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Genetic Variation in Stress Resistance According to Some Blood Values in Iraqi Sheep

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Abstract: **This study was conducted to examine the Neutrophil/Lymphocyte ratio (N/L) as an indicator to seasonal (winter and summer) stress and criterion for selection for stress resistance in sheep. Four genetic groups were included: Local Awasi (L), Karradi (K), Local x Karradi (L x K) and Local Awasi x Arrabi (L x A). Mean group N/L ratio was calculated from all individual's value. Mean N and L also calculated.**

The Lower 95% confidence Limit method of selection was used to separate individuals, within each genetic group, into Resistant (R) and sensitive (S); defining all individuals with an N/L ratio over the 95% lower limit as (S) and these below as (R). Such limit was for the four genetic groups, 1.01, 0.79, 0.78 and 0.70 respectively. Within each genetic N winter mean are significantly higher than summer ones while the L went in the opposite direction.

Winter overall mean N/L (before separation into R and S) were significantly higher than summer values for all the four groups. Such values were (1.18 and 0.61), (0.95 and 0.53), (0.88 and 0.71) and (0.99 and 0.60) for L, K, LxK and LxA respectively. Within genetic group, the R mean ratio did not differ significantly from summer to winter (p>0.05), while in the S, winter values were about two folds those of summer ones.

The regression of daughter /dam N/L values indicated a reasonable heritability; it was (0.44) for the overall genetic groups.

The consistently higher N/L ratio in winter for the four groups, and the consistently higher S values compared to R value, within genetic groups, in winter too and the close ratio in summer might indicate that winter stress, in our region, is more effective than summer stress; this point worth further investigation.

The overall results could be taken to reflect that the N/L ratio is affected and is an indicator to seasonal stress and could be used as a criterion for selection for stress resistance.

Keyword: **Neutrophil, Lymphocyte, Iraqi Sheep**

I. Introduction

Many worker has established the adverse effect of ambient temperature on production and reproduction in sheep [1,2,3]. It was also reported that stress is an immune-suppressive factor [4], because stress increases cortisol concentration, which in turn, reduce the response of the immune system [5,6,7,8]. Many studies [9, 10,11] revealed that lymphocytes and neutrophils are affected by different stressors, including diseases and heat; total number of neutrophils increases and that of lymphocytes decreases. Woodard et al. [12] showed that neutrophils deficiency, in sheep, has an adverse effect on the neutrophil activity. The total White Blood Cells (WBCs), and their differential count, is also reported to be affected by age [9], by nutritional level [13] and by breed [14, 15].

Research results showed that immune response and resistance to stressors, in different animal species, are under the genetic control and that there is a sizable genetic variability for such traits and suggested the use of this trend to establish genetically resistant lines and breeds [16,17].

Al-Murrani*et al.*[16] used the hetrophil/lymphocyte ratio, in poultry. As a selection criterion for heat stress resistance and succeeded to separate a flock of 1100 parent stock into a resistant (R) or sensitive (S) group; the R significantly excelled S in egg size (4% more) and production (21.6% higher); mortality of R was much lower too (18.6% vs 28.4%).

It was decided to investigate, in this study, to use of neutrophil/lymphocyte ratio (N/L), in sheep, as an indicator for environmental (winter and summer) stress and as a quickly and easily diagnosed criterion for selection to stress resistance. Our hypothesis leans on the fact that both its components, the neutrophils and the lymphocytes, were known to be affected by stress and they have an important role in immune response. This work is an extension to the use of Hetrophil/Lymphocyte ratio as an indicator to heat stress and criterion for selection for heat stress resistance in poultry [16]. The neutrophils in domestic animals plays a similar role to heterophils in poultry.

II. Materials and Methods

The Sheep:

This study was conducted in privet sheep farm near Baladrooz. Four breeding sheep groups were included, namely: Local Awasi, Karradi, local Awasi x Karradi and local Awasi xArrabi crosses. The age of adult ranged between 2-3.5 years and offspring 6-8 months and of rams 4-4.5 years at the start of the study. Family records were not kept and the only genetic information available is the construction of the genetic groups the purebred Local and Karradi and the two crosses, and the daughter/ dam relation. Birth weights were very close and ranged between 3.85_3.94kgm.

Direct blood smears were prepared from the ear vein, fixed with methyl alcohol for one minute and kept till stained and read within 3 days. Sampling was done two times from ewes (winter and summer), one time from offspring (summer) and from rams (winter).

Number of ewes sampled in summer and winter were in table (1). Number of offspring sampled were 16 for L, K and L x K and 9 for L x A. Rams were 12 of the first three genetic groups.

Differential count and N/L ratio:

Within three days of sampling, the fixed smears were stained using Wright's stain; differential count was done according to [18]. 100 cells were differentially counted for each individual and out of these the number of Neutrophils was divided by that of Lymphocytes to get the N/L ratio. Mean Neutrophils and Lymphocytes were also calculated.

