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Correlation between Leptin Encoding Gene and Some Haematological and Biochemical Parameters in Awassi Sheep

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Abstract
The present study was scanned 104 of different genotypic Iraqi Awassi sheep to determine the relation of genetic polymorphism for leptin gene with several biochemical and haematological features. The results of this study were revealed that most of blood characters were homologous for the genotypes AA, AB, AH, AM, AR, and AS except for RBCs and WBCs count since there were significant differences (P< 0.05) between them, and the following genotypes AH and AB were surpassed on the two genotypes AM and AB concerning the number of RBCs, and the two genotypes AB and AH were surpassed on the genotype AM concerning the number of WBCs. Most of biochemical characters were nonhomologous except for the concentration of urea, since significant differences (P< 0.05) among the genotypes were observed with respect to glucose, protein, cholesterol, and triglycerides. The AH genotype was surpassed the two genotypes AB and AR concerning the glucose ratio. The AB genotype was surpassed the two genotypes AM and AS, the two genotypes AA and AH were surpassed the AB genotype concerning cholesterol ratio, and the AB genotype was surpassed the AH genotype concerning the level of triglyceride. In conclusion, the leptin gene diagnostic tool could be used for selection process to enhance the production levels in Awassi sheep through eliminating the animals that were correlated with the genotypes of some undesired characters in early breeding times.
Keywords; leptin, gene, Awassi, parameter, blood, biochemistry

1. Introduction

As a result of the revolution of molecular biology, it was possible to determine the genetic markers that have high correlation with many aspects of DNA structure particularly for the genes that have major effect on the economical features (Field, 2007). Recently, there were much more concern with leptin gene as a key for the biological control that were bound with the important characteristics in animal breeding, such as feeding, lipid content in the meat, and the quality of meat (Geary *et al*., 2003). On the other hand, the genetic character of this gene was considered as a "marker" that is capable to refer on the relative differences among individuals, and the markers of this gene were became a portion of the commercial genotypic determination plan to design what is known as "marker assisted selection" in several types of animals (Nkrumah *et al*., 2005; Schenkel *et al*., 2005).

The importance of some haematological criteria in the evaluation of the animal activity was reflected on age, sex, race, and other factors (Iida and Tachibana, 1996; Xie et al., 2013). It was found that age and weight possess an obvious role in the number of WBCs too (Chen et al., 2002). Add to that, this phenomenon can be applied on glucose level, hemoglobin, and platelets (Iida and Tachibana, 1996; Xie et al., 2013). Moreover, it was revealed that cholesterol concentration in the blood was affected by its amount in the food, type, age, and the particular physiological stage of the animal and the level of hormones (Randal et al., 2001). On the other hand, since the protein components constitute an important portion of blood components, it was known that any change in protein concentration was reflected on the hygienic and metabolic situation of the animal (Al-Hasani et al., 2005).

In addition to the above mentioned criteria, the estimation of triglyceride ratio was also included on in this study since the concentration and production of leptin in plasma was correlated with the amount of the adipose tissues produced triglycerides (Chilliard *et al*., 2005). Moreover, the concentration of blood urea gives an indication for the performance of the animal to its physiological function. The level of blood urea concentration depends on the balance between the type of food and its level of protein (Wootton, 1974).

Since much more attention were revealed recently in the genetic markers for middle east sheep breeds (Aslaminejad *et al.*, 2010; Barzehkar *et al.*, 2009), this study was performed in order to pave the way for the observation of several parameters that correlated with the animal genotypic performance and to select the suitable genotypes to the local environment. Therefore, this study was taken place to monitor the effects of polymorphism of leptin gene marker on several factors related with the productive and physiological criteria.

2. Materials and Methods

2.1. Experimental animals

One hundred and four randomly selected Awassi sheep of six genetically determined genotypes were chosen in this study. The chosen sheep were bred in the grazing lands that belonging to college of agriculture of Al-Qasim Green University. Standard program of breeding was applied.

2.2. Blood collection

Only 5ml of blood was aspirated from jugular vein of each individual sheep. Then, 2ml was placed in EDTA tubes, while the residual portion was placed in EDTA free tubes. Centrifugation at 3000rpm for 15 min was performed. The serum was place in 1.5ml centrifuge tubes. The samples were kept at -20 ℃ until further processing.

2.3. Blood analysis

All blood analysis were performed in the same day of bringing samples to the lab to avoid any expected damage in the blood. The total number of RBCs and WBCs were estimated by haemocytometer counting chamber (Schalm *et al.*, 1975; John and Lewis, 1984). Haemoglobin level was estimated by using haemometer Sahli according to Schalm and his colleges (1975). The packed cell volume (PCV) was estimated according to Archer method (Archer, 1965).

