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Molecular detection of transferrin genetic formation as a marker for weight and growth hormones in male Arabi sheep

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ABSTRACT

Background: Blood proteins have been widely used to characterize animal breeds, as most are genetically determined and follow simple genetic rules. Therefore, the transferrin protein has been studied based on its various alleles. Transferrin is a type of protein that binds two iron atoms to each transferrin molecule. Sheep meat production can be increased through genetic improvement.
Aim: The aim of the study is to predict the live weight and growth hormone (GH) concentration of lambs by identifying transferrin gene alleles and selecting them for meat production or breeding.
Methods: This study was conducted at the Research Station of the College of Agriculture, University of Basrah. Blood samples were taken from 74 Arabi sheep, and the genetic makeup of the iron transporter protein (transferrin) was studied using polyacrylamide gel electrophoresis in a variable buffer solution with black amido dye. The relationship between gene expression, lamb live weight, and GH concentration was investigated.
Results: indicated the detection of six transferrin genotypes AA, AB, AC, BB, BC, and C were detected based on their electrophoretic mobility in basal medium. Three alleles, A, B, and C, accounted for these genotypes. The results showed that the AA and BB genotypes were associated with lamb live weight and GH concentration, accounting for 48.61% and 16.66 % of the genotypes, respectively. The average lamb weights were 38.15 and 37.22 kg, and the GH concentrations were 9.97 and 9.51 ng/ml, respectively. In contrast, the genotypes AB and AC accounted for 20.83% and 11.11%, respectively, with an average weight of 36.20 and 36.05 kg, and a GH concentration of 8.97 and 8.85 ng/ml, respectively. As for the genotypes AC, BC their percentage reached 1.33%, with an average weight of 35.65 and 35.35 kg, respectively, and a GH concentration of 8.75 and 8.45 ng/ml, respectively, as these traits are recessive.
Conclusion: From the results, we conclude that there is a correlation between body weight, GH concentration in lambs, and the genotype AA and BB, indicating that it is the dominant trait over the other genotypes.
Keywords: Genetic transferrin, Live weight, Growth hormones, Genetic polymorphism.

Introduction

Blood proteins have been widely used to characterize clans of animal lineages because most of these proteins are genetically formed and follow simple heredity laws. Therefore, these proteins have been studied in several animal species using various techniques. Most of these studies were valuable, particularly in the areas of heredity, clan inheritance, clinical diagnosis, and genetic maps. Transferrin is a class of protein that binds to two atoms of iron for each transferrin molecule. It acts as an oxygen carrier, and its concentration in natural plasma ranges from 240 to 280 mg/100 ml (Tulloh, 1991). There are also several names for the transferrin protein, and these names depend on the origin of the protein; for example, if it is found in the blood, it is called transferrin (Evan *et al.*, 1956). Ovotransferrin in birds constitutes approximately 12% of the egg white protein (Williams, 1968). Lactoferrin plays a significant role in the transport of iron ions in milk (Baker and Rumball, 1987). Melanotransferrin is found in human tissue (Richardson, 2000).

Many researchers have taken advantage of this advantage of the huge biological diversity of alleles and started studying the relationship between these alleles and some economic traits in sheep, including birth weight and weight gain (Bildik and Yur, 1999; Dellal and G, 2002; Yadva et al, 2013), wool production (Dellal, 2001), milk production in goats and sheep (Sultan., 2019; Younis *et al.*, 2024) milk production in goats and sheep (Sultan., 2019; Younis *et al.*, 2024), and reproductive performance (Steppa *et al.*, 2007; Jaafar Ahmed *et al.*, 2018). Bashar (2015) identified six genotypes for the transferrin protein, comprising three homozygous genotypes AA, BB, and

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CC and three heterozygous genotypes AB, AC, and BC, resulting from the presence of three alleles A, B, and C. The frequency of the A allele was higher than that of the B and C alleles. Sheep meat production can be increased through genetic improvement combined with a selection program. Recently, molecular selection involving functional genes has been used to obtain sheep productivity genetic markers (Bowles, 2015).

The growth hormone (GH) gene is a promising candidate for genetic selection in farm animals. GH has various biological activities, including somatic and lactogenic activities, and plays a major role in increasing growth performance or milk production. GH is responsible for milk formation and lactation continuity, growth stimulation, and fat stores reduction. The use of modern genetic selection programs and the development of new breeding techniques allow the concentration of desired genes within a breeding population (El-Mansy *et al.*, 2023; Putra *et al.*, 2024). This study aimed to predict the live weight and GH concentration of lambs by identifying transferrin gene alleles and selecting them for meat production or breeding.

Materials and Methods

Seventy-four blood samples (10 ml per specimen) from Arabi sheep were obtained from the jugular vein. Blood samples were placed in ethylenediaminetetraacetic acid free tubes to determine the genetic structures of transferrin. The electrical relay was performed according to the polyacrylamide gel electrophoresis SDS-PAGE method SDS, also known as sodium dodecyl sulfate, with some modifications, using a vertical electrophoresis relay apparatus, supplied by Cleaver Scientific, UK. Separation gel and stacking gel solutions were prepared to separate blood proteins, as shown in Table 1.

