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EFFECT OF SUPPLEMENTING TWO LEVELS OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.) POWDER IN BROILER DIETS ON HISTOLOGICAL PARAMETERS OF SMALL INTESTINAL SEGMENTS

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Abstract

The study was conducted to determine the broiler intestine effectiveness by supplementing two levels of Jerusalem artichoke (*Helianthus tuberosus* L.) powder in the diets. A total of 210 one-day-old broiler chicks were allocated to seven treatments, with three replicates per treatment and 30 birds per replicate. Control treatment was feed on stander diets and Jerusalem artichoke (JA) was supplemented as following: 2.5% (T2) and 3.5% (T3) in starter diet. 2.5% (T4) and 3.5% (T5) in grower diet. Also, 2.5% (T6) and 3.5% (T7) in finisher diet. Six birds per treatment at the marketing age were isolated based on the mean treatment weight and euthanized to perform the necropsy. The birds were slaughtered and the duodenum, jejunum and ilium cross sections were measured by an ocular micrometer after stained by Haematoxylin and Eosin stain. The improvement percentage in the duodenum wall parameters were 7-17% in villi length, 3-11% in crypt depth and 4-12% in the total wall thickness. While in jejunum the improvement percentages were 2-24% in villi length, 6-19% in crypt depth and 1-18% in the total wall thickness. Similarly, in ilium the improvement percentages were, 1-33% in villi length, 3-30% in crypt depth and 2-23% in the total wall thickness. It can be conclude that JA cased significant ($p<0.05$) improvement in the histological parameters of the broiler intestine and beset results were reported in T2, T3 in duodenum, T3, T4, T5 in jejunum, T2, T3 in Ilium.

Key words: Jerusalem artichoke, Inulin, *Helianthus tuberosus* L.

Introduction

The *Helianthus tuberosus* L., (Jerusalem artichoke) is an erect, rhizomatous perennial herb, up to 3-4 m height. Though perennial, it is mainly grown as an annual and is considered as one of the prebiotic sources (Snel *et al.*, 2002). The dominant prebiotics are fructo-oligosaccharide products (FOS, oligofructose, inulin) (Patterson and Burkholder, 2003); gluco- oligosaccharides, stachyose, malto-oligosaccharides, and oligochitosan, and have also been investigated in the broiler chickens (Zhan *et al.*, 2003; Gao and Shan, 2004; Jiang *et al.*, 2006; Huang *et al.*, 2007). Inulin belonged to a class of fructose-based, highly soluble polysaccharides collectively called fructans. Fructans are the major non-structural carbohydrates in many plant species, particularly in the prevalent and evolutionarily advanced orders of Asterales, Liliales and Poales eg. chicory, onions, wheat (Hendry, 1993).

Small intestine consists of duodenum, jejunum and ileum with no demarcation on gross observation between duodenum and jejunum. While, Mickel diver- ticulum is often used as a landmark to separate the jejunum and ileum (Dorman and Deans, 2000; Cervantes, 2006; Jawad *et al.*, 2015). Furthermore, intestinal wall histologically consist of four layers: mucosa, sub- mucosa, muscularis and serosa. Mucosal layer of small intestine forms villi which project into the lumen and greatly increase the overall absorption surface area of the organ. The surface epithelium of the villi is small columnar epithelium with numerous goblet cells. Intestinal absorptive cells have

