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CROSSBREEDING AND POTENTIAL TO DISEASE RESISTANCE 1- BREED DIFFERENCES AND CELLULAR IMMUNE RESPONSE IN SHEEP

(With 7 Tables)

By

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أثر الخلط فى الأغنام وأحتمالية مقاومة الأمراض
١- العلاقه بين اختلاف السلالات والأستجابه المناعيه الخلويه

بدير عجاج ، عصام شحاته ، دولت أمين
نعيمه عفيفى ، عزت عفيفى

أجريت هذه الدراسه على ٤٨ حولى عمر ٤ شهور، قسمت الى سبعة مجموعات مكونـه من كل من الرحمانى المحلى والفنلندى الاجنبى ونتاج الخلط بينهما (٢/١ فنلندى رحمانى جيل أول وجيـل ثانى و ٤/١ فنلندى رحمانى جيل أول وجيل ثانى و٨/٣ فنلندى رحمانى جيل أول) وتم حقن هذه المجموعات بالألتراكورن والكوفاكسين -٨ وذلك لدراسة الاستجابه المناعيه الخلويه والتى تمثلت فى العد الكلى والنوعى لكرات الدم البيضاء والعد الكلى لخلايا الليمفوسيت المكونـه للروزيت بالأضافه الى أختبار حساسية الجلد البطئ وقد أسفرت هذه الدراسه عن إثبات وجود أجيال خليط لا تقل كفاءة من ناحية أستجابتها المناعيه عن السلاله المحليه وتزيد كفاءتها عن السلاله الأجنبيه تحت الظروف المحليه وهى الجيل الثانى من كل من ٢/١ و ٤/١ فنلندى رحمانى.

SUMMARY

The aim of the work was to investigate breed differences in cellular immune response to simultaneous immunization with Ultra-corn and Covexin-8, and its relation to disease resistance. Finnish Landrace and Rahmani lambs, as well as ½, ¼ and 3/8 F Rfl lambs were included in this trial. All lambs used responded well to Ultra-corn and multivalent clostridial vaccines, as

indicated by the increased values of total leucocytic counts, lymphocytes, monocytes, erythrocyte rosette forming lymphocytes, and skin delayed type hypersensitivity. The crossbreds ½ FR_{F2} and ¼ FR_{F2} lambs were shown to have an efficiently stimulated cell mediated immune response as recognized for the local Rahmani lambs while 3/8 FR and the foreign breed Finn lambs showed the lowest response.

Key words: Crossbreeding Cellular Immune Response in Sheep

INTRODUCTION

Domestic animals, over the centuries, have been attacked by infectious and parasitic diseases. Accordingly, for disease control, selection for resistance must have instance (Spooner, 1975). Resistance to infectious and parasitic organisms is a product of interactions among the pathogen, the host and the environment. The establishment of population generally resistant to a particular infectious or invasive diseases become a necessity as other approaches, such as veterinary care, hygienic measures and improvement of the environmental conditions fail to provide effective control (Hamori, 1983).

Several literature have documented that some breeds possess either genetic resistance or susceptibility to a disease. Withers (1959) found that Channel Island breed cows is more susceptible to Johne's disease than other breeds. Gedymin et al. (1964) reported the inheritance of resistance to tuberculosis in sow and boar lines. The incidence of tuberculosis differed significantly among resistant and susceptible families and lines. The inheritance of congenital immunity to Brucella millitenses was demonstrated in goats by Leon and Guerbero (1969). Genetic variation among sheep breeds in resistance to worm has been evidenced by Le Jambre (1978). Natural resistance to foot rot has been claimed for some sheep breeds. British breeds showed less severity and shorter infection duration than that observed in Merino sheep (Emery et al., 1984 and Stewart et al., 1985). They postulated that the mechanism of such resistance might be in the inter-digital skin which is an integral and active element of the immune system.

Immunological protection against pathogens could be reinforced by intra-breed selection or by cross-breeding with resistanct breeds, in addition to other veterinary measures. In Canada, the first crosses made among four breeds of sheep resulted in a decrease in lamb losses from 25% to 17%, and to 14% after double crossing were made (Hamori, 1983).

