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Evaluation of the Efficacy of Asparagus (*Asparagus officinalis* L.) Root Powder and Aqueous Extract on the Physiological and Immunological Performance of Broilers

Rasool A.A. Al-Subaihawi and Rabia J. Abbas[1]*

Ministryof Agriculture, Directorate of Maysan Agriculture, Maysan, Iraq
[1]Department of Animal Production, College of Agriculture, Basrah University, Iraq
**E-mail: rabia.jaddoa@uobasrah.edu.iq*

***Abstract*:** The 315 one -day old broiler chicks (Ross- 308) were randomly assigned to seven treatments with three replication. The first treatment was fed a basal diet (control). Second, third, and fourth treatments were fed basal diet supplemented with the Asparagus(*Asparagus officinalis* L.) root powder (ARP) at 5, 10, 15 (g kg^{-1}), while aqueous extract of ARP was administrated to drinking water at 5, 10, 15 (ml l^{-1}) in fifth, sixth, and seventh treatments respectively. There was significant improvement in number of RBC, WBC (ARP extract at 5, 10, and 15 ml l^{-1}), hemoglobin, PCV compared to control, and ARP (5 g kg^{-1}). Serum total protein, globulin, and albumin were improved significantly in comparison with control, ARP (5 and 10 g kg^{-1}), while a significant decrease was observed in serum glucose, cholesterol, and triglyceride compared to control. Phagocytic activity, phagocytic index, immune response, and value of antibodies titer against Newcastle and Gumboro disease were significantly better than control. In comparison with control, feeding Asparagus improved lymphocyte and decreased heterophils and H/L ratio. The study concluded that the best results were achieved at level 15 (g kg^{-1}) of the ARP and level 15 ml l^{-1} of its aqueous extract in improving the physiological and immune performance of broilers.

***Keywords*:** Broilers, *Asparagus officinalis*, Blood parameters, Immune traits

Asparagus officinalis L. is a perennial vegetable, which was classified under the family *Liliacea* in the past, while at present it comes under the *Asparagaceae* family (Joanna et al 2019). *Asparagus officinalis* L. is a perennial herb with various bioactivities and has been widely used as medicine and food since ancient times, and it originated from the eastern Mediterranean and Asia Minor and has been cultivated for more than 2000 years (Guo et al 2020). *A. officinalis* rich in bioactive compounds including antioxidants and the most important characteristic of asparagus is a vegetable that offers multiple health benefits due to containing flavonoids, alkaloids, phenols, saponins, tannins, which possess strong antioxidant properties (Minh et al 2019), which is a precursor to many pharmacologically active stimulants. Roots and leaves are important parts to use medicinally. Besides that, a recent study suggested that asparagus may constitute a good source of natural antioxidants to be used in our diet, as well by industries for functional food preparations (Joanna et al 2019). The *A. racemosus* (Shatavari) is used to enhance the body's resistance against infections and improve the immune system, and it is widely used for the treatment of various ailments as it contains many different phytochemicals (Singh et al 2018). Asparagus is also, contains various phytochemical compounds such as polysaccharides, polyphenols, anthocyanins, and saponins, which exhibit anticancer, antitumor, antioxidant, immunomodulatory, hypoglycemic, antihypertensive, and antiepileptic effects through *in vitro*, and *in vivo* experiments (Guo et al 2020). The root powder of *A. racemosus* is used as an herbal feed supplement in poultry feed, Shatavari augments the appetite and stimulates the liver (Shukla et al 2018). Shatavari possess anabolic properties viz., growth promotion, increase the production potential in broilers by using herbs possessing therapeutic potential (Gaikwad et al 2018). Several studies have been undertaken to assess the influence of dietary supplementation of *Asparagus racemosus* (Shatavari) root powder in broilers, to improve body weight, feeding efficiency, conformation traits and carcass yield (Chikwae t al 2018), immunity (Dahale et al 2014), removed cold stress and improves immune status of broiler chicks(Kant et al 2014). Due to the rarely of studies that deal with Asparagus (*Asparagus officinalis* L.) as a feed additive in poultry. The study was designed to investigate the effect of *A. officinalis* L. root powder, and its aqueous extract as dietary supplementation on some haemato-serobiochemical parameters, and the immunologic status of broiler chickens.