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extensive microvilli on its apical surface. Goblet cells are scattered between the absorptive cells and produce mucous. Intestinal glands (Crypts of lieberkuhn) extend from the base of the villi into the underlying lamina properia. Undifferentiated epithelial cells located in the glands divide and migrate up to renew the glandular and surface epithelium every 24-48 h. Acidophilic granular cells (paneth cells) are present in the epithelium at the base of the gland, these cells produce peptidase and lysozyme and may be phagocytic. Enteroendocrine cells are also present in the epithelium of the intestinal gland. Tunica sub mucosa is very thin in chicken with the absence of bronner gland, tunica muscularis is characterized by two layers of smooth muscle, the inner layer of circular muscle fibers are surrounded by an outer of longitudinal folds. Myentrice plexus are often present between muscle layers. A typical tunica serosa lies outside the tunica muscularis as the outermost layer of the organ (Elizabeth and Fredric, 2001; Jawad *et al.*, 2016; Naji *et al.*, 2016; Naji *et al.*, 2017; Hasan *et al.*, 2017). The current study was aimed to elucidate the effect of *H. tuberosus* on broiler production performance. In a previous reports, it was hypothesized that the mechanism resulted in an improvement in the body performance of the chicken fed on diet supplemented by *H. tuberosus* (Jawad *et al.*, 2017a, b) due to an increase in the level of benefit bacteria in the gastro-intestinal trunk.

Materials and methods

Chicken husbandry and experimental design

The farm experiment was conducted in animal house number 14, Agricultural Research Station, Poultry Department, Baghdad, Abi-Gharib, for the period from 22th September to 2nd November 2019 for a period of 42 days to see the effect of adding the almaze powder at the level 2.5 and 3.5% to the broiler feed (the starter, Growth and ultimate) on productive performance, carcass traits and histological and microbial characteristics of the intestine. This study consists of 210 Day-Old broiler (Ross 308), floor bred. They were randomly assigned to seven treatment groups by 30 birds/ treatment, and each treatment consisted of three replicates of 10 birds/ replicate. Feed was prepared and offered ad libitum the same diets (1–10 days: starter; 11-24 days: grower; 24 day-slaughter: finisher) (Table 1) with continuous water. Furthermore, constant lighting and continuous ventilation were provided. All birds were kept under uniform management conditions throughout the experimental period of six weeks. About 60 kg of JA were purchased from the market and processed by slicing, drying and grinding. JA powder was supplemented at levels 2.5%, 3.5% to the starter diet of T2, T3 and to the grower diet of T4, T5 and to the finisher diet of T6, T7 respectively. Its worthy to mention that chicken in T1 fed on dry food without supplementation.

Table 1: Composition of the basal diet

S. No	Items (%)	Basal diet		
		1 to 10 d	11 to 24 d	24 to 42 d
1	Corn	47.52	50.89	54.84
2	Wheat	10	10	10
3	Soybean meal (44%)	32	28	24
4	Protein concentrated	5	5	5
5	Sunflower oil	3	4.15	4.3
6	Limestone	1.2	1.1	1.1
7	Dicalcium phosphate	0.7	0.5	0.4
8	Salt	0.1	0.1	0.1
9	Methionine	0.24	0.13	0.13
10	Lysine	0.24	0.13	0.13
	Total	**100**	**100**	**100**
Calculated analysis				
1	Crude protein (%)	22.5	19.33	19.4
2	Metabolism energy (kilo calorie per kg. diet)	3060	3227	3227
3	Crude fiber (%)	2.70	2.55	2.55
4	Methionine + Cysteine (%)	1.10	0.90	0.90
5	Lysine (%)	2.70	1.18	1.18
6	Calcium (%)	0.97	0.84	0.844
7	Phosphorus (%)	0.48	0.48	0.41

* according to NRC (1977)

Histometrical assay

Six, randomly selected, birds per treatment (two bird/ replicate) at the marketing age were slaughtered and small intestinal organs were collected. It is worthy to mention that selected birds were within the average treatments weight. A 1 cm segment from the midpoint of the duodenum, jejunum and ileum were immediately fixed in formalin (10%) for 24 h. Then, dehydrated with increasing concentration (70-100%) of ethyl alcohol and passed into two content of xylol. After that the samples were embedded in paraffin and sectioned by the rotary microtome at 5 µm. The sections were fixed on the glass slides and remoistening process was done by passing the sections through decreasing concentration (100-70%) of ethylic alcohol and in xylol. The histological slides were stained by hematoxylin and eosin stain (Munro, 1971; Jawad *et al.*, 2016). Morphological parameters were measured by a computerized image analyzer (Leica DM LB2, Germany). An ocular micrometer was used to measure the thickness of the intestinal wall layers. Variation ration was counted based on the formula which were reported by Jawad *et al.* (2015).

