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INFLUENCE OF THE ADMINISTRATION OF DRIED CALF THYMUS OR CALF THYMUS EXTRACT ON PERFORMANCE AND IMMUNITY OF BROILERS

By

F.F.MOHAMED [1] ; JAKEEN EL-JAKEE[2] and G.S. ESSAWY[3]

1- Department of Nutrition and Clinical Nutrition.
2- Department of Microbiology.
3- Department of Physiology.
Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

SUMMARY

The effect of administration of dried calf thymus (DCT) or calf thymus extract (CTE) with protein concentration 6 mg/ml was studied using 4 groups of one-day old Cobb chicks. The first group was fed on a diet contained 1% (DCT), the second group received orally 1.5 ml/bird (CTE), the birds in the third group received 0.3 ml/bird (CTE) by I/P injection, while the fourth group was the control one. The treatments were administered daily for 20 days starting from the second day of the study. Production performance and immune parameters used were; body weight, body gain, feed consumption, feed efficiency, total and differential leukocytic count, total serum proteins, albumin, globulins, relative weights of thymus, bursa and spleen, percent of active phagocytes in the peripheral blood, phagocytic index, HI antibody titer against NDV as well as wattle dermal testing using oil adjuvant killed NDV vaccine. Results showed that treated groups had significantly higher body weights and gain as well as better feed efficiency. The injected group had higher total leukocytic count, serum globulins and percent of active phagocytes. The relative weights of bursa and thymus were significantly higher in the (DCT) group only. The obtained results augment the supposition of presence of growth factor (s) in calf thymus extract and its (their) possible involvement in modulating immunity in broilers.

Key words: broilers, thymus, thymus extract, performance, humoral immunity, cell mediated immunity.

INTRODUCTION

The thymus is an important organ which plays a crucial role in the formation of the lymphoid structures in the embryonal and early postnatal period of life and in orchestrating the lymphoid system throughout life. Different chemically defined polypeptides have immuno-modulating activities were isolated from calf thymus (Burstein et al., 1988). Various studies with the thymus extract have indicated a variety of beneficial effects. The extracts of the thymus of poultry (Murthy and Ragland, 1984) and mammals (Schulof et al., 1989) have immunomodulating effects. The thymus extract increased lysozymes activity, T-lymphocytes, total blood protein and haemoglobin in claves (Nikitenko et al., 1984), restored the depressed function of the humoral and cellular systems of

alloxan-diabetic mice (Hadzija et al., 1987) and resulted in a partial regression of metastatic hepato-cellular carcinoma in a case report (Palmieri et al., 1990). The thymus extract also enhanced the blastogenic response of peripheral blood lymphocytes to PHA and Con A mitogens in chickens (Murthy and Ragland, 1992). It has been recognized that a small but highly significant increase (4.2%) in weight gain in chicks fed diets contained 5.3% fresh calf thymus (1.06% based on dry matter contents) as stated by Ross et al., (1955). Also, the injection of the oily extract of buffalo calves thymi and/or feeding dried glandular tissues (after extraction) increased egg number, percent of egg production, average egg mass as wel as egg weight (Abd El-Aziz, 1977). This study was carried out to determine the effect of supplementation of dried calf thymus or calf thymus extract on growth performance and some immunity parameters in borilers.

MATERIAL AND METHODS

Birds: One hundred and sixteen, commercial, one day old, Cobb chicks were randomly allocated into four groups each of twenty nine. The chicks were floor reared in an electrically heated room provided with clean feeders and waterers and kept under standard hygienic and management conditions. The birds were fed ad-libitum on unmedicated , mycotoxin free starter diet (Table 1) with constant access to fresh water.

Thymic preparation: Fresh calf thymi were obtained from the abattoir and transported directly to the laboratory. Fat, membranes as well as blood clots were removed . The glandular tissue was then homogenized. For preparation of the dried calf thymus, thin layers of the homogenized glandular tissue were left at warm air (38°C) until drying. The dried glandular tissue was then ground into powder. The calf thymus extract was prepared by the method described by Plakhotin, (1982). The protein content was estimated (Bradford, 1976) and adjusted to be 6 mg/ml.

Vaccinal Strain of NDV: The lentogenic NDV LaSoto strain vaccine produced by Intervet International B.V. Boxmeer-Holland (Batch # 80536A) was found to have a titer of 10^9 EID_{50}/ml. The vaccine was preserved at 4°C until date of vaccination.

