Contact Sensitivity to Dinitrochlorobenzene as a Marker Trait in the Indirect Selection for Body Mange and Coccidiosis Resistance in Broiler Rabbits

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ABSTRACT : To determine the effects of genetic and environmental influences on cell mediated immune (CMI) responses in broiler rabbits, contact sensitivity to 2,4-Dinitrochlorobenzene (DNCB) was assessed in three temperate broiler breeds of rabbits, namely Soviet Chinchilla, New Zealand White and Grey Giant. The feasibility of using the contact sensitivity to DNCB as a marker trait in selection for disease resistance was examined. There were highly significant differences between breeds ($p<0.01$) in initial skin thickness and contact sensitivities to DNCB at 24, 48 and 72 hours. Initial skin thickness was greatest in the Soviet Chinchilla breed (mean 2.2484 mm), and was significantly greater ($p<0.01$) in males (2.4963 mm) than in females (1.7846 mm) ($p<0.01$). Highest contact sensitivity to DNCB was in the New Zealand White breed with mean increase in skin thickness of 1.1884, 0.9072 and 0.5879 mm at 24, 48 and 72 hours post challenge respectively. Delayed type hypersensitivity (DTH) reaction to DNCB at 24 hours post challenge had a highly significant association ($p<0.01$) with the incidence of body mange in rabbits. The results indicated a lowered contact sensitivity to DNCB at 24 hours post challenge was associated significantly ($p<0.01$) with an increase in incidence and severity of body mange, suggesting its potential value as a marker. The correlation s among contact sensitivities at 24, 48 and 72 hours were positive and highly significant ($p<0.01$); correlations between initial skin thickness and contact sensitivities were negative and highly significant ($p<0.01$). Another notable significant correlation was between body weight and delayed type hypersensitivity at 24 hours indicating that an enhanced CMI might be associated with better growth rate and general wellbeing. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 165-168*)

Key Words : Contact Sensitivity, Dinitrochlorobenzene (DNCB), Mange, Coccidiosis and Marker Trait

INTRODUCTION

Broiler rabbit production possesses tremendous potential as an alternate source of meat for the developing tropics, but temperate broiler breeds introduced into the tropics have poor growth and viability. There is a high prevalence of diseases like coccidiosis, body mange and respiratory infections, and heavy neonatal mortality has also imposed severe restrictions to the development of broiler rabbit industry (Mukundan et al., 1992; Sundaram and Bhattacharya, 1992; Nandakumar and Thomas, 1998).

Tropical stresses appear to have an adverse effect on the immune responses predisposing rabbits to diseases leading to heavy mortality; a defective immune status does predispose animals to both high morbidity and high mortality (Leitner et al., 1992). It has been reported that diseases such as coccidiosis and body mange in poultry and dogs are modulated by cell mediated immunity (Rose et al., 1990; Wilkie et al., 1979). Information on the genetics of cell mediated immunity (CMI) and its association with diseases is scanty, but Harmon et al. (1985) have demonstrated the effective genetic regulation of cell mediated immunity to *Brucella abortus* in cattle resistant to brucellosis.

According to Burton et al. (1989) delayed type hypersensitivity (DTH) reaction to Dinitrochlorobenzene (DNCB) could find use as a marker trait in selection for disease resistance. The immune response to contact allergen like DNCB is a type IV or DTH following percutaneous absorption of hapten in sensitised animals. DNCB skin tests have been widely used to assess the function of human CMI responses (Friedman et al., 1983). It has also been used in dogs to assess the CMI status (Wilkie et al., 1991). DNCB skin tests were reported to be comparable with other *in vivo* DTH reactions using phytohaemagglutinin (PHA) or Johnin in cattle.

The aims of the present investigation were to ascertain the contact sensitivity status of broiler rabbits to DNCB, and to assess the feasibility of using contact sensitivity tests as a marker trait in selection for disease resistance in broiler rabbits.

MATERIALS AND METHODS

The animals used were 131 adult broiler rabbits of both sexes aged 6-12 months and belonging to three temperate breeds, namely New Zealand White (n=44), Grey Giant (42), and Soviet Chinchilla (45). They were maintained under identical conditions of feeding and management for a period of 6 months. Body weights were recorded at the beginning of the experiment.

