to the control group. The thickness of the muscular layer and the number of goblet (G) cells increased compared to the control. The intracellular lymphocytes increased markedly in this group which means that nucleotides enhance immunity (Figure5).

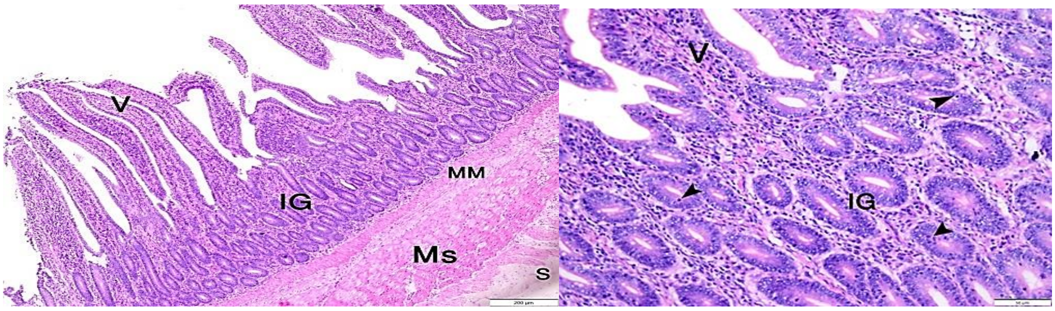

Fig.5: H&E sections showed jejunal mucosa of broilers in the third group supplemented with nucleotides

The fourth group fed a diet supplemented with Echinacea and nucleotides showed a degeneration and desquamation in the epithelium lining the villi (waved arrows). The infiltration of lymphocytes (waved arrow) was more obvious in this group. A small number of the intestinal villi showing normal arrangement, however, the number of the goblet cells (G) and lymphocytes increased markedly in these villi. The intestinal gland showed normal organization (IG) with large number of lymphocytes (arrowheads). The thickness of the muscular layer increased in this group (figure 6).

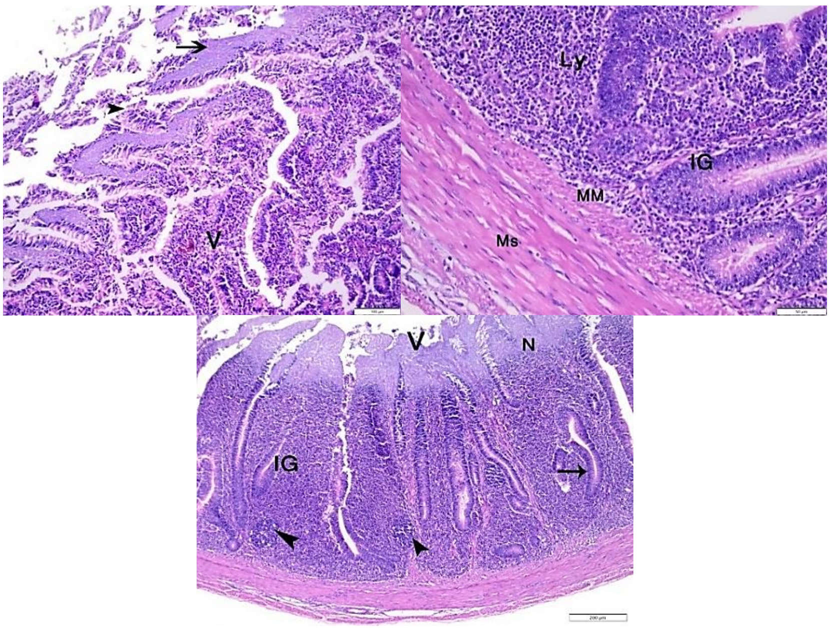

Figure 6: H&E sections showed the jejunal mucosa of broilers in the fourth group supplemented with *Echinacea purpurea* and nucleotides

