



Original Contribution

**BREED, GENDER AND SEASONAL VARIATIONS OF
BLOOD PHAGOCYtic ACTIVITY IN LOCAL SHEEP BREEDS
REARED IN BULGARIA**

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ABSTRACT

The studies were performed in 2008 on 168 sheep and 114 rams. Investigations were carried out on 6 sheep and 6 rams from the following breeds: Karakachan, Tsigay, Replyan, Panagyurishte, Copper-red Shoumen, Karnobat, Pleven Blackhead, White Maritsa and Patch-faced Maritsa, Stara Zagora and Romanov. The phagocytic activity was determined via two parameters – phagocytosis index (PI) and phagocytic number (PN). PI was determined as a ratio of phagocytized leukocytes and the total leukocyte counts, multiplied by 100. PN was calculated as the number of bacteria, phagocytized by one leukocyte.

During the spring, the highest PI was found out in Replyan, Copper-red Shoumen and Stara Zagora sheep (79.33%). The lowest PI values were those of Karnobat (76.0%) and Karakachan (76.67%) sheep. Among studied rams, the highest PI was established in Tsigay and Romanov breeds – 79.33%, whereas the lowest – in Copper-red Shoumen rams – 72.67. During the summer, the highest PI was obtained in Karakachan sheep – 79.33 and Romanov sheep – 79.20, and on the contrary, Karnobat sheep had the lowest PI ($p < 0.05$). During the autumn, Karakachan sheep had the highest PI again – 80.67, and the lowest phagocytic index (75.33) was determined for Copper-red Shoumen sheep ($p < 0.01$).

During the spring, Replyan sheep had the highest PN – 1.607, and those from the Panagyurishte breed – the lowest – 1.500 ($p < 0.05$). The highest PN was obtained in Romanov and Stara Zagora rams' the lowest PN – in Pleven Blackhead rams ($p < 0.01$).

There was a tendency for highest PI values in sheep during the spring, a certain reduction in the summer and elevation during the autumn. For rams, PI exhibited a general increase during the summer and autumn with variable changes for the different breeds. For both sheep and rams, PN was the highest during the spring, lower in summer and thereafter elevated during the autumn.

Key words: sheep, rams, breeds, phagocytic activity – phagocytic index, phagocytic number

INTRODUCTION

The earliest form of cellular natural immunity in animals is phagocytosis. In the view of Ado & Mayanski (1) and Andonova & Gundasheva (2), in vertebrates and men, phagocytosis depends on polymorphonuclear granulocytes and cells from the mononuclear-phagocytic system, thus reflecting the status of systemic defense and adaptation capacity.

During the recent years, phagocytosis is widely used to test the systemic reactivity under the influence of various factors. Age-, breed-,

gender-, and genetic variations of phagocytic activity have been reported in men (3, 4, 5), fowl (6, 7, 8), sheep, goats (9, 10), calves (11, 12, 13, 14), cattle (15, 16), swine (17, 18, 19, 20), dogs (21), rabbits (20, 22) and mice (23).

The purpose of this study was to investigate the breed-, gender-related and seasonal traits of phagocytic activity in local sheep breeds reared in Bulgaria.

MATERIALS AND METHODS

The studies were performed in 2008 on 168 sheep and 114 rams. Investigations were carried out on 6 sheep and 6 rams from the following breeds: Karakachan, Tsigay, Replyan, Panagyurishte, Copper-red Shoumen, Karnobat, Pleven Blackhead, White Maritsa

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and Patch-faced Maritsa, Stara Zagora and Romanov. The animals were owned by the Institute of Mountain Animal Stockbreeding and Agriculture– Troyan, the Agricultural Institute in Karnobat, The Kabiyuk State Enterprise - Shoumen, the Experimental Farm of the Trakia University – Stara Zagora and private farmers from Chirpan, Saedinenie, Vidin, Panagyurishte, Veliko Tarnovo.

Fasting blood samples were aseptically obtained in the morning from the jugular vein. The phagocytic reaction was determined by the method of Valchanov (24). For this purpose, a 24 h microbial suspension of *St. aureus* strain 209 with a density of $2 \times 10^9/\text{cm}^3$ was used. The phagocytic activity was determined via two parameters – phagocytosis percentage – PI (% phagocytosis) and phagocytic number (PN). PI was determined as a ratio of phagocytized leukocytes and the total leukocyte counts, multiplied by 100. PN was calculated as the number of bacteria, phagocytized by one leukocyte.

The data were statistically processed by one-way analysis of variance (ANOVA) with fixed effects of the factor using the statistical software Statistica (StatSoft Inc.).

RESULTS

Table 1 presents the blood phagocytic index percentages during the different seasons in sheep and rams from studied breeds.