Specific and effective immune responses may help to provide an applicable means for selection for disease resistance in sheep. The present work used the difference in cellular immune response to vaccination with Ultra-corn and Convexin-8 as a tool to evaluate the genotype differences in disease resistance and the possibility of immunity transfer from the local breed to the crossbreds.

In this study the cellular immune response parameters include; total leucoytic counts, differential leucocytic counts, erythrocyte rosette forming lymphocytes (T-lymphocytes) and skin delayed hypersensitivity.

MATERIAL AND METHODS

Animals: A total of 48 male and female lambs of about 4 months old were used. The lambs represented seven different breed groups;

6 Rahmani (R),
6 Finn Landrace (F),
12 ½ Finn x ½ Rahmani (½ FR_{F1}),
6 Inter se mated FR_{F1} (½ FR_{F2})
6 ¼ Finn x 3/4 Rahmani (¼ FR_{F1})
6 Inter se mated ¼ FR_{F1} (¼ FR_{F2}),
6 3/8 Fx 5/8 R (½ Fr x ¼ FR)- (3/8 FR_{F1})/

All animals were apparently healthy and free from both external amd internal parasites as proved by parasitological examination.

VACCINES:

A) Covexin-8: is a combined Clostrdial vaccine containing *Cl perfringens types B, C and D,C l. septicum, Cl. chauvoei, Cl. tetani and Cl. novyi* types B and D. It is produced by Wellcome Foundation Ltd., England.

B) Ultra-corn: Is an ultrasonically lysated suspenion of strains of Corynebacterium cutis strains at 20 mg/ml concentration. It is manufactured by Virbac laboratories, France. Two ml dose of Covexin-8 was inoculated subcutaneously in the neck site and repeated after 6 weeks, while 2 ml of Ultra-corn was given intramuscularly in the limb site.

Tuberculin: The purifred protein derivative (Mammalian PPD tuberculin) used for single intradermal tuberculin test was obtained from the Serum and Vaccine Research Institute, Cairo, Egypt.

Skin Test: The intradermal tuberculin test was perfomed by injecting 0.1 ml (10000 units) of tuberculin into the skin (I/D) at the mid-third part of the neck. The reaction was measured after 72 hours.

Blood sampling: Heparinized blood samples were collected from each lamb before and after vaccination to determine the total and differential leucoytic counts as well as percentages of erythrocytes and rosette forming lymphocytes.

METHODS

The total and differential leucocytic counts were determined by the techniques described by **Coles (1986).** Absolute values were calculated by multiplying the obtained percentage values from the differential count by the total leucocytic count. Assessment of the percentage of erythrocyte rsette forming lymphocytes was done according to Chanorin (1989). Statistical analysis was adopted according to Berly and Lindgren (1990).

RESULT

The results obtained in this investigation were demonstrated in tables (1-7).

The results represented that the crossbreeds ½ FR_{F2} and ¼ FR_{F2} showed the best immune response as indicated by increased values of total leucocytes (Table, 1) lymphocytes (Table, 3), monoytes (Table, 4), erythrocytes rosette forming lymphocytes (Table, 6) and delayed type hypersensitivity (Table, 7). The same response was recorded in Rahmani lambs, while Finn and 3/8 FR lambs were shown to have the lowest immune response.

DISCUSSION

Data presented in table (1) shows the mean values of total leucocytic counts in lambs before and after vaccination with Ultra-corn and Covexin-8. The total leucocytic counts were significantly increased in Rahmani ½ FR_{F2} and ¼ FR_{F2} lambs than Finn lambs ($P < 0.05$) at 4 weeks post -vaccination. The absolute values of lymphocytes (Table, 3) showed a significant increase in Rahmani, ½ FR_{F2} and ¼ FR_{F2} lambs than in Finn and 3/8 FR_{F1} at 2 and 4 weeks post-vaccination.