Skin sensitisation with DNCB

The cutaneous response to DNCB was assessed as described by Wilkie et al. (1991) with the following modifications. A two per cent solution (W/V) of 2,4-Dinitrochlorobenzene (Sisco Research Laboratory) in acetone was prepared freshly every two weeks. An area of skin of about four cm^2 on the dorsal thorax was clipped and cleansed with alcohol. A metal ring of about 1 cm diameter was placed on the clipped area and 0.1 ml of DNCB solution was applied drop by drop to the area circumscribed by the metal ring and was

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blown dry. Two weeks later a different site on the dorsal thorax was prepared as before and 0.05 ml of one per cent (W/V) solution of DNCB was applied as before. The double skin fold thickness at the site was measured prior to challenge and at 24, 48 and 72 hours post challenge. The post challenge increase in skin thickness in mm was determined.

Disease incidence and severity

The experimental animals were screened at the beginning and at regular intervals for the coccidial oocyst output and clinical signs of body mange. Only rabbits with zero output and no clinical signs were chosen for the experiment.

Based on the severity and nature of clinical signs of mange, rabbits were scored as detailed below:

Severity and clinical signs	Score
Clinical signs absent and no lesions	0
Mild clinical signs, occasional lesions on muzzle and ear	1
Moderate clinical signs, lesions extending on ear and muzzle	2
Severe clinical signs, lesions covering the entire face, muzzle and ears	3
Generalised and severe clinical signs, lesions covering the entire body, muzzle, face and ears	4

Based on the coccidial oocyst output after examining 10 microscopic fields following preparation with the floatation technique, rabbits were scored as shown below:

Oocyst output	Score
Absent	0
Oocysts in less than five microscopic fields	1
Oocysts in more than five microscopic fields	2
Oocysts in all ten fields with more than 2-3 oocysts per field	3

The prevalence of coccidiosis and mange among the experimental animals were worked out.

Statistical analysis

Least squares analysis (Harvey, 1985) was performed to assess the effects of breed, sex and incidence of mange and coccidiosis on the initial skin thickness, contact sensitivity to DNCB and adult body weight using the following model.

$$Y_{ijklm} = \mu + b_i + s_j + Ma_k + Cc_l + e_{ijklm}$$

Where,

Y_{ijklm} = Skin thickness or DTH to DNCB (mm)
μ = Overall population mean
b_i = effect of i^{th} breed
S_j = effect of j^{th} sex
Ma_k = prevalence of body mange (k=0,1,.....4)
Cc_l = prevalence of coccidiosis (l=0,1,....3)
e_{ijklm} = random error

Phenotypic correlation between skin thickness, contact sensitivity at 24, 48 and 72 hours and adult body weight were calculated.

RESULTS AND DISCUSSION

The overall incidence of coccidiosis among all the breeds of broiler rabbits studied was 37.4 per cent and that of mange was 34.46 per cent. Overall mean initial skin thickness among broiler rabbits was 1.9296 mm and contact sensitivity at 24, 48 and 72 hours was 1.1028, 0.7822 and 0.5074 mm respectively.

Least squares analysis of variance for the association of breed, sex, mange and coccidiosis with initial skin thickness, contact sensitivity to DNCB at 24, 48 and 72 hours, and adult body weight are presented in table 1. Differences between breeds in skin thickness, and in the DTH reaction to DNCB at 24, 48 and 72 hrs were significant ($p<0.01$). The only variable significantly different between sexes was initial skin thickness ($p<0.01$). Contact sensitivity at 24 hours post challenge had a highly significant effect on the incidence of body mange ($p<0.01$).

Least squares means for the effect of breed, sex, incidence of mange and coccidiosis on skin thickness, the DTH reaction to DNCB at 24, 48 and 72 hours post challenge, and adult body weight are shown in table 2. Soviet Chinchilla had the greatest skin thickness with a mean of 2.2484 ± 0.0747 and Grey Giant had the lowest with a mean of 2.0728 ± 0.0799 mm. Skin thickness in males, mean 2.4963 ± 0.876 mm, was significantly greater ($p<0.01$) than in females (1.7846 ± 0.0693 mm). The incidences of body mange and of coccidiosis were not significantly related to initial skin thickness.

