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EFFECT OF YUCCA SCHIDIGERA EXTRACT ON IMMUNE PARAMETERS OF AMMONIA STRESSED BROILER CHICKENS.

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SUMMARY

This study was carried out in a private broiler chicken farm at Sharkia Governorate .The chickens were reared under bad ventilation system, overcrowding (28 birds/m2) and high levels of humidity and ammonia (70-100 ppm). Birds were 35 days old with weight range of 800-900 gm. The chickens were divided into two equal groups in the same house with plastic and wire screen, group (B) left untreated and group (C) treated with Yucca schidigera extract (100mg/kg of feed) for one week. The control group (A) was obtained from another farm of the same age, breed, ration and protocol and reared under good condition with nearly normal level of ammonia (15-20 ppm). The results revealed that chickens under high levels of ammonia showed poor feed conversion and significant decreases in the body weight gain, feed intake, plasma interleukein-2 (IL-2), serum total, α and γ globulins in addition to leucocytopenia associated with lymphocytopenia and eosinopenia. However it showed significant increase in the levels of plasma adrenocorticotrophic hormone (ACTH). Our results pointed out that administration of Yucca schidigera extract to chickens under high level of ammonia minimized the adverse effect of ammonia on body weight, feed intake, feed conversion, immuno-endocrine parameters (ACTH & IL-2), globulins and leucogram.

INTRODUCTION

Yucca schidigera plant contains three steroid saponins (sarapogenin, smilogenin and hecogerim) that inhibit the intestinal ammonia which is highly correlated with ascitis syndrome (Podogorski et al., 1996). Feeding of Yucca schidigera inhibits

urease activity and reduces ammonia contents in small and large intestine, in the litter and environment (Tymczyna et al., 1996), increases weight gain and improves feed conversion, reduces mortality and improves hazardous effect of ammonia on the broiler chickens health (Koral et al., 1995 and Selim, 2001).

High levels of ammonia increase the incidence of diseases (Oyetunde et al., 1976), decrease growth rate (Quarles and Kling, 1974 and Deaton et al., 1984) and it is harmful to the nervous system and pathogenesis of hepatic encephalopathy (Rabbe, 1987 and Cornelis et al., 1993).

Biological responses to stress can involve physiological mediators that regulate activity between neuroendocrine and immune systems (Dietert and Golemboski, 1994). The neuroendocrine system can exert immediate feedback regulation on the immune system as well as control specific aspects of immune differentiation and development, furthermore, the primary lymphoid organs, immune and accessory cells are known to produce a variety of secreted products or cytokines that have the potential role not only for the regulation of immune function but also for mediating neuroendocrine activities (March and Scanes, 1994).

One of major cytokines is interleukin-2(IL -2)-produced mainly by T-helper lymphocytes that affects many facets of the immune response, as augmenting proliferation and activity of cytotoxic T-cells , enhancement of gamma interferon production, activation of natural killer cells and participation in T -dependent B -cells responses (Farrar et al., 1982).

Virtually, immune assessment relative to environmental-immune interactions can enhance the efficiency of the production operation and produce the welfare of the animals during the production cycle (Dietert and Golemboski, 1994).

The present study was carried out to clarify the effect of feed addition of Yucca schidigera extract on body gain, feed conversion and immunoendocrine status of chickens exposed to high levels of environmental ammonia. The measured immunoendocrine parameters were adrenocorticotrophic hormone (ACTH), interleukin- 2 (IL-2), serum total protein, albumin and total, α, B and γ-globulins. In addition to total and differential white blood cells count.

MATERIAL AND METHODS

- Yucca schidigera extract powder obtained from JEFO Nutrition Inc., Canada.

Field Trial:

The trial was carried out in a private chicken farm at Sharkia Governorate suffering from overcrowding and bad ventilation system recording high levels of humidity and ammonia (70-100 ppm) . Ammonia was recorded by ammonia analyzer bendix-Gastec. Birds were 35days old with

weight range of 800-900 gm . The chicken house was divided by a plastic and wire screen into two sections (Groups B and C) with separate feed and water supplies, so that there was similar conditions for all birds. The group (B) left untreated, meanwhile, group (C) received Yucca schidigera extract 100mg/ kg feed for one week. One hundred of healthy chickens (control group, A) of the same age and breed were obtained from another farm having the same protocol but reared under good ventilation system with normal level of ammonia (15-20 ppm). Weight gain was recorded for all birds of group (A) and 100 random birds from groups (B and C) on days 35 and 42 .Feed consumption and feed conversion rate were also calculated .

