Vet.Med.J.,Giza. Vol.53, No.2. (2005): 311-328.

EFFECT OF CHLORTETRACYCLINE AS A GROWTH PROMOTER AND LEVAMISOLE ON THE IMMUNE RESPONSE OF CHICKENS AGAINST NEWCASTLE DISEASE VACCINE.

NAGWA, A. SAID *) ; H.A., ABDEL-KHALIK GAMMAZ.** M.F.M. HASSAN.** and DALIA, A.ELSHAZLY.***)

Immunology Dept.,Animal Health Research Institute, Dokki, *

Pharmacology Dept., Faculty of Vet. Medicine, Suez Canal University:**

Biochemistry Dept., Animal Health Research Institute, Ismaillia, Egypt.***

Received: 16.3.2005.

Accepted: 23.3.2005.

SUMMARY

310 one day old Ross chicks were used to study the effect of Chlortetracycline as a growth promoter (200 gm/ton ration) and/or Levamisole (20 mg/kg b.w.) on the immune response of chickens either non-vaccinated or vaccinated against Newcastle disease virus. Chlortetracycline was found to increase the body weight and food consumption, and to improve the feed conversion ratio and feed effeciency, but it decreased the cellular immune response represented by decreased lymphocyte stimulation index against phytoheamagglutinin, phagocytosis percentage and phagocytic index. Also, it decreased the humoral immune response represented by decreased antibody titre (ELISA titre) and decreased protection rate of the Newcastle vaccine against challenge with virulent ND virus. While, levamisole increased all the parameters of the cellular and humoral immune response in the chickens treated with both chlortetracycline and levamisole, thus assuring the levamisole immunorestorative properties.

Key words: Chlortetracycline, Levamisole, Immune response, Immunosuppression, Immunomodulator, chickens.

INTRODUCTION

Poultry industry is facing increasing problems due to emergence of more virulent forms of pathogens causing severe infections and mortality in commercial flocks, for example; chickens are very sensitive to a wide range of viral, bacterial and parasitic diseases, such as New castle disease, Salmonellosis and Coccidiosis (Buerstedde et al., 1999) and the two main mechanisms by which disease is controlled involve the use of vaccines and antimicrobials. Vaccines aim to offer protection against particular pathogens mainly

viral pathogens. While, antimicrobials provide protection against bacterial diseases and this protection require their continual use even in the absence of apparent disease especially when having a growth promoting activity (Lowenthal, 2001).

Many antibiotics are capable of depressing the immune system especially when used at high levels and/or for extended periods and this immunosuppression contributes to a variety of health problems (Giambrone, 1994) and the most obvious signs of immunosuppression-health problems include an observed increase in susceptibility to infection, poor performance and often accompanied by decreased antibody responses, which may be seen as suboptimal vaccine responses or vaccine failure (Adair, 1996).

Based on these facts, the present investigation aims to Study the effect of Chlortetracyclin as a growth promoter on the development of cellular and humoral immune response against Newcastle disease vaccine in chickens and to assess the immunomodulatory effect of Levamisole in chlortetracycline treated chickens as well as in healthy, vaccinated chickens.

MATERIALS AND METHODS

Chickens:

Three hundreds and ten (310) one day old, commercial (Ross) broiler chicks from Ismaillia-Misr Poultry Company were used in this study.

The chicks were reared under hygienic conditions in batteries and fed on a balanced commercial ration free from antimicrobial agents and watered ad-libitum.

The ration given to Chlortetracycline (CTC) treated groups was supplemented with CTC 20% by 1kg/ton (200 gm CTC pure/ton ration) starting from one day old till age of 5 weeks (Yeo and Kim, 1997).

Drugs:

Chlortetracycline (Chlortetracycline 20%): It is used in the study as 20 % powder (Amoun Pharmaceutical Co. Egypt) and Levamisole (Ucimisole)Æ is used in the study as 10% solution produced by (Amoun Pharmaceutical Co. Egypt).

Biological Agents:

NDV vaccine

Hitchner B1 (Intervet.Holland) was used at the 7th day of age as eye drops and Lasota (Intervet.Holland) was used at the 18th day of age in drinking water.

NDV challenge strain

Velogenic Viserotropic strain of NDV (VVNDV) was kindly supplied by Dr. Abeer Sayed, immunology department, Animal Health Research Institute (AHRI) Dokki, Egypt. The challenge dose was 10^6 EID_{50}/0.2 ml/bird via intramuscular injection.

Antigen for phagocytosis

Candida albicans was obtained from Mycology Department, Animal Health Research Institute (AHRI) Dokki, Egypt. The yeast has been grown on Sabaroud dextrose agar medium 24 hours before use. It was used to determine the phagocytic activity and the percentage of chicken peripheral blood monocytes.

