Dept. of Pharmacology,
Fac. of Vet. Med. (Kafr El-Sheikh), Tanta Univ., Egypt.

INFLUENCE OF SOME ANTIBIOTICS USED AS GROWTH PROMOTORS ON IMMUNE RESPONSE OFCHICKENS

(With 5 Tables)

By

M.I. ABD EL-AZIZ and A.E. AGAG*

*: Virology department, Animal Health Research Institute,
Dokki, Cairo, Egypt.

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تأثير بعض المضادات الحيويه المستخدمه كمنشط للنمو على الاستجابه المناعيه فى الفراخ

مجدى عبد العزيز ، أحمد عجاج

تم دراسة تأثير عقار الفيرجينياميسن والافوبارسين على الوزن المكتسب وكذا الاستجابه المناعيه الخلويه والمصليه فى ديوك الهبرد. لوحظ زيادة فى الوزن الكلى ووزن الكبد فى كل الطيور المغذاه على عليقه مضاف اليها كلا العقارين. وجد نقص معنوى فى مجموعة الطيور المعالجه بالأفوبارسين فى كلا من تركيز الجاماجلوبيولين والاستجابه المصليه لخلايا الدم الحمراء للخروف، والتفاعل الجلدى لمشتق البروتين النقى وكذلك عدد خلايا الايزينوفيل والهيتروفيل فى الدم. أما فى المجموعه المعالجه بالفيرجينياميس فقد سجلت تأثير طفيفا على الاستجابه الخلويه والمصليه.

SUMMARY

The effect of virginiamycin and avoparcin on body weight gain, humoral and cell-mediated immune response in Hubbard Cockerels was investigated. In all treated birds, body weight gain and liver weights were increased. A significant decrease in the weight of bursa of Fabricius was recorded in Cockerels fed on avoparcin treated ration. There was a significant decrease in serum concentrations of gamma globulins; humoral response to sheep RBCs; cutaneous reactions to purified protein derivative or heterophil and

eosinophil counts in avoparcin treated group. Virginiamycin had a slight effect on humoral and cell mediated responses.

Key words: Antibiotics-Growth promotors-immune response- chickens

INTRODUCTION

Poultry industry is of a great importance for Egyptian consumer with somewhat cheep source of animal protein. Chickens are raised under highly intensive conditions that often lead to crowding and spreading of infectious agents. Under the assumption of controlling subclinical and clinical diseases and stimulating the growth, antibiotics are used. Although the beneficial effects of antibiotics have been widely acclaimed, their effects on the immune system in birds were largely lgnored.

LAWRENCE et al., (1979) and BEFUS et al., (1980) reported that bursa of fabricius, gut associated lymphoid tissues in birds along with the lymphoid tissues of the lung help in maintenance of serum antibody content. So, it is logical that any factor that impairs lymphoid tissues function, delays its maturity or affects its normal cell denisty would affect on the immune capability of the bird.

WEISBERGER et al. (1966); PANIGRAPHY et al. (1979); TU. (1980); FINCH (1980) and HAUSER and REMINGTON (1982) reported that several antibiotics were found to have effect on various immune functions in man and animals. In rabbits, specific antitoxin production was suppressed when an antibiotics such as oxytetracycline or gentamycin were administered simultaneously *LOCHMAN et al., 1979).*

Virginiamycin and avoparcin are among the antibiotics which have been used as feed additives to improve body weight gain *(MARCH et al., 1978; CANALE et al., 1980; PENSACK et al., 1982; MILES et al., 1984 and SHIHATA et al., 1989).*

Lack of literatures on their effects on immune response in chickens encouraged us to investigate their effect on protein fractions and immunological response in chickens. Also, the effects of virginiamycin and avoparcin on body weights in chicks were studied.

on differential leucocyte counts and delayed hypersensitivity; to purified protein derivative. They were maintained on control, virginiamycin and avoparcin rationss respectively for two weeks. Five birds in each group were inoculated intradermally (sensitised) with 0.05 ml of Bacille Calmette Guerin (BCG, 1 mg protein/ml) at the dorsum of the neck.

