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THE HAEMOGLOBIN, TRANSFERRIN, CERULOPLASMIN AND GLUTATHIONE POLYMORPHISM OF NATIVE GOAT BREEDS OF TURKEY, I- ANGORA AND HAIR

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Abstract

This study has been carried out in order to determine the polymorphic traits of various biochemical parameters in goat breeds which are native to Turkey. For this purpose, Angora and Hair goat breeds were chosen as live materials. Two different herds for each breed were selected from Ankara and Antalya, respectively. Blood samples were taken from a total of 120 goats aged between 2 and 4 which was made up of 60 Angora goats and 60 Hair goats. All which derived equally from 4 lots of herds. Analyses were performed for the polymorphic determination of the haemoglobin (Hb), transferrin (Tf), ceruloplasmin (Cp) and glutathione (GSH). Hb types were determined by starch gel electrophoresis and Tf types were detected by SDS-PAGE electrophoresis. Furthermore, Cp and GSH analyses were performed by spectrophotometrically. Following the analysis, Hb types were found as 3 genotypes (AA, AB, BB) controlled by 2 allel genes (Hb^A, Hb^B). Tf types were found as 6 genotypes (AA, AB, AC, BB, BC, CC) controlled by 3 allele genes (Tf^A, Tf^B, Tf^C). Findings for Hb were in line with the Hardy-Weinberg Equilibrium (HWE) in Angora goats while the Hair goat was not found to be in line. Moreover, Tf was found in line with the HWE for 2 separate goat breeds. The levels of Cp and GSH of two breeds were significantly different from other ($P<0.0001$). The findings are recorded as a source of reference for prospective polymorphism studies.

Key words: *Electrophoresis, genetic resource, goat, spectrophotometer.*

INTRODUCTION

Living things are adapted to a particular region, maintained presence here and widely grown. This condition is referred as genetic diversity. Moreover, native gene pool is consisted in species belonging to the races. Protection of the shrinking and verge of extinction domestic breeds are protected by suitable reclamation works. On the other hand, the creation of superior efficiency of the herds is essential.

According to FAO (2012), the data of the presence of goats in Turkey asset share in the world in the past 40 years (1961-2011) were declined as 0.719 % from 7.073 % in the past 40 years. One reason of this is also the genetic erosion of kinds. For this reason, identification and control of genetic resources are great importance in terms of biochemical polymorphic features Turkey's livestock. Goat with 90% pregnancy rates is the most efficient type of domestic animal (Ince, 2010). Better quality breeding may be possible with the selection of high-yielding goats among the population (Kurnianto, 2009).

All over the world, Angora goats (*Capra hircus ancyrensis*) known as "Angora" goats homeland is Central Asia. This breed was grown in Turkey from the 13th century (Porter, 1996) until the middle of the 19th century. Breeding of Angora goats were began with 1 pairs of Angora goat and 12 kids in South Africa (Anonymous, 2013).

The importance of this breed utilized from meat, skin and rarely milk is mohair. A type of lint known as "Mohair" in the World is a valuable raw textile materials with long, soft and shiny fiber-like structure. Mohair produced abroad has not reached the same levels of produced in Turkey in terms of important features such as delicacy and softness. For this

reason, Turkey's role in the World market is very important at mohair.
In our country, the majority of the race has been spread to mainly in Ankara, Central Anatolian Plateau on especially in Anatolia. The purest samples collected on breed characteristics (Fig. 1a, b) are located in the Ankara region.

Figure 1. Angora Goats a) 1st herd b) 2nd herd

In order to improve the efficiency of our ruminants, define the genetic structure of populations and know the herds for the yield quality are necessary. On this case, it is heavily studied for making use of some blood parameters in order to obtain a new generation of high efficient. Biochemical parameters and introduction of genes and their mechanisms as well as detailed studies on the molecular level are very often used for the recognition of different biomolecules structures of herd.
Hair goat is another goat breed commonly found in Turkey. They are known "Black goat" or "Ordinary goat" among the people. These animals benefit from scrub areas, they are resistant to diseases and their adaptability are quite high.
Hair goat (Fig. 2a, b) is grown by Turkmens and Nomands as substantially domesticated animals and cover of the Nomand's tent is weaved from hair of these goats. Spread area of the Mediterranean Region and in the Antalya, Konya, Isparta triangle areas are mainly inhabited by Nomads. Especially rich marquis of vegetation, steep mountains and rugged construction of Mediterranean region are effective for goat breeding. Hair goat known as hot and cold-tolerant breed, its contribution to the local economy is very large and production of meat and hair are significant. Goat meat is higher preferred than the sheep meat by people living in the Antalya Region.