Antigen Used For HI Test: The lentogenic LaSota vaccinal strain of NDV was passaged 2 times in the allantoic sac 9-11 days old Embryonated chick egg (ECE) before being used. Eight HA units suspended in PBS with pH. 7.2 were used.

Antigen Used for dermal Reaction: NDV killed virus vaccine (Pestikal oily vac, inactivated. Batch # 2411112 Pliva, Zagreb, Croatia).

Blood Samples : At the age of 35 and 42 days, Two mls of blood were obtained via cardiac puncture from each bird in a sterile plastic centrifuge tube containing heparin (20 iu/ ml) for determination of total and differential leukocytic count and for separation of lymphocytes and phagocytosis assay. Three mls of blood were also obtained from each bird in sterile tubes for serum separation for assessing the HI titer, total protein and albumin at age of 35 days.

Media, Stains and Reagents:

- RPMI 1640 tissue culture medium (Flow Lab, UK)
- Ficol Hypaque (Sigma, USA) with 1.077 density.
- Sterile Foetal Calf Serum ((Gibco Ltd, UK, Batch # 6208A) which was kept at -20°C after

heat inactivation at 56°C for 30 min. It was added at a final concentration of 10-15% to the RBMI medium.

- Phosphate Buffer Saline (PBS, pH. 7.2).
- Washed Sheep RBCs for determination of phagocytic activity and percent of peripheral blood monocytes.
- Trypan Blue Stain (Difco Lab, UK) to determine vitality of lymphocytes.
- Giemsa stain (Sigma, USA).

Methods:

Experimental Design: The birds were allocated into four groups . The first group was fed on a diet contained 1% DCT, the second group received orally 1.5 ml/bird CTE, the birds of the third group received 0.3 ml/bird CTE by I/P injection, while the birds of the fourth group were kept as control. Treatments were administerd daily for 20 days staring from the second day of the study (two days old).

Body weight Development: Birds were weighed individually every week. Feed consumption and conversion as well as weight gain were calculated.

Vaccination with NDV Vaccine: The chicks were vaccinated on day 14 and 28 by eye drop instillation method using a standard dropper (0.5 ml/drop containing $10^{5.8}$ EID_{50}), each bird received 2 drops; one in each eye.

Hemagglutination Inhibition Test (HI): was carried out according the standard procedure (Majiyabe and Hitchner, 1977).

Serum Total protein and albumin were assessed using commercial kits (BioMerieux, ref # 6 1602, BioMerieux, ref # 1105; respectively).

The total leukocytic count was performed according to the method of Nutt and Herrick, (1952), while the differential leukocytic count was performed by the method of Schleicher, (1962). The separation and enumeration of circulating mononuclear cells from peripheral blood was carried out according to Lee, (1978 & 1984).

The blood monocyte culture and phagocytosis assay were performed as described by Chu and Dietert, (1989). Percentage of phagocytosis, phagocytic activity as well as phagocytic index were calculated. The lymphoid organ/body weight index was calculated for thymus, bursa and spleen according to Montogomery et al., (1985) as follows:

Organ weight/body weight multiplied by 10,000.

Dermal Reaction: At the age of 7 weeks, 5 birds from each treated group were injected each, intradermally at the right wattle with 0.1 ml of killed oil adjuvant NDV vaccine, while the left side was left as control. Five birds from the control group were injected each, with 0.1 ml autoclaved normal saline. All birds were kept under observation and were examined for reactivity at different time intervals. The interpretation was based on the difference between the thickness of indurated areas injected and control wattle in each bird.

Statistical Analysis: The obtained data were analyzed by one way analysis of variance using Minitab Data Analysis Soft Ware (1986).