Research design and data analysis

This research used one way complete random sampling. The gained data which was resulted were analyzed by one way analysis of variance (ANOVA). If the treatment significantly affected the chicken, LSD and Duncan's (1955) Multiple Range would be applied (DRMT) (Vincent, 1991; Genstat, 2003). Differences between treatments subjects were considered significant level at P<0.05.

Results and discussion

The effects of supplementing 2.5 and 3.5% of JA powder in the broiler diet on the histological parameters of small intestine segments at week 6 of age are presented in Table 2. The duodenum wall of the chicken in T2, T3, T5 and T7 were significantly (P<0.05) higher in thickness compared with other treatments. That take place because of increase the villi length (17, 17, 12, 11%), total mucosa thickness (14, 14, 11, 11%) respectively.

Table 3 show the jejunum wall thickness effectiveness by supplementing JA in broiler diets. It was significantly (P<0.05) increased in T4, T3, T5, T6 and T7 (18, 13 15, 10, 10) in order to improve the villi length (24, 21, 19, 17, 13%) and total mucosa thickness (23, 18, 19, 13, 12%) respectively.

Furthermore best improvement values compared with control group in the ilium wall thickness were reported in

Table 2: Effect of JA on histometrical parameters of broiler duodenum at week six of age

Trt	VIL	LEB	MM	MUC	SM	M	S	TOTAL
T1	5.42±0.09d	1.18±0.03	0.1±0	6.7±0.1c	0.1±0	0.84±0.01	0.52±0.02	8.15±0.1c
T2	6.33±0.1a	1.24±0.03	0.1±0	7.67±0.13a	0.1±0	0.83±0.01	0.52±0.02	9.11±0.13a
T3	6.35±0.13a	1.21±0.02	0.1±0	7.66±0.14a	0.1±0	0.83±0.01	0.53±0.02	9.12±0.14a
T4	5.63±0.17cd	1.23±0.03	0.1±0	6.97±0.2c	0.1±0	0.84±0.01	0.54±0.02	8.45±0.2c
T5	6.08±0.09ab	1.29±0.04	0.1±0	7.47±0.11a	0.1±0	0.81±0.01	0.51±0.02	8.88±0.11ab
T6	5.7±0.11bcd	1.23±0.05	0.1±0	7.03±0.11bc	0.1±0	0.84±0.01	0.56±0.02	8.53±0.12bc
T7	6±0.17abc	1.31±0.04	0.1±0	7.41±0.17ab	0.1±0	0.81±0.01	0.57±0.01	8.93±0.16ab

Y Mean values with (a, b, c) common superscript in column differ significantly (P<0.05). D, J and I compared separately between TRT.
Y TRT: treatment; D: duodenum; J: jejunum; I: ileum; Vl: villi length; DL: Depth of Crypts of Lieberkuhn; MM: Muscularis Mucosa; TMT: Total Mucosa Thickness (VL+DL+MM); SM: Submucosa; MA: Muscularis; S: Serosa; TWT: Total Wall Thickness (TMT+SM+MA+S).

Table 3: Effect of JA on histometrical parameters of broiler jejunum at week six of age

Trt	VIL	LEB	MM	MUC	SM	M	S	TOTAL
T1	4.07±0.28c	1.07±0.05b	0.1±0	5.24±0.3b	0.1±0	0.82±0.01	0.56±0.02abc	6.72±0.31b
T2	4.15±0.1bc	1.08±0.04b	0.1±0	5.33±0.13b	0.1±0	0.82±0.02	0.55±0.02bc	6.79±0.13b
T3	4.91±0.2a	1.16±0.02b	0.1±0	6.17±0.19a	0.1±0	0.84±0.01	0.51±0.02c	7.62±0.19a
T4	5.05±0.21a	1.27±0.04a	0.1±0	6.42±0.25a	0.1±0	0.82±0.01	0.56±0.01ab	7.91±0.25a
T5	4.85±0.3a	1.28±0.03a	0.1±0	6.23±0.31a	0.1±0	0.83±0.01	0.54±0.02bc	7.7±0.31a
T6	4.75±0.17ab	1.07±0.04b	0.1±0	5.92±0.17ab	0.1±0	0.82±0.01	0.53±0.02bc	7.37±0.18ab
T7	4.61±0.18abc	1.13±0.03b	0.1±0	5.85±0.19ab	0.1±0	0.83±0.01	0.6±0.01a	7.38±0.2ab