Media:

Roswell Park Memorial Institute (RPMI 1640) tissue culture medium with L-glutamine was purchased from EuroClone-Europe, Lymphocyte separation medium (Ficol hypaque) with density of 1.077 gm/ml, was obtained from Biochrom KG, Berlin, Foetal Bovine Serum (F.B.S) obtained from Biochrom KG, Berlin.

Reagents

Phytoheamagglutinin-L (PHA-L) (Biochrom Berline), It was obtained as lyophilized powder and reconstituted in 5 ml RPMI-0-1640 medium and used as non specific mitogen in the lymphocyte proliferation test.

Glucose reagent liquicolor used for evaluation of the lymphocyte activity.

NewCastle disease virus Antibody Kit

Commercial ELISA system (IDEXX laboratories, USA) was used according to the manufacturer's instruction.

Experimental design:

310 one day old commercial (Ross) broiler chicks were classified into 2 groups:

1. Control non-treated group (155 chicks) fed on a balanced commercial ration free from antimicrobials.
2. Treated group (155 chicks) fed on a balanced commercial ration supplied with Chlortetracycline (CTC) as a growth promoter in a dose of 200 gm/ton ration for 5 weeks.

At age of 7 days each mean group was devided into 2 subgroups (75 birds in each) as follows:

1. Control non-treated, non-vaccinated group.
2. Treated group with chlortetracycline and non-vaccinated.
3. Non-treated, vaccinated group with Hitchner B1 as eye drops.
4. Treated with CTC and vaccinated group.

At age of 17 days old, each of the four groups was further devided into 2 groups one of them was treated with Levamisole as 20 mg/kg body weight in the drinking water (Dutta et al., 1999) for 3 days, the 17^{th}, the 18^{th} and the 19^{th} day of age.

At age of 18 days old, all the vaccinated groups were vaccinated with Lasota in the drinking water. Where groups became as follows:-

1. Non-treated, non-vaccinated group.

2. CTC-treated-non-vaccinated group.
3. Non-treated-vaccinated group.
4. CTC-treated-vaccinated group.
5. Levamisole-treated-non-vaccinated group.
6.Levamisole+CTC-treated-non-vaccinated group.
7.Levamisole-treated-vaccinated group.
8.Levamisole+CTC-treated-vaccinated group.

At age of 5 weeks, the rest of birds (20 birds in each group) were fed on ration free from antimicrobials after stoppage of CTC for the treated groups, and at age of 6 weeks; all the birds in each group were challenged by the Velogenic Newcastle Virus and kept under daily observation for 3 weeks.

Blood samples

2 blood samples were collected from each bird each time (5birds from each group at 1, 2, 3, 4, and 5 weeks old); one sample was collected with heparine anticoagulant for the cellular immune response assay and used for assessment of :

Lymphocyte stimulation index against non-specific mitogen phytohaemagglutinin (PHA): A modified method of Lee (1974), (1977) and (1984), Charles et al., (1978) was carried out.

Phagocytosis percentage and phagocytic index were assessed according to Richardson and Smith (1981) a&b, Anthony et al., (1985) and Antley and Hazen (1988), While the 3rd sample did not include any anticoagulant and was centrifuged to obtain serum for humoral immune response assay, measuring the antibody titre by ELISA test.

RESULTS

Effect on the growth performance parameters:

Supplementation of ration with CTC, induced significant increases in body weights till the marketing age (6 weeks) as shown in table (1), and feed consumption/bird and as well, improved significantly feed conversion rate and feed efficiency as shown in table (2).

Effect on the cellular immune response :

a-Effect of chlortetracycline:

CTC decreased significantly the lymphocyte stimulation index against (PHA) as shown in table (3), the phagocytosis percentage and the phagocytic index in both CTC-treated-non-vaccinated and vaccinated chickens at 2-5 weeks post treatment as shown in table (4) and (5).

b-Effect of levamisole :

The drug showed significant increases in the lymphocyte stimulation index against (PHA) (table 3), the phagocytosis percentage (table 4) and the phagocytic index (table 5)in the groups treated with levamisole+CTC and either vaccinated or non-vaccinated and also in the levamisole-treated-vaccinated group after treatment with levamisole by 1, 8, and 15 days.

Table (1): Effect of Chlortetracycline (200gm/ton ration) given for 5 weeks on the weight (gm) in non-vaccinated and vaccinated, levamisole treated and non-treated chickens throughout the fattening period (6 weeks).

