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Multivariate Discriminant Analysis on Differentiating Sheep Breeds Based on Live Body and Carcass Measurements

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Meat tracing, Canonical discriminant analysis, Lamb

ABSTRACT

The multivariate technique analysis was utilized for assigning and discriminating three Saudi sheep breeds, Naemi, Najdi and Hari based on live body and carcass traits. The traits were body height at wither and rump, body length, heart girth, body depth, head length, ear length, body weight at slaughtering, empty body, dressing percentage, hot carcass, cold carcass, and head weight which had a significant effect on breed. The phenotypic associations between the traits were also studied and strong associations between economically important traits were reported. The analyses of principal components were efficient in showing the total variation of 13 traits accumulated in linear combinations of four traits of most discriminant power body depth, ear length, body height at the rump, and head length. About 77% of the total variation between the breeds. Overall, the analysis of canonical discriminant was very successful in verifying the carcass of each breed considering thus previously mentioned 13 traits. It is recommended to disseminate the findings of this study as a guideline to assign slaughtered sheep and its carcass to its own breed when other means impossible to take place.

INTRODUCTION

Sheep breeds are characterized and differentiated on phenotypic traits and molecular genetic data (Al-Atiyat *et al.*, 2018) for taxonomy, conservation, and breeding purposes. The information provided so far describes breed differentiation from other breeds based on the genetic structure of their gene pools considering geographic, reproductive, and gene flow (Al-Atiyat *et al.*, 2018). Thus, breeders use different techniques to assign individual sheep to a specific breed. Butchers and consumers, on the other hand, find it difficult to compare or assign sheep individuals slaughtered based on carcasses to their specific breeds. Animal phenotype is without a doubt the oldest method in breed taxonomy studies (FAO, 2007). However, some phenotypic traits, such as meat characteristics, are linked to the quality of sheep meat. Several studies and reviews have associated the quantity

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and quality of sheep carcasses with breeds (Markovi´c *et al.*, 2019; Prache and Schreurs, 2022).

There are specific preferences for sheep meat carcasses and their quality in each country. These preferences have also been noticed by consumers for sheep meat (FAO, 2012). The sheep carcass and meat quality were linked to production system characteristics such as farm or grassland-based systems (Prache and Schreurs, 2022). Quality has been particularly linked to commerciality, which stems from consumer preferences for specific breeds of sheep. Many researchers were successful in using various tools to assess and predict sheep breeds based on live body and carcass measurements (Markovi'c *et al.*, 2019; Al-Atiyat and Al-Dawood, 2021; Al-Atiyat *et al.*, 2021; Suliman *et al.*, 2022). Simple statistical techniques, such as correlations on live bodies and carcass measurements allowed for speculation on their past and origin. In fact, many researchers have reported successful comparisons between breeds based on carcass measurements, considering factors such as slaughter body weights, ages, and feeding systems (Silva and Pires, 2016; Pinheiro and Jorge, 2010). These researchers used statistical methods based on quantitative, qualitative, and behavioral characteristics that provide reliable genetic discriminants. For example, Dillon and Goldstein (1984) described statistical multivariate discriminant analysis, which does not limit the number of monitored variables while also confirming the discriminatory capacity point of view in sheep (Yadav *et al.*, 2017; Hailemariam *et al.*, 2018). Studies have reported that the combination of multiple traits and multivariate analysis techniques were extremely efficient for carcass characteristics (Medeiros *et al.*, 2009; Bezerra *et al.*, 2012; Urbano *et al.*, 2015).

The necessity and importance of the study provide evidence ability of multivariate and discriminant analyses to identify carcasses of preferred sheep breed when others DNA based test or other tests are unavailable or expensive. Thus, butchers and consumers would be skilled to assign sheep carcasses to authentic breeds after slaughtering avoiding any fraud. This study aimed to apply multivariate and discriminant analyses to identify live body and carcass traits have the best discriminatory power in assigning sheep to their breeds.

MATERIALS AND METHODS

Live body and carcass measurements of sheep breeds

The body performance of twenty-four intact 12-months old males from three major Saudi sheep breeds Najdi (NJ), Neaimi (NM) and Harri (HA) was measured in terms of live body biometrics, slaughter weight, and carcass. Eight male lambs from each breed, each used as an experimental unit, were selected randomly for this study. The sample size was determined using two approaches. The first is to take into account the similar number suggested by previous studies that dealt with similar work and found significant differences (Gaili, 1993; Al-Haidary, 2004). The second approach was using PASS 13 (Hintze, 2013) software in order to determine the best sample size. The sample size of 80 sheep from each breed were sufficient to provide reliable results using PASS 13 software. The lambs were as homogeneous as possible in terms of age (3 months old) and weight (15 kg).