During the spring, the highest PI was found out in Replyan, Copper-red Shoumen and Stara Zagora sheep – 79.33%, and the lowest PI – in Karnobat (76.0%) and Karakachan (76.67%) sheep. For all breeds, PI ranged between 76.00 and 79.33% (on the average 77.78 ± 1.03).

Among studied rams, the highest PI was established in Tsigay and Romanov breeds – 79.33%, whereas the lowest – in Copper-red Shoumen rams – 72.67 ($p < 0.01$). For rams from the other breeds, PI was within 75.00-78.7%. In Karakachan, Copper-red Shoumen, Karnobat, Patched-face Maritsa and Romanov breeds, PI of rams were higher as compared to those of sheep, whereas the contrary was observed for Tsigay, Plevan Blackhead, White Maritsa and Stara Zagora breeds where rams exhibited insignificantly higher PI values than sheep. In general, spring PI was lower in rams (75.67 ± 1.09) as compared to that in sheep.

During the summer, the highest PI was obtained in Karakachan sheep – 79.33 and

Romanov sheep – 79.20, and the lowest PI – in Karnobat sheep ($p < 0.05$). The PI in Stara Zagora sheep was 78.67, in Plevan Blackhead and Patched-face Maritsa – 77.33, in Tsigay, Stara Planina Tsigay and Copper-red Shoumen sheep was equal (76.67%); in White Maritsa – 76.00%. In general, breeds reared in semi-mountainous regions (Troyan) had higher PI values, as well as those reared in the Experimental University Farm in Stara Zagora.

Among rams, those from the Copper-red Shoumen and Romanov breeds had the highest PI values – 79.00% and 78.00%, respectively, whereas the lowest PI (76.00%) was determined in Tsigay rams. The PI in Karakachan and Karnobat rams was 76.67% but the differences were not statistically significant ($p > 0.05$). There was a tendency for higher PI in rams vs sheep in Copper-red Shoumen and Karnobat breeds, and higher PI in sheep vs rams in Karakachan, Tsigay and Romanov breeds.

During the autumn, Karakachan sheep had the highest PI again – 80.67, and the lowest phagocytic index (75.33) was determined for Copper-red Shoumen sheep ($p < 0.01$). The PI in Romanov sheep was also high – 79.33, in Tsigay and Plevan Blackhead sheep PI was equal – 78.00, as in Karnobat and Stara Zagora breeds – 76.67.

From all studied breeds, Tsigay rams were with highest PI (79.33), whereas Karnobat rams – with the lowest one (75.33). The PI in Copper-red Shoumen rams was 78.67, followed by Karakachan rams (77.33). In Tsigay and Copper-red Shoumen rams, PI was higher than that of sheep from the same breed, but in Karakachan and Karnobat males was lower than that of respective females ($p > 0.05$).

Phagocytic numbers in studied sheep breeds and for the ovine species as a whole are given in **Table 2**.

During the spring, Replyan sheep had the highest PN – 1.607 ($p < 0.05$), and Panagyurishte sheep – the lowest (1.500; $p < 0.05$). In sheep from the other breeds, PN values were close to the average value for the species (1.523 ± 0.029).

Table 1. Breed-related and seasonal traits of blood phagocytic index (%) of sheep and rams from local breeds reared in Bulgaria

Breeds	Spring			Summer			Autumn		
	n	$\bar{X} \pm S\bar{X}$	Level of significance	n	$\bar{X} \pm S\bar{X}$	Level of significance	n	$\bar{X} \pm S\bar{X}$	Level of significance
Sheep									
Karakachan	6	76,67 ± 2,40	-	6	79,33 ± 1,606	p	6	80,67 ± 1,909	a
Tsigay	6	78,00 ± 2,25	-	6	76,67 ± 1,606		6	78,00 ± 2,477	a
Replyan	6	79,33 ± 1,91	-	-	-		-	-	
Stara Planina Tsigay	-	-		6	76,67 ± 2,813		-	-	
Panagyurishte	6	78,00 ± 2,25	-	-	-		-	-	
Copper-red Shoumen	6	79,33 ± 2,81	-	6	76,67 ± 2,404		6	75,33 ± 2,171	
Karnobat	6	76,00 ± 2,92	-	6	74,67 ± 2,231	pr	6	76,67 ± 2,404	
Pleven Blackhead	6	78,67 ± 2,67	-	6	77,33 ± 2,459		6	78,00 ± 3,055	
White Maritsa	6	78,00 ± 1,37	-	6	76,00 ± 2,309		-	-	
Patch-faced Maritsa	6	77,33 ± 2,86	-	6	77,33 ± 1,978		-	-	
Stara Zagora	6	79,33 ± 2,62	-	6	78,67 ± 1,978		6	76,67 ± 1,606	
Romanov	6	78,00 ± 1,71	-	6	79,20 ± 1,905	r	6	79,33 ± 1,909	
Total	66	77,78 ± 1,03	-	60	77,25 ± 1,743		42	77,81 ± 1,831	
Rams									
Karakachan	6	75,33 ± 2,40	pq	6	76,67 ± 1,230		6	77,33 ± 3,041	
Tsigay	6	79,33 ± 2,40	aprst	6	76,00 ± 2,733		6	79,33 ± 2,616	
Copper-red Shoumen	6	72,67 ± 1,91	abcu	6	79,00 ± 1,612		6	78,67 ± 2,459	
Karnobat	6	75,20 ± 1,90	drv	6	76,67 ± 2,404		6	75,33 ± 2,404	
Pleven Blackhead	6	76,00 ± 2,31	uw	-	-		-	-	
White Maritsa	6	78,67 ± 2,23	bvxy	-	-		-	-	
Patch-faced Maritsa	6	75,33 ± 2,40	sxe	-	-		-	-	
Stara Zagora	6	75,33 ± 1,91	fty	-	-		-	-	
Romanov	6	79,33 ± 1,91	cdefqw	6	78,00 ± 1,713		6	76,00 ± 2,921	
Total	54	75,67 ± 1,09	-	30	77,27 ± 2,010		30	77,33 ± 2,015	
statistical significance		a, b, c, d, e, f	p < 0,01						
		p, q, r, s, t, u, v, w, x, y	p < 0,05		p, r	p < 0,05		a	p < 0,01