RESULTS AND DISCUSSION

The body weight development data are shown in

Table (2). Results indicated that treated groups were significantly higher compared to the control group regarding the body weights starting from the third week of the study in the DCT and CTE (I/P) groups but from the fourth week in the CTE (oral) group. Old data (Ross et al., 1955) pointed out that chicks fed diet contained 5.3% fresh calf thymus (1.06% based on dry matter content) had a small but highly significant increase (4.2%) in weight gain confirming earlier report on the presence of a chick growth factor (s) in calf thymus. Data of the total gain, feed consumption and conversion (Table 3) indicated that treated groups had better gain, better feed conversion and relatively less feed consumption. Although the injection of thymus homogenate increased the total serum blood protein in calves and piglets (Nikitenko et al., 1984), yet data shown in Table (4) indicated that birds supplemented with DCT or CTE (orally or by injection) had no significant difference in their total serum proteins, albumin or HI antibody titer against NDV. However, the CTE (I/P inj.) group had a significantly ($P < 0.05$) higher serum globulins. The thymus homogenate S/C injection increased lysozyme activity, T-lymphocytes in calves and piglets (Nikitenko et al., 1984). Moreover, immunomodulation and increments in several parameters of cellular immunity were observed in cases of chronic non-A, non-B hepatitis following bovine thymus extract therapy (Civeira et al., 1989). Also, calf thymus extract (TFX) ameliorated the symptoms and signs of several diseases including primary immuno-deficient diseases, bone marrow failure, autoimmune disorders as well as recurrent viral and bacterial diseases (Skotnicki, 1989). Thymostimulin (TP.1), a polypeptide thymus extract, treated cases of severe atopic eczema (Harper et al., 1991). In the present study, the oral supplementation with DCT or CTE significantly ($P < 0.01$) inreased the % of active phagocytes (Table 5), while the injection of CTE significantly ($P < 0.05$) increased the % of active phagocytes and the % of phagocytosis. Moreover, the data on the wattle reaction to injection of inactivated NDV (Table 5) indicated that treated groups were significantly ($P < 0.05$) more sensitive compared to control. The total and differentaial leukocytic count data are shown in table (6). The total leukocytic count was significantly ($P < 0.05$) higher in the injected CTE group. However, the differential leukocytic count was not significantly affected by any of the treatments. Data of the lymphoid organ weight index (Tabl 7) indicated that oral supplementation of DCT significantly ($P< 0.05$) increased the indices of the thymus as well as the bursa. The immunomodulation produced by administration of DCT or CTE could be attributed to its nucleoproteins accumulated in its cells. The several thymic factors including thymic hormone are probably, at least partially, responsible for thymus immunomodulating activity. The effect of thymus administration appears more obvious in cases of primary or secondary immunodeficiency.

Conclusion:

DCT or CTE stimulates growth and generally inproves immunity in broilers.

CONCLUSION: DCT or CTE stimulates growth and generally improves immunity in broilers

Table(1): The Composition of the Diet Used in the Study

Ingredient	%
Yellow corn	63.3
Soybean oil meal (44 % protein)	31
Meat and bone meal (60 % protein)	3
Bone meal	1.6
Ca Co_3	0.38
Nacl	0.35
Vitamin & Mineral mixture*	0.3
D L methionine	0.07
ME kcal/kg	2910
CP %	21
Calorie/protein ratio	138

* Nutrient supplied per kilogram: Vit. A 7700 IU; Vit. D_3 1650 ICU; Vit. E 5 mg; Vit. K_3 30 mg;vitamin B_2 4.5 mg; Niacin 28 mg; Pantothenate 6.6 mg; Vit. B_1 15 mg; folic acid 0.44 mg; Biotin 750 mg, Choline 60 mg; Vit. B_{12} 9 ug; zinc 60 mg;selenium 1 mg; manganese 75 mg; copper 4 mg; iron 40 mg menadione sodium bisulfite 2.2 mg; ethoxyquin 62 mg and iodine 1 mg.

Table (2): Effect of Dried Calf Thymus or Calf Thymus Extract On Body Weight Development of Broilers (g.).

Group Age	DCT	CTE, oral	CTE, I/P inj	Control
Initial Wt	38	41	39.3	39.1
First Week	101.5 ±2.6	100.8 ±2.9	102.3 ±2.5	96.9 ±2.9
Second Week	255 ±8.6	209 ±7.3	219.6 ±5.2	201.6 ±8.5
Third Week	367.1* ±13.3	343.1 ±10.8	357.9* ±9.6	327.6 ±9.4
Fourth Week	601.9* ±20.5	610* ±15.6	623** ±14.6	552.3 ±17.4
Fifth Week	939.4** ±26.1	963.2** ±24.4	928.6** ±20.8	810.3 ±23.7
Sixth Week	1303.5** ±36.3	1310.5** ±44.8	1247.5* ±29.3	1127.1 ±30.1

-Values are means ± standard deviation.
* Significantly differ at P<0.05 compared to control.
** Significantly differ at P<0.01 compared to control.

Table (3): Effect of Dried Calf Thymus or Calf Thymus Extract on Overall Performance of Broilers .