Upon arrival at the study site, the experimental farm of the Department of Animal Production of the College of Food and Agricultural Sciences at King Saud University, all animals were ear-tagged and treated against internal and external parasites. They were divided into three groups of eight animals each. The feeding period extended for 90 days, preceded by a 14-day adaptation period during which alfalfa (*Medicago sativa*) hay and a graded amount of the experimental diet were provided to the lambs. The experimental diet was a concentrate mixture formulated isocalorically and isonitrogenously to satisfy all the nutrient requirements of the animals according to the NRC (1985). The daily ration was served twice a day (ad lib, twice daily system), at 8:00 in the morning and 15:00 in the afternoon. Drinking water and salt licks were available around the clock. Throughout the feeding period, all performance parameters were measured and recorded. The live body measurements of this study were final body weights (at slaughtering), external linear body measurements, body length, body height at withers and rump, heart girth, body depth, head length, and ear length. The carcass measurements were slaughter weight, empty body weights, hot and cold carcass weights, dressing percentage, and head weight. An electronic measuring balance was used for measuring weights, and measuring plastic tape was used to measure length and girth traits. In detail, lambs were weighed as live body weight in the morning of the slaughtering day after a 16-h fast using an electronic small animal scale for lambs. After slaughtering, hot and cold body carcass weights were taken using the same electronic balance and recorded to determine the dressing percentage. Carcass and non-carcass components, including the head, were weighed immediately after slaughter, and the weight of the digestive contents was computed as the difference between the full and empty digestive tract. The empty body weight was computed as the difference between the slaughter weight and the weight of digested content. On the other hand, measuring the length of the lamb's body was done using measuring tape from the point of the shoulder to the pin bone. Heart girth was measured around the heart girth and in relation to the location of the lamb's heart. It was ensuring an accurate measurement during these measurements by compressing the sheep's wool so that the measure reflected the body and did not include the body plus the wool.

The statistical multivariate discriminant analysis

Data for continuous quantitative variables were collected and constructed into a SAS format file. Using SAS program version 9.2 (SAS, 2012), the SAS file was subjected to various procedure runs of discriminant and clustering analyses. The SAS procedures were calculating means (PROC MEANS), and using simple discriminant analysis (SAS DISCRIM) to discriminate probabilities of including or excluding lamb in a predefined breed. In addition, the stepwise discriminant procedure (STEPDISC) was applied to determine the body variables used in the final clustering analysis. The canonical discriminant analysis procedure (CANDISC procedure), was used to perform uni- and multivariate analysis to derive canonical variables (CAN) for the best match breed or strain (Sneath and Sokal, 1973; SAS, 2012). Furthermore, Mahalanobis distances, which measure genetic square distances, were also generated. Finally, MEGA software used Mahalanobis distances to reconstruct a dendrogram (Tamura *et al*., 2013). Shapiro-Wilk test was performed to assess the normality of the variables.

RESULTS AND DISCUSSION

The thirteen traits were significantly affected by breed and had a wide range of values. In fact, the statistical descriptions of studied phenotypic traits for lamb breeds are presented in Table I. Table I showed higher values for lambs of various breeds and patterns. As a result, the thirteen traits were chosen for pearson's correlation and stepwise selection for statistical analysis. Some traits were significantly ($P<0.05$) higher in Najdi when compared with Hari, except for heart girth, body depth, and dressing percentage. Furthermore, Najdi breed had a significantly ($P<0.05$) high body height at the withers, body height at the rump, and dressing percentage than Naemi breed. The results of the Shapiro-Wilk test showed that the distribution of body height at wither, body height at rump, head length, and ear length departed significantly ($P<0.05$) from normality (see supplementary file). On the other hand, performing the Shapiro-Wilk test for the remaining nine variables did not show evidence of non-normality (see the supplementary file).

Traits showed significant correlation coefficients associated with each other and carcass traits (Table II). Height at wither and rump, for example, correlated significantly with each other as well as with head length, head weight, and cold carcass. Body length was positively correlated with all studied traits except body depth and dressing percentage. In addition, heart girth was correlated with all traits except height traits. The important traits in differentiating sheep breeds were head length and weight, which showed significant correlation with most economically important traits except dressing percentage as expected. Live body weight at slaughter, as one of the most important traits, was significantly correlated with

Table I. Live body and carcass variables with (Mean±SEM) studied sheep breeds.

