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EFFECT OF GENOTYPES FOR GROWTH HORMONE GENE IN AWASSI EWES ON MILK PRODUCTION AND COMPONENTS

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ABSTRACT

This study was conducted in the Department of Animal Production/College of Agriculture and Forestry/University of Mosul from 3/4/2022 to 3/2/2023, by using forty Awassi ewes aged between 3-5 years and with an average weight of (42 kg). The ewes were distributed into two groups depending on the structure of growth hormone genes (AA,AG) to study the effect of genetic structure on milk yield and composition by using PCR-RFLP technique. The growth hormone gene was detected by amplifying the gene based on the genome primer. The number of ewes that are born and have AA genotype were 17ewes, while those have AG gene and were 7 ewes only. Results revealed that ewes that have AA structure of growth hormone gene produced more milk significantly ($p \leq 0.01$) the total milk yield during 90 days was 25.19 kg as compared with those that had AG structure for growth hormone which was 19.48 kg. This increase in milk production was accompanied with increase in milk fat significantly at the first and third months of lactation period. Otherwise, the other milk components of protein, lactose and solid non-fat were not affected by the difference in growth hormone genotype. We conclude from the results in this study that the genotype of growth hormone has an important role in causing changes in the amount of milk produced and its fat content in the local Awassi ewes, and this has an additional effect on the growth of lambs and its ability to survive to the weaning.

INTRODUCTION

The rapid progress in molecular biology and the development of molecular genetic techniques made it possible to identify differences between genetic structures of the DNA. Recently, candidate genes that affect economic traits have been studied and have received considerable research attention due to their use as an adjunct in genetic testing and for the delineation of evolutionary relationships in different breeds of animals (Afifi *et al* 2014). This technique had been widely used and accepted as testing tools in various animal species such as sheep (Bastos *et al*., 2001) and goats (Neelam *et al.*, 2007). The growth hormone gene is a candidate gene for animal genetic testing due to its important role in influencing the productive performance such as milk production (Etherton and Bauman, 1998; Ishag *et al.*, 2010). (McMahon *et al*., 2001) found that growth hormone gene stimulate of increased milk production in cows and other animals, enhance growth and reduces stored fat by exploiting it when needed as an energy source, the growth hormone gene (GH) in sheep consists of 5 exons and 4 introns like other mammals (Maniou *et al.,* 2001). The production of milk in ewes is one of the main factors affecting the growth of lambs after birth and even weaning because of their dependence on it during this period, it was observed that the growth and survival of lambs during the first 3-4 weeks, also, at 8 weeks of lambs age the differences between the weights of lambs begin to appear and affect subsequent weights (Morgan *et al.,* 2007). And regardless of environmental factors that can be controlled the genetic factor is the important factor affecting milk production and composition. Genetic structure affects the growth of lambs, which are transmitted equally between selected parents, which are characterized by quality performance, appropriate milk quantities, and a set of non-genetic factors (Al-Dabbagh, 2009 and Al-Jawari, 2011). Therefore, the genetic makeup has a great impact on productive performance, which is done through genetic improvement and through the selection of parents. body weights and body gain lambs, milk yield, milk fat% and milk protein%, increased significantly by the effect of the body weight and litter size. Heavy and twins lambing result more milk yield, while single lambs have a heavier weaning weight (Sultan,2019). effect BCS on ewes and their lambs with high BCS had a linearly higher BW to compared with those with low BCS (Sultan and Abdul-Rahman,2023). feeding high degradable protein could effect in weekly, monthly and commercial yield and milk components (fat, protein, lactose) during sucking and after weaning (Al-Hafz and Ali.,2019). The result of (Raoof, 2017) study showed that there were a highly significant effects of breed and BCS on milk yield and birth weight. Therefore, we studied the genetic make-up of the growth hormone gene in Awassi sheep and stood on their productive performance of milk and its components.

MATERIALS AND METHODS

Ethical approval

The study and sample collection were carried out with the agreement of the ethical and animal welfare committee Under the number UM.VET.2022.080 and with the date of April 1, 2022, of the College of Veterinary Medicine, University of Mosul.

Experimental animals

This study was conducted in the animal field at the department of animal production/ college of agriculture and forestry/ university of Mosul for the period 3/4/2022 to 3/2/2023, forty Awassi ewes were selected with an age ranging from (3-5) years and a weight of (42 kg), veterinary treatments and vaccines were given according to the approved vaccine schedule.

Polymerase chain reaction (PCR)

The variation in the growth hormone (GH) gene was detected, in which the reaction kit for PCR technology, primers for the gene, and extracted DNA samples were used. The results of the PCR reaction were carried forward with the presence of the volumetric guide, DNA Ladder. It was confirmed that the amplification of the required piece was successful. Treatment with the cutting enzyme HaeIII Amplification of specific fragment (950 bp) of Awassi ewes growth hormone gene was performed by PCR-RFLP using specific primer Table (1) resulted in reaction products of 296 bp, 202 bp, and 150 bp in the blood samples of sheep (mothers., and the results were showed two genetic combinations AA 25 ewes and AG 15 ewes.

Table (1): Primers sequence of sheep growth hormone gene

Primer	**Sequence**
GH-F	GGAGGCAGGAAGGGATGAA
GH-R	CCAAGGGAGGGAGAGACAGA

Nutrition

Ewes were fed on a standard diet its components and chemical analysis are shown in Table (2). Within each genetic group, ewes were divided into two groups and fed daily with 1.5 kg per head of concentrate feed for one month (flushing period). Then sponges saturