STRESS MANAGEMENT FOLLOWING VACCINATION AGAINST COCCIDIOSIS IN BROILERS

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ABSTRACT

The present project was designed to detect the stress following vaccination (Eimeria vaccine) against coccidiosis in broilers and its management by using multivitamins and aspirin. The parameters studied were heterophil/lymphocyte ratio, total serum protein, serum glucose and serum cholesterol estimation, for three consecutive post vaccination days. At the end of experiment, on 42nd day, adrenal gland/body weight ratio and histopathology of adrenal gland was performed to assess any change. The results showed no statistically significant difference among difference groups. However, the group that was given multivitamins showed maximum weight gain and minimum stress, while the aspirin therapy did not show any significant difference.

Key words: Stress, vaccination, coccidiosis, *Eimeria*

INTRODUCTION

Several different approaches have been suggested to control coccidiosis in chickens including production of genetically resistant strains, quarantine, chemotherapy, diets with high fatty acids, feed enzymes, sanitation and immunization (McDougald and Reid, 1998). Among these, immunization is an attractive alternative. It has been reported that a small number of oocysts administered as a primary infection are capable of producing immunity in birds and protect them against repeated exposure to challenge infection (Long, 1984). These oocyst suspensions are inoculated onto feed or into the drinking water at 7-14 days of age. Since a single infection does not produce prolonged immunity to some *Eimeria sp.*, the success of controlled exposure immunization programmes depends on oocysts production in bird, fecal excretion of oocysts, sporulation of these oocysts and ingestion by the bird. It has been shown that multiple infection by small number of oocysts, called 'trickle' infection induces a more profound immune response than a single infection by a large number of oocysts (Glisson and Kleven, 1993). Owing to repeated administration of vaccine against coccidiosis, the birds are undergoing stress under the influence of oocyst suspensions which may lead to negative effects on the growth traits of the broiler flocks. It is also reported that the vaccination stress disturbs the immune response to coccidiosis (Pierson *et al.*, 1997). The present project is designed to evaluate the role of various substances for stress management following vaccination against coccidiosis in broilers. The results of this study will help in choice of medication in post-vaccination period.

MATERIALS AND METHODS

Experimental birds:

One hundred and sixty day-old broiler chicks were obtained from local hatchery and were reared under standard managemental conditions in research experimental house of Pathology Department, University of Veterinary and Animal Sciences (UVAS), Lahore. A commercial broiler feed without any anti-coccidial agent was provided *ad libitum* to the birds along with 24 hours supply of clean drinking water.

Experimental materials:

a) Coccidial vaccine (Eimeria vaccine) containing the inactivated sporulated oocysts prepared by Parasitology Department UAVS, Lahore.
   Isolation of coccidial oocysts was done from the caecal contents of chicken infected with *Eimeria tenella.* Oocysts were treated with 3% formalin for 48 hours for preparation of vaccine and were washed with nor/mal saline afterwards to neutralize the latter effects of formalin (Hashmi *et al.*, 1994).
   Dose: 1000 occysts per bird orally
b) Vety-stresscheck (Vetycare) containing multivitamins.
   Dose: 1 gm/4 lit. of drinking water.
c) Aspirin (Shamsi Pharma) containing acetyl salicylic acid.
   Dose: 180 mg/lit. of drinking water.

Experimental design:

Chicks were randomly divided into four groups (A, B, C and D) having forty chicks each on day-1 of the experiment. Experimental design is shown in Table-1.

Collection of Samples:

Blood samples from 10 randomly selected birds of each group were collected after vaccination from day 6-8 and from day 13-15 for serum separation. Fresh blood smears from all samples were also prepared. Adrenal glands were collected from 10 birds of each group after slaughtering at 42nd day of the experiment.

Experimental parameters:

a) Heterophil/lymphocyte ratio (Benjamin, 1978).
b) Estimation of serum biochemical substances i.e. total serum protein (Gornell *et al*., 1949), serum glucose (Trinder, 1969) and serum cholesterol (Richmond, 1973).
c) Determination of adrenal/body weight ratio (Giamborne and Closser, 1990).
d) Gross and histopathological examination of adrenal gland (Drury and Wallington, 1980).
e) Economics of the flock (Oyekole, 1984).

Statistical analysis:

Data thus collected was analyzed statistically (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Results are tabulated in Tables 2-7.