MATERIAL AND METHODS

Animals and experimental diets: The present study was

conducted for 35 days from Dec 16, 2020. Three hundred and fifteen (315) one-day-old mixed-sex broilers (Ross 308) were used on a completely randomized design (CRD) in seven treatment groups each having 45 chicks and each group was further divided into three replicates containing 15 chicks each. The chicks in the control group (T1) were given no supplement, whereas in treatments T2, T3 and T4, chicks were supplemented with Asparagus (*A. officinalis*) root powder (ARP), at 5, 10, 15 (g kg^{-1}) of feed, respectively. The aqueous extract of ARP was administrated to drinking water at levels 5, 10, 15 (ml l^{-1}) in T5, T6, and T7 respectively. The feeding program consisted of a starter diet, which was fed until day 21, and a grower diet, which was fed from day 22 until 35 and was based on yellow corn, wheat and soybean meal as shown in Table 1. The diets were formulated to meet the nutrient requirements of the broiler (commercial recommendation). The chicks had free access to feed and water throughout the experiment period.

Collection of plant material: The roots of the Asparagus plant were collected from the Eastern Island of Teeb, East of Maysan Governorate. The roots were cleaned from the dust, washed with tap water, cut into small pieces, dried in the shade, and ground into powder. The fine powders were stored in black plastic bags at room temperature (25°C) until the extraction process was performed.

Table 1. Ingredients and nutrient composition of broiler starter and grower diets

Ingredient (%)	Starter diet 1-21 days	Grower diet 22-35 days
Yellow maize	56.20	61.00
Wheat	04.00	04.00
Soybean meal (48%)	32.00	26.50
Vegetable oil	1.50	2.50
[1]Broiler protein concentrates (40%)	5.00	5.00
Limestone	0.80	0.50
Premix	0.25	0.25
Common salt	0.25	0.25
Total	100	100
Calculated composition[2]		
Metabolizable energy (Kcal Kg^{-1})	3023	3137
Crude protein (%)	22.70	20.47
Crude fat (%)	2.77	2.89
Crude fiber (%)	2.36	2.22
Calcium (%)	0.61	0.49
Phosphorus available (%)	0.28	0.24
Lysine (%)	1.21	1.15
Methionine + Cysteine (%)	0.88	0.81
Calorie: protein ratio	133.17	153.24

[1]Broiler protein concentrate (Brocorn-5 special W), Exported by (Wafi B.V., Alblasserdam – Holland),supplied per kilogram of feed: Crude protein 40%, 2017 kcal/kg M.E, 5% fat, 2.20% crude fiber, 7.10% moisture, 28.30% ash, 4.20% calcium, 2.65% total phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine+ cysteine, 0.42% tryptophan, 1.70% threonine, 2.50% sodium, 4.20% chloride, 200 mg/kg copper, 1.600 mg/kg manganese, 2.000 mg/kg zinc, 2.000 mg/kg iron, 20.00 mg/kg iodine, 5.00 mg/kg selenium. [2]Was calculated according to the chemical composition of feedstuff contained in NRC (1994)

Preparing of *Asparagus officinalis* root aqueous extracts: The aqueous extract was prepared according to the method described by Sharma et al (2012). Five grams (5g) of dry fine powder of Asparagus root was extracted with distilled water (500 ml), and then the solution mix well to obtain a homogeneous mixture then leave for 4 hours in a horizontal shaker at medium speed, after which the sample is left to stabilize for an hour and then filter the mixture using filter paper (Whatman No.1). After that, the mixture was evaporated by using the drying oven at a temperature of 37°C for a period of seven days, after which the concentrated extract obtained in a thick viscous form, scrape the product, and store in the refrigerator for future use.