The New Zealand breed of rabbit showed the highest DTH to DNCB at 24, 48 and 72 hours. The mean contact sensitivity values were 1.1884, 0.9072 and 0.5879 mm respectively. The lowest contact sensitivity to DNCB was for the Grey Giant breed with 0.7903, 0.4869 and 0.2764 mm respectively. DTH to DNCB was significantly associated ($p<0.01$) with the incidence of body mange. The trend indicated that a decrease in DTH to DNCB was associated with an increase in severity of body mange. At 24 hours post challenge, rabbits showing mild clinical signs of body mange had a mean contact sensitivity of 1.4184 mm; animals showing moderate clinical signs had a mean value of 0.8883 mm, and with rabbits showing severe clinical signs it was only 0.5591 mm. Body weight was not found to have any significant association with the incidence of body mange or of coccidiosis.

Significant variation between breeds in CMI responses to PHA has already been documented in pigs and poultry (Van Der Zijpp, 1983; Buschmann and Meyer, 1990; Benda et al., 1990), but there are few

Table 1. Least Square Analysis of variance for the association of breed, sex and adult body weight, body mange and coccidiosis in the contact sensitivity to DNCB in broiler rabbits

Source of variation	df	Initial skin thickness MSS	24 hrs Post challenge MSS	48 hrs Post challenge MSS	72 hrs Post challenge MSS	Adult body weight
Breed	2	0.3670**	1.6051**	1.7604**	0.9830**	0.1852NS
Sex	1	8.8770**	0.5958NS	0.0612NS	0.0147	0.2077NS
Mange	4	0.1328NS	0.9098**	0.1377NS	0.0533NS	0.0884NS
Coccidiosis	3	0.0623NS	0.0697NS	0.0981NS	0.1326NS	0.0540NS

** Significant at 1% level; * Significant at 5% level; NS-Nonsignificant; MSS-Mean sum of squares.

reports on the association of breed with contact sensitivity to DNCB and this may be the first report on this effect in broiler rabbits. Though basically PHA responses and contact sensitivity to DNCB are measures of CMI, contact sensitivity operates via Langerhans cells and affects the presensitised lymphocytes (Wilkie et al., 1991). The results of the present study point out to the feasibility of utilising DNCB skin tests to assess the CMI in rabbits also.

The reports on the association of sex, on the contact sensitivity to DNCB are scarce though sex has been reported to modulate DTH response to PHA in poultry (Cheng and Lamont, 1988).

There have been reports for several animal species, but not for broiler rabbits, of an association between lowered CMI status and several diseases, including transplantable fibrosarcoma in chickens (Edelmann et al., 1986), coccidiosis in poultry (Benda et al., 1990), goat pox (Deshmukh et al., 1990) and atopic dermatitis in dogs (Wilkie et al., 1991). Initial skin thickness and contact sensitivity at 24, 48 and 72 hours post challenge had no significant association with the incidence and severity of coccidiosis (table 1). However the least squares mean for contact sensitivity at 24 hours post challenge was the highest for rabbits with no faecal oocyst output and showed a declining trend as the output began to increase; further investigation is required. In this context it should be noted that coccidiosis resistance in mice and poultry has been reported to be associated with cell mediated immune responses (Benda et al., 1990; Rose et al., 1990). The present study did establish an association between lowered contact sensitivity and incidence of body mange in broiler rabbits. This observation is in agreement with the report of Wilkie et al. (1979) on the incidence of demodectic mange and CMI response status in dogs. Body mange is a menacing problem among broiler rabbits and the results of the present studyshow that it is feasible to use contact sensitivity to DNCB as a marker trait in selective breeding.