DISCUSSION

1- Growth performance

Results of growth performance parameters including body weight gain and feed conversion ratios are supported by the finding of Kruger and Werf (2018), Salah *et al.* (2019), Mohamed *et al.* (2020) and Kamel *et al.* (2021) who found that dietary supplementation of nucleotides improved weight gain and FCR. The positive effect of nucleotides in the current study is mainly due to its amelioration effect of intestinal histomorphology by the increased length of intestinal villi and increased number of intestinal glands also increased thickness of the muscular layer. Addition of *Echinacea purpurea* improved broiler's body weight gain, this finding was in agreement with Hashem *et al.* (2020), Shen *et al.* (2020) and Bagno *et al.* (2021). On the other hand, Nasir and Grashorn (2010), Lee *et al.* (2012) and Nosrati *et al.* (2017) found that supplementation of Echinacea did not have a significant effect on growth performance parameters. The adverse effect of the addition mixture of *Echinacea purpurea* and nucleotides in the fourth group may be due to the low inclusion level (0.5%) of Echinacea and also downregulation of the IL-10 expression which led to increased immune destruction of host tissues in case of inflammatory reaction and that explain the excessive necrosis and degeneration of intestinal epithelium.

Feed intake of broilers is confirmed by Kamel *et al.* (2021) who recorded that nucleotides supplementation decreased broilers' feed intake and Nasir (2009) who reported that addition of *Echinacea purpurea* decreased the feed intake of broilers. Values of feed conversion ratios were in line with Kruger and Werf (2018) and Salah *et al.* (2019) who demonstrated that nucleotides improved feed conversion ratios. Numerous studies supported the theory that the beneficial outcomes were brought about by nucleotides' exceptional capacity for promoting the division of intestinal cells, which led to enhanced digestion and nutritional absorption. On the contrary, Kamel *et al.* (2021) recorded that the addition of nucleotides to broiler diets increased feed conversion ratios. *Echinacea purpurea* supplementation improved feed conversion ratios of broilers, this result is supported by the finding of Dehkordi and Fallah (2011), Lee *et al.* (2012) and Bagno *et al.* (2021). Nosrati *et al.* (2017) stated that *Echinacea purpurea* did not have any effect on FCR. The strengthening of the digestive system's functioning was achieved by the phytogenic substances, which increased the feed conversion ratio (Przybilla and Weiss, 1998).

2- Carcass traits

Data on carcass traits matched the findings of Nosrati *et al.* (2017), Bagno *et al.* (2021) and Pourasghar *et al.* (2021) whore ported that *Echinacea purpurea* had no effect on the carcass yield percentage or relative weight of some internal organs. Shen *et al.* (2020) found that *Echinacea purpurea* had a positive effect on slaughter performance which was observed with the low level of inclusion than the high level. Nucleotides supplementation did not affect carcass yield % or relative weight of internal organs and this result was supported by the finding of Pelícia *et al.* (2010). Addition of *Echinacea purpurea* to broiler diets showed a significant increase in the relative weights of the bursa. Our results were supported by the results of Dehkordi and Fallah (2011), Rahimi *et al.* (2011) and Salah *et al.* (2019) who reported that the inclusion of nucleotides in broilers diets significantly increased the relative weights of the bursa. Many studies have confirmed that the absorption of an exogenous source of nucleotides in the intestine and then emigrate to immune organs as bursa, subsequently, nucleotides enhanced the immunity of broilers (Hess and Greenberg, 2012).

3- Serum biochemical parameters

Dietary supplementation of *Echinacea purpurea* increased the serum total protein of broilers, this results is in line with Gilani *et al.* (2018) who found that phytobiotics numerically increased serum total protein. Our results of lipid profile were supported by the findings of Rahimi *et al.* (2011) and Nosrati *et al.* (2017) who stated that *Echinacea purpurea* supplementation significantly increased serum level of HDL and decreased cholesterol and LDL. This may be due to the anti-atherosclerotic properties of phytobiotics (Sharifi *et al.*, 2013). Addition of nucleotides significantly increased serum total protein level, this result is in agreement with the findings of Gopi *et al.* (2020), who found that nucleotides significantly decreased cholesterol and LDL, while increased serum level of HDL in broiler chickens. These results were supported by the findings of Kannan *et al.* (2005) and Rafique *et al.* (2020) who reported that the addition of yeast-derived nucleotides reduced serum level of cholesterol because yeast could participate to control serum cholesterol level by de-conjugation of bile acids.