Haematological parameters and serum biochemistry: At 35 days old, blood samples were taken from the brachial vein from three birds of an average weight selected from each treatment (chick/replicate) randomly. Blood samples were used for a fresh blood count. The hematological analysis includes red blood cell (RBC) count, white blood cells (WBC) count, hemoglobin (Hb), packed cell volume (PCV). Blood smears were also performed to count lymphocytes (L) and heterophils (H), which made it possible to determine the H: L ratio. For serum biochemical indices, a blood sample was drawn and allowed to stand for an hour at room temperature (18°C) to serum collection. Serum was separated by centrifugation at a speed of 3000 rpm for 15 minutes and then stored at −20°C for further analysis. Serum total protein (TP) and albumin were analyzed by a colorimetric method using commercial kits (Spinreact, Spain). Sera globulin was calculated by subtraction from TP. Blood serum glucose, cholesterol, and triglyceride were calculated by using special kits (Biolabo AS, France).

Relative weight of lymphoid organs and immunoglobins: At the end of the experimental (35 days) period, three birds of an average weight selected from each treatment (chick/replicate) randomly. After the birds were manually eviscerated and dressed. Lymphoid organs (spleen and bursa of Fabricius) were carefully removed, weighed and expressed as a percentage of the live weights. Thereafter, the bursa index was calculated. Immunoglobulin tests were performed by means of the ELISA test (Enzyme-Linked Immune Sorbian Assay) to determine the concentration of immunoglobulin's IgM, IgA, and IgG. Phagocytosis and Phagocytosis index was measured

according Erhard et al (1992). The determination of the Titer for serum antibody directed against the Newcastle disease and infectious bursal disease (IBD) (Gumboro) HI test (Hemoagglutination Inhibition Test) according to Enzyme-Linked Immuno Sorbent Assay (ELISA) was used to determine the titer of antibodies.

Statistical analysis: Data were analyzed as a completely randomized design by using SPSS program software (2015).

RESULTS AND DISCUSSION

Hematological and biochemical parameters: The hematological parameters of red blood cell (RBC), white blood cell (WBC in T5, T6 and T7),) hemoglobin (Hb), and pack cell volume (PCV), were significantly improved in broilers fed with Asparagus root powder diet compared to the control and T2 (Table 2). The level of 15 ml l^{-1} of *A. officinalis* extracts was achieved the highest values in these parameters. This positive effect of these parameters may be attributed to the vital function of the bioactive compounds in the root of the *Asparagus* plant that offers multiple health benefits owing to containing flavonoids, phenolic, alkaloids, saponins, and tannins compounds, which own strong antioxidant properties (Minh et al 2019), hence higher values indicate a greater potential for these function and a better state of birds health, which reflects positively on increasing hematological attributes. Kant et al (2014) reported a significant improvement in RBC count, Hb, and PCV with respect to Shatavari and vitamin E treated groups than the control group of broilers. On other hand, Shukla et al (2018), showed an improvement in the health, physiological, immunological traits, and haematobiochemistry characteristics of the blood after supplement *Asparagus* root powder to a broiler diet. As well, in a study on rats, Chaudhary et al (2016) noted that methanol extract from *A. racemosus* root powder led to a significant improvement in RBC count and hemoglobin concentration compared to the control, when anemic rats were treated.

The biochemical changes observed in the study included significant improvements (with 15 g kg^{-1} and ARP extract groups) in total protein, globulin, and albumin concentrations, as compared to control, T2 and T3, while glucose, cholesterol, and triglycerides levels were significantly (decreased by the dietary treatments of broilers (Table 3). Improvements in total protein, globulin, and albumin in birds fed ARP and its aqueous extract may be due to asparagus root powder that supports the immune system of birds (Rekhate et al 2010, Kant et al 2014), which was positively reflected in the improvement of these serum parameters. The reduction in the levels of serum total glucose when the chicks supplemented with ARP may be related to the main active constituents of the roots of Asparagus (steroidal saponins and sapogenins), which have antidiabetic properties. Mathews et al (2006) illustrated that aqueous extract of *A. adscendens* have antidiabetic potentials as it stimulated both the secretion and action of insulin as well as inhibiting starch digestion in the clonal pancreatic β cell line. Additionally, Visavadiya and Narasimhacharya (2007) observed that the phytosterol and saponin contents of *A. racemosus* root besides polyphenols, flavonoids, and ascorbic acid could be responsible for increased fecal sterol excretion and decreased cholesterol levels in the hyperlipidemic rats. Bhardwaj et al (2009) observed a significant increase in serum total protein due to the inclusion of ARP in broiler diets.In accordance with the present results, Yadav et al (2018) concluded that supplementation of the diet with Shatavari (*A. racemosus)* meal at 0.5% and above reduced plasma cholesterol in coloured chicken. Similar findings were reported by Ashwini et al (2019), that roots of *A. racemosus* of broiler diets decreased serum cholesterol.