Figure 2. Hair goats a) 1st herd b) 2nd herd

Polymorphism studies made in goats hold common part of the genetic studies. This case means the differences between individuals in a population. Bushman and Schmid (1968) have drawn attention to the rare allele frequency in the population at least two alleles of a locus when defining the genetic polymorphism. Rare alleles relative frequency in the population should be at least 0.05 or 0.01 levels for a gene locus to be polymorphic.
Balanced polymorphism is an important term of polymorphism. This case is expressed as a heterozygote and homozygote mixture provided that with progeny mixture and opposite the selection pressure. Balanced populations are desirable in HWE. On the other hand, genes frequency of comprising population in the gene pool remains constant from insemination to fertilization. Also this type of populations is named as stable (balanced) population. Thus, HWE is achieved.
Hb which a large and complex protein is one of the polymorphic system that the most widely studied in vertebrates (Alphonsus et al., 2012). The presences of Hb in different structures were detected in the sheep bloods for the first time by Harris and Warrien (1955) and Cabannes and Serai (1955) (Dogrul 1985). Genetic variation of iron-binding protein was found for the first time in serum Tf.
In mammals, 90-95% of plasma Cu are found as Cp form which depending on α2-globulin (Burtis and Ashwood, 1999). There are significant positive correlations between the level of Cp and Cu levels of plasma, serum and whole blood (Herbert and Ravin, 1991).
GSH concentration in erythrocytes is controlled by one pair of autosomal allele gene. Genes which controlled by high levels of glutathione (GSH^H) are thought to be dominant versus to genes that controlled by low level of glutathione (GSH^h) (Rizzi et al., 1988).

MATERIALS AND METHODS

Sample collection phases of this study were done at Ankara-Ayas for Angora goat and Antalya-Akseki for Hair goat. Independently herds of goats have been determined in firstly. But similar care and feeding conditions are preferred for these goats in semi-intensive breeding system. Herds width were not allowed to be fewer than from 150 goats.

Totally, 120 goats in which 30 goats from each herds were studied on Angora and Hair goat breeds that native genetic resources grown in Turkey. A random selection among the other animals was made for adult female goats (2-4 years old) that appeared clinically healthy and showed the most obvious characteristics as phenotypic in the selected herds. Blood samples were taken at 8 o'clock in the morning from hungry goats which have done last feeding before one night.

The principle of Hb type assay is based on the separation of polymorph properties in the heredity structure of Hb with direct current power in starch gel plates (Soysal, 1983). Sample's Hb type in this study were studied in erythrocytes with continuous buffer system that using the horizontal starch gel electrophoresis system (Meyer, 1963; Braend, 1971). Also Hb genotypes were directly determined on the gel after than individual genotypes were noted. They were defined as the quick-moving type: Hb^A and the slow-moving type: Hb^B (Ustdal, 1976).

Serum samples were used for Tf analysis. Dashed SDS-PAGE method was adapted to BioRad Mini Protean Tetra Cell system for Tf system in these analyzes. And so, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zacharius et al., 1969; Racusen, 1979; Jay et al., 1990) was performed (Laemmli, 1970). Then, Tf types were read considering the electrophoretic rapids. Gel which carried samples was scanned and passed from the scanner and determined the genotype of each individual was noted in the computer environment. Bands of Tf were defined as the fastest-moving type: Tf^A, the slowest-moving: TF^C (Dogrul, 1985). All bands based on their fast were listed as Tf^A, Tf^B and Tf^C, from fast to slow.

Hb and Tf gene and the genotype frequencies were determined by direct counting method (Nei, 1987; Russell, 1992). Chi square ($\chi 2$) test was performed (Pembeci, 1978; Duzgunes et al., 1987; Yeh et al., 1997) for genetic equilibrium control of populations (importance of between the observed and expected genotype differences - HWE).