The remaining 5 birds in each group (non sensitised) were similarly treated but given saline instead of BCG. Feeding on the same ration was continued for further 4 weeks. The thickness of both wattles was measured using a skinfold callipar and each bird was injected with 0.1 ml of purified protein derivative (PPD; Egyptian Organisation for Biological Products and Vaccines) into the left wattle and an equal volume of sterile saline was injected intradermally into the right wattle, then wattle thickness was measured at 0, 6, 24 and 48 hours following injection with PPD. The method described by *PRESCOTT et al., (1982)* was used to measure the increase in left wattle thickness after subtracting the increase in the thickness of the right wattle injected with saline (non specific). Blood films were taken from wing vein of sensitised birds at 0, 1, 2, 4 weeks following PPD injection for differential leucocytic count *(HUDSON and HAY, 1980).*

The obtained data were statistically analysed according to *SNEDECOR (1967).*

RESULTS

As shown from table 1 addition of virginiamycin for 6 weeks significantly increased prealbumin. However, prealbumin and albumin were significantly increased with a significant reduction ($P< 0.05$) in gamma globulin levels in avoparcin treated chickens. Also, the results cleared that albumin/globulin (A/G) ratio seems to ride largely ($P< 0.05$) by continous feeding on avoparcin ration.

Chickens in the two treated groups had significant increase in body weight compared with control group. Liver weight expressed as percentage of total body weight in both virginiamysin and avoparcin treated groups were significantly increased over the control. A significant decrease ($P< 0.05$) in the weight of bursa of fabricius was recorded in avoparcin treated birds. On the other hand insignificant changes in kidney and spleen weights were shown in virginiamycin and avoparcin treated chickens (table 2).

Table 3 showed a significant decrease ($P< 0.05$) in anti-sheep RBCs hemagglutinin titres (log 2) between birds fed on avoparcin ration (4.92 ± 0.32) and control group (6.04 ± 0.41). However, the reduction in anti-

sheep RBCs hemagglutinin titres in chickens fed on virginiamycin ration was insignificant.

Table 4 showed that all sensitized birds with BCG responded to intradermal injection of PPD, the peak response was at 24 hours. A significant reduction in wattle thickness was observed in avoparcin treated chickens compared with those in non medicated birds. Injection of wattle of all unsensitized birds with PPD induced increased wattle thickness of 6.2±1.8% which nearly similar to the reaction induced by injecting saline solution (5.5±2.1%). These results indicated that the increase in wattle thickness of sensitized birds was a specific delayed hypersensitivity reaction.

The present results revealed a significant decrease in eosinophils count in chickens fed on virginiamycin mixed ration However, a significant decrease in both heterophils and eosinophils counts was observed in avoparcin treated group (table 5).

DISCUSSION

In the present investigation, the results showed insignificant changes in total plasma protein level in the two treated groups. These results are in agreement with that obtained by *NEMTEAN et al., (1971) and FATMA (1986)* and disagree with *TOURNET et al., (1964) and CZARNCKLE et al., (1981).* Also the results revealed a significant increase in prealbumin in birds treated with either virigniamycin or avoparcin. However plasma albumin level was not significantly affected by addition of virginiamycin, these results are in agreement with *FATMA (1986).*

A decrease in gamma globulin fractions in avoparcin treated chickens with a rising of albumin/globulin ratios were recorded. These results could be attributed to interference of the drug with synthesis of immunoglobulin. *COOK et al., (1984)* mentioned that antibiotics have been shown to decrease the immunoglobulin bearing cells in turkeys. In this respect, interference in protein or immunoglobulin synthesis lowered phagocytosis and reduced exposure to antigens have been indicated as possible mechanisms of immunosuppression of antibacterial drugs *(FINCH 1980, HAUSER and ROMINGTON, 1982).* On the other band *IBRAHIM et al., (1989)* stated that feeding of female fayoumi pullets on virginiamycin mixed ration increased globulin levels. These differences may be due to sex difference of the used chickens as reported by *UREST et al., (1958)* who mentioned that oestrogen activity increases globulin fractions.

