Effect of of supplementation of different levels of Hayicinth (*Einhhornia Crassipes*) powder on villi of Jejunum of Broilers Chicks

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Abstract. This study was conducted to investigate the effect of addition the different levels of Hayicinth (*Einhhornia Crassipes*) instead of barely in diet on Jejunum of villi of broilers, The experiment was carried out in the Animal Production Department, Faculty of Agriculture, University of Kufa, associated with the department of medical laborites Sciences / laboratory of Histology and Anatomy. A total of 300 broiler Ross type, one day age, were divided into 5 groups, each group contain 60 birds. They were subjected to five treatments as following: group (T1): control in which they were given normal diet without any additions, Group (T2): were given 25 % of Hayicinth (*Einhhornia Crassipes*) in diet daily, Group (T3): were given 50 % of Hayicinth (*Einhhornia Crassipes*) in diet daily, Group (T4): were given 75 % of Hayicinth (*Einhhornia Crassipes*) in diet daily and Group (T5): which were given 100 % of Hayicinth (*Einhhornia Crassipes*) in diet daily. The treatment started at the beginning of one day old of age till 5 weeks. At the end of treatment a portion of Jejunum was taken for histological examination of villi. The histological examination revealed that Group (T1) control showed normal structure for the villi in the 4 main layer, while, Group (T2) had long villi, mucosa had damage in some metaplasia, epithlicl villi was damaged and serosa with smooth muscle was regular and edematous. Group (T3) revealed long, thick hypertrophy villi, mucosa and sub mucosa was edematous and damaged, while, serosa had irregular fiber smooth muscle, intestinal glands was irregular in shape and epithlicl villi was damaged. However, Group (T4) deducted long thin lamina properia with complete damage of mucosa, Filamentous sub mucosa without muscle fiber and intestinal glands were irregular in shape, while, Group (T5) showed sever damage of mucosa and sub mucosa, filamentous lamina properia, intestinal glands showed sever hypertrophy and irregularity in shape with some glands were damaged with cytoplasm lost its appearance and nucleus lost its shape while, serosa showed irregular muscle fiber and edematous. It was concluded that the addition of Hayicinth (*Einhhornia crassipes*) had adverse effect on the structure of villi of Jejunum of broiler chicks which was a dose dependent.

1. Introduction
Hayicinth (*Einhhornia crassipes*) is water plant with long life and free generation, it belongs to family (pontedericeac) water herb, distributed over more than 80 countries during last decades.
There are seven types from it, but the most dangerous and more distributed one is found in Iraq known as (*Einhornia crassipes*) [1]. This herb is used for feeding of animal instead of some grains like Barely to its high level of protein which is about 22.8 % protein and ash 12.4 %. Moreover. It is used in synthesis of some of drugs. Leaves contains feinulat and flafuned which have biological active against the virus, fungus, tumor and Bacteria [2 ; 3], also it contains some anti-oxidant enzymes [4].

Sapogenin is one of the secondary metabolic products of this herb which had effect on insects and produce foam [5], also it has biological effect on permeability of cell membrane as form analysis RBC, because there is link between the glycan with phospholipid in cell and produce complex structure [6]. Sapogenin possessed a toxic effect especially on snake and fish because it caused damage for respiratory organ there for used in fashion in dosage 5 mg in water, show low level of RBC, Hb, and PCV % and high level consume of O2 and high of ammonium and damage in intestine. Low level of sugars in the blood during the active of Insulin production or low absorption of glucose from the intestine to the blood and decrease transverse the glucose by villi [7 and 8]. Sapogenin have effect on lower level of cholesterol in human and animal [9]. Tannin, Alkaloid and Flavonoid is also found in this plant [10].

The incorporation of Hayicinth (*Einhornia crassipes*) in the diet of chicks for 5-8 weeks had been found to lead to lower body weight of chicks [11]. Lopez [12] investigated that the addition of different levels of Hayicinth (*Eichhornia crassipes*) in the diet of chicks instead of soybean caused a low feed consumption of chicks, and that high levels of Hy. Powder had negative effect on starter diet feeding more than growth diet. Moreover, Malik et al. [11] showed that the use of Hy. Powder in the diet of broiler chicks instead of barley had no significant effect on consumption of diet.

The aim of study was to investigate the effect of addition of different levels of Hayicinth (*Eichhornia crassipes*) on the villi of Jejunum when used instead of barley in diet of broiler chicks.