Electrophoresis sample analysis

The samples were injected using a Micropipette, where 15 µl of Bromophenol blue dye was thoroughly mixed with 5 µl of blood plasma and thoroughly mixed. Then, 3 µl was taken and placed in the gel pits, after which the device was turned on at 90 V for minute. Then, raise the voltage to 90 V for 3 hours. After the deportation time, the electrodes were lifted, the device was opened, and the gel was removed from the glass slides. The gel was then placed in a plastic basin and washed with distilled water to remove of the electrophoresis solution remnants. Then, the washing water was discarded, and the gel was added the Coomassie blue tincture at a concentration of 0.05 g per 100 ml of distilled water. Leave it for 24 hours, then wash with distilled water to remove the dye residues.

The gel was placed in the washing solution (7% acetic acid) to remove the dye and detect the packages. The gel was then placed on a glass slide and photographed.

Statistical analysis

We used one-way analysis of variance ANOVA with the least significant difference LSD or Duncan multiple range test, considering differences significant at $p < 0.05$, to statistically analyze the data using SPSS 27.0 (SPSS, 2021).

Ethical approval

The research was approved by the College's Ethical Committee for Animal Research.

Results

Figure 1 illustrates the most important serum proteins in sheep blood. At the top, the presence of albumin, which is the largest protein in sheep blood, is noted. A simple genetic makeup is observed in some samples, but it does not meet the ideal standard because the method is specific to the transferrin gene. Transferrin, which is in the middle, is followed by albumin, consistent with its neutral amino acid content. We notice the

Table 1. Electric relay system for transferrin blood of sheep (PH 9.8).

Separation gel			Concentration gel			
No. solution	**Quantity of solution/100 ml**	**Amount of solution in the mixture**	**No. solution**	**Quantity of solution/100 ml**	**Amount of solution in the mixture**	**Quantity of solution/100 ml**
1	Hydrolytic acid 0.48 ml	One part	4	Aqueous Phosphate 25.6ml	One part	≥6 g
	Gel 36.6g			Gel 5.7g		
	Timid 0.5ml			Timid 1g		
2	Acrylamide 30g	Two parts	5	Acrylamide 30g	Two parts	Glycin 28.8 g
	Methylene bis acrylamide 1g			Methylene bis acrylamide 2.5g		
3	Ammonium persulfate 0.28 g	Four parts	6	Riboflavin 4 mg	One part	
	Urea 9 Molar		7	Sucrose	Four parts	

Jaayid *et al.* (2011).

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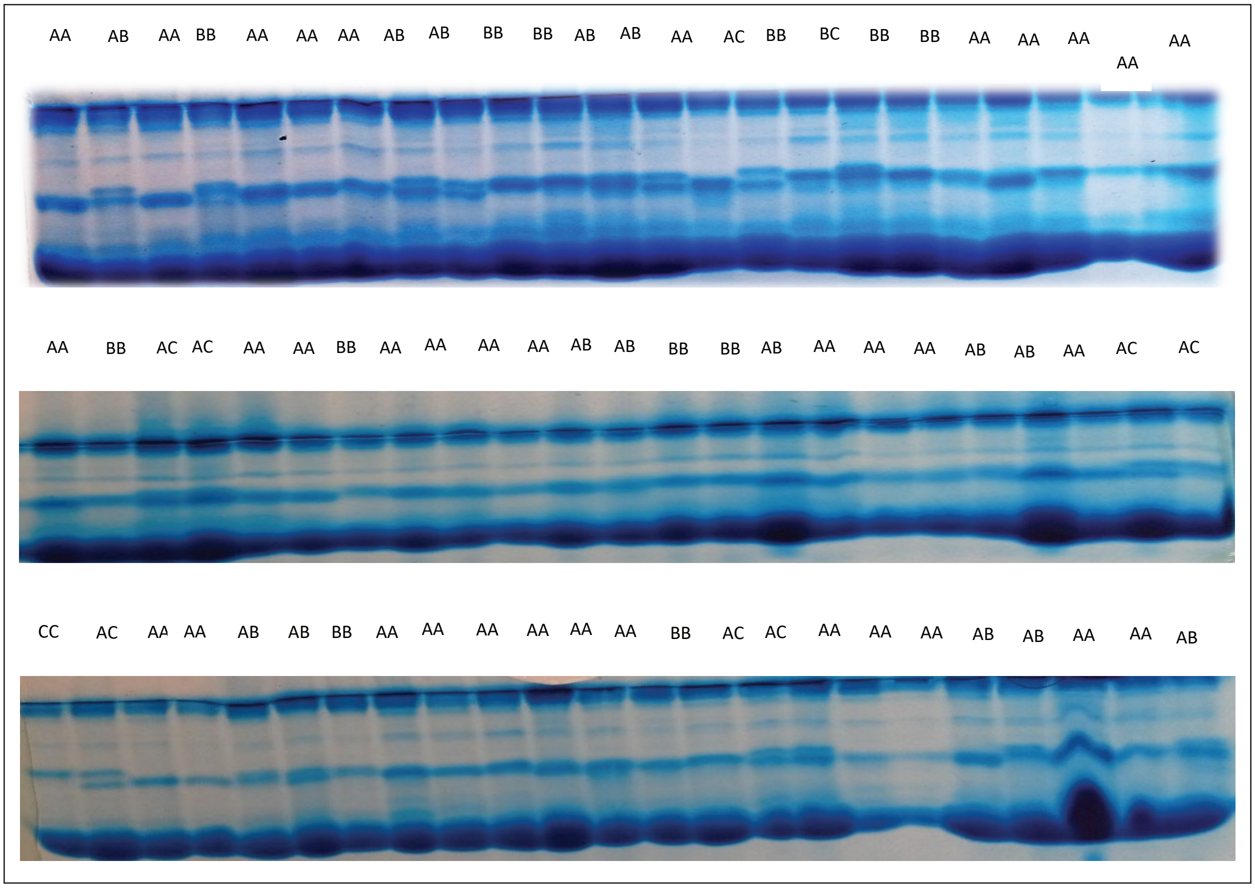