Age \ Group	Non-treated non-vaccinated	CTC treated-non-vaccinated	Non-treated vaccinated	CTC treated-vaccinated	Lev-treated non-vaccinated	Lev CTC-treated vaccinated	Lev - treated-vaccinated	Lev CTC-treated-vaccinated
1 day	31.25 ±0.81	31.25 ±0.81	----	----	----	----	----	----
1 week	78.4 ±9.35	87.8 ±8.27	-------	-------	-------	-------	-------	-------
2 week	189.5 a ±18.7	239.8 B ±6.03	186.3 ± 17.2	244.3 B ±5.66	------	------	------	------
3 weeks	373.2 a ±21.6	475.6 b ±9.9	367.8 ±21.7	476.8 b ±5.83	372 a ±19	478.2 b ±6.6	369.4 a ±12.3	480.6 b ±7.63
4 weeks	649.6 a ±23.9	863.2 b ±6.58	643.8 a ±22.6	863.4 b ±4.91	648.6 a ±16.5	864.2 b ±6.28	647.6 a ±20.3	867.4 b ±4.84
5 weeks	971.4 a ±23.6	1317.4 b ±6.2	966.8 a ±17.5	1315 b ±8.6	974.4 a ±16.2	1320.8 b ±5.6	975.8 a ±11.8	1323.4 b ±4.6
6weeks	1263.4 a ±22.4	1614.2 b ±7.3	1260 a ±15	1613.4 b ±6.1	1265 a ±15.4	1619 b ±5.1	1268.4 a ±8.3	1622.8 b ±5.7

CTC=Chlortetracyclin Lev=Levamisole (Mean±S.E) N=5

Means having different capital superscripts (a,B) are significantly different at ($p<0.05$) within the same raws, and

Means having different small superscripts (a,b) are significantly different at ($p<0.01$) within the same raws

Table (2): Effect of Chlortetracycline (200 gm/ton ration) given for 5 weeks old on The Feed Consumption, Feed Conversion .Ratio and Feed Efficiency in non-vaccinated and vaccinated, levamisole treated and non treated chickens at 6

Parameter / Group	Feed Consumption (gm./bird)	Feed Conversion Ratio (gm/gm)	Feed Efficiency (gm/gm)
Non=treated-non-vaccinated	2569.9	2.086 ± 0.039^{a}	0.479 ± 0.008^{a}
CTC-treated-non vaccinated	2760.2	1.744 ± 0.041^{b}	0.573 ± 0.003^{b}
Non-treated-vaccinated	2546.4	2.07 ± 0.054^{a}	0.482 ± 0.0058^{a}
CTC-treated-vaccinated	2722.1	1.719 ± 0.027^{b}	0.581 ± 0.0079^{b}
Lev-treated-non-vaccinated	2573.3	2.084 ± 0.03^{a}	0.479 ± 0.007^{a}
Lev+CTC-treated-non-vaccinated	2784.6	1.752 ± 0.01^{b}	0.570 ± 0.0024^{b}
Lev-treated vaccinated	2549.5	2.056 ± 0.021^{a}	0.486 ± 0.0046^{a}
Lev+CTC-treated vaccinated	2758.8	1.732 ± 0.042^{b}	0.577 ± 0.0021^{b}

CTC=chlortetracycline Lev=levamisole (Mean±S.E). N=5

Means having different superscripts (a, b) are significantly different at ($p<0.01$) within same column.

'able (3): Effect of chlortetracycline (200 gm/ton ration) given for 5 wks and / or levamisole (20 mg / kg b.w) given for 3 s sive days on the lymphocyte stimulation index to phytohaemagglutinin (PHA) in non-vaccinated and vaccinated chic

Group / Age	Non-treated non-vaccinated	CTC treated-non-vaccinated	Non-treated vaccinated	CTC treated-vaccinated	Lev-treated non-vaccinated	Lev +CTC-treated-non-vaccinated	Lev - treated-vaccinated	Lev +CTC-treated-vaccinated
i day	1.682 ±0.058	1.585 ±0.069	----	----	----	----	----	----
2 week	1.684 a±0.035	1.148 b ±0.0092	2.597 c ±0.0054	1.821 d ±0.0157	-------	-------	-------	----
3 week	1.733 a ±0.01	1.078 b ±0.0025	3.072 c ±0.04	1.907 d ±0016	1.753 a ±0.032	1.784 a ±0.0035	3.599 e ±0.037	3.089 c ±0.0414
4 weeks	1.833 a ±0.023	1.053 b ±0.0018	2.881 c ±0.0081	1.823 a ±0.011	1.843 a ±0.020	1.818 a ±0.013	3.214 d ±0.05	2.877 c ±0.025
5 weeks	1.749 a ±0.017	1.027 b ±0.0028	2.566 c ±0.017	1.737 b ±0.0152	1.821 a ±0.055	1.801 b ±0.034	2.817 d ±0.0101	2.57 c ±0.0169

CTC=chlortetracycline Lev=levamisole (Mean ± S.E) N=5

Means having different superscripts (a, b Ö.) are significantly different at ($p<0.01$) within the same raws.