GSH levels in whole blood and Cp levels in serum were determined with spectrophotometrically. Absorbance of the colored product was based on P-phenylene diamine dichloride (PPD)-induced serum samples with acetate buffer (pH 5.2 and 37°C). Studied samples at 550 nm were analyzed for Cp levels (Ceron and Subiella- Martinez, 2004). Then according to the described method in the reference literature (Curzon and Vallet, 1960), Cp levels were calculated. GSH measurement was based on the analysis of the measured resultant yellow color as spectrophotometrically (Beutler et al., 1963; Rizzi et al., 1988). The amount of GSH in these colored compounds optical density at wavelength of 412 nm was evaluated by determining (Burtis and Ashwood, 1999). SAS 9.3 statistical analysis software package was used in GSH and Cp levels in the calculation as statistically.

RESULTS AND DISCUSSIONS

Three genotypes (AA, AB, BB) in Hb bands were obtained with starch gel electrophoresis in Angora and Hair goats. Frequencies of these genotypes and homologous / heterologous genotypes were presented in Table 1.

Difference between observed and expected gene frequencies in Angora goats was found to be non-significant at Hb electrophoresis. For this reason, The 1st herd, the 2nd herd and intra-breed population of Angora goats were in HWE in terms of Hb genotypes.

Difference between observed and expected gene frequencies of Hair goat in Hb electrophoresis were significant at the 1st herd ($P<0.05$), the 2nd herd ($P<0.01$) and intra-breed population ($P<0.0001$). HWE was found in this regard with respect to Hb genotypes (Fig. 3).

Table 1. Hb genotypes, allele frequencies and homologous / heterologous genotypes

		Angora Goat			Hair Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
N / GENES		30	30	60	30	30	60
GENOTYPE	HbAA	14	13	27	12	17	29
		%	%	%	%	%	%
		46.67	43.33	45.00	40.00	56.67	48.33
	HbAB	12	15	27	8	6	14
		%	%	%	%	%	%
		40.00	50.00	45.00	26.67	20.00	23.33
	HbBB	4	2	6	10	7	17
		%	%	%	%	%	%
		13.33	6.67	10.00	33.33	23.33	28.33
FREQUENCY							
	HbA	40	41	81	32	40	72
		0.666	0.683	0.675	0.533	0.666	0.600
		7	3	0	3	7	0
	HbB	20	19	39	28	20	48
		0.333	0.316	0.325	0.466	0.333	0.400
		3	7	0	7	3	0
GEN	Hb AA,B B	18	15	33	22	24	46
		%	%	%	%	%	%
		60.00	50.00	55.00	73.33	80.00	76.67
	Hb AB	12	15	27	8	6	14
		%	%	%	%	%	%
		40.00	50.00	45.00	26.67	20.00	23.33

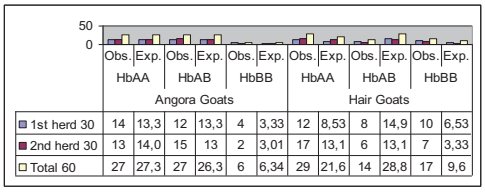


Figure 3. Observed and expected values of Hb frequencies in Angora and Hair goats

Six genotypes (AA, AB, AC, BB, BC, CC) of Tf were obtained in SDS-PAGE electrophoresis of Angora and Hair goat bloods. However, in Angora goats, AC genotype was not observed in the 1st herd. Homologous/heterologous genotypes and % frequencies of these genotypes were given in Table 2.

Observed and expected Tf gene frequencies differences were not significant in the electrophoresis of Hair goats. In this regard, the 1st herd, the 2nd herd and intra-breed population were in HWE in terms of Tf genotypes (Fig. 5).