2.4. Biochemical analysis

In blood serum several biochemical tests were performed. Cholesterol concentration measurements were taken place using Biolabo SA kit (France). Total glucose concentration measurement was estimated by Linear Chemicals kit (Spain). Total protein concentration was estimated by Biolabo SA kit (France). Urea concentration was measured using Bio Merieux SA (UK). Triglyceride concentration was measured in blood serum using Biolabo SA kit (France).

2.5. Biostatical analysis

The data of the current study (haematological and biochemical criteria) were analyzed using SPSS analysis system (Landau and Everitt, 2004).

3. Results

Leptin relation with some haematological and biochemical criteria was clarified as follows;

3.1. Haematological criteria

The results of this study for some haematological criteria revealed the presence of a significant effect ($P < 0.05$) between AR genotype and AB and AM genotypes in the number of RBCs. The average of AR genotype (11.195×10^6 corpuscle/cm^3) compared with AB genotype (9.933×10^3 corpuscle/cm^3) and AM genotype (9.215×10^3 corpuscle/cm^3) respectively. Whereas a significant difference ($P < 0.05$) was not obtained among the genotypes with respect to haemoglobin and platelets (table 1).

3.2. Biochemical criteria

The table (2) shows significant differences ($P < 0.05$) among the genotypes with respect to glucose, protein, cholesterol, and triglycerides levels for the studied cases, in such a way AH genotype was surpassed on AB and AR genotype in relation to glucose level, and the averages were 0.117, 0.059, and 0.062 gm/cm^3 respectively. Whereas AB genotype was surpassed AM and AS genotypes with respect to the total protein ratio, in which the ratios were reached the averages 8.461, 5.879 and 4.764 gm/100 cm^3 respectively. The AA and AH genotypes were surpassed on AB genotype concerning cholesterol level, in which the ratios were reached the averages 0.105, 0.092, and 0.065 gm/cm^3 respectively.

It was noticed according to table (2) the presence of a significant difference ($P < 0.05$) between AB and AH genotypes with relation to the triglyceride levels, in which the averages were 0.565 and 0.283gm/100cm^3 respectively. Add to that, the table (2) refers to the existence of significant differences ($P < 0.05$) among the genotypes according to the level of urea in the blood.

4. Discussion

Several haematological and biochemical parameters of different genotypes of Awassi sheep were tested

extensively in this study.

4.1. Haematological Criteria

The results of this study were revealed the presence of a significant effect ($P < 0.05$) between AR genotype and AB and AM genotypes in the number of RBCs. Whereas no significant differences among genotypes concerning haemoglobin, amount of platelets were found. It was referred to the presence of a proportional relationship between haemoglobin feature and the amount of platelets and the number of RBCs (Fernie *et al*., 1994). The increasing of metabolic activities in the high weight animals leads to more increasing demands of oxygen in which haemoglobin was responsible about its transfer into the cells in the body (Dickerson and Geis, 1983; Ulanowicz *et al.*, 1970).

The reason behind the increasing of RBCs number might be attributed to the resistance to the stress that was occurred as a result of the environmental conditions (Evans *et al*., 1999). Since the animals require oxygen because of the continuous stress, the number of RBCs might be increased (Whitfield and Martin, 1985). These results were shown the presence of significant differences between AB and AH genotype and AM genotype with respect to the number of RBCs. This was attributed to the fact that AB and AH genotypes have a state of adaptation capable of withstanding the harsh environmental conditions in such a way it was possible to increase the number of RBCs as an evidence of such stress.

4.2. Biochemical criteria

The results of this study revealed the presence of significant differences ($P < 0.05$) among the genotypes according to the levels of glucose, proteins, cholesterol, and triglycerides, in which AH genotype surpassed AB and AR genotypes according to glucose level. Whereas AB genotype was overcome AM and AS genotypes. Add to that, AA and AH genotypes were surpassed AB genotype concerning cholesterol level. It was noticed the presence of a significant difference ($P < 0.05$) between AB and AH in relation to triglyceride levels, in which the averages were 0.565 and 0.283gm/100cm^3 respectively. Thus, the decrease and increase of glucose concentration could not be relied on in ruminants as a source or as indicator for energy since it was variable, and a portion of glucose was converted to create cholesterol and triglycerides, instead the acetate was relied on. Therefore, it was noticed that some genotypes have the ability to convert glucose into cholesterol and triglycerides (Glowinska and Oler, 2013). Thus, glucose concentration was reduced in both AB and AR genotypes and increased in AH genotype. The reason behind this was the animals might not get their food requirements entirely. Because of weakness of the reduced amount of food in the providing energy to adipose tissues the animals recruit alternative sources in releasing energy from these tissues in such a way it leads to elevated levels of cholesterol (Mazur *et al*., 2009).