Table (4): Effect of chlortetracycline (200 gm/ton ration) given for 5 weeks and / or levamisole (20 mg / kg b.w) given for 3 successive days on the phagocytosis percentage (%) in non vaccinated and vaccinated chickens .

Age \ Group	Non-treated non-vaccinated	CTC treated-vaccinated	Non-treated vaccinated	CTC treated-vaccinated	Lev-treated non-vaccinated	Lev +CTC-treated-non-vaccinated	Lev - treated-vaccinated	Lev +CTC-treated-vaccinated
1 day	34.6 ±0.678	34.2 ±0.860	----	----	----	----	----	----
2 week	35.2 a ±0.917	26.8 b ±0.489	39.2c± 0.663	36.3a± 0.51	-------	-------	-------	----
3 week	36.0 a ±0.837	25.2 b ±0.8	41.6c± 0.927	30.5d± 0.509	37.8a± 1.16	37.2 a ±1.28	73.6 e ±0.927	42.4 c ±1.08
4 weeks	35.4 a ±0.927	24.6 b ±1.029	40.4c± 1.03	28.7a± 0.837	36.2a± 1.068	36.4 a ±0.86	60.8 d ±1.16	41.8 c ±1.07
5 weeks	34.8 a ±0.763	24.2 b ±0.91	39.8c± 1.067	27.9d± 1.077	36a± 1.14	36.2 a ±0.927	52.2 e ±0.86	40.6 c ±0.678

CTC=chlortetracycline **Lev**=levamisole (Mean ± S.E) **N**=5

Means having different superscripts (a, b Ö.) are significantly different at ($p<0.01$) within the same raws.

Table (5): Effect of chlortetracycline (200 gm/ton ration) given for 5 weeks and / or levamisole (20 mg / kg b.w) given for 3 successive days on the phagocytic index in non-vaccinated and vaccinated chickens

Age \ Group	Non-treated non-vaccinated	CTC treated-non-vaccinated	Non-treated vaccinated	CTC treated-vaccinated	Lev+treated non-vaccinated	Lev +CTC-treated-non-vaccinated	Lev - treated-vaccinated	Lev +CTC-treated-vaccinated
1 day	1.595 ±0.013	1.584 ±0.02	----	----	----	----	----	----
2 week	1.624 ^{a} ±0.022	1.179 ^{b} ±0.025	1.778 ^{c} ±0.012	1.606 ^{a} ±0.015	-------	-------	-------	----
3 week	1.669 ^{a} ±0.041	1.080 ^{b} ±0.018	1.987 ^{c} ±0.015	1.350 ^{d} ±0.014	1.743^{a}± 0.043	1.716 ^{a} ±0.02	3.122 ^{e} ±0.12	2.01 ^{c} ±0.102
4 weeks	1.672 ^{a} ±0.044	1.065 ^{b} ±0.017	1.896 ^{c} ±0.016	1.199 ^{a} ±0.019	1.723^{a}± 0.028	1.736 ^{a} ±0.025	2.476 ^{e} ±0.11	1.932 ^{c} ±0.085
5 weeks	1.678 ^{a} ±0.012	1.039 ^{b} ±0.013	1.818 ^{c} ±0.014	1.129 ^{d} ±0.01	1.706^{a}± 0.031	1.69 ^{a} ±0.032	1.988 ^{e} ±0.023	1.851 ^{c} ±0.055

CTC=chlortetracycline **Lev**=levamisole (Mean ± S.E) N=5

Means having different superscripts (a, b Ö.) are significantly different at ($p<0.01$) within the same raws.

Table (6): Effect of chlortetracycline (200 gm/ton ration) given for 5 weeks and / or levamisole (20 mg / kg b.w) given for 3 successive days on the antibody titre in vaccinated chickens using ELISA technique

Age	Time post vaccination	Non-treated-vaccinated	CTC-treated-vaccinated	Lev-treated-vaccinated	Lev+CTC-treated-vaccinated
1 week	Zero time	423 ±31.9	428 ±27.2	-----	-----
2wweks	1wk post 1ry vaccine	1171^{a} ±50.4	959.2^{b} ±108.4	-----	-----
3weeks	3d post 2ry vaccine	572.25^{a} ±86.85	395.75^{a} ±65.95	869.3^{c} ±106.03	628^{a} ±79.3
4weeks	10d post 2ry vaccine	3181^{a} ±166.4	1264.8^{b} ±295.5	3543^{c} ±92.8	3023^{c} ±181.6
5weeks	17d post 2ry vaccine	2778^{a} ±196.96	881.8^{b} ±124.48	3170^{c} ±73.9	2590^{c} ±190.2

CTC=chlortetracycline **Lev**=levamisole (Mean ± S.E) N=5

Means having different small superscripts (a,b,c) are significantly different at ($p<0.05$) within the same raws, and

Means having different capital superscripts (a,B) are significantly different at ($p<0.01$) within the same raws.