Fig. 1. Protein degradation products on the separation gel polyarylamide by the transferrin protein electrophoretic migration in the blood of Arabian sheep.

presence of more than one genetic makeup, based on the presence of one or two bands in each sample, as well as the distribution of these bands, whether they are high or low in the gel medium, and so on. Research has indicated the presence of a hybrid genetic makeup through the presence of two bands and the presence of a homogeneous genetic makeup through the presence of a single band (Ashton, 1958; Jaayid *et al.*, 2011). The results of the study, as shown in Table 2, showed that six genetic structures of the transferrin protein were discovered, namely AA, AB, AC, BB, BC and CC according to their electrophoretic movement in the basic medium. Three alleles A, B and C are responsible for these six genetic structures, and their inheritance follows codominants according to Mendel laws of inheritance (Jaayid, 2003). The results showed a significant $p < 0.05$ superiority of the genotype AA in lamb weight, as it accounted for 40.83% of the genotypes and an average weight of 38.15 kg. In comparison, the genotype BB accounted for 16.66% and an average weight of 37.22 kg, and the genotypes AB and AC accounted for 20.83% and 11.11% and an average weight of 36.20 and 36.05 kg, respectively. As for the genotypes CC and BC there, percentages were 1.33% and an average weight of 35.65 and 35.35 kg, respectively, as these genotypes are recessive traits, according to Table 2.

Table 2 shows a significant superiority $p < 0.05$, for the AA genotype in terms of GH concentration levels, as it accounted for 40.83% of the genotypes, with an average GH concentration of 9.97 ng/ml. The genotype BB accounted for 16.66%, with an average GH concentration of 9.51 ng/ml. The genotypes AB and AC accounted for 20.83% and 11.11%, and an average GH concentration of 8.97 and 8.85 ng/ml, respectively. The genotypes CC and BC accounted for 1.33%, with an average GH concentration of 8.75 and 8.45 ng/ml, respectively.

Discussion

Through these results, a correlation was found between the body weight and GH concentration of lambs and the transferrin protein genotypes. The two alleles AA and BB outperformed the other alleles. The results were consistent with those of (Yadva *et al.*, 2013), who reported a relationship between lamb birth weight and

Table 2. Genetic alleles, their ratios, average weights, and GH levels of lambs.

Alleles	Number oflambs	The ratio%	Averageweights(kg)	Average GH (ng/ml)
AA	35	48.61	38.15±0.13 **a**	9.97±0.10 **a**
BB	12	16.66	37.22±0.27 **b**	9.51±0.10 **ab**
AB	15	20.83	36.20±0.37 **c**	8.97±0.30 **b**
AC	8	11.11	36.05±0.70 **c**	8.85±0.41 **b**
BC	1	1.33	35.65	8.75
CC	1	1.33	35.35	8.45
Sig.			*	*

abc indicates a significant difference at $p \leq 0.05$; T = treatment effect in the same column.

transferrin protein in Carolean sheep. These results were also consistent with those of (Steppa *et al.*, 2009), who found a relationship between milk production and transferrin protein in sheep. However, the results were inconsistent with those of (Dellal, 2002), who showed no relationship between genotype and lamb birth weight in sheep. There is also a direct relationship between body weight and other productive and physiological traits. These traits improve as the body weight increases (Bashar and Dhafir, 2012; Sultan, 2019). Putra *et al.* (2024) found a relationship between body weight and GH levels. In crossbred Merino sheep, the higher the body weight, the higher the GH concentration. El-Mansy *et al.* (2023) found a relationship between body weight, carcass characteristics, and GH concentration. The results were consistent with those of previous studies.

Conclusion

We conclude from the results that there is a correlation between body weight, GH concentration in lambs, and the genotype AA and BB, indicating that it is the dominant trait over the other genotypes. No correlation was found between the genotype AB and lamb weight. The genotype AC and CC is a recessive trait. The genotype can predict the lamb weight.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

All authors equally contributed to the study design, data analysis and interpretation, and manuscript preparation.

Data availability

All data supporting this study's findings are available in the manuscript.

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