Table 2. Tf genotypes, allele frequencies and homologous/ heterologous genotypes

		Angora Goat			Hair Goat		
		1st Herd	2nd herd	Total	1st Herd	2nd herd	Total
N / GENES		30	30	60	30	30	60
GENOTYPE	Tf AA	1	2	3	1	4	5
		%	%	%	%	%	%
		3.33	6.67	5.00	3.33	13.33	8.33
	TfAB	1	11	12	9	10	19
		%	%	%	%	%	%
		3.33	36.67	20.00	30.00	33.33	31.67
	TfAC	-	1	1	1	2	3
		%	%	%	%	%	%
		0.00	3.33	1.67	3.33	6.67	5.00
	TfBB	12	7	19	12	6	18
		%	%	%	%	%	%
		40.00	23.33	31.67	40.00	20.00	30.00
	TfBC	12	7	19	6	5	11
		%	%	%	%	%	%
		40.00	23.33	31.67	20.00	16.67	18.33
	TfCC	4	2	6	1	3	4
		%	%	%	%	%	%
		13.33	6.67	10.00	3.33	10.00	6.67
FREQUENCY	TfA	3	16	19	12	20	32
		0.050	0.266	0.158	0.200	0.333	0.266
		0	7	3	0		7
	TfB	37	32	69	39	27	66
		0.616	0.533	0.575	0.650	0.450	0.550
		7	3	0	0	0	0
	TfC	20	12	32	9	13	22
		0.333	0.200	0.266	0.150	0.216	0.183
		3	0	7	0	7	3
GEN	TfAA,B B CC TfAB AC BC	17	11	28	14	13	27
		%	%	%	%	%	%
		56.66	36.66	46.66	46.66	43.33	45.00
		13	19	32	16	17	33
		%	%	%	%	%	%
		43.33	63.33	53.33	53.33	56.66	55.00

Gen frequency differences of observed and expected in Tf electrophoresis were found significant ($P<0.01$) at the 1st herd of Angora goats. This herd in terms of Tf genotypes was not in the HWE. However, the 2nd herd and intra-breed population were not detected in HWE (Fig. 4).

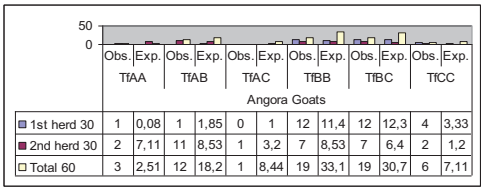
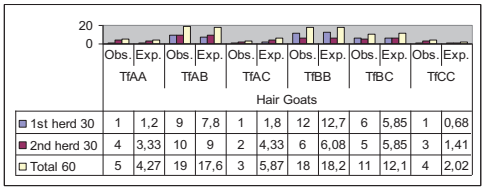


Figure 4. Observed and expected values of Tf frequencies in Angora goats.



1st herd 30	1	1,2	9	7,8	1	1,8	12	12,7	6	5,85	1	0,68
2nd herd 30	4	3,33	10	9	2	4,33	6	6,08	5	5,85	3	1,41
Total 60	5	4,27	19	17,6	3	5,87	18	18,2	11	12,1	4	2,02

Figure 5. Observed and expected values of Tf frequencies in Hair goats.

Significant differences (P<0.001) were detected in the 1st and the 2nd herds of Angora and Hair goats for analyzed Cp results. Fairly high level significant difference (P<0.0001) was found between breeds in terms of the Cp (Tab. 3).

Table 3. CP and GSH values in Angora and Hair goats.

		Angora Goat			Hair Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
CP	X	2.608	1.864	2.236	1.685	0.591	1.138
	±Sx	±0.14 9	±0.11 3	±0.10 4	±0.17 3	±0.07 0	±0.54 7
	Xmin	0.943	0.314	0.314	0.157	0.157	0.157
	Xmax	5.500	2.986	5.500	3.614	3.043	3.614
	P	<0.001			<0.001		
GSH	x±Sx	43.49 3±0.5	33.24 4±0.5	38.36 8±0.7	35.22 8±0.6	38.21 9±0.6	36.72 3±1.4
		94	93	86	29	90	92
	Xmin	37.33	28.80	28.80	29.33	32.26	29.33
	Xmax	48.53 3	39.46 7	48.53 3	42.66 7	46.13 3	46.13 3
	P	<0.001			<0.01		

Also scatter diagram of Cp values at the 1st and the 2nd herds were given in Fig. 6.

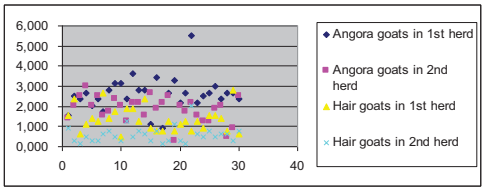


Figure 6. Cp scatter diagram

Also, scatter diagram of GSH levels was given for totally two herds from each breed in Fig. 7.

