Effects of Cyclamen and Marjoram Medicinal Plants on the Immune Response of Broilers against Newcastle Disease

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ABSTRACT

This study was conducted to investigate the effect of 2 medicinal plants: *Cyclamen persicum* and *Marjoram origanum* which are available in Local Iraqi markets on the immune response against Newcastle disease in broiler for both sexes. The type of chicks used was English Hebrd (220 chick) at one day old. They were divided into four equal groups. First group was fed basic ration, 2nd group was fed basic ration with the addition of 2% *C. persicum*. The 3rd group was fed basic ration with 2% *M. origanum* and the 4th group was fed basic ration with 2% *C.persicum* and 2% *M. origanum*. The effects of the above mentioned nutritional treatments for both sexes on the different groups were measured to achieve certain objectives for evaluation of the efficiency of the immune response against Newcastle virus and comparison between both sexes. Results indicate significant variation in the immune response; the males were demonstrating high significant responses over the females. Compatibility and synergistic effects detected and positive results obtained when chicken in group 4 fed ration with a combination of both (*Cyclamen persicum* & *Marjoram origanum*) powder.

***Keywords: Cyclamen persicum, Marjoram origanum*, Newcastle disease, immunity, medicinal herbs.**

1. Introduction

Following the natural infection or inoculation of live or dead vaccine against Newcastle disease, the preliminary immune response will be shown within 2-3 days (Alexander, 1997) and reach the peak time after 2 weeks. Then start to decline in the 3rd and 4th weeks. Following the inoculation of Newcastle vaccine, the humeral secondary immune response will appear within 6-10 days. The level of serum antibodies depend on the strain used which can reach a highest peak on 3-4 weeks post vaccination, antibodies production start to decline after weeks, this lead to a secondary response (Davis and Sell, 1989; Khalifa, 2000). The situation of immune response to Gumboro disease virus is different from that of vaccination

(Cullen and Wyth, 1975), the first preliminary response appears after natural infection is within 6-28 days while the secondary immune response shown after 3 days (Cook, 1991).

John Gerard has narrated that the extract of this plant is totally safe to use as Antispasmodic, stimulant of the nervous system, the treatment of brain diseases and headaches due to cold. He recommended not to be used during pregnancy because it acts as uterine tonic. Few researches comment that the usage for two continuous months at the dose of 5 mg/kg of body weight did not affect the liver, kidney or blood morphology which makes it safe without any side effects. It is also useful in cases of asthma, colds, sinus congestion and mouth ulcers. It has an antioxidant effect which prevents destroying the cells. The key elements of Marjoram is the perfume oil (Alsabinin) as well as the substances of Althymol, Alcavacrol and resinoids and Kavoria. It is also consisting of flavonyat, acid caffeine and linalool. The plant contains anti inflammatory agent called EBCP (E- beta caryophyllene). Studies on the plant Marjoram had indicated that it contains many elements such as potassium, iron, phosphorus, sodium, calcium, zinc, and magnesium and free of any poisoning elements (Baratta, 1998).

2. Materials and Method

2.1. Plants: The medicinal plants were provided by Al-Attar Co. for Medical supplies. *Cyclamen Perciscum*: An herbal plant can live more than a century, breeding by tubers of brown colours and storing the food as well (Al-Rawi and Chakravarty, 1988; (AOFAD, 1988). The leaves are oval with heart-shape base of serrated sides, the length is 3-12cm and width is 2-7cm of dark green colour. Flowers like crowns and have several colours of red, white and dark or light pinks. It is also has several usages, such as tubers are crushed and folded in a piece of bondage to dress on the infected wounds, festering boils, infected eyes, feet fungal, all skin diseases, and an impact on the purgative and intestinal antihelmintic. It is extract can cure rheumatism, headache, goitre, and heart disease. Whilst the leaves have the important roles, when it is dried and crushed, used in curing the slight skin burns, skin cancer and sneaks bites.

Marjoram origanum: Used from the old times as an effective herbal medicine in curing so many diseases, such as gases-free, soothing coughs, phlegm, colic, gastro-intestinal as well as treatments of eczema, colds, convulsions and high blood pressure, insomnia and finally rheumatic pain; which persuaded many researchers in the medical fields in studying the advantages of this plant (AOFAD, 1988).

2.2. Experimental design: Two hundred and twenty chickens, type English Hebrd had been used at the age of one day and were non-sexed. The birds were divided into 4 groups each group of 55 non-sexed birds. After one week, each group was divided into 2 subgroups in order to avoid problems created by crowding. At the age of 16 days the birds were sexed and divided into 5 duplicate groups for males and females. The system of duplicates was adopted in the study. The birds during the experimental period were freely feed ad-libtum. Starting at the first day of age, all Chicken at the groups were fed a fundamental ration (table 1) (Ibrahim, 1987) and maintained to the control group during the experiment. The other three rations prepared with addition of *C. Perciscum* powder forming 2% of the 2nd ration and *M. origanum* powder forming 2% of the 3rd ration. The 4th ration composed of the two

medicinal plants in ratio of 1% for each plant. The aforementioned rations with the nutritive additives had been used to feed experimental chicken groups 2, 3 and 4 respectively.

Table 1: The constituents of the fundamental ration used in the experiment.