Table 2. Least squares means for the association of breed, sex, incidence of mange and coccidiosis with the skin thickness, contact sensitivity to DNCB at 24, 48 and 72 hrs post challenge and adult body weight

Classes	n	0 hrs Mean±SE	24 hrs Mean±SE	48 hrs Mean±SE	72 hrs Mean±SE	Adult body weight Mean±SE
Breed		(p=0.002)**	(p=0.009)**	(p=0.005)**	(p=0.0010)**	(p=0.1642)NS
New Zealand White	44	2.1003±0.0861	1.1884±0.1254	0.9072±0.1265	0.5879±0.0992	2.8500±0.0861
Grey Giant	42	2.0728±0.0799	0.7903±0.1164	0.4869±0.1174	0.2764±0.0921	2.9627±0.0799
Soviet Chinchilla	45	2.2484±0.0747	1.0332±0.1088	0.6782±0.1098	0.3938±0.0861	2.9726±0.0747
Sex		(p=0.0000)**	(p=0.0980)NS	(p=0.5973)NS	(p=0.7412)NS	(p=0.1542)NS
Male	29	2.4963±0.0826	1.0962±0.1276	0.6612±0.1287	0.4049±0.1010	2.8740±0.0876
Female	102	1.7846±0.0693	0.9118±0.1009	0.7203±0.1017	0.4339±0.0798	2.9829±0.0692
Incidence of mange		(p=0.2686)NS	(p=0.0030)**	(p=0.6410)NS	(p=0.8104)NS	(p=0.4809)NS
0	86	2.1824±0.0772	1.0850±0.1124	0.7971±0.1134	0.4327±0.0890	2.9175±0.0772
1	22	2.0593±0.0885	1.4184±0.1289	0.8104±0.1301	0.4601±0.1020	2.9349±0.0885
2	14	2.1950±0.1089	0.8883±0.1587	0.7648±0.1601	0.3400±0.1256	2.7661±0.1089
3	4	2.3315±0.1748	0.5591±0.2549	0.4338±0.2568	0.3148±0.2014	2.9918±0.1747
4	5	1.9342±0.1458	1.0689±0.2124	0.6477±0.2143	0.5494±0.1680	3.0320±0.1458
Coccidiosis		(p=0.6094)NS	(p=0.8089)NS	(p=0.7215)NS	(p=0.4023)NS	(p=0.6633)NS
0	82	2.1097±0.0664	1.0902±0.0968	0.6714±0.0977	0.4976±0.0766	2.9997±0.0665
1	37	2.0549±0.0749	1.0436±0.1090	0.7340±0.1100	0.5439±0.0863	2.9640±0.0749
2	7	2.2076±0.1323	1.0086±0.1927	0.5451±0.1944	0.3835±0.1525	2.9521±0.1323
3	5	2.1897±0.1576	0.8735±0.2295	0.8126±0.2315	0.2525±0.1817	2.7980±0.1576
Overall mean		1.9296±0.0381	1.1028±0.0442	0.7822±0.0423	0.5074±0.0330	2.9559±0.0279

** Significant at 1% level; * Significant at 5% level; NS-Nonsignificant.

Table 3. Phenotypic correlation between contact sensitivity to DNCB at 24, 48 and 72 hrs and adult body weight in broiler rabbits

	Initial skin thickness	Contact sensitivity at 24 hrs	Contact sensitivity at 48 hrs	Contact sensitivity at 72 hrs	Body weight
Initial skin thickness	1.0000	- 0.2961**	- 0.3896**	- 0.3645**	0.1130
Contact sensitivity at 24 hrs		1.0000	0.6376**	0.5226**	0.1812*
Contact sensitivity at 48 hrs			1.0000	0.8030**	0.0804
Contact sensitivity at 72 hrs				1.0000	0.0130
Body weight					1.0000

** Significant at 1% level; * Significant at 5% level.

The phenotypic correlations between initial skin thickness, contact sensitivity and adult body weight are shown in table 3. It is interesting to note that initial skin thickness had a highly significant ($p<0.01$) negative correlation with contact sensitivity at 24, 48 and 72 hours. This might possibly be due to interference in the percutaneous absorption of hapten through the thick skin of rabbits which might reduce the contact sensitivity to the hapten. The correlations between contact sensitivity at 24, 48 and 72 hours were highly significant ($p<0.01$), indicating the intensity of contact sensitivity is correlated with its persistency. Another notable association was the significant ($p<0.05$) correlation of 0.1812 between contact sensitivity at 24 hours and adult body weight. This is suggestive of a general wellbeing of rabbits with higher contact sensitivity at 24 hours leading to the higher body weight. Further studies are required before arriving at a generalised conclusion to use this trait in selective breeding for enhanced growth in rabbits.

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