Table (7): Effect of chlortetracycline (200 gm/ton ration) given for 5 weeks and / or levamisole (20 mg / kg b.w) given for 3 successive days on the protection against challenge with virulent NDV in ND vaccinated and non-vaccinated

Item \ Group	Non-treated non-vaccinated	CTC treated-non-vaccinated	Non-treated vaccinated	CTC treated-vaccinated	Lev+treated non-vaccinated	Lev -CTC-treated-non-vaccinated	Lev - treated-vaccinated	Lev +CTC-treated-vaccinated
Total No. of birds	20	20	20	20	20	20	20	20
No. of dead birds	20	20	2	11	20	20	1	2
No. of survived birds	0	0	18	9	0	0	19	18
Protection rate	0%	0%	90%	45%	0%	0%	95%	90%

CTC = chlortetracycline Lev= levamisole

Levamisole did not affect significantly on any of the measured parameters in the levamisole-treated-non-vaccinated group.

Effect on Humoral immune response:

a- Effect of chlortetracycline:

CTC significant reduced the antibody titre measured by ELISA test, in both CTC-treated-non-vaccinated and vaccinated chickens at 2-5 weeks post treatment as shown in table (6).

b-Effect of levamisole :

It increased significantly the antibody titre measured by ELISA test, in the levamisole-treated-CTC-treated group and also in levamisole-treated-vaccinated group at 1, 8, and 15 days post levamisole treatment (table 6).

Challeng test :

The use of CTC in the study as a growth promoter led to decreases in the protection rate of the ND vaccine against challenge of birds with the Velogenic viserotropic ND virus. As the protection rate of the vaccine in the CTC-treated-vaccinated group was 45% and in the non-treated-vaccinated group 90% as shown in table (7).

The use of levamisole increased the protection rate of the ND vaccine against challenge with the virulent strain of ND virus, and protection rate in levamisole-treated-CTC-treated-vaccinated group was 90% while in levamisole-treated-vaccinated group was 95% (table 7).

DISCUSSION

Regarding the effect of CTC (as 200 gm /ton ration for 5 weeks starting from 1 day old) on the body weight, feed consumption, feed conversion ratio and feed efficiency, the present results revealed significant improvement on all parameters. These results agreed with those obtained by Shojadoost et al., (2001) and Li et al., (2002) in broilers, and these effects on the growth performance parameters give CTC the advantage of promoting growth when is given as a feed additive. The growth promoting effect of CTC could be regarded according to Visek, (1978) to the CTC effect as an antimicrobial on digestive tract micro-organisms either stimulating the proliferation of microorganisms that positively affect nutrient utilization or inhibiting the proliferation of micro-organisms that may produce antigrowth factors or through the digestive tract thinning and the associated effects on nutrient absorption. Also, this effect could be explained according to the hypothesis suggested by Hathaway et al., (1996) regarding CTC effect on growth to an endocrine axis; increasing plasma concentration of Insulin-like-Growth Factor-I (IGF-I) which positively enhance growth, or could be attributed to the explanation presented by Rumsey et al., (2000) suggesting that the sub therapeutic feeding of CTC attenuate the response of pituitary gland to

Thyroid Releasing Hormone (TRH) giving lower plasma thyroxin level and tend to increase energy utilization, or as suggested recently by Tong et al., (2002) that dietary CTC promotes the growth performance by blocking the immune system and supported by the illustration presented by Sauber et al., (1999) who revealed that when the bird is faced by an infectious microbe, the immune system's white cells are then activated and release cytokines such as Interleukine-1 (IL-1) and Tumer Necrosis Factor (TNF) which mediate a series of metabolic adjustments that include; an elevated basal metabolic rate and body temperature, greater rates of gluconeogenesis and glucose oxidation, reduction in circulating concentrations of anabolic hormones including Growth Hormone (GH), IGF I and prolactin and stimulated release of catabolic hormones including glucocorticoids. These endocrine shifts tend to depress feed intake, tissue growth rates and efficiency of feed utilization in growing chicks as a result of clinical or subclinical infections. When birds fed sub therapeutic antibiotics, these antibiotics do not kill microbes but work by blocking the previously mentioned cytokine pathway and consequently result in birds grow rapidly but with weakened immune systems.