Heterophil/lymphocyte (H/L) ratio seemed to be higher after 48 hours of 1st shot of vaccination for all the groups. Post 2nd shot, H/L ratio was higher after 48 hours for group A but for groups B, C and D it was higher after 72 hours, but there was no significant difference among the four groups. Our results are not in line with John and Ringer (1962) and McFarlane and Curtis (1989), who described H/L ratio as a good indicator of stress in birds due to administration of ACTH. Prabhakarana *et al.* (1997) stated H/L ratio as a useful index of response of hypothalamus-hypophyseal adrenal axis to stress. This difference might be due to transient change in ACTH level as a result of mild vaccination stress in this study. Our findings are favoured by Freeman and Manning (1984).

Mean total serum protein for groups A, B, C and D was not significantly different from each other. Our findings do not agree with Shukla and Pachauri (1995), who reported that stress due to aflatoxin caused decrease in total serum protein but this difference might be due to different stressor. Our findings are in accordance with Duneva and Dimitrova (1987). Theodorakis *et al.* (1988) also indicated that levels of some classes of heat-shock protein mRNA might not be significantly different between stressed and unstressed cells.

Mean serum glucose level had no significant difference between the groups. Freeman and Manning (1980) reported that birds became hypoglycemic within one hour but afterwards they became normoglycemic within 24 hours. Similarly, Freeman and Manning (1984) reported that changes in serum glucose levels were transient following vaccination. But in this study no observation was made in first 24 hours and this might be the possible reason for the normal serum glucose level in our study. Freeman *et al.* (1979) observed that glucose level was within the normal range after 24 hours after the injection of corticotropin. Our findings are in line with the results of above workers but differ with Siegel and Beane (1961) and Latour *et al.* (1996) because these research workers used ACTH as stressor without mentioning the time limit of hyperglycemia. Stress in this study was of mild nature while change in serum glucose concentration for longer period was possible only in severe and persistent type of stress (Freeman and Manning, 1978).

Serum cholesterol levels were not significantly different among groups. These results do not agree with the findings of Siegel and Beane (1961), Shukla and Pachauri (1995), Mate *et al.* (1996), Latour *et al*, (1996) and Puvadolpirod and Thaxton (2000) possibly due to different stress factors and climatic conditions but corroborate with Freeman and Manning (1984) and John and Ringer (1962).

On 42nd day of age, mean adrenal gland/body weight ratio was not significantly different among birds of four groups. Our results are in line with the findings of Siegel and Beane (1961), John and Ringer (1962) and Freeman and Manning (1982), who concluded from their experiments that adrenal weight determination, as a stress indicator was unreliable. The finding of this study differs from Conner (1959), who reported enlargement of adrenal glands due to sever stress. This difference was probably due to mild nature of vaccination stress in this study.

No significant gross and histopathological lesions were observed in adrenal glands of birds of four groups. It might be due to two reasons; firstly stress was of mild nature, which might have caused changes in adrenal gland at molecular and ultra structural level which were not detectable in this experiment and secondly the adrenal gland was studied at the end of experiment and till that time the birds might have recovered from the effects of mild stress. Our results are similar to Freeman and Manning (1982), who also reported that

Table 1: Experimental design

Treatment	Experimental day	Groups			
		A	B	C	D
Vaccination against coccidiosis	5th	-	+	+	+
	12th	-	+	+	+
Vety-stress check	5-7th	-	-	+	-
	12-14th	-	-	+	-
Aspirin	5-7th	-	-	-	+
	12-14th	-	-	-	+

Table 2: Mean values (± S.E.) of heterophil/lymphocyte ratio of experimental and control groups

Groups	Shot	Post vaccination period (hrs) 24	48	72
A	1st	0.50 ± 0.02	0.52 ± 0.02	0.51 ± 0.03
	2nd	0.52 ± 0.03	0.57 ± 0.02	0.55 ± 0.03
B	1st	0.45 ± 0.02	0.51 ± 0.02	0.49 ± 0.03
	2nd	0.46 ± 0.04	0.43 ± 0.02	0.47 ± 0.03
C	1st	0.54 ± 0.02	0.58 ± 0.03	0.55 ± 0.03
	2nd	0.60 ± 0.03	0.62 ± .0.02	0.63 ± 0.03
D	1st	0.51 ± 0.03	0.57 ± 0.03	0.54 ± 0.04
	2nd	0.55 ± 0.03	0.58 ± 0.02	0.60 ± 0.03