The result of T-lymphocyte counts as determined by erythrocyte rosette formation technique were demonstrated in (table, 6). Statistical analysis of these data revealed that there were more increases in the percentages of T-lymphocytes in Rahmani, and ½ FRF2 lambs, than in Finn and ¼ FR_{F1} lambs.

The importance of lymphocytes in the immune response has been clarified by several authors; Bogan, et al., 1983; Tizard, 1987; Kaneko, 1989; Mackay and Mackay, 1989. They stated that immune responses are mediated by two major classes of lymphocytes (T-andB-lymphocytes). T-lymphocytes are responsible for cell mediated immune functions including cell mediated killing of virus infected cells and tumor cells, allograft rejection and expression of delayed type lypersensitivity. Cell mediated killing is important for defense against viruses and intracellular bacteria or protozoa. Whereas, B-lymphocytes are responsible for the synthesis of circulating immunoglobulins that neutralize both bacterial toxins and viruses.

It is evidnced from table 4 that the absolute values of monocytes in Rahmani and ¼ FR F2 lambs were significantly increased than those of Finn and 3/8 FR_{F1} lambs throughout the experiment and ½ FR_{F1} and ¼ FR_{F1} only at 4 weeks post -vaccination. Monocytes are immature cells in the circulation that become tissue macrophages after maturation (Kaneko, 1989). Macrophages play an important role in non-specific immunity by ingesting and killing microorganisms and by releasing soluble factors which contribute to the host defense and to the inflammation (Quinn et al., 1994). Table (5) illustrates that the absolute values of eosinophils showed an increase in ½ FR_{F2} and ¼ FR_{F2} lambs than in Fin, ¼ FR_{F1} and 3/8 FR_{F1}, at 4 weeks post-vaccination. Although, the increase in eosinphils is endesirable this increase might be the result of total leucocytic increase in these lambs.

Regarding with delayed type hypersensitivity reaction, if we consider an increase of 5 mm in skin thickness as positive tuberculin reaction in sheep (Kelly, 1984) the difference in the skin reaction recorded in this investigation (table, 7) was negative for all groups of lambs. Similar results were obtained by Salama, et al (1996) where, they found that BCG vaccinated lambs gave clear reactions. while those vaccinated with Ultra-corn have negative reaction. This is because the sensitized lymphocytes that will transfer immunity and hypersensitivity can be attained most effectively by viable organisms (Rosenthal, 1980). In spite of the negative reaction of the skin test (less than 5 mm), there were clear difference in the skin thickness among the different groups of tested lambs. Rahmani, ½ FRF2 and ¼ FRF2 lambs have significantly higher skin reaction than that detected in Finn lambs (Table, 7). This indicate that the cell mediated immune response or delayed type hypersensitivity (DTH) was more efficient in those lambs. The DTH reaction is the product of the recognition of a tuberculin antigen by delayed type hypersensitivity (TDH) cells, followed by an immigration reaction and activation of macrophages (Bogan, et al., 1983). These cells provide

protective immunity against bacteria which grow in macrophages such as Mycobacteria, Listeria and Brucella (Bogan et al., 1983).

The obtained results in the present work illustrate that all groups of lambs vaccinated with Ultra-corn and Covexine-8, simultaneously showed better cellular responses, as indicated by the increased values of total leukocytes, lymphocytes, monocytes, erythrocyte rosette forming lymphocytes and delayed type hypersensitivity reaction. This response was more apparent in Rahmani, ½ FR_{F2} and ¼ FR_{F2} lambs when compared with Finn and 3/8 FR_{F1} lambs. This indicates that those lambs had generally good immune response and consequently have good resistance to infections. These results could be confirmed by Leon and Guerbero, (1969), LeJambre, (1978); Emery et al., (1984) and Stewart et al., (1985) who found genetic variation in relation to disease resistance.

On the other hand, the lower cellular immune response observed in the exotic pure Finn lambs may be responsible for the higher susceptibility of this breed to viral and intracellular bacterial infection. This might explain the increase in the mortality rate of Finn lambs than their crossbreeds under our environmental conditions as pure bred lambs requires strict hygienic measures and periodical vaccination for their maintenance and survival.