Immunological Parameters

Supplementation of Asparagus root powder in treatment

Table 2. Hematological analysis of broilers under different levels of *A. officinalis* root powder and its aqueous extract at 35 days of age

Treatments	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g 100 ml^{-1})	PCV (%)
T1	3.62^{d} ±0.09	21.68^{d}±0.17	9.66^{d} ±0.26	28.99^{d}±0.79
T2	3.84^{cd} ±0.07	22.32^{cd} ±0.03	10.18^{cd} ±0.16	30.55^{cd} ±0.49
T3	3.98^{bc}±0.01	22.38^{cd} ±0.08	10.62^{bc} ±0.04	31.87^{bc} ±0.13
T4	4.03^{ab}±0.09	22.65^{bcd}±0.09	10.75^{abc}±0.25	32.25^{abc} ±0.76
T5	3.95^{bc}±0.08	23.12^{abc}±1.07	10.53^{bc} ±0.23	31.61^{bc} ±0.69
T6	4.16^{ab}±0.10	23.92^{ab}±0.44	11.09^{ab} ±0.27	33.27^{ab} ±0.81
T7	4.24^{a}±0.02	24.14^{a}±0.26	11.31^{a} ±0.06	33.94^{a} ±0.19

*Values in the same column with no common superscript differ significantly ($p \leq 0.05$). ** T1 - control; T2, T3 and T4 - Asparagusroot powder at 5, 10 and 15 (g kg^{-1}) in basal diet; T5, T6 and T7 Asparagus aqueous extract - at 5, 10 and 15 (ml l^{-1}) in drinking water, respectively

groups caused significant improvement in phagocytosis, phagocytosis index, and immunoglobulin's levels (IgM, IgA, and IgG) as compared to control (Table 4). The improvement in the immune response of broiler chicks may be attributed to saponins (active principle) properties. Saponins have been ascribed to a number of pharmacological actions, such as immunomodulatory potential via cytokine interplay, and have tremendous cytotoxic (Sun et al 2009). Shaha and Bellankimath (2017) reported that the tuberous roots of this *A. racemosus* plant are widely applied in pharmaceutical preparations as well on the biotechnology scale, in the preparation of various herbal preparations for they possess a distinct potential and a defense system. Singh et al (2018), Shatavari can enhance the body's resistance against infections and improve the immune system. Wang et al (2020) concluded that the pectic-like polysaccharides from white asparagus (*A. officinalis*) skin can be potentially used as an immunomodulatory agent in functional foods. Kumari et al (2012) concluded that the extract from the *A. racemosus*, could significantly increase the humoral and cell-mediated immune responses and had immuno-modulatory effects of the treated birds.

Experimental groups showed higher antibody titers against Newcastle (ND), and IBD (Gumboro) disease as compared to the control group (Table 5). The maximum was observed in group T7 (3246.00), and (2738.33) for ND and IBD disease respectively, which was administered with 15 (ml l^{-1}) *A. officinalis* root extract drinking water. T1 showed the lowest value reached (2652.67) and (1893) for both diseases respectively. The improvement in titer value against ND and IBD disease for supplementary treatments may be due to the herbs like Asparagus contain different a variety of active components that affect the properties of cell-mediated

Table 5. Effect of treatments on serum titers of Newcastle and Gumboro disease in broiler chickens at 35 days of age