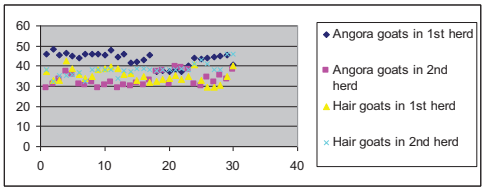


Figure 7. GSH scatter diagram

GSH levels were evaluated on the basis of herd. Goats below average were divided as GSH^H and the others were determined as GSH^h. These two groups were analyzed independently in themselves. The lowest GSH^h average (30.948 ± 0.268 mg/dl) was observed in the 2nd Angora goat herd. However the highest GSH^H average (39.903 ± 0.735 mg/dl) was found in the 1st herd of Angora goat (Tab. 4).

Table 4. GSH^h and GSH^H levels in Angora and Hair goats

		Angora Goat		Hair Goat	
		1st Herd	2nd herd	1st herd	2nd herd
N		30	30	30	30
GSH^h	X	39.903	30.948	32.879	35.980
	±Sx	±0.735	±0.268	±0.412	±0.589
	X_{min}	37.333	28.800	29.333	32.267
	X_{max}	43.467	33.067	34.667	38.133
GSH^H	X	45.572	36.689	38.555	40.644
	±Sx	±0.265	±0.595	±0.590	±0.851
	X_{min}	44.000	33.600	35.733	38.400
	X_{max}	48.533	39.467	42.667	46.133

In this study, 4 different polymorphic parameters, Hb, Tf, Cp and GSH were studied in Angora and Hair goats. Hb system was determined by 2 codominant alleles (Hb^A, Hb^B), when Tf system was controlled by 3 codominant alleles (Tf^A, Tf^B, Tf^C) in investigated goats. Also, Cp and GSH levels were determined in the expected physiological values.

A study on the 186 Hair goats was done in Antalya province by Karabag (2000). Accordingly, 126 HbAA and 40 HbAB genotypes were in his samples. Hb genotypes had been done for aimed at determining the Hb genotype frequencies of domestic Hair goats by Boztepe et al. (1993) in a different study. And Hb genotype variants have determined as HbAA: 0.50, HbAB: 0.48 HbBB: 0:02. In the same study, gene frequencies have been identified as Hb^A: 0.74 and Hb^B: 0:26.

Alphonsus et al. (2012) have come across Hb^C allele in goats reared in Abuja Nigeria in their study. 4 genotypes (AA, AB, BB, AC) managed by 3 allele genes (Hb^A, Hb^B, Hb^C) have been identified in the same study executed on 94 goats. But the HbBC and HbCC genotypes were not detected. Allele of the Hb^C was found at very low frequency as 0.0640 while HbAC genotypic frequency was 0.1227. Hb^A allele has been determined with the highest frequency of 0.6010.

In this study, Angora goats HB^A and Hb^B allele frequencies were calculated as 0.6667 and 0.3333 in the 1st herd, as 0.6833 and 0.3167 in the 2nd herd, respectively. Hb^A and Hb^B allele frequencies of Hair goats were detected as 0.5333 and 0.4667 in the 1st herd, as 0.6667 and 0.3333 in the 2nd herd, respectively. Two alleles were also determined in different study conducted on 54 Norduz goats by Aygun (2006) Also, 48% HbAA, 48% HbAB and 4% HbBB genotypes have been identified. Allele frequencies have been reported as 0.7222 and 0.2778 for Hb^A and Hb^B, respectively. Although there were different breeds, these results were similar to this study.

In this study, two allele genes (HB^A, HB^B) controlled by three hemoglobin genotype (AA, BB, AB) were determined. Three genotypes (AA, BB, AB) were also detected in the presence of both Angora and Hair goat herds. Common allele frequencies were less than 95%, so Hb system has been considered polymorphic in this study conducted in Angora and Hair goats.

Hair goats were not in HWE with regard to Hb genotype frequencies. We could be said to protect of stability of gene and genotype structures under the Panmixia conditions for these goats. Such as the used a small number of male goat could be effective on this case. On the other hand, small herd and used their own males could be caused to departing from genetic stability. It was sufficiently believed that population size and such case as chances factor could be effective on Angora goats.