Concerning the effect of CTC on the lymphocyte Stimulation index against (PHA), these results revealed that CTC induced significant decreases in the lymphocyte stimulation index at 2, 3, 4, and 5 weeks post CTC treatment in both non-vaccinated and vaccinated chickens, indicating that CTC induced depression in the proliferation of T-lymphocytes against in vitro stimulation with PHA. The present data were in harmony with that of Tong et al., (2002) and may be explained according to Forsgren et al., (1980) who investigated the effect of oxytetracycline on lymphocyte proliferation in vitro and suggested that oxytetracycline may be able to penetrate lymphocytes and inhibit its protein and DNA synthesis and thereby its proliferation.

The results concerning the effect of levamisole on the lymphocyte Stimulation index against (PHA) showed that levamisole induced significant increases in CTC-treated chickens either vaccinated or non-vaccinated at 1, 8, and 15 days post levamisole treatment. These results were in consistent with those obtained by Kayatas (2002). Likewise, these findings also agreed with Drews (1990) who defined levamisole as an immunorestorative drug having the ability to restore hypofunctional lymphocytes to the level of normal function and this can be attributed to the levamisole enhancement of the intracellular concentration of cGMP (cyclic Guanosine Mono Phosphate) which results in stimulatation of protein and nucleic acid synthesis in antigen- and mitogen-stimulated lymphocytes.

Levamisole according to the present results induced significant increases in lymphocyte stimu-

lation index against PHA in levamisole-treated-vaccinated chickens after 1, 8, and 15 days post levamisole treatment. These results were supported by those obtained by Dutta et al., (1999) and attributed to the previous explanation.

Regarding the effect of CTC on the phagocytosis Percentage and the phagocytic index, the obtained results showed that CTC induced significant decreases at 2, 3, 4, and 5 weeks of CTC treatment in both vaccinated and non-vaccinated chickens. The present results were in harmony with those of Forsgren and Gnarpe (1982) who stated that tetracyclines may alter polymorphnuclear functions as a result of alteration in its morphology leading to inability of leukocytes to phagocytose yeasts and bacteria. Kucers et al., (1987) tried to investigate the adverse effect of tetracycline on the phagocytic activity and explained that by the increased urinary secretion of vitamin c during tetracycline therapy depleted leukocytes of ascorbic acid which is valuble for its phagocytic function.

Concerning the effect of levamisole on the phagocytosis percentage and phagocytic index, the resulted data showed that levamisole induced significant increases at 1, 8, and 15 days post levamisole treatment in levamisole-treated-CTC-treated chickens either vaccinated or non-vaccinated to a level comparable with that of the corresponding control non-levamisole-treated-non-CTC-treated groups. These results were confirmed by those obtained by Zhamsaranova and Lebedeva (2003) in mice immunosuppressed by herbicide 2,4-D and can be regarded to the explanation presented by Drews (1990) in which he suggested that levamisole can enhance production and secretion of IL-2 (Interleukin-2) and interferon.

According to Sheehan (1997), IL-2 and interferon-gamma are secreted by TH1 (T-helper-1) and function to promote activation of Tc (T-cytotoxic) cells, NK (Natural Killer) cells and macrophage, and consequently phagocytic activity.

The results concerning the effect of levamisole on the phagocytosis percentage and phagocytic index in levamisol-treated-vaccinated chickens showed that levamisole induced significant increases at 1, 8, and 15 days post treatment and agreed with those obtained by Afifi (1990) and may be regarded to the previous explanation.

In concern to the effect of CTC on the antibody titre, these results showed that CTC induced significant decreases in CTC-treated-vaccinated group at 2, 3, 4, and 5 weeks post treatment. These findings were in harmony with those obtained by Rzedezicki et al., (1991) and Tong et al., (2002) in chickens against ND vaccine and can be explained according to Naqi et al., (1984) who regarded this effect to the lower number of Ig-positive cells in gut associated lymphoid

tissues as a result of tetracyclines treatment, or can be attributed according to Booth and McDonald, (1988) to the inhibition caused by CTC on lymphoid tissue proliferation and reduction of splenic live cell nuclei.

Regarding the effect of levamisole on the antibody titre of immunosuppressed chickens (treated with CTC) and vaccinated, the obtained data revealed that levamisole induced significant increases at 1, 8, and 15 days post treatment, and restored the antibody level of chickens to a level comparable with the non-CTC-treated-vaccinated chickens. These results were confirmed by those obtained by Panda and Rao (1994) in chickens immunocompromised with infectious bursal disease virus.

In respect to the effect of levamisole on the antibody titer in levamisole-treated-ND-vaccinated chickens, the present study showed that levamisole induced significant increases at 1, 8, and 15 days post treatment. The results obtained by Rao et al., (1996) using infectious bronchitis vaccine, and Kalita and Dutta (1999) using ND vaccine confirmed the present results. This agreed with the findings expressed by Zhang et al., (1999) who revealed that levamisole enhanced B-lymphocyte differentiation, and supported by Sun et al., (2003) who suggested that levamisole may modulate serum Intreleukin-6 (IL-6) level which is according to Sheehan (1997), secreted by TH2 (T-Helper-2) and can promote B-cell activation, proliferation and differentiation into antibody producing plasma cells.