Treatment	Newcastle disease	Gumboro disease
T1	2652.67^{e} ±50.53	1893^{f} ±2.30
T2	3066.00^{d} ±4.72	2247.33^{e} ±5.54
T3	3077.67^{bc}±2.02	2518.33^{d} ±3.48
T4	3084.33^{c} ±2.90	2583.00^{c} ±4.72
T5	3078.00^{bc}±2.08	2555.67^{bc} ±3.93
T6	3125.67^{b} ±4.66	2681.67^{b} ±5.20
T7	3246.00^{a} ±9.50	2738.33^{a} ±18.35

See Table 2 for details

Table 3. Biochemical parameters in broilers supplemented to *A. officinalis* root powder and its aqueous extract at 35 days of age

Treatments	Total protein (g $100ml^{-1}$)	Albumin (g $100ml^{-1}$)	Globulin (g $100ml^{-1}$)	Glucose (mg $100ml^{-1}$)	Cholesterol (mg $100ml^{-1}$)	Triglycerides (mg $100ml^{-1}$)
T1	4.20^{d}±0.03	1.77^{c}±0.06	2.42^{d}±0.02	254.28^{a} ±1.00	168.68^{a}±1.85	95.14^{a}±0.91
T2	4.44^{d}±0.04	1.80^{c}±0.08	2.62^{cd}±0.11	245.76^{b} ±1.43	161.4^{b} ±0.71	85.03^{b} ±0.22
T3	4.51^{cd}±0.20	1.84^{c}±0.04	2.67^{bcd}±0.03	239.61^{bc}±1.14	156.29^{cd}±1.18	82.85^{c} ±0.74
T4	5.28^{b}±0.01	2.19^{b}±0.01	3.09^{ab}±0.16	231.90^{cd} ±4.81	156.12^{cd} ±1.28	77.27^{d} ±0.54
T5	4.77^{c}±0.17	1.84^{c}±0.07	2.93^{abC}±0.13	239.75^{bc}±3.93	158.44^{bc} ±0.67	79.23^{d} ±1.01
T6	5.52^{ab}±0.05	2.24^{b}±0.04	3.27^{a}±0.29	225.67^{de} ±2.11	153.9^{de} ±0.44	75.00^{e} ±0.33
T7	5.75^{a}±0.03	2.51^{a}±0.03	3.24^{a}±0.16	222.10^{e} ±1.77	152.10^{e}±0.35	73.89^{e} ±0.63

See Table 2 for details

Table 4. Effect of *A. officinalis* root powder and its aqueous extract on phagocytosis and immune response of broiler chickens

Treatment	Phagocytosis (%)	Phagocytic index	Immune response		
			IgG (mg ml^{-1})	IgA (mg ml^{-1})	IgM (mg ml^{-1})
T1	42.00^{f} ±0.57	49.83^{d} ±1.27	1.95^{e} ±0.02	2.26^{d} ±0.01	2.61^{f} ±0.03
T2	44.67^{e} ±1.45	53.28^{bc}±1.22	2.08^{d} ±0.03	2.68^{bc}±0.01	3.36^{e} ±0.04
T3	46.33^{d} ±1.45	53.71^{bc}±1.39	2.19^{c} ±0.01	2.75^{c} ±0.03	4.44^{c} ±0.02
T4	50.33^{c} ±2.84	62.59^{ab}±1.21	2.31^{ab}±0.01	3.42^{b}±0.03	4.49^{b}±0.01
T5	47.33^{d} ±2.33	58.72^{c} ±3.20	2.17^{bc}±0.02	3.33^{ab}±0.03	3.50^{d} ±0.03
T6	52.33^{b} ±1.45	63.54^{b} ±0.96	2.35^{b}±0.02	3.44^{b}±0.01	4.30^{c} ±0.04
T7	55.01^{a} ±1.66	67.62^{a} ±0.49	2.81^{a}±0.03	3.82^{a}±0.01	4.58^{a}±0.01

See Table 2 for details

Table 6. Effect of treatments on the relative weight of lymphoid organs, lymphocytes, and heterophils cell in broiler chicken at 35 days of age