The addition of virginiamycin and avoparcin to the diet of Habbard chickens resulted in a significant increase in body weight. These results agree with those reported by *MARCH et al., (1978); BERGAMASCHI (1979); GRIFFIN (1980) and SHALABY et al. (1991)* on their studies on chickens fed on virginiamycin mixed ration. Growth response to virginiamycin may attributed to its action exerted through the gut. Virginiamcin decreases the intestinal weight *(HENRY et al., 1986)* leading to increase in the absorptive capacity of the intestine *(MARCH, 1978)* and increase in the amount of metabolized energy *(HAUSER, 1951)*. Virginiamycin has also been shown to enhance the utilization of sulpher containing amino acids in pullets and broilers *(MILES et al., 1984)* and turkeys *(HARMRS and MILES, 1983)*.

Organ weights expressed as a percentage of total body weight of cockerels showed increase in liver weights in both treated groups. The significant increase in organ weights may be attributed to faster growth rate in the experimental group as a result of protein sparing effect of growth promotors *(MILS et al., 1984)*. However, decreased weight of the bursa of fabricius in cockerels fed avoparcin is in agreement with that reported by *COOK et al., (1984)* who recorded that normal development of the bursa of fabricius was retarded in turkeys fed antibiotics. This result could be attributed to the immunodepressant effect of the drug. This suggestion is supported by slightly decrease in lymphocyte counts in the same group in response to PPD injection.

The present results indicate that avoparcin interferes with humoral and cell mediated immune response in chickens as this substance significantly decreased the humoral response to injection of sheep erythrocytes and caused reduction in delayed hypersentivity reaction to PPD. Methods used in our present study to evaluate humoral and cell mediated immune response have been used by other investigators to elucidate the effect of enviromental pollutants on immune status of chickens *(ROSZKOWSKI, 1979; Prescontt et al., 1982)*.

Insignificant changes in humoral mediated immunity of cockerels fed on virginiamycin mixed ration agree with those reported by *SHALABY et al., (1991) and KHILFA et al., (1994)*.

In conclusion, our results revealed that the degree of immune depresant of virginiamycin was very slight and cell mediated responses were insignificant. On the other hand, depression of cellular and humoral immune response of chickens fed on avoparcin was more recorgnized. If the using of antibiotics is reducing infection risk and, at the same time, is reducing immunity, the benefit and risk situation should be evaluated carefully.

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Table (1): Changes in total protein (TP) and electrophoretic pattern of serum proteind of chickens fed on ration mixed with virginiamycin and avoparcin in concentration of 20 ppm for successive weeks (n=12).

Group	Time of sampling	T.P g/dl	electrophoretic pattern of protein (g/dl) pre-albumin	albumin	alpha-globulin	Beta-globulin	gamma globulin	A/G ratio
Control	0	3.55±0.19	0.51±0.02	1.54±0.05	0.40±0.02	0.70±0.06	0.40±0.02	1.02±0.04
	2	3.60±0.31	0.60±0.03	1.54±0.04	0.43±0.02	0.70±0.05	0.33±0.02	1.05±0.05
	4	3.70±0.20	0.67±0.04	1.40±0.05	0.51±0.03	0.77±0.06	0.34±0.02	0.86±0.03
	6	3.95±0.36	0.66±0.02	1.53±0.03	0.53±0.03	0.89±0.05	0.32±0.02	0.87±0.03
Virginiamycin	0	3.44±0.21	0.53±0.02	1.47±0.08	0.36±0.03	0.63±0.04	0.44±0.03	1.03±0.05
	2	3.78±0.20	0.58±0.03	1.59±0.07	0.41±0.03	0.80±0.07	0.37±0.02	1.01±0.05
	4	3.87±0.34	0.60±0.04	1.55±0.06	0.51±0.05	0.83±0.04	0.38±0.02	0.90±0.03
	6	4.11±0.28	0.76±0.03*	1.60±0.06	0.53±0.03	0.87±0.04	0.35±0.01	0.91±0.03
Avoparcin	0	3.57±0.36	0.52±0.04	1.56±0.07	0.37±0.03	0.71±0.06	0.41±0.03	1.05±0.03
	2	3.77±0.20	0.54±0.02	1.68±0.06	0.41±0.02	0.74±0.03	0.40±0.03	1.08±0.05
	4	3.90±0.23	0.66±0.03	1.60±0.08*	0.48±0.05	0.86±0.05	0.30±0.02	0.97±0.04*
	6	4.04±0.24	0.79±0.04**	1.62±0.03*	0.47±0.04	0.92±0.06	0.26±0.01*	0.96±0.03*