Sampling:

At the 42nd day of life, 10 random birds were collected from each group where two blood samples were collected from each bird. The first sample was collected on EDTA as anticoagulant for leucogram study and plasma collection where the second blood sample was used for serum 2nd collection. The separated plasma and serum were stored at - 20°C until used for biochemical analysis.

Biochemical Analysis

Plasma Adrenocorticotrophic hormone (ACTH) was estimated using radioimmunoassay kits obtained from Diagnostik product Corporation, Los Anglos according to Hendricks et al . (1995), plasma Interleukin-2 (IL-2) using ELIZA kits obtained from Genzyme Immunobiologicals. Cambridge-USA after Kuziel and Green (1990). Serum total protein (Peterst, 1968). Electrophoresis for protein fractionation (albumin, α, B and γ globulins) according to Laemmli (1970).

Leucogram Studies:

Total and differential white blood cells count were performed using Natt and Herricks diluent solution and Wright stain after Coles (1986).

Statistical Analysis:

The obtained results were analysed using ANOVA computerized programme according to Snedecor and Cochran (1980).

RESULTS

Chickens reared under high level of ammonia exhibited depression, under weight and respiratory manifestations with significant decrease in body weight, body gain, feed intake and feed conversion when compared with the control chickens or Yucca schidigera extract treated chickens. (Table. 1).

High levels of ammonia (70-100ppm) resulted in significant increase in plasma ACTH, significant decreases in plasma IL-2 and serum total, α and γ globulins without marked changes in either total protein, albumin or B-globulin.

Chickens fed ration with Yucca schidigera and exposed to high level of ammonia (group C) showed values around those of control group (Tables ,2 &3).

Results of total and differential leucocytic count in Table (4) showed leucocytopenia, lyomphocytopenia and eosinopenia in chickens under high ammonia level (group B), however treatment with Yucca schidigera extract (group C) having leucocytic picture around the control level but induced monocytosis.

Table (1): Body weight gain, feed intake and and conversion rate of 42 days old chickens under high ammonia with or without Yucca schidigera extract (YSE) in feed.(n =

Group	Parameters / Treatment	Body weight (gm)	Body gain (gm)	Feed intake (gm)	Conversion rate
A	Control	1081.3 a ± 36.08	243.75a ± 20.40	540	2.22
B	Ammonia	906.25b ± 32.87	98.75b ± 18.2	380	3.85
C	Ammonia and YSE	1037.5a ± 40.4	200.0a ± 20.56	500	2.5

Mean ± SE

Means in the same column followed by different letters are significantly different ($P < 0.05$).

Table (2): Plasma levels of Adrenocorticotrophic hormone (ACTH) and Inter-lukein-2 (IL-2) of 42 days old chickens reared under high ammonia with or without Yucca schidigera extract (YSE) in feed(n = 10)

Group	Parameters / Treatment	ACTH ng/ml	IL-2 Pg/ml
A	Control	58.2 ± 2.15b	163.4 ± 4.31b
B	Ammonia	57.0 ± 4.47b	179.8 ± 5.33^{a}
C	Ammonia and YSE	98.6 ± 1.9a	138.4 ± 6.34^{c}

Mean ± SE

Means in the same column followed by different letters are significantly different ($P < 0.05$).

Table (3): Total protein, albumin, Total globulins and its fractionations of 42 days old chickens reared under high ammonia with or without Yucca schidigera extract (YSE) in feed (n = 10).

Group	Parameters / Treatment	Total proteins	Albumin	Total globulin	α globulin	β globulin	γ globulin
		Gm/dl					
A	Control	6.1±0.36	3.08±18	3.02±0.2a	1.14±0.09a	1.07±0.06a	0.80±0.05a
B	Ammonia	5.6±0.56	3.23±0.34	2.37±0.18b	0.84±0.09b	0.90±0.09a	0.64±0.04b
C	Ammonia and YSE	6.01±0.34	3.29±0.2	3.72±0.13a	0.93±0.06a	1.0±0.06a	0.79±0.04a

Mean ± SE
Means of the same column followed by different letters are significantly different ($P < 0.05$)

Table (4): Total and differential leucocytic counts of 42 days old chickens reared under high ammonia with or without Yucca schidigera extract (YSE) in feed(n = 10)

Group	Parameters / Treatment	TLC $10^3/\mu l$	Differential leucocytic count $10^3/\mu l$					H/L
			L	H	M	E	B	
A	Control	18.7a±1.14	7.45a±0.53	10.3b±0.86	0.32b±0.05	0.31a±0.04	0.28a±0.08	1.50b±0.16
B	Ammonia	11.6b±1.08	2.3b±0.22	8.22b±0.76	0.24b±0.05	0.17b±0.05	0.26a±0.06	3.57a±0.19
C	Ammonia and YSE	19.0a±1.14	6.69a±0.34	11.1a±0.68	0.72a±0.06	0.30b±0.05	0.19a±0.01	1.66b±0.18

L: Lymphocytes H: Heterophils M: Monocytes E: Eosinophils B: Basophils
Mean ± SE
Means in the same column followed by different letters are significantly different ($P < 0.05$).