Treatment	Spleen (%)	Bursa of fabricius (%)	Bursa Index	lymphocytes (%)	Heterophils (%)	H/L
T1	0.15±0.008	0.09^{a} ±0.003	1.00^{a}±0.000	66.08^{c} ±0.86	25.24^{a} ±0.41	0.382^{a} ±0.0097
T2	0.16 ±0.008	0.09^{a} ±0.003	1.02^{a}±0.040	67.23^{bc} ±1.59	23.18^{b} ±0.48	0.346^{b}±0.0155
T3	0.15±0.005	0.09^{ab} ±0.004	0.98^{a}±0.047	71.36^{a} ±0.52	22.23^{bc} ±0.62	0.311^{c}±0.0064
T4	0.15±0.005	0.08^{bc} ±0.002	0.86^{b}±0.032	71.49^{a} ±2.12	22.89^{bc} ±0.91	0.320^{bc}±0.0108
T5	0.18±0.006	0.07^{c} ±0.001	0.79^{b}±0.012	72.36^{a} ±0.57	23.13^{b} ±0.52	0.319^{bc}±0.0075
T6	0.16 ±0.013	0.07^{c} ±0.006	0.80^{b}±0.068	70.56^{ab} ±1.39	21.39^{c} ±0.30	0.303^{c}±0.0061
T7	0.17 ±0.003	0.06^{d} ±0.001	0.66^{c}±0.016	71.83^{a} ±1.26	21.83^{bc} ±0.27	0.304^{c}±0.0097

See Table 2 for details

immunity, such as lipopolysaccharides, polymers, saponins. Saponins-based adjuvants have the ability to stimulate the cell-mediated immune system, as well as to enhancing antibody production and it has the advantage that only a low dose is required for auxiliary activity (Rajput et al 2007). Kumari et al (2012) observed that the use of *Asparagus racemosus* extract in broilers feed has significant positive effects in both humoral and cell mediated immune responses of the birds which were evident by increased antibody titer after HI test after they study the immunomodulatory effects of *A. racemosus* extract against Newcastle disease in one week old normal and immunocompromised broiler chicks. Patil et al (2012) reported that the activating action is attributed to the isoprinosine content of the asparagus root plant, which has been shown to increase cytokine production, increase active T cells and induce T-cell surface markers on protein cells, besides increase lymphocyte proliferation. The findings in the current study are supported by Tekade et al (2008) who recorded higher antibody production than normal due to more stimuli to the immune system in broilers treated with *A. racemosus* alone as well as in different combinations with *Sida cordifolia* was and *Levamisole* starting from 28th day of age for 2 weeks. Similar observations were reported by Yadav et al (2018), that dietary supplementation of Shatavari root meal @ 0.5% improves immunity in coloured chicken (*Chabro*).

No significant differences were observed in the relative weight of the spleen among treatment groups (Table 6). However, there was a significant difference in the bursa of Fabricius (%), bursa index, lymphocytes (L), heterophils (H) populations and H/L ratio. ARP and ARP extract significantly decreased bursa relative weight and bursa index (except in T2 and T3), heterophils, and H/L ratio, whereas the lymphocytes population was increased significantly as compared to control. The *Asparagus racemosus* plant has immunostimulating properties (Sharma and Sharma 2013), also modulates the action of the immune system (Shaha and Bellankimath 2017). Mishra et al (2017) indicated that extracts and formulations prepared from *A. racemosus* aids in increases in white cell counts, absolute neutrophil counts, haemagglutinating, and contribute to increasing the phagocyte activity of infected laboratory mice, thus eliminating the negative effects and preserving on the titer of antibody to blood in mice, also showed that asparagus is an immune stimulant, along with *Withania somnifera*, *Tinospora cordifolia* and *Picrorhiza kurroa*, significantly suppresses the chemotactic activity and production of interleukin-1 and TNF-α by macrophages.

CONCLUSION

The supplementation of 15 g kg^{-1} of *A. officinalis* root powder and (15 ml l^{-1}) of aqueous extract to drinking water, improve the heamatobiochemical parameters, phagocytosis, immunoglobulin's (IgM, IgA, IgG), and antibody production against ND and IBD, and improve lymphocyte, while it reduced heterophils and H/L ratio of broiler chicks.

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