Table 2. Breed-related and seasonal traits of blood phagocytic number of sheep and rams from local breeds reared in Bulgaria

Breeds	Spring			Summer			Autumn		
	n	$\bar{X} \pm S\bar{X}$	Level of significance	n	$\bar{X} \pm S\bar{X}$	Level of significance	n	$\bar{X} \pm S\bar{X}$	Level of significance
Sheep									
Karakachan	6	1,513 ± 0,072	-	6	1,460 ± 0,042		6	1,513 ± 0,050	a
Tsigay	6	1,507 ± 0,060	-	6	1,413 ± 0,063		6	1,427 ± 0,046	
Replyan	6	1,607 ± 0,052	p	-		p	-		
Stara Planina Tsigay	-			6	1,367 ± 0,050		-		
Panagyurishte	6	1,500 ± 0,069	p	-			-		
Copper-red Shoumen	6	1,523 ± 0,068	-	6	1,440 ± 0,060		6	1,400 ± 0,065	abc
Karnobat	6	1,533 ± 0,072	-	6	1,400 ± 0,045		6	1,447 ± 0,066	
Pleven Blackhead	6	1,513 ± 0,068	-	6	1,447 ± 0,061		6	1,507 ± 0,071	b
White Maritsa	6	1,553 ± 0,066	-	6	1,440 ± 0,048		-		
Patch-faced Maritsa	6	1,553 ± 0,060	-	6	1,413 ± 0,074		-		
Stara Zagora	6	1,527 ± 0,054	-	6	1,453 ± 0,047		6	1,460 ± 0,041	
Romanov	6	1,507 ± 0,084	-	6	1,456 ± 0,053		6	1,520 ± 0,061	c
Total	66	1,523 ± 0,029	-	60	1,429 ± 0,039		42	1,468 ± 0,059	
Rams									
Karakachan	6	1,487 ± 0,056	-	6	1,400 ± 0,046		6	1,427 ± 0,055	a
Tsigay	6	1,507 ± 0,063	-	6	1,413 ± 0,056		6	1,507 ± 0,086	b
Copper-red Shoumen	6	1,433 ± 0,076	pq	6	1,480 ± 0,015	p	6	1,520 ± 0,051	d
Karnobat	6	1,432 ± 0,068	ar	6	1,447 ± 0,024		6	1,413 ± 0,074	c
Pleven Blackhead	6	1,407 ± 0,051	bc	-	-		-	-	
White Maritsa	6	1,427 ± 0,047	ds	-	-		-	-	
Patch-faced Maritsa	6	1,440 ± 0,050	tu	-	-		-	-	
Stara Zagora	6	1,540 ± 0,064	bprst	-	-		6	1,760 ± 0,080	abcde
Romanov	6	1,553 ± 0,081	acdqu	6	1,380 ± 0,027		6	1,533 ± 9,126	e
Total	54	1,457 ± 0,029	-	30	1,424 ± 0,041		36	1,527 ± 2,143	
statistical significance	in sheep	p	p < 0,05				in sheep	a,b,c	p < 0,05
	in rams	a, b, c, d	p < 0,01				in rams	d, e	p < 0,01
		p, q, r, s, t, u-	p < 0,05		p	p < 0,05		a,b,c	p < 0,05

In rams, highest PN values were exhibited by Romanov and Stara Zagora breeds, and lowest – by the Pleven Blackhead breed ($p < 0.01$). It should be noted that rams in general had lower PN values than sheep – 1.457 vs 1.523 ($p < 0.01$), largely due to lower PN of rams from individual breeds with the exception of Tsigay rams (whose PN was the same as that of sheep) and Stara Zagora and Romanov rams (with higher PN than those of respective females).