4- Antioxidant status

Results of enzymatic antioxidant activity supported by Abdel-wahab *et al.* (2019) who reported that yeast supplementation (as a nucleotides source) in quails increased serum SOD while Lee *et al.* (2012) found that addition of *Echinacea purpurea* increased the level of SOD. Malondialdehyde (MDA) was significantly decreased in Echinacea and nucleotides supplemented groups. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells, if free radicals increase leads to over production of MDA (Gaweł *et al.*, 2004). Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) are the most important antioxidant enzymes. GPX enzyme in the cell is the primary hydrogen peroxide scavenging enzyme which mutates hydrogen peroxide to water, SOD enzyme is accountable for the removal of superoxide radicals and stimulates the dismutation of hydrogen peroxide and molecular oxygen (Guruprasad *et al.*, 2012). Addition of Echinacea, nucleotides single and in a combination enhanced the antioxidant capacity of broilers.

5- Gene expression

A cytokine with strong anti-inflammatory effects is interleukin 10 (IL-10) which plays an essential role in preventing inflammatory and autoimmune pathologies, thereby preventing the tissue damage (Iyer *et al.*, 2012). Our finding is commensurate with the results of Kamel *et al.* (2021) who reported that nucleotides supplementation increased the expression of (IL-2 and INF-γ) genes, while Alizadeh *et al.* (2016) and Leung (2018) found that dietary addition of nucleotides caused down-regulation of (IL-10). The protein encoded by (IGF) gene is like insulin in function and structure also this protein is one of a family of proteins included in mediating growth and development. Results in the second and fourth groups were in agreement with the finding of Gilani *et al.* (2021) who found that the addition of phytobiotics to broiler diets downregulated ghrelin (ghrelin hormone stimulates brain structures having growth hormone receptors).

6- Intestinal histomorphology

Echinacea purpurea supplementation in the second group showed an improvement in intestinal morphology which was supported by the findings of Basit *et al.* (2020) and Gilani *et al.* (2021) who reported that the dietary supplementation of phytobiotics in broiler diets improved gut morphology. As well as results of nucleotides supplemented group are in accordance with the findings of Alizadeh *et al.* (2016), Leung (2018), Wu *et al.* (2018), Khedr and Ahmed (2020) and Kamel *et al.* (2021) who demonstrated that nucleotides improved broilers intestinal morphology. Jejunal histomorphological examination showed degeneration and necrosis in the fourth group, and this finding was in line with Gurbuz *et al.* (2010) who reported that the jejunal villi height and width as well as the muscular layer thickness was lower in cichoric-acid-fed group than the control. The difference in the current study may be due to the variable levels of chicoric acid and other active constituents among different types of phytobiotics.

CONCLUSION

The addition of nucleotides (0.05%) or *Echinacea purpurea* (1.0%) to broilers' diets had a beneficial effect on growth performance, gene expression, immune and antioxidant status, as well as an obvious improvement in intestinal histomorphology of broiler chickens. Feeding broilers on diet supplemented with a combination of Echinacea and nucleotides had an adverse effect on growth performance and intestinal histomorphology.