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Table (1) Average total leucocytic counts (x $10^3/mm^3$)in the peripheral blood of lambs, before and after vaccination.

Breed	Before vaccination	After vaccination			
		2 weeks		4 weeks	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	8.60 ± 0.25	13.46 ± 0.84	56.51	10.76 ± 0.55	25.12
Finn	8.58 ± 0.49	12.15 ± 0.32	41.60	9.25 ± 0.30	7.81
½ FR_{F1}	8.48 ± 0.27	13.03 ± 0.34	53.66	9.81 ± 0.41	15.68
½ FR_{F2}	8.14 ± 0.28	13.93 ± 0.50	71.13	10.62 ± 0.50	30.47
¼ FR_{F1}	8.86 ± 0.33	12.63 ± 0.49	42.55	9.72 ± 0.34	9.71
¼ FR_{F2}	9.20 ± 0.33	13.16 ± 1.15	43.04	11.02 ± 0.54	19.78
$^3/_8$ FR_{F1}	8.13 ± 0.28	12.21 ± 0.42	50.18	9.51 ± 0.30	16.97

4 weeks post vaccination.

	F	3/8 FR
R	*	
1/2 FR_{F2}	*	*

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test

Table (2) Average Neutrophil counts (in absolute value/mm^3) in the peripheral blood of lambs, before and after vaccination.

Breed	Before vaccination	After vaccination			
		week-2		week-4	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	4483 ± 384	5440 ± 382	21.35	4527 ± 318	0.98
Finn	3921 ± 298	5399 ± 124	37.69	4102 ± 318	4.62
½ FR_{F1}	3668 ± 197	5541 ± 273	51.06	4062 ± 210	10.74
½ FR_{F2}	3413 ± 62	5785 ± 172	69.49	4600 ± 335	34.78
¼ FR_{F1}	4191 ± 228	5195 ± 294	23.96	4311 ± 276	2.86
¼ FR_{F2}	3635 ± 315	5106 ± 376	40.47	4128 ± 363	13.56
$^3/_8$ FR_{F1}	3627 ± 220	5213 ± 391	43.72	4252 ± 277	17.29

Table (3) Average lymphocytic counts (in absolute value/mm^3) in lambs, before and after vaccination.

Breed	Before vaccination	After vaccination			
		2 weeks		4 weeks	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	4287 ± 379	7069 ± 254	64.89	5202 ± 192	21.84
Finn	4104 ± 276	6150 ± 215	49.85	4357 ± 209	6.16
½ FR_{F1}	4266 ± 161	6728 ± 353	57.71	4936 ± 253	15.71
½ FR_{F2}	4167 ± 221	7318 ± 295	75.62	5072 ± 210	21.72
¼ FR_{F1}	4172 ± 213	6800 ± 341	62.99	4610 ± 174	10.50
¼ FR_{F2}	4930 ± 172	6948 ± 284	40.93	5741 ± 358	16.45
$^3/_8$ FR_{F1}	4023 ± 115	6309 ± 212	58.98	4590 ± 127	14.09

	2 weeks	4 weeks		
R	F	F	¼ F_1	$^3/_8$ FR
F	*	*		
½ F1		*		
½ F2	*	*		
¼ F2		*	*	*

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test

Table (4) Average monocyte counts (in absolute value/mm^3) in lambs, before and after vaccination.