During the summer, the tendency of change in PN values was similar to that observed for PI values. The highest PN was established for Karakachan (1.46), Romanov (1.456) and Stara Zagora sheep (1.453). Then followed Pleven Blackhead – 1.447, White Maritsa and Copper-red Shoumen (pigmented Tsigay) – 1.440, Patched-face Maritsa and Tsigay – 1.413, Karnobat – 1.400 and Stara Planina Tsigay – 1.367. In rams, highest PN values were found out in the Copper-red Shoumen breed (1.48) followed by Karnobat (1.447), Tsigay (1.413), Karakachan (1.400) and Romanov (1.380) breeds. Phagocytic numbers of blood in rams from the Karnobat and Copper-red Shoumen breeds were higher than respective values in sheep whereas such a difference was not observed for Karakachan and Romanov breeds.

During the autumn, Romanov (1.520) and Karakachan (1.513) sheep showed that highest PN values whereas Copper-red Shoumen sheep – the lowest (1.400; $p < 0.05$). Pleven Blackhead sheep had higher PN as compared to the Copper-red Shoumen breed – 1.507 ($p < 0.05$). The PN values tended to be higher in the descendants of the Tsakel breed. In Stara Zagora sheep, PN was on the average 1.460. In Tsigay (1.427) and Karnobat sheep (1.447) the blood phagocytic activity was lower. Among studied rams, those from the Stara Zagora and Romanov breeds had the highest PN values – 1.76 and 1.533, respectively and those from the Karnobat breeds – 1.413 ($p < 0.05$). Lower PN was observed in Tsigay (1.507), Karakachan (1.427) and Karnobat (1.413) rams. As compared to sheep from the same breed, Tsigay, Copper-red Shoumen, Stara Zagora and Romanov rams had higher PN values, and those from the Karakachan, Karnobat and Pleven Blackhead breeds - lower PN.

DISCUSSION

The differences in phagocytic activity of sheep from various breeds are possibly a consequence of immune system adaptation to

environmental conditions in the dwelling areas and the local antigens. Being descendants to old Karakachan and Tsigay sheep, contemporary sheep carry at a various extent the hereditary information of their progenitors that could be responsible for the different phagocytic activity. Most probably, phagocytic activity in sheep is related to their production type as well as to environmental factors such as ambient temperature, humidity, altitude, pasture sward, soil type, air and water status etc.

As shown by presented results, there was a tendency for highest PI in the spring, reduced values in the summer and another increase in the autumn in local sheep. For rams, PI increased in the summer and the autumn, but the changes were not consistent in the individual breeds. For the species in general, phagocytic numbers were the highest in spring, decreased during the summer months and increased again in the autumn.

Variations in blood phagocytic activity due to the season, breed, and gender were also found out in goat kids, goats and bucks (25, 26). The highest PI in studied goats was observed during the summer ($80.97 \pm 0.867\%$), and the lowest – in winter ($78.53 \pm 0.801\%$). There was a tendency towards highest PI values during the spring (1.416 ± 0.020) and towards gradual decrease during the other seasons, with lowest values in winter (1.373 ± 0.020). In bucks, PI tended to be the lowest in spring and highest in summer ($81.11 \pm 1.364\%$). The phagocytic number in bucks was higher during the spring, reduced in summer and autumn, and the highest in winter (1.449 ± 0.027). For the different studied goat breeds, seasonal patterns of phagocytic activity changes were various.

Gender- and breed-related differences were observed in previous studies of ours in chickens (8). Three-month old White Rhode Island pullets had higher phagocytic activity than cockerels, whereas the Red Rhode Island pullets – a lower one (6). In pigs, phagocytic activity changed with age and depended upon the breed and the breeding technology (20). In cattle, phagocytic activity increased to the 2nd-3rd year of age (14) and was the most reduced by the 8th year of age. Phagocytic activity was exhibited at a various extent in the different breeds (16). Breed-related variations were also reported in sheep (9).

The phagocytic activity is related to the other factors of immunity (lysozyme, complement, properdins etc.) as well as to blood composition, thus being a subject of interest for more detailed studies.

According to our results, there were variations related to gender and breed in sheep and they should be therefore taken into consideration when interpreting their immune status, health condition and productivity.

CONCLUSIONS

There was a tendency for highest PI values in sheep during the spring, a certain reduction in the summer and elevation during the autumn. For rams, PI exhibited a general increase during the summer and autumn with variable changes for the different breeds. For both sheep and rams, PN was the highest during the spring, lower in summer and thereafter elevated during the autumn.

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