The procedure of identifying Resistance (R) and Sensitive (S) animals:

The 95% Confidence Interval Limit (CL), methods of selection described by [1] was used to separate sheep into R and S. After defining the 95% Lower Confidence Limit depending on winter value of N/L ratio, sheep within each genetic group were assigned to R or S; they are not bred selectively for resistance prior to measuring their N/L ratio. Individuals having a ratio less than the Lower 95% Confidence Limit were considered Resistance and those above Sensitive.

Statistical analysis:

Total number of observations for all genetic groups in summer and winter was 441. Analysis of variations, the F-test of significance, the LSD multiple comparison test, the regression of offspring on dam N/L ratio and other statistical manipulations were carried according to [19].

III. Results and Discussion

Mean Neutrophils and Lymphocytes:

This study is limited to be genetic one with the objective of testing the N/L ratio as a quickly diagnosed marker for stress and criterion for selection for stress resistance in sheep. Though the N and L were used to produce the ratio their physiology was not addressed.

Table (1) contains the mean of Lymphocytes and Neutrophils in Winter and summer. Within each genetic group, the N winter mean values are significantly higher than summer values; the L mean showed the reverse trend, summer ones are consistently and significantly higher. Between genetic groups comparison showed that the Local Awasi has significantly higher N and Lower L than the other groups in winter; no such trend was noticed during summer.

The Method of selection:

The 95% Confidence Limit of Selection used in this study is based on the assumption that the low N/L ratio, at the prevailing stress conditions, indicates resistant individuals, while those with high N/L ratio are sensitive. This physiology is built on the fact that Neutrophils increases on stress while Lymphocyte decreases [9,10,11]. The high N and Low L means in winter and the reverse direction in summer shown in table (1) are constant with that. Blunt [20] in his review to the blood of sheep gave a normal range of (10-50)% for N and (40-70)% for L. This might be an evidence for genetic variability that we used in this study. The lower ram's ratio in winter compared to that of ewes could be attributed to adaptation to stress being of older age and selected from many available males on the bases of performance and health record.

Table 1: Mean Neutrophil and and Lymphocyte in summer and winter and significance of differences between the two season within genetic groups and betweengenetic groups within seasons

Genetic group	Mean ± SE				Significant level of difference of :	
	Winter		Summer			
	N	L	N	L	N	L
Local Awasi	50.36a ± 1.83	45.07a ± 1.65	35.15ab ± 1.64	59.52 ± 1.41	<0.0001	<0.001
	47		36			
Karradi	44.98b ± 1.87	50.04b ± 1.75	31.87a ± 1.04	60.76a ± 1.00	<0.001	<0.001
	50		34			
Local Awasi X Karradi	43.81b ± 1.39	51.05b ± 1.33	37.89b ± 1.6	56.09b ± 1.68	<0.01	<0.01
	86		44			
Local Awasi x Arrabi	44.94b ± 2.41	49.05b ± 2.04	35.91b ± 2.33	59.45b ± 2.50	<0.05	0.05
	32					

Mean carrying different letters (within column) are significantly different at 5% level.

In this study, we have chosen the lower 95% Confidence Limit which was built on winter values, as a border line, though one could use the upper 95% C.L. or any other limit. Such choice depends on the intensity of selection aimed at and population size. By choosing the lower 95% C.L. just to be sure, as far as possible, that only those individuals with very low N/L value, so the must stress resistant, will be included in the (R) group.

The N/L ratio:

Table (2) show that values, before separation into R and S, for ewes and rams for all the breeding groups, ranged from 1.18 to 0.88 in winter and between 0.53 to 0.71 in summer. Local Awasi N/L ratio was significantly ($p<0.05$) higher than that of the other groups in winter, while that of Local Awasi x Karradicrossbred was the highest in summer ($p<0.05$). Rams winter N/L values were significantly much lower than those of ewes in winter too. Within breeding groups, differences were significantly ($p<0.01$). Winter N/L values, in this study are also much higher than that which was recalculated for some exotic breeds, which ranged from 0.44-0.56 [10], 0.54 [14] and 0.65 [21]. Such values are very close to summer values of this study. Recalculation was done by dividing mean N on mean L% quoted by the authors.

Table 2: Overall mean N/L ratio during winter and summer for ewes (before separation into resistant and sensitive) and rams values in winter

Breeding groups	N/L ratio of Ewes X ± SE		Level of sig.	N/L ratio of Rams(winter)	Sig. level Ram(winter)
	Winter	Summer			
Local Awasi	1.18± 0.08b	0.61± 0.05a	**	0.68±0.20a	*
Karradi	0.95±0.07a	0.53±0.3	**	0.67±0.10a	*
Local Awasi x Karradi	0.88±0.05a	0.71±0.05b	**	0.68±0.20a	*
Local Awasi x Arrabi	0.99±0.11a	0.60±0.05a	**	------	

* <0.05; ** <0.01; Means within a column with different letters differ significantly at 5%

The high N/L values in winter might indicate that winter stress is more effective and has an adverse effect on sheep than summer stress. It was noted that changes in blood constituents occur in winter and early spring [22]. Research results also indicated that exposure of sheep to low temperatures leads to similar changes [23.10]. It was also found that lymphocytes and neutrophils respond to all kinds of stressors, including environmental temperature[9,8]. Slee[1] found high lambs mortality due to decrease in ambient temperature.