Table 3: Mean values (± S.E.) of total serum protein of experimental and control groups (mg/dl)

Groups	Shot	Post vaccination period (hrs) 24	48	72
A	1st	5.75 ± 0.21	5.52 ± 0.16	5.58 ± 0.19
	2nd	5.61 ± 0.11	5.48 ± 0.16	5.56 ± 0.21
B	1st	5.34 ± 0.17	5.53 ± 0.13	5.47 ± 0.23
	2nd	5.31 ± 0.21	5.47 ± 0.16	5.39 ± 0.19
C	1st	5.56 ± 0.24	5.51 ± 0.14	5.59 ± 0.16
	2nd	5.59 ± 0.14	5.49 ± 0.23	5.62 ± 0.16
D	1st	5.61 ± 0.33	5.50 ± 0.23	5.56 ± 0.30
	2nd	5.57 ± 0.15	5.32 ± 0.21	5.60 ± 0.17

Table 4: Mean values (± S.E.) of total serum glucose levels (mg/dl)

Groups	Shot	Post vaccination period (hrs) 24	48	72
A	1st	182.56 ± 4.2	188.91 ± 2.7	186.12 ± 2.3
	2nd	184.32 ± 3.1	177.17 ± 1.9	185.95 ± 4.1
B	1st	172.45 ± 2.8	185.23 ± 3.5	189.62 ± 2.9
	2nd	169.48 ± 2.8	179.38 ± 3.5	173.66 ± 3.3
C	1st	178.10 ± 2.1	191.52 ± 4.2	196.73 ± 3.9
	2nd	180.25 ± 2.1	177.26 ± 2.7	182.92 ± 3.2
D	1st	175.39 ± 2.7	189.51 ± 3.1	192.53 ± 2.9
	2nd	179.97 ± 2.3	175.42 ± 3.0	180.16 ± 3.1

Table 5: Mean values (± S.E.) of total serum cholesterol of experimental and control groups (mg/dl)

Groups	Shot	Post vaccination period (hrs) 24	48	72
A	1st	152.92 ± 4.2	154.12 ± 3.3	155.86 ± 5.1
	2nd	158.92 ± 2.7	166.26 ± 3.2	162.91 ± 3.8
B	1st	151.79 ± 2.6	153.53 ± 4.1	150.68 ± 3.8
	2nd	161.25 ± 2.1	170.20 ± 3.3	164.17 ± 4.1
C	1st	158.62 ± 3.6	159.42 ± 2.9	151.78 ± 3.3
	2nd	158.42 ± 4.3	160.98 ± 3.6	161.91 ± 3.8
D	1st	160.35 ± 3.9	152.28 ± 2.6	148.66 ± 2.9
	2nd	164.21 ± 2.9	155.62 ± 3.4	150.84 ± 3.9

Table 6: Adrenal/body weight ratio of experimental and control groups

Groups	Means (±SE). on day 42nd
A	0.11 ± 0.01
B	0.07 ± 0.01
C	0.09 ± 0.01
D	0.13 ± 0.05

Table 7: Total profit and profit per bird (Rs.)

Groups	No. of birds in experiment Start	End	Total input	Total output	Profit	Profit per bird
A	40	37	1677.00	2109.00	432.00	11.67
B	40	36	1677.00	1915.20	238.20	6.61
C	40	38	1681.32	2310.40	629.08	16.55
D	40	36	1688.34	2052.00	363.66	10.10

corticotrophin hormone did not cause hypertrophy of the adrenal cells.

Highest profit was recorded in group C, which was treated with multivitamins preparation. From this it can be assumed that multivitamins therapy had positive effect on the health of the bird. The findings are similar to that of Bains 1996) and Bashir *et al.* (1998), who reported that birds, which received rations with vitamins along with vaccination, had higher mean body weight. However, it is rather difficult to give clear conclusion regarding the economics because number of birds in each group was quite limited. Aspirin therapy showed no significant weight gain.

It was concluded from this study that vaccination against coccidiosis (Eimeria species) caused no significant stress in broiler chickens. Multivitamin supplementation had a beneficial effect on weight gain whereas aspirin had no such effect. From the future study point of view it is recommended that sample be taken after 1st hour post vaccination and varying up to 24 hours. Detection of hormones should also be done and morphological changes in adrenal glands should be examined immediately after the application of stressor.

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