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أثر تغذية الاشينيسيا والنيوكليوتيدات على الأداء الإنتاجي و التركيب النسيجي للأمعاء والتعبير الجيني في الدجاج اللاحم

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أجريت هذه الدراسة لتقييم تأثير تغذية مسحوق أوراق الاشينيسيا بروبريا والنيوكليوتيدات منفردة أو مجتمعة على كفاءة الأداء الإنتاجي وصفات الذبيحة والتركيب النسيجي للأمعاء والتعبير الجيني والقياسات البيوكيميائية والحالة المناعية ونشاط إنزيمات الأكسدة في دجاج التسمين. تمت الدراسة على عدد ١٠٨ كتوت (روس ٣٠٨) غير مجنس عمر يوم وتم توزيعها عشوائيا على أربعة مجموعات متساوية (٢٧ كتكوت لكل مجموعة) في ثلاثة مكررات بكل منها ٩ كتاكيت. غذيت الكتاكيت في المجموعة الأولى على العليقة الضابطة الأساسية (العليقة الأولى) بدون أي إضافات غذائية, بينما الطيور في المجموعة الثانية غذيت على العليقة الثانية والتي تحوي على ١ % من مسحوق أوراق الاشينيسيا بروبريا. الطيور في المجموعة الثالثة غذيت على العليقة الأساسية الضابطة (العليقة الأولى) مع إضافة ٠,٠٥% نيكليوتيدات (نيكليوفورس) في حين ان الطيور في المجموعة الرابعة غذيت على عليقة الثالثة والتي تحتوي على خليط من الاشينيسيا (٠,٥%) و النيكليوفورس (٠,٠٥%) .
وتم تسجيل النتائج المطلوبة وقد أظهرت هذه النتائج أن هناك زيادة معنوية في وزن الجسم (٢٠١٢,٦٠ ± ٧٥,٨٢ جم و ٢٠٠٥,٤٠ ± ٣٨,١٩ جم) وتحسن في نسب التحويل الغذائي (١,٤٤ ± ٠,٠٥ & ١,٤٥ ± ٠,٠٢) مقارنة بالمجموعة الضابطة و المجموعة الرابعة. إضافة مسحوق الاشينيسيا أدى إلى زيادة الوزن النسبي للجراب والغدة الزعترية بينما انخفض الوزن النسبي للطحال والكبد في حين ان إضافة النيوكليوتيدات إلى علائق بدارى التسمين أدى إلى زيادة معنوية في مستوى البروتين الكلي والدهون الثلاثية والبروتينات الدهنية عالية الكثافة في مصل الدم ، بينما انخفضت نسبة الكوليسترول والبروتينات الدهنية منخفضة الكثافة. أدت إضافة إيشينسيا بروبريا إلى زيادة معنوية في البروتين الكلي لمصل الدم والبروتينات الدهنية عالية الكثافة وانخفاض نسبة الكوليسترول والبروتينات الدهنية منخفضة الكثافة. انخفض MDA معنويا في جميع المجموعات المعالجة مقارنة مع المجموعة الضابطة ولكن زادت إنزيمات GPX و SOD بشكل ملحوظ في المجموعتين الثالثة والرابعة.
أظهرت المجموعتين الثانية والثالثة تحسنا ملحوظا في التركيب النسيجي للأمعاء ، بينما أظهرت المجموعة الرابعة تنكرس ونخر وتأكل في التركيب النسيجي للأمعاء.
أدت إضافة النيوكليوتيدات إلى علائق الدجاج اللاحم إلى تنظيم كل من جين الإنترلوكين-١٠ وجين عامل النمو الشبيه بالأنسولين.
خلصت هذه الدراسة إلى ان إضافة نبات القنفذية (الايشينسيا) أو النيوكليوتيدات الى علائق الدجاج اللاحم حسن بشكل ملحوظ الأداء الإنتاجي والمناعة ونشاط إنزيمات الاكسدة واثر بشكل ايجابي على التركيب النسيجي للأمعاء في حين ان التركيب النسيجي للطيور التي غذيت على مزيج من الإيشينسيا بوربوريا والنيوكليوتيدات اظهر تنخر وتنكرس وتأكل في الأمعاء بالإضافة إلى ضعف الأداء الإنتاجي للدجاج.