The 95% Lower Confidence Limit of N/L and R and S means:

Within genetic group between seasons comparision in table (3) shows a clear trend; the N/L ratios, for the (Resistant), of all genetic groups, did not differ significantly between winter and summer, while the (Sensative) values differed ($p<0.01$), being much higher in winter. Within group and season comparisons of R and S values showed that the (S) values were about more than two folds that of the (R) in winter, while no trend was noticed during summer.

The validity of the N/L ratio as an indicator to stress is based on results presented in tables (2,3). Table (2) show that the overall mean winter values, before separation into R and S, are consistently and significantly higher than summer means for all the genetic groups. Environment with all management and feeding regimes were the same for all individuals; the difference is the total winter and summer effects where the most prominent feature is the high and low ambient temperature respectively, especially when feed was supplemented. When animals were separated into R (having lower N/L values than the 95% lower C.L. border line) and s (having higher N/L ratio), table (3), two points were clear. First: within each breeding group, seasonal variation was not significant in R; the N/L ratio remained approximately the same while in the S group, winter values are significantly much higher than summer values. Secondly: during winter, S values were around 2 folds R values, while the difference in such values during summer is only trivial and showed no tend. This could be taken to reflect that the N/L ratio is affected and is an indicator to stress. Animals, whether of R or S type, probably experience a mild stress during summer. This might also potentiate our previous conclusion that stress, in our region, is mainly in winter.

Sheep maintains a stable body temperature usually within 2˚C. of the normal 39.5˚C. Response to cold exposure commences when the ambient temperature falls below (critical) which is as high as 28˚C. For adult shorn sheep; the animal then is obliged to raise its metabolic ratio in order to maintain its deep body temperature [1]. Temperature in winter ,inthis study, ranged between 4.7-17.6˚C. with many days below zero; such temperatures are far below the critical. The common practice of housing or sheltering, in the region, which cares much for summer rather than winter, should be reversed or revised.

The tolerance of summer stress might reside, among other mechanisms, with the physiology of perspiration, evaporative cooling and condensation. The sensitivity to winter stress involves many complicated mechanisms that are beyond the scope of this study.

Slee[1] descried results on cold resistance in sheep using climate chambers: this character was found to be variable within and between breeds but had a high repeatability within individuals. He also pointed that cold resistance may have a fairly high heritability of about 30%. Progeny from selected high resistant rams showed slightly better survival rates than those from low resistant rams though differences were not significant.

The offspring/dam regression and h^2 estimates:

The regression of daughter on dam N/L ratios of summer values indicated a reasonable heritability. The overall h^2 is (0.44); it was 0.38, 0.14 and 0.44 for the local Awasi, Table (4).

The different N/L values of R and S, within and between the breeding groups (table 3) together with the intermediate regression coefficients though contains some dam effect [24] and h^2 s (overall estimated h^2 = 0.44). table (4) indicate a reasonable genetic variability available for selection for stress resistance using the N/L ratio as an indicator to stress and as a criterion for selection in sheep. The 95% Lower Confidence Limit, or any chosen limit, method of selection was found feasible in separating sheep into R and S groups.

Table 3: The 95% Lower confidence limit, mean N/L ratios for resistant and sensitive during Summer and Winter, % of R individuals

Breeding groups	N/L 95% Lower C.L.	Mean N/L ± SE			Level of sig.	%R Individuals
		Category	Winter	Summer		
Local Awasi	1.01	R	0.62± 0.05	0.66±0.03	NS	38
		S	1.52±0.09	0.58±1.0	***	
	Sig. Level		***	*		
Karradi	0.79	R	0.55±0.3	0.49±0.03	NS	51
		S	1.36±0.10	0.56±0.04	***	
	Sig. Level		***	*		
LocalAwasi xKarradi	0.78	R	0.55±0.3	0.69±1.0	NS	49
		S	1.20±0.06	0.75±0.10	**	
	Sig. Level					
Local AwasixArrabi	0.70	R	0.48±0.03	NA		
		S	1.29±0.10			
	Sig. Level					

** p<0.05, ** p<0.01 , *** p< 0.001 , NS not significan*

Table 4: Mean N/L ratioof dams, offspring and their regression coefficients and h^2 estimates (summer values)

Breeding groups	n	Mean of N/L ratio		H^2 estimate	
		dam	Offspri-ng	Regression offsp./dam	
Local Awasi	16	0.61	0.57	0.19	0.38
Karradi	16	0.53	0.75	0.07	0.14
Karradixocal Awasi	16	0.71	0.65	0.22	0.44
Overall estimate	48			0.22±0.08	0.44

We cannot define the nature of the mechanism(s) connected with N/L ratio whereby sheep are either R or S, probably that might be associated with high or low glucosteroid production on stress or some other mechanisms that help in adaptation. This point which was not dealt with in this study, worth a specially designed experiment.

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