Variable	P > F	Breed		
		Harri	Najdi	Neami
Body height at wither (cm)	0.0097	66.13±0.97b[+]	69.69± 0.97a	65.25± 0.97b
Body height at rump (cm)	0.0141	68.50± 1.17b	72.31± 1.17a	67.13± 1.17b
Body length (cm)	0.0108	67.25± 1.02b	72.06± 1.02a	70.25± 1.02ab
Heart girth (cm)	0.0002	82.56± 0.49a	84.88± 0.49a	86.00± 0.49a
Body depth (cm)	0.0001	38.13± 0.37a	38.38± 0.37a	40.63± 0.37a
Head length (cm)	0.0069	21.50± 0.42b	23.63± 0.43a	22.81± 0.43a
Ear length (cm)	0.0002	14.75± 0.55b	18.25± 0.55a	18.31± 0.55a
Body weight at slaughtering (kg)	0.0031	45.19± 1.02b	49.54± 1.02a	50.53± 1.02a
Empty body (kg)	0.0022	41.72± 0.87b	45.45± 0.87a	46.48± 0.87a
Dressing%	0.0146	54.98± 0.75a	54.37± 0.75a	51.76± 0.75b
Hot carcass (kg)	0.0490	22.93± 0.48b	24.69± 0.48ab	24.04± 0.48a
Cold carcass (kg)	0.026	19.22± 0.43b	20.93± 0.43a	20.54± 0.43a
Head weight (kg)	0.0133	1.59± 0.06b	1.87± 0.06a	1.82± 0.06a

* SE: Standard Error

Table II. Pearson correlation coefficients of live body and carcass traits for studies sheep breeds.

	Body height at rump	Body length	Heart girth	Body depth	Head length	Ear length	Body weight at slaughtering	Empty body	Dress-ing%	Hot carcass	Cold carcass	Head weight
Body height at wither	0.95	0.60	0.15	-0.1	0.63	0.2	0.36	0.31	0.04	0.39	0.46	0.6
	<.0001	<.001	NS	NS	<.001	NS	NS	NS	NS	NS	0.02	<.001
Body height at rump		0.55	0.16	-0.06	0.61	0.26	0.32	0.29	0.04	0.36	0.46	0.57
		0.01	NS	NS	<.001	NS	NS	NS	NS	NS	0.02	<.001
Body length			0.55	0.16	0.73	0.48	0.61	0.63	-0.31	0.48	0.67	0.65
			0.01	NS	<.0001	0.02	<.001	<.001	NS	0.02	0	<.001
Heart girth				0.64	0.53	0.56	0.79	0.82	-0.45	0.59	0.76	0.54
				<.001	0.01	<.001	<.0001	<.0001	0.03	<.001	<.0001	0.01
Body depth					0.13	0.39	0.47	0.47	-0.39	0.24	0.34	0.29
					NS	NS	0.02	0.02	NS	NS	NS	NS
Head length						0.45	0.77	0.77	-0.32	0.63	0.67	0.7
						0.03	<.0001	<.0001	NS	<.001	0	<.001
Ear length							0.57	0.56	-0.32	0.4	0.5	0.62
							<.001	<.001	NS	NS	0.01	<.001
Body Weight at Slaughtering								0.98	-0.44	0.77	0.85	0.74
								<.0001	0.03	<.0001	<.0001	<.0001
Empty body									-0.5	0.75	0.86	0.67
									0.01	<.0001	<.0001	<.001
Dressing%										0.19	-0.21	-0.29
										NS	NS	NS
Hot carcass											0.81	0.56
											<.0001	<.001
Cold carcass												0.62
												<.001

Table III. Univariate test statistics of live body and carcass traits for studies sheep breeds.