DISCUSSION

Ammonia is considered the main pollulant in poultry houses due to overcrowding and bad ventilation (Wathes et al., 1997). The range of ammonia in the present study was 70 - 100 ppm, the exposed chickens suffered from depression, respiratory troubles, with significant decrease in both body weight and body gain due to the high reduction in feed intake and feed conversion (Table,1). In the same respect decreased growth, weight gain, feed conversion and egg production was recorded also by Quarles and King (1974), Reece et al. (1980) and Deaton et al. (1984) in poultry exposed to high levels of ammonia referring that to the catabolic effect of the prolonged elevation of corticosteroids in these stressed birds.

As for the effect of ammonia on the measured immuno endocrine hormones it was noticed a significant increase in plasma ACTH accompanied with significant decrease in plasma IL-2 (Table,2). Stress conditions evoked elevated plasma ACTH in white leghorn cockerels after antigen challenge (Mashaly et a., 1993), also infection raised the levels of glucocorticoids and epinephrine (Nockles, 1996). Abdel Hady et al. (1998) recorded significant decrement in plasma IL-2 in stressed rats. There is feed back mechanism between ACTH and IL-2 as inflammatory immune stress produces peripheral cytokines like IL-1, IL-6 and tissue necrosis factor (TNF) which firstly causes appropriate stimulation of leukocytes to secrete ACTH (Mashaly et al., 1993), also causes direct stimulation to hypothalamic corticotrophin releasing factor to release pituitary ACTH (Constanti et al., 1998). Then ACTH counteracts secretion of IL-2 by feed back mechanism (March and Scanes, 1994), by the inhibitory action on both cytokine genes expression and targeT-cells (Costas et al. 1996). It is an immunosuppressor as it impairs the production of IL-2 (lymphokines) by T-cells. Thus elevation of plasma ACTH and decrement of plasma IL-2 is a phenomenon of immuno suppression, this was previously emphasized by Pages and Costa (1985) and Kempf et al. (1987) who reported immuno suppression effect of ammonia on broilers.

High levels of ammonia (70-100 ppm) didnít cause any significant changes in the levels of total protein, albumin and B globulin, meanwhile, there were significant decrease in the levels of total, and α-globulins of exposed chickens (Table, 3) ,these results were in consistent with that of Ali and Hassan (1986) and El-Dirdiri (1987) who reported that a significant decrease in total globulins in animal under toxic stress.

The recorded leucocytopenia, lymphocytopenia and eosinopenia (Table, 4) denoted a case of stress on chickens exposed to ammonia (70-100 ppm). These results are in agreement with William et al. (1973) and Coles (1986) who recorded leucocytopenia, lymphocytopenia and eosinope-

nia. Morage (1989) reported leucocytopenia and Gross and Siegel (1993) registered elevated avian heterophil / lymphocyte ratio during stress such as cachexia, irradiation, chemical or plant intoxication. They attributed such decrease to the elevated ACTH and gluococorticoids by a mechanism of depressing the lymphopoiesis arresting lymphocyte formation and also producing lysis of these cells. So that in severe stress the lymphatic tissues are depleted. Isobe and Lillehoj (1992) and Mashaly et al. (1993) suggested that elevated glucocorticoids cause redistribution of circulatory T-cells to secondary lymphoid tissues to share in immune response. Also March and Scanes (1994) recorded that the early release of ACTH by activated leukocytes may be a critical event in early lymphocyte redistribution events that facilitate the initiation of immune response.

Yucca Shidigera extract improved the chickens proteinogram, leucogram and the measured immunoendocrine parameters towards the control levels except presence of monocytosis (Tables 1,2,3 &4). These results coincided with Tymczyna et al. (1996) who attributed that to the inhibitory action of Yucca schidigera on urease activity reducing the ammonia conterntration in small and large intestine and in the environment. Also the result of Koral et al. (1995) and Selim (2001) sustained these results. The increased number of monocytes may be due to presence of damaged tissues from ammonia intoxication.

This study concluded that adding of Yucca schidigera to the ration of ammonia stressed broilers is valuable in alleviating the adverse action and prevents the possible immunosuppressive effect of ammonia.

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