2. Materials and Methods
A total of 300 broiler Ross type, one day old age, 40 g / chick housed in cages (1.5 × 1.5 m) and 26 chick in each repeaters after the hall is equipped with a mattress of sawdust in addition to their manholes and manger plastic 5 litter of manholes and 5 kg .for crib were divided into 5 groups, each 60 birds. They were subjected to five treatments as follows:-Group (T1): control in which they were given normal diet without any additions, Group (T2): were given 25 % of Hayicinth (*Einhornia Crassipes*) in diet daily, Group (T3): were given 50 % of Hayicinth (*Einhornia Crassipes*) in diet daily, Group (T4): were given 75 % of Hayicinth (*Einhornia Crassipes*) in diet daily and Group (T5): which were given 100 % of Hayicinth (*Einhornia Crassipes*) in diet daily as shown in Table 3 and 4. The treatment started at the beginning of one day old of age till 5 weeks. At the end of treatment, all the groups of chicken were slaughtered and examined macroscopically at first then a portion of Jejunum was taken for histological examination for the changes in the structure of villi. The chemical composition of Hayicinth crassipes shown in Table 1 (A.O.A.C.1990), while Table 2 showed the active groups percentage.
Table 1. The chemical composition of Hayicinth crassipes.

| Metal  | Amount (ppm) |
|--------|--------------|
| Fe     | 328.5        |
| Cd     | 6.3          |
| Zn     | 22.8         |
| Pb     | 1.4          |
| Cu     | 21.22        |
| Co     | 1.00         |
| Mg     | 4937         |
| Se     | Very little  |
| Mn     | 116.1        |
| Ca     | 140.4        |

Table 2. Active group in Hayicinth crassipes

| Active group | Percentage |
|--------------|------------|
| Alkaloids    | 1.7 %      |
| Tannin       | 12.5 %     |
| Saponin      | 22.7 %     |
| Flavonoid    | 0.53 %     |

Table 3. The composition of diet for studied groups

| Substance            | T1       | T2       | T3       | T4       | T5       |
|----------------------|----------|----------|----------|----------|----------|
| Maize                | 44.90    | 44.68    | 44.58    | 44.18    | 44.10    |
| Soybean (47 %)       | 38.0     | 37.72    | 37.42    | 37.22    | 36.80    |
| Wheat                | 10.0     | 7.50     | 5.0      | 2.50     | ----------|
| Powder Hay.crip.     | -------- | 2.50     | 5.0      | 7.50     | 10.0     |
| Premix *             | 2.50     | 2.50     | 2.50     | 2.50     | 2.50     |
| Maize oil            | 3.0      | 3.50     | 3.90     | 3.50     | 5.0      |
| Phosphat Calcium**   | 1.20     | 1.20     | 1.20     | 1.20     | 1.20     |
| L. lysine ***        | 0.10     | 0.10     | 0.10     | 0.10     | 0.10     |
| DL. Methionine ****  | 0.30     | 0.30     | 0.30     | 0.30     | 0.30     |

*Premix M-25 =each 1/kg= Vit. A 400.000 IU, Vit.D3=0.000160 IU, vit. E 1600 IU, vit. K =80 mg Vit.B1=240 mg, cal.p.A =5200mg, niacin =1400mg, vit. B6=1200mg, Bayotin =2mg, folic acid=40mg, vit. B12=0.4mg, Phosphat Dicalcium=120 mg., phytes=4.000mg, oil=20.000mg, Carbonat calcium =422 000mg, Cholin=20.000mg., Protein=20 %, Energy K.K/kg =3000cal/k., lysine digest=5.71. Methionine digest=8.2., Nacl=5.92

**Phosphat Dicalcium=22 % calcium unorganic and Phosphors unorganic=18 %

*** L-Lysine (Hcl) Purity =98.5 %, **** DL-Methionine Purity=99 %.

Table 4. The Chemical composition of the diet

| Substance          | T1       | T2       | T3       | T4       | T5       |
|--------------------|----------|----------|----------|----------|----------|
| ME Kcal /kg        | 3020.0   | 3020.0   | 3017.0   | 3020.0   | 3021.0   |
| Crude protein %    | 23.93    | 23.97    | 23.97    | 23.96    | 23.92    |
| Total Calcium %    | 1.00     | 1.00     | 1.00     | 1.00     | 1.00     |
| Aval. Phosphor %   | 0.64     | 0.64     | 0.64     | 0.64     | 0.64     |
| Crude Fiber %      | 5.05     | 4.72     | 4.37     | 4.02     | 3.67     |
| Lysin %            | 1.43     | 1.43     | 1.43     | 1.43     | 1.43     |
| Methionine+systin %| 1.07     | 1.07     | 1.07     | 1.07     | 1.07     |
| C / P Ratio .Energy| 126.20   | 126.00   | 125.86   | 126.04   | 126.29   |
2.1 Preparation of Biopsy
The Jejunum Part of the intestine of chicken was taken then cut part from it. The biopsy was put in specific container have 10% formalin for fixation then the tissue is out from the formalin and cut by the knife in to small part and placed in cassettes in which the following steps then done.