Variable	Total standard deviation	Pooled standard deviation	Between standard deviation	R square	R-square/ (1-RSq)	F value	P
Body height at wither	3.28	2.75	2.35	0.36	0.55	5.82	0.010
Body height at rump	3.88	3.32	2.69	0.33	0.50	5.26	0.014
Body length	3.42	2.89	2.43	0.35	0.54	5.66	0.011
Heart girth	1.96	1.37	1.75	0.55	1.24	13.03	0.000
Body depth	1.52	1.04	1.38	0.57	1.34	14.08	0.000
Head length	1.46	1.20	1.07	0.38	0.61	6.37	0.007
Ear length	2.26	1.57	2.04	0.56	1.29	13.57	0.000
Body weight at slaughtering	3.64	2.90	2.84	0.42	0.73	7.69	0.003
Empty body	3.14	2.45	2.50	0.44	0.79	8.34	0.002
Dressing%	2.48	2.12	1.71	0.33	0.50	5.20	0.015
Hot carcass	1.48	1.34	0.89	0.25	0.33	3.49	0.049
Cold carcass	1.38	1.21	0.90	0.29	0.42	4.37	0.026
Head weight	0.21	0.18	0.15	0.34	0.51	5.34	0.013

those of the same economic importance to the market and consumers: Hot and cold carcass weights. The results showed the expected results of no correlation between hot and cold carcass weight and dressing percentage. In general, high phenotypic correlation coefficients were found between the majority of the body and carcass weight traits studied.

On the other hand, the univariate procedure within multivariate discriminant analysis tested all variables using reliable racial discriminants and confirmed the discriminant power of each trait (Table III). Table III showed that all the phenotypic variables were significantly

Table IV. Stepwise selection summary of most discriminanat power live body and carcass traits.

Variable	Partial R-square	F value	Pr > F	Wilks' Lambda	Pr < Lambda	Average squared canonical correlation	P
Body depth	0.573	14.08	0.000	0.427	0.0001	0.286	<.0001
Ear length	0.494	9.76	0.001	0.216	<.0001	0.516	<.0001
Body height at rump	0.428	7.1	0.005	0.124	<.0001	0.626	<.0001
Head length	0.351	4.86	0.021	0.080	<.0001	0.685	<.0001

Table V. Function, eigen value, variance percentage and canonical correlation.

Canonical correlation	Adjusted canonical correlation	Approximate standard error	Squared canonical correlation	Eigenvalues of Inv(E)*H = CanRsq/(1-CanRsq)			
				Eigen value	Difference	Proportion	Cumulative
0.94	0.91	0.023	0.89	7.99	5.65	0.77	0.77
0.84	0.76	0.062	0.70	2.35		0.23	1.00

different (P<0.05). Therefore, those traits were utilized more in multivariate discriminant analysis. Four traits (body depth, ear length, body height at the rump, and head length) were most significant in discriminating each lamb individual into its own breed. The average squared Canonical correlation (P 0.0001), R^2, Wilks lambda, and F-values of these traits were higher than those of the other traits studied (Table IV). Furthermore, those traits were selected because they presented high eigenvalues (Table V). The eigenvalue (7.99) of the four traits in canonical function 1 (CAN 1) explained 77% of the total variation of data; the other eigenvalue (7.99) of the remaining traits explained 23% of the total variation in canonical function 2 (CAN 2).

The canonical functions CAN 1 and 2 assigned animals to their breed as the percentage of correct assignment (Table VI). In CAN 1, the most discriminating traits were live body measurements: Heart girth, body depth, ear length, empty body, and body weight at slaughter. While the most discriminating traits in CAN 2 were head length, body length, hot carcass, body height at wither, body height at rump, weight, and ear length (Table VI), In details, the result revealed those traits had the highest loading (0.76-0.62 and 0.69–50 in functions 1 and 2, respectively). In a similar vein, Harkat *et al*. (2015) stated that the loading value showed similar findings for the correlation of each variable with the discriminant function. The highest loading of traits suggested that the correlation between them was the function that discriminated between the individuals in discriminant function. In addition, Figure 2 presents the discriminant relationship of lambs in the form of multiple correspondence analyses with data obtained from canonical discriminant analysis, whose structure is shown in Table VI. In Figure 1, the canonical variable CAN1 generated significant traits on the x-axis (p 0.0001) (Table VI),

Table VI. Total canonical structure the CANDISC procedure.