* Significant at $P < 0.05$

** Significant at $P < 0.01$

Table (2): Effect of virginiamycin and avoparcin (20 ppm) added to ration fed to cockerels for 6 successive weeks on weight of organs expressed as percentage of body weight. (n=12).

Grouping	Body weight in grams	Liver %	Kidney %	Spleen %	Bursa %
Control diet	1,352± 50	2.84±0.18	0.81±0.04	0.18±0.008	0.39±0.02
Virginiamycin diet	1,586±68*	3.66±0.21**	0.92±0.04	0.19±0.006	0.37±0.02
Avoparcin diet	1,492±42*	3.75±0.32*	0.94±0.05	0.20±0.007	0.31±0.03*

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table (3): Serum anti-sheep RBCs hemagglutinine titre of chickens fed on control (no drug); virginiamycin and avoparcin rations for 6 weeks, (n=8).

Group	Anti-sheep RBCs titre (log 2)
Control ration	6.04 ± 0.41
Virginiamycin ration	5.59 ± 0.58
Avoparcin ration	4.92 ± 0.32*

* Significant at $P < 0.05$

Table (4): Effect of virginiamycin and avoparcin added to rations fed to cockerels on delayed hypersensitivity to purified protein derivative (PPD), (n=5).

Group fed on	Increased wattle thickness in mm			
	0	6 hours	24 hours	48 hours
Control ration	0.84±0.03	2.13±0.05	2.26±0.06	1.98±0.05
Virginiamycin ration	0.90±0.04	2.01±0.06	2.09±0.06	1.92±0.03
Avoparcin ration	0.92±0.03	1.98±0.04*	2.02±0.05*	1.86±0.05

* Significant at $P < 0.05$

Table (5): Effect of virginiamycin and avoparcin ration (20 ppm) fed to cockerels previously sensitised with Bacille Calmette Guerin (BCG) for 6 successive weeks on leucocytic percent responses to purified protein derivative (PPD), n=5.

Group fed on	Parameters	Weeks after PPD injection			
		0	1	2	4
Control ration	Lymphocytes	59.80±1.0	63.00±1.41	64.00±2.12	60.40±2.70
	Heterophils	34.00±1.70	31.00±1.41	29.10±1.12	30.80±1.40
	Eosinophils	2.00 ±0.10	1.80±0.08	1.50±0.04	1.60±0.05
Virginiamycin ration	Lymphocytes	60.70±2.12	61.50±2.14	61.70±3.74	60.30±2.04
	Heterophils	33.80±1.17	30.80±2.81	29.07±1.86	29.88±1.98
	Eosinophils	1.89±0.07	1.78±0.06	1.69±0.08	1.36±0.09*
Avoparcin ration	Lymphocytes	59.20±2.08	59.80±2.26	60.10±2.19	58.80±2.64
	Heterophils	34.50±1.16	29.10±1.84	25.50±1.43	26.20±1.10*
	Eosinophils	1.92±0.07	1.84±0.08	1.31±0.05*	1.32±0.06**

* Significant at $P < 0.05$

** Significant at $P < 0.01$