Most of the blood characters are homologous for AA, AB, AH, AM, AR, and AS genotypes except RBCs and WBCs were found significant differences ($P < 0.05$) among them, where AR genotype was surpassed AB and AM genotypes concerning the number of RBCs, while AB and AH genotypes were surpassed AM genotype concerning the number of WBCs. Thus, most of the observed biochemical characters for Awassi sheep were nonhomologous except for the concentration of urea, since there were significant differences ($P < 0.05$) among the genotypes with respect to the glucose, protein, cholesterol, and triglycerides levels. Furthermore, AH genotype was surpassed AS and AR genotypes with respect to glucose level, while AB genotype was surpassed AM and AS genotypes, AA and AH genotypes were surpassed AB genotype concerning the cholesterol level, and AB genotype was surpassed AH genotype concerning the level of triglycerides. Consequently, the leptin gene could be used as a selective tool for the selection process in order to enhance the averages of production in Awassi sheep by ruling out the unwanted genotypes in this race through the undergoing the necessary blood and biochemical assessments.

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Table (1). The averages of blood criteria according to genotypes (± standard error) for the samples under the study of Awassi sheep.

Character	Average ± standard error	number	genotype
number of RBCs x 10^6 corpuscle/cm^3	10.050^{ab} ± 0.44	12	AA
	9.932^{ab} ± 0.20	19	AB
	10.195^{ab} ± 0.22	43	AH
	9.748^{b} ± 0.23	16	AM
	11.195^{a} ± 0.73	7	AR
	10.694^{ab} ± 0.69	7	AS
number of WBCs x 10^3 corpuscle/cm^3	9.673^{ab} ± 0.20	12	AA
	10.594^{a} ± 0.31	19	AB
	10.136^{a} ± 0.26	43	AH
	9.215^{b} ± 0.21	16	AM
	9.400^{ab} ± 0.16	7	AR
	10.071^{ab} ± 0.81	7	AS
Haemoblobin (Hb) gm/cm^3	11.366 ± 0.23	12	AA
	11.157 ± 0.16	19	AB
	10.993 ± 0.11	43	AH
	11.362 ± 0.35	16	AM
	11.014 ± 0.30	7	AR
	11.357 ± 0.46	7	AS
Amount of platelets (PVC) %	35.075 ± 0.69	12	AA
	34.457 ± 0.49	19	AB
	33.995 ± 0.34	43	AH
	35.018 ± 1.02	16	AM
	34.042 ± 0.90	7	AR
	35.085 ± 1.38	7	AS

* The lowercase letters indicate the presence of significant differences

Table (2). The averages of the biochemical components of the blood serum according to genotypes (± standard error) for the samples under the study of Awassi sheep.

character	Average ± standard error	number	genotype
Glucose gm/cm^3	0.074^{ab*} ± 0.009	12	AA
	0.059^{b} ± 0.006	19	AB
	0.117^{a} ± 0.015	43	AH
	0.083^{ab} ± 0.008	16	AM
	0.062^{b} ± 0.007	7	AR
	0.119^{ab} ± 0.016	7	AS
Protein gm/cm^3	7.451^{ab} ± 1.766	12	AA
	8.461^{a} ± 0.948	19	AB
	7.031^{ab} ± 0.516	43	AH
	5.879^{b} ± 0.513	16	AM
	5.357^{ab} ± 0.223	7	AR
	4.764^{b} ± 0.409	7	AS
Cholesterol gm/cm^3	0.105^{a} ± 0.026	12	AA
	0.065^{b} ± 0.002	19	AB
	0.092^{a} ± 0.007	43	AH
	0.077^{ab} ± 0.011	16	AM
	0.097^{ab} ± 0.010	7	AR
	0.092^{ab} ± 0.007	7	AS
Triglycerides gm/cm^3	0.387^{ab} ± 0.058	12	AA
	0.565^{a} ± 0.186	19	AB
	0.283^{b} ± 0.035	43	AH
	0.347^{ab} ± 0.062	16	AM
	0.246^{ab} ± 0.075	7	AR
	0.285^{ab} ± 0.078	7	AS
Urea gm/cm^3	0.051 ± 0.004	12	AA
	0.052 ± 0.003	19	AB
	0.049 ± 0.003	43	AH
	0.053 ± 0.001	16	AM
	0.047 ± 0.005	7	AR
	0.044 ± 0.003	7	AS

* The lowercase letters indicate the presence of significant differences