Variable	Can 1	Can 2
Heart girth	0.76	0.25
Body depth	0.76	-0.30
Ear length	0.66	0.50
Empty body	0.65	0.31
Body weight at slaughtering	0.62	0.33
Head weight	0.44	0.49
Cold carcass	0.39	0.48
Head length	0.35	0.62
Body length	0.34	0.59
Hot carcass	0.29	0.50
Body height at wither	-0.17	0.69
Body height at rump	-0.21	0.65
Dressing%	-0.59	0.17

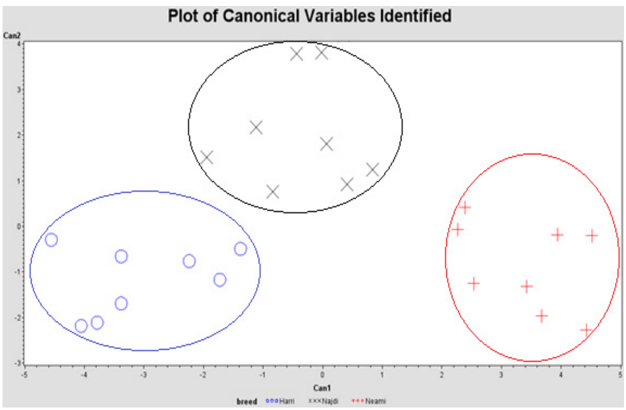

Fig. 1. Cluster analyses for of the three Saudi sheep breeds; Naemi (+), Najdi (X) and Hari (O).

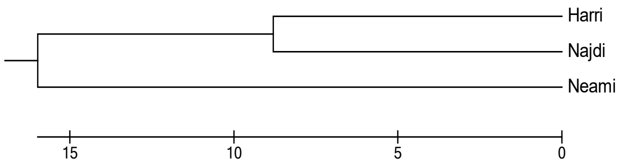

Fig. 2. Evolutionary genetic tree of Saudi sheep breeds based on live body and carcass measurements.

with those of CAN2 on the y-axis. Thus, the multiple correspondence analysis showed that the total variation was explained by two canonicals (CAN1 and CAN4). It is clear from the figure that sheep individuals were separated from each other and from three distinct groups. The groups were predefined breeds of Neami, Najdi, and Hari. It was noted that Najdi sheep were closer to each breed. While there is significant discrimination between Neami and Hari individuals. The result is expected, bearing in mind that Najdi and Hari share the same genetic origin. On the other hand, it would be expected considering the discrimination power of major discriminating traits that show less variation between Najdi and Hari. Figure 2 shows that longer divergence can be seen within breeds as a few sheep move away from each other into the circle. It can also be seen from a closer distance of others into the circle of each breed. In other words, the multiple correspondence analysis assigned the sampled sheep into the separated clusters representing their own predefined breeds of Naemi, Najdi, and Hari. Overall, the canonical discriminant analysis proved its success in verifying the breed of lamb and its carcass based on the previously mentioned 13 traits. Furthermore, the results help fully describe the genetic distances (Mahalanobis distances) between studied breeds as an indicator of the genetic tree (Fig. 2). The evolutionary genetic tree is the degree of genetic distance between breeds, species, or populations measured by the numerical method (Dauda *et al*., 2018). Tamura *et al*. (2013) indicated for tree design that the tree is drawn with branch lengths in the same units as those of the evolutionary distances used to recognize the phylogenetic tree. The distances between all pairs were significant (P<0.0001). Figure 2 shows the sheep evolutionary history inferred using the UPGMA method, in which one cluster had both Hari and Najdi breeds. The Najdi breed was found in the middle, reflecting the optimal tree's close evolutionary history, with a branch length sum of 22.16. The evolutionary history of the best tree between the Naemi and Hari breeds was estimated to be branch length = 40.76, indicating a distance nearly twice that of the Najdi.

CONCLUSION

It is objectively noble to conclude that live linear body and carcass traits that had a significant effect between breeds with discriminatory power would be able to assign slaughter animals and their carcasses to their own breeds. Furthermore, the principal components analysis efficiently confirmed the total variation of the 13 traits accumulated in linear combinations to discriminate the studied sheep individuals. In the event that all of the studied traits were difficult to handle at the farm or market, the four traits with the greatest discriminant power body depth, ear length, body height at the rump, and head length which explained 77% of the total variation present in the breeds would be able to discriminate breeds' carcasses. Overall, the discriminant analysis proved its success in identifying the breed of any carcass based on the previously mentioned traits. It is recommended to disseminate the findings of this study as a guideline to assign slaughtered sheep and their carcasses to their own breed when other means make this impossible.

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Ethical statement and IRB approval

The experiments conducted in this research study adhered to the guidelines for experiments involving animals as established by the research Ethics Committee (REC) of King Saud University. The study received ethical approval with the reference number: (KSU-SE-20-17).

Statement of conflict of interest

The authors have declared no conflict of interest.

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