Breed / crossbred	Before vaccination	After vaccination			
		2 weeks		4 weeks	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	520 ± 31	733 ± 73	40.96	862 ± 84	65.77
Finn	426 ± 28	484 ± 58	13.62	625 ± 32	46.72
½ FR_{F1}	413 ± 33	639 ± 51	54.72	595 ± 63	44.07
½ FR_{F2}	422 ± 39	639 ± 86	51.42	712 ± 59	68.72
¼ FR_{F1}	360 ± 51	528 ± 53	46.67	629 ± 75	74.72
¼ FR_{F2}	482 ± 59	793 ± 99	64.52	908 ± 91	88.38
$^3/_8$ FR_{F1}	334 ± 25	558 ± 33	27.07	523 ± 59	56.58

	2 weeks			4 weeks			
	F	¼ F_1	$^3/_8$ FR	F	½ F1	¼ F_1	$^3/_8$ FR
R	*		*	*	*	*	*
¼ F2	*	*	*	*	*	*	*

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test

Table (5) Average Eosinophilic counts (in absolute value/mm^3) in lambs, before and after vaccination.

Breed / crossbred	Before vaccina	After vaccination			
		2 weeks		4 weeks	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	110 ± 23	118 ± 33	7.27	169± 25	53.64
Finn	129 ± 40	117 ± 31	0	148 ± 21	14.72
½ FR_{F1}	133 ± 24	118 ± 28	0	217 ± 20	63.16
½ FR_{F2}	138 ± 31	190 ± 34	37.68	228 ± 27	65.22
¼ FR_{F1}	135 ± 36	107 ± 28	0	170 ± 19	25.93
¼ FR_{F2}	152 ± 46	270 ± 68	77.63	243 ± 41	59.87
$^3/_8$ FR_{F1}	149 ± 24	121 ± 36	18.79	141 ± 19	0

	F	¼ F1	¼ F2
1/2 F2	*	*	*
¼ F2	*	*	*

4 weeks

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test

Table (6) Average percentage of erythrocytes rosette (ER) forming lymphocytes in the peripheral blood of lambs, before and after vaccination.

Breed / crossbred	Before vaccination	After vaccination			
		2 weeks		4 weeks	
		Mean ± S.E.	Average increase %	Mean ± S.E.	Average increase %
Rahmani	17.20 ± 1.21	25.20± 0.91	46.51	22.00 ± 1.57	27.91
Finn	15.80 ± 1.25	20.40 ± 1.00	29.11	17.60 ± 0.61	11.39
½ FR_{F1}	17.50± 0.97	23.00 ± 0.68	23.91	20.00 ± 0.91	14.29
½ FR_{F2}	18.50 ± 0.96	27.00 ± 0.88	45.95	22.20 ± 1.16	19.84
¼ FR_{F1}	15.67 ± 0.77	22.30 ± 0.77	40.31	17.80 ± 0.83	13.78
¼ FR_{F2}	16.83 ± 0.98	23.00 ± 0.60	36.67	19.40 ± 0.96	15.26
$^3/_8$ FR_{F1}	17.20 ± 0.77	22.40 ± 1.71	30.23	19.60 ± 1.31	13.95

	2 weeks				4 weeks	
	F	½ F1	¼ F_1	$^3/_8$ FR	F	¼ F_1
R	*		*		*	*
½ F2	*	*	*	*	*	*

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test

Table (7): Average skin fold thickness (in mm) before and 72 hours after tuberculin testing in lambs.

Breed/ crossbred	Before mammalian tuberculin application	After mammalian tuberculin application	Difference	Average increase %
Rahmani	2.98 ± 0.13	4.94 ± 0.30	1.96 ± 0.31	65.77
Finn	2.76 ± 0.07	3.82 ± 0.13	1.06 ± 0.11	38.41
½ F. R (F1)	2.83 ± 0.07	4.45 ± 0.36	1.59 ± 0.35	56.18
½ F. R (F2)	3.07 ± 0.11	5.10 ± 0.27	2.03 ± 0.28	66.12
¼ F. R (F1)	2.87 ± 0.12	4.32 ± 0.12	1.43 ± 0.17	49.83
¼ F. R (F2)	2.90 ± 0.08	4.72 ± 0.22	1.82 ± 0.16	62.76
3/8 F. R (F1)	2.88 ± 0.12	4.32 ± 0.21	1.44 ± 0.29	50.00

4 weeks

	F
R	*
½ F2	*

* Significant between groups at $P < 0.05$ using L.S.D. as comparative of means and ANOVA test