	DCT	CTE, oral	CTE, I/P inj	Control
Initial Wt (g.)	38	41	39.3	39.1
Final Wt (g.)	1303.5	1310.5	1247.5	1127.1
Total Gain	1265.5	1269.5	1208.2	1088
Total Feed Consumed (g.)	2790	2760	2790	2850
Feed:Gain Ratio	2.2	2.17	2.3	2.61

Table (4) Effect of Dried Calf Thymus or Calf Thymus Extract on Serum Total Proteins, Albumin, Globulins as well as on the Mean HI Antibody Titer Against NDV Vaccine.

Assay	DCT	CTE, oral	CTE, I/P inj	Control
Total Protein g.%	4.05 ±0.55	4.3 ±0.15	4.52 ±0.2	4.17 ±0.13
Albumin g.%	2.1 ±0.19	2.22 ±0.21	2.08 ±0.16	2.44 ±0.18
Globulin+ g.%	2.0 ±0.3	2.14 ±0.5	2.5* ±0.7	1.75 ±0.1
HI titer	24.4 ±7.3	26.8 ±6.6	28.8 ±9.9	26.6 ±7.4

- Values are means ± standard deviation.
- n=8
* Significantly differ at $P<0.05$ compared to control.
+ Calculated by subtracting the albumin value from total serum protein.

Table (5): Effect of Dried Calf Thymus or Calf Thymus Extract on Phagocytic Activity %, Phagocytosis %, Phagocytic Index And Wattle Reaction to NDV Inoculation.

Assay	Age - Days	DCT	CTE, oral	CTE, I/P inj	Control
Phagocytic Activity %	35	59.4* ±4.2	55.3* ±6.7	60.3* ±9.9	39.3 ±8.4
	42	55.3* ±8.1	54.9* ±3.9	58.5* ±3.6	39.7 ±8.9
% of Phagocytosis	35	45.9 ±7.0	44.4 ±8.7	71.2** ±16.9	37.2 ±8.3
	42	45.7 ±9.4	45.2 ±8.8	63.4** ±15.2	38.3 ±9.1
Phagocytic Index	35	0.24 ±.07	0.32 ±.17	0.28 ±.05	0.35 ±.09
	42	0.33 ±.09	0.25 ±.09	0.31 ±.18	0.33 ±.12
Wattle Reaction† (mm) 24 h. after inoculation		1.9* ±.4	1.76* ±.32	1.6* ±.3	0.82 ±.25

- Values are means ± standard deviation.
- n=8
† n=5
* Significantly differ at P<0.05 compared to control.
** Significantly differ at P<0.01 compared to control.

Table (6): Effect of Dried Calf Thymus or Calf Thymus Extract on the Total and Differential Leukocytic Count of Broilers at 42 days

Group Item	DCT	CTE, Oral	CTE, I/P inj	Control
WBCs $x10^3$/ul	24.9 ±5.4	27.8 ±4.6	53.2** ±6.7	23.3 ±3.23
Lymphocytes %	68.38 ±3.34	69.38 ±2.26	72.00 ±2.39	70.75 ±4.10
Monocytes %	5.25 ±1.04	5.0 ±1.2	5.0 ±1.31	4.75 ±1.39
Heterophils %	22.75 ±4.65	20.88 4.29	16.50 ±3.42	19.50 ±4.14
Eosinophils %	3.00 ±1.69	4.13 ±1.39	5.38 ±1.06	4.25 ±1.91
Basophils %	0.63 ±.74	0.5 ±.53	0.63 ±.74	0.75 ±.89

-Values are means ± standard deviation.
** Significantly differ at P<0.01 compared to control.
- n=8

Table (7) Effect of Dried Calf Thymus or Calf Thymus Extract on the Lymphoid Organ Weight Index

Lymphoid Organ	Age in Days	DCT	CTE, oral	CTE, I/P inj	Control
Thymus	35	56.2* ±16.4	48.2 ±10.3	47.2 ±9.4	42.6 ±7.4
	42	42.8* ±10.7	36.2 ±9.4	32.6 ±4.4	30.5 ±8.7
Bursa	35	38.6* ±2.6	25.02 ±7.7	26.2 ±10.8	22.4 ±10.8
	42	32.4* ±7.7	26.9 ±8.4	24.2 ±6.3	23.2 ±3.6
Spleen	35	15.8 ±1.2	13.7 ±1.2	14.5 ±2.3	14.4 ±4.1
	42	13.5 ±3.1	11.6 ±2.4	10.0 ±1.8	12.4 ±1.2

- Values are means ± standard deviation. - n=8
* Significant at P<0.05 compared to control.

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