Regarding the effect of CTC on the protection rate of ND vaccine after challenge with VVND virus, the present data showed that mortality rate in CTC-treated-vaccinated group were (11/20) 55% in compare with (2/20) 10% in non-CTC-treated vaccinated group, giving evidence of CTC to decrease efficiency and protection rate of ND vaccine from 90% to 45% as showen in the present experiment.

Concerning the effect of levamisole on the Protection rate of ND vaccine after challenge with VVND in levamisole-treated-CTC-treated group, the present study showed marked decrease in mortality rate from (11/20) 55% to (2/20) 10% and consequently increase in protection rate of the vaccine from 45% to 90%. These results agreed with those obtained by Rao and Chakravarty (1999) in chickens immunosuppressed by low doses of aflatoxine B1.

In regard to the effect of levamisole on the protection rate of ND vaccine after challenge with VVND virus in levamisole-treated-non-CTC-treated group, the present results showed marked elevation from 90 % in non-levamisole-treated-vaccinated chickens to 95% in levamisole-treated-vaccinated chickens and this was supported by the

results obtained by Dutta et al., (1993).

REFERENCES

Adair, B.M. (1996): Poultry Immunology. P.301-315,Carfax Publishing Company ,New York.

Afifi, M.A.A. (1990): Studies on the role of some immunostimulants in using Poultry vaccines. PhD thesis presented to Fac. Of Vet. Med., Cairo University.

Anthony, T.W.; Irwin, K.M.; Erin, M.W. and Michael, E.M. (1985): Phagocytic and killing capacities of uterine derived leukocytes from mares resistant and susceptible to chronic endometritis.Am J. Vet. Res. 46 (9); 1938-1940

Antley, P.P. and Hazen, K.C. (1988): Role of yeast cell growth temperature on Candida albicans virulence in mice. Infect. Immmunity 56, 2884-2890

Booth, N.H. and McDonald, L.E. (1988): Veterinary Pharmacology and Therapeutics. 6th Ed. P. 813-820 and 897-904, Iowa State University Press/Ames.

Buerstedde,J.M. ; Chausse, A.M. ; Mckay, J. ; Marechal, D. ; Bumstead, N.; Bernardi, G. and Young, J.R. (1999): Improving chicken immunity. The chicken image project. EUROPA Research.

Charles, R.; Carpenter, A.B.; Henry, R. and Bose, J.R. (1978): Suppression of the mitogen-stimulated blastogenic response during reticuloendotheliosis of virus induced tumorigenesis. J. Immunol. 120(4) 1313-1320

Drews, J. (1990): Immunopharmacology Principles and Prespectives. P. 274-277, Springer Verlage, Berline, New York, London, Paris.

Dutta, T.C.; Kalita, C.C. and Dutta, G.N. (1993): Effect of Biovet as an immunomodulator in Newcastle disease vaccination. Journal of Assam Veterinary Council. 3 : 53-54

Dutta, T.C.; Kalit, C.C. and Dutta, G.N. (1999): Immunomodulatory effect of levamisole on cell-mediated immune response in broiler chicks vaccinated against Newcastle disease.Indian J. of Poultry Science. 43 : 3, 384-386

Forsgren, A.; Banch, G.; Backman, H. and Bellashene, A. (1980):Antibiotic host defense interaction in vitro and in vivo. Scand. J. Infet. Dis. 24 (suppl):195-203

Forsgren, A. and Gnarpe, H. (1982):The effect of antimicrobial agents on the association between bacteria and leukocytes. Scand. J. Infect. Dis. 33: 115-118

Giambrone, J.J.(1994):Immunosuppression in poultry: Causes and Prevention. The Alabama Cooperative Extension Services. Auburn University.

Hathaway, M.R.; Dayton, W.R.; White, M.E.; Henderson, T.L. and Henningson, T.B. (1996): Serum Insulie like Growth Factor-I (IGF-I) concentration are increased in pigs fed antimicrobials. J. Anim. Sci. 74 : 1541-1547

Kalita, D.N. and Dutta, G.N. (1999): Immunomodulatory effect of levamisole upon Newcastle disease, pigeon pox and Marek's disease vaccination in broiler chicks. Indian Veterinary Journal. 76:6, 490-492

Kayatas, M. (2002): Levamisole treatment enhances protective antibody response to hepatitis B vaccination in hemodialysis patients. Artif. Organs. 26 (6) :492-496

Kucers, A.; Bennett, N,M. and Kemp, R.J. (1987): The Use of Antibiotics. 4th Ed., P. 879-1025. J.B. Lippincott Company, philadilphia.