TfAA, TfAB and TfBB genotypes in Norduz goats were detected by Aygun (2006). He had been identified as 0.787 and 0.213 for Tf^A and Tf^B in same study, respectively. Nozawa et al. (1978) had been detected Tf^A, Tf^B and Tf^C alleles in a different study which in Japanese goats. In the same study, Tf^A was reported as monomorphic. Tf^A in the Spanish domestic goat was identified as predominant by Tunon et al. (1987). Additionally, Tf^C was detected in only Negra Serrana breed by them.

Levels of Tf frequencies were detected as 0.0500 and 0.2667 for Tf^A, 0.6167 and 0.5333 for Tf^B, 0.3333 and 0.2000 for Tf^C in this study conducted in the 1st and the 2nd herds of Angora goats, respectively. Tf frequencies in the 1st and the 2nd herds of Hair goats were calculated as Tf^A: 0.2000 and 0.3333, Tf^B:0.6500 and 0.4500, Tf^C: 0.1500 and 0.2167, respectively. However, TfAC genotype could not be determined in the 1st herd of Angora goats. We have thought to be associated with scarcity of Tf^C allel in this breed. On the other hand, the effect of aleatory factor was not ignored for the selected animals.

In general, three allel genes (Tf^A, Tf^B, Tf^C) which controlled by the 6 Tf genotype (AA, AB, AC, BB, BC, CC) were determined in the goat herds. Presence of 6 genotypes was detected in both Hair goat herds. However, TfAC genotype was not determined in the 1st herd of Angora goat. Due to chance factor could be effective on this case. On the other hand, lack of one genotype was usual if we have thought that 6 genotypes detected in 30 goats.

Fewer male goats were probably used at researched materials. So, Tf genotype frequencies in populations and herds (the 1st herd of Angora goats, the 2nd herd of Hair goats) were not in HWE. Breeders have not applied any selection in investigated goat breeds. In general, the main reasons for departing from the genetic stability could be listed as migration, the effect of breeding systems, genetic mutations and absence of sufficient size of herd. Also, lots of high mating and using male goats with high reproductive performance and fertility could be leaded to some problems in terms of breeding. This condition might be brought genetic problems due to inbreeding such inadequate herd size and various chance factors were thought to be effective on the HWE.

Allele number of features which have been considered to be effective on analyses of blood characteristics with genetic variation (such as Hb and Tf ...). If the number of your samples is sufficient in study, homologous and heterozygous genotypes can obtain higher chances for determining by two allele genes (A, B) with three genotypes (AA, AB, BB) in different populations. Otherwise you may not have the chance to identify all of homologous and heterologous genes. Blood Hb and Tf electrophoresis were studied on 30 goats of each herd in this planned study. We have considered that obtained genotypes were enough for sufficient to define the whole

population. With more than expected number of genes alleles (3 and more) characteristics of the herd size might be more effective incoming research results. Otherwise, we would not have any chance for identify and describe the entire of population.

Investigated study, the average Cp values in Angora and Hair goats were detected as 2.236 ± 0.104 mg/dl and 1.138 ± 0.547 mg/dl on average, respectively. High level significant differences were detected in Angora and Hair goat herds when Cp levels were examined. It was thought to be associated with nutrition of goats and physiological states because blood samples were taken in February. Thus, the weak pasture composition might be effect on blood mineral levels.

Gurcan et al. (2011) have used Saanen X Malta crossbred goats in their erythrocyte GSH study. GSH^H and GSH^h allele gene frequencies have been reported as 0.43-0.57 in the same study by them. Otherwise in this study, average GSH levels were detected as 38,368 ± 0786 mg/dl in Angora goat herds and as 36,723 ± 1,492 mg/dl in Hair goat herds. Differences between Hair goat herds were found significant when GSH levels were examined on the basis of herds. On the other hand, high significant level difference was detected between two herds of Angora goats. This might be related with the result of different care and feeding conditions.

CONCLUSIONS

As a result, we can say that there was probably enough number of male goats for this study. About these goats may be said to be homogenous dispersion in herd and hold regular election especially for cases where the equilibrium provided. Other studies showed that numerical values obtained in polymorphic characters such as Cp and GSH were seen sufficient number of samples in the study. But, polymorphism studies with more numerous samples may be appropriate for multiple allele system examination.

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