1.	Protein	**%12**	Total Gross: Row Protein = 21.5% Representative Energy = 3020 KC/KG
2.	Soybean	**%20**	
3.	Corn	**%28**	
4.	Wheat	**%40**	
5.	Lime Stone	**%0.7**	
6.	Food Salt	**%0.3**	

3.3. Vaccination programmes have been conducted as preventive measures by the following vaccines (Table 2).

Table 2: Vaccination programmes used to protect experimental chickens.

Vaccine Type	Age of chickens at Vaccination	Methods
Newcastle B1 strain	Day one	Spraying
Newcastle Lasota strain	16 & 26 days	Intracroup drunch
Cumboro Lukert strain	12 & 22 days	Intracroup drunch

3.4. Immunological tests: ELISA test was performed to detect the immune response. Blood was collected at the age of 6–7 weeks for testing by the indirect method of ELISA to measure the titer of antibodies in the serum using special Newcastle disease kits.

3.5. Challenge test and protective Index: Samples have been taken from 10 birds of each experimental group (5 birds from each repetitive) at the age of 7 weeks. They have been infected with virulent Newcastle virus with a titer of $0.1/10^7$ /ml-ELD_{50} at a dose of 2 ml by dropping in the eyes, mouth and nostrils. Clinical signs were observed and recorded with necropsy of the dead birds over two weeks post exposure to the challenge dose. The protective index has been calculated as per the following equation:

$$\text{Protective Index} = \frac{\text{\% Dead Birds of Control Group - \% Dead Birds of Each Group}}{\text{\% Dead Birds of Control Group}}$$

3.6. Statistical analysis: applied using the software (SAS, 2001).

3. Results and discussion

3.1. ELISA tests: The results of this test demonstrate the existence of significant differences between chicken groups fed with nutritive additives in the ration and sexes in relation to the standard titer of antibodies formation to Newcastle disease, this finding was not in accord with Cook (1996). The values of groups (2, 3 and 4) were higher when compared to the control group, while Group 4 was of a much higher antibody titer in broilers at 5 and 7 weeks old. Males surpassed the females in the rate of antibodies to Newcastle disease virus (Table 3).

Table 3: ELISA test Results for all study variables at weeks 5 and 7.

Study variables	Week 5	week7
Group 1	27751.50	0030780
Group 2	31149.50	0042620
Group 3	0031162	0035342
Group 4	0040446	0047365
Sex		
Male	35100	7238920
Female	30752.25	60408.75
LSD Value		
T	101.52	110.52
S	115.43	118.11
S*T	104.50	112.71

3.2. Challenge Test and Protective Index: Clinical signs appeared in the control group after 3 days from the time of exposure to the challenge dose. And characterized by lack of appetite, lethargy, ruffled feathers and lack of feathers arranged accompanied by signs of respiratory difficulty and sneezing. Signs of diarrhea also observed on some birds. The neurological signs had appeared after six days of challenge and testing, seen as a sprained or torticulosis of the neck, paralysis and inability of birds to move. Deaths of birds started at day 5 after challenge. Gross lesions in the postmortem, dead and ill bird, at all groups showed the presence of hemorrhagic ulcers and erosions in the proventriculus mucosal epithelial lining, ceacal tonsils enlargement, necrosis and ulceration along the mucosa of small intestine and presence of mucous in the congested trachea (Al-Sheikly and Carlson, 1975). The experimental groups differ among each other when compared with the control group at the time of appearance of the first clinical signs, death of birds and the rate and percentage of immune protection. In general, the level of protection was characterized by males higher than females (Tables 4, 5, 6). It seems useful to encourage poultry farmers to add Cyclamen powder 2%

and Marjoram 2% as nutritive additives to the main ration for better health protection which has been indicated for other dietary substances (Al-Homidan et al. 2002). Conduct future studies on the genetic factors to promote the medicinal herbs in the improvement and transfer of genetic traits related to immune status and productivity of the birds. Further research studies are recommended for the possibility of using the plants and medicinal herbs for other species of bird's diet to observe the effect on growth performance and in protection against other common, commercially and economically important disease of poultry industry (Samia et al. 2012)

Table 4: Indicate the data for clinical signs appearance and death time of experimental birds post challenge with Newcastle disease virus.

Aspects	Appearance of Clinical Signs (Days after Challenge Dose)		Death Time (Days after Challenge Dose)	
Studied Factors	Male	Female	Male	Female
Group 1	4	4	6	6
*Group*2	7	6	11	9
*Group*3	7	5	1	8
*Group*4	12	11	13	10

Table 5: Death toll and protection percentage in both sexes of the experimental groups.

Aspects	Death Toll		Protection Percentage %	
Studied Factors	Male	Female	Male	Female
*Group*1	10/5	10/6	50	40
*Group*2	10/4	10/5	60	50
*Group*3	10/2	10/4	80	60
*Group*4	10/0	10/3	100	70

Table 6: Indicate the protective index in both sexes and for the different experimental groups.

*Aspects Studied Factors	Protective Index in % (Male)	Protective Index in %` (Female)
*Group*2	20	16
*Group*3	60	33
*Group*4	100	50
Sex		
Male	60	-
Female	-	33

* Statistical significance between groups and sexes in a rate of $(P \leq 0.05)$.

4. Conclusion

The powder of Cyclamen 2% and Marjoram 2% have a positive impact in raising the level of humeral immune response of chickens against Newcastle disease. Consensus believe that nutritive additives of the medicinal plants used in this experiments had a positive influence and Synergistic function when used in combined (Cyclamen and Marjoram) which reflects greater efficiency of immunity to the birds. There are no side effects of medicinal plants detected towards elevated protection of birds against Newcastle disease.

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