Lee, Lucy F. (1974): Stimulation of avian peripheral lymphocytes. Avian Dis.,18,4:602-609

Lee, Lucy F. (1977): Chicken lymphocyte stimulation by mitogens: A microassay with whole blood cultures. Avian Dis., 22, 2:296-307

Lee, Lucy F. (1984): Proliferation response of chicken T and B-lymphocytes to mitogens. Chemical Regulation of Immunity in Vet. Med. 15:44-52

Li, Y.Y.; Li, P.G.; Tang, S.L.; Zhang, X.Z.; Fan, X.Z.; Shi, Q.M. and Ma, Z.J. (2002): Application effects of avian serum immunoglobuline agents. China Poultry. 24:8, 15

Lowenthal, J.W. (2001): New Therapeutics for Poultry. Rural Industries Research and Development cooperation. No. 01/149

Naqi, S.A.; Sahin, N.; Wagner, G. and Williams, J. (1984): The adverse effects of antibiotics on the development of gut associated lymphoid tissues and the serum immunoglobulin in chickens. Am. J. of Vet. Res. 45:7,1425-1429

Panda, S.K. and Rao, A.T. (1994): Effect of levamisole on chicken infected with infectious bursal disease (IBD) virus. Ind. Vet J. 71:5, 427-431

Rao, A.T. and Chakravarty, B.S. (1999): Effect of levamisole on immune status in experimental aflatoxicosis in chickens. Ind. J. of Vet. Pathol. 23:1-2, 33-35

Rao, T.V.S.; Jaiswal, T.N. and Mishra, S.C. (1996): Effect of levamisole on immune response in chicks vaccinated with infectious bronchitis virus. Indian J. of animal Sciences. 66:11, 1095-1099

Richardson, M.D. and Smith, H. (1981 a): Resistance of virulent and attenuated strains of Candida albicans to intracellular killing by human and mouse phagocytes. The J. of Infectious Disease. 144, 6:557-564

Richardson, M.D. and Smith, H. (1981 b): Production of germ tubes by virulent and attenuated strains of Candida albicans. The J. of Infectious Disease. 144, 6:665-669

Rumsey, T.S.; Mcleod, K,; Elsasser, T.H.; Kahl, S. and Baldwin, R.L. (2000): Performance and carcass merit of growing beef steers with chlortetracycline modified sensitivity to pituitary releasing hormones and fed dietry protein levels. J. Anim. Science. 78:11, 2765-2770

Rzedzicki, J,; Cybulska, R.; Trawinska, B.; and Jaworska-admu, J. (1991): Effect of some antibacterial agents on the immune response and intestinal mucosa of chickens.(polish), Zeszyty Naukowe Akademii Rolniczejwe Wroclawiu, Weterynaria. No.49, 179-189

Sauber, T.E.; Stahly, T.S. and Nonnecke, B.J. (1999): Effect of level of chronic immune system activation on the lactational performance of sows. J.Anim. Scien. 77:1985-1993

Sheehan, C. (1997): Clinical Immunology, Principles and Laboratory Diagnosis. Lippincott, Philadilphia, New York.

Shojadoost, B.; Bozorgmehri, F.M.H. and Tabalabayi, A.H. (2001): Study the effect of some antibiotics on performance of broilers. J. of Faculty of Vet. Med., University of Tahran. 56:1, 32-36

Sun, A.; Chia, J.S.; Chang, Y.F. and Chiang, C.P. (2003): Levamisole and Chinese medicinal herbs can modulate the serum interleukin-6 level in patients with recurrent aphthous ulcerations. J. Oral Pathol. Med. 32(4): 206-214

Tong, J.M.; Zhang, R.J.; Sa, R.N.; Pan, S.Y.and Huang, Y. (2002): Immunosuppressant effect of long term and

subtherapeutic chlortetracycline in broiler. Agricultural Sciences in China 1:4, 472-476

Visek, W.J. (1978): The mode of growth promotion by antibiotics. J. Anim. Scien. 46:1447-1469

Yeo, J. and Kim, K. (1997): Effect of feeding diets containing an antibiotic on growth and intestinal urease activity in broiler chicks. Poultry Scien. 67: 331-335.

Zhamsaranova, S.D. and Lebedeva, S.N. (2003): Correction of immunodeficiency status caused by herbicide 2,4-D in experiment Gig. Sanit. May-Jun. ;(3): 55-57

Zhang, R.J.; Tong, J.M. and Huang, Y. (1999): Effects of levamisole in diets on immune function in broiler chicks. Chinese J. of Animal Science. 35:5, 5-8