2.2 Dehydration
Used ethanol in different concentration to remove the water because the wax is Hydrophopic and not soluble in water, so embedded the tissue in in five beakers each have ethanol but in different concentration (30%, 70%, 80%, 90%, 100%) for 1.5 hours to each one.

2.3 Clearing
Use xylene that removed ethanol because wax is more soluble in xylene.

2.4 Infiltration
Embedded the tissue in paraffin wax in three beakers each one for one hour to remove xylene and take place by paraffin, and then the tissue is transferred to embedding site by used wax machine fill the cassette with wax and then placed the tissue in -4 °C until the wax is harden. The tissue was then placed in microtome for trimming the wax in 15-20 um and then cutting the tissue in 3-5um its optimal thickness then placed in water bath at 48-50 °C and then take the floating section placed in slide by forceps, then oven is used to dry the slide and remove access wax. The slide is embedded in three beaker of xylene each one for 10 minutes and then in three beaker have ethanol in different concentration (100%, 90%, 70%) each one for 15 minutes and rinse in distilled water for 5 minutes.

Hematoxylin and eosin were used to stain the slide, the slide is stain by hematoxylin for 10 minutes and then wash by tap water and then stain by eosin for 15 minutes and wash by xylene for 5 minutes to remove access stain. The slides were then examined by light microscope line (10).

3. Results and Discussions
There are less studies about the effect of water herb Hayicinth on the digestive tract specially the intestines. so; this study was down to know the effect of incorporation of water Hy. with different levels in the diet of chicken on the intestine specially the villi.

There are damage and changes in the structure of Jejunum in all treated groups according to the amount of water herb Hy. in the diet, in which group T2 and T3 showed less damage than T4 and T5 respectively as shown in figure 2-6. This could be attributed to the presence of tannin in the plant that used in current experiment about (12.5 %) which consider as ant nutrient if present in the diet of animals, because of the linkage between protein and tannin resulting less digestion process, decrease growth rate and decrease diet consumption [13]. Moreover, Ortiz et al. [14] stated there were atrophy in villi due to present of tannin in the diet which causes irritation and damage for the mucosa of intestine due to sharp needle of calcium oxalate which causes damage for intestine mucosa. Bolenz et al. [15] found lower nutrient conversion when increase amount of water herb Hyacinth in the diet instead of barley, because the plant contains toxic substance which effect on the villi and intestine mucosa so result in low body weight and more mortality rate.
Figure 1. Show the macroscopic appearance of intestine of chicken which was dark red in color and in some cases there was hemorrhage and thin serosa.

Figure 2. Show the villi of T1 Group which was normal in shape with the main layers distinguished mucosa (A) which lined by simple columnar epithelium, sub mucosa (B), lamina propria with lymphoid cell and smooth muscle (C), serosa with 2 layer smooth muscle and adipose cell (D).

Figure 3. Show the villi of T2 Group which was long villi(A), mucosa was damaged in some metaplasia(B), epical villi was damaged(C) and serosa with smooth muscle was regular and edematous (D).

Figure 4. Show the villi of T3 Group which was short, thick villi “hyper atrophy” (A), mucosa and sub mucosa was edematous and damaged(B), serosa with irregular fiber smooth muscle(C), intestinal glands were irregular shape(D) and epical villi was damaged(E).
Figure 5. Show the villi of T4 Group which was long, thick villi with complete hyperplasia of lamina properia (A) and intestinal glands were irregular in shape (B).

Figure 6. Show the villi of T5 Group which had severe damage of mucosa and sub mucosa(A), filamentous lamina propria(B), intestinal glands had severe metaplasia with irregular shape while cytoplasm showed loss in appearance and also nucleus loss its shape (C), and serosa with irregular muscle fiber and edematous (D).

4. Conclusions
It was concluded that the addition of Hayacinth (Einhornia crassipes) had adverse effect on the structure of villi of Jejunum of broiler chicks which was a dose dependent.

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