Y Mean values with (a, b, c) common superscript in column differ significantly (P<0.05). D, J and I compared separately between TRT.
Y TRT: treatment; D: duodenum; J: jejunum; I: ileum; Vl: villi length; DL: Depth of Crypts of Lieberkuhn; MM: Muscularis Mucosa; TMT: Total Mucosa Thickness (VL+DL+MM); SM: Submucosa; MA: Muscularis; S: Serosa; TWT: Total Wall Thickness (TMT+SM+MA+S).

Table 4: Effect of JA on histometrical parameters of broiler ilium at week six of age

Trt	VIL	LEB	MM	MUC	SM	M	S	TOTAL
T1	2.64±0.08d	0.9±0.05e	0.1±0	3.64±0.1d	0.1±0	0.82±0.01	0.5±0.02d	5.06±0.11d
T2	3.5±0.12a	1.08±0.04bc	0.1±0	4.68±0.14a	0.1±0	0.85±0.01	0.53±0.02cd	6.16±0.15a
T3	3.47±0.16a	1.18±0.03a	0.1±0	4.74±0.18a	0.1±0	0.84±0.01	0.56±0.02abc	6.24±0.17a
T4	2.81±0.13cd	1±0.02cd	0.1±0	3.91±0.13cd	0.1±0	0.82±0.01	0.58±0.02ab	5.41±0.14cd
T5	2.67±0.09d	0.93±0.03de	0.1±0	3.7±0.11d	0.1±0	0.83±0.01	0.56±0.03abc	5.18±0.12d
T6	3.26±0.1ab	1.14±0.02ab	0.1±0	4.49±0.1ab	0.1±0	0.82±0.02	0.6±±0.01a	6.01±0.11ab
T7	3.05±0.14bc	0.98±0.03de	0.1±0	4.13±0.15bc	0.1±0	0.8±0.01	0.61±±0.02a	5.64±0.15bc

Y Mean values with (a, b, c) common superscript in column differ significantly (P<0.05). D, J and I compared separately between TRT.
Y TRT: treatment; D: duodenum; J: jejunum; I: ileum; Vl: villi length; DL: Depth of Crypts of Lieberkuhn; MM: Muscularis Mucosa; TMT: Total Mucosa Thickness (VL+DL+MM); SM: Submucosa; MA: Muscularis; S: Serosa; TWT: Total Wall Thickness (TMT+SM+MA+S).

T3, T2 and T6 (23, 22, 19%) (Table 4). This results take place as a consequence to increase the villi length (31, 33, 23%) and the total mucosa thickness (30, 28, 19%) of these treatments respectively.

The results of present research is constant with Rehman *et al*. (2007) observation that supplementing 1% inulin in broiler diet notically improved the intestinal villi height and crypt depth. Whereas, Rebole *et al*. (2010) found that inclusion of 10, 20 g/kg inulin did not affect the jejunal histomorphology measured in broilers at 35 d of age, especially, villus height and crypt depth or microvillus length, width, density and amplification factor. However, a significant increase in the villus height: crypt depth ratio could be observed in the group receiving the diet containing 10 g of inulin/kg. Further- more, Konosonoka *et al*. (2015) reported that adding 0.5 and 1% *H. tuberosus* in broiler diet did not significantly effect on the villi length in duodenum and ilium while increased the depth of the crypt. In the same regard, Nabizadeh (2012) found that 0.5 and 1% inulin had no impact on histological structure of broiler duodenum and ilium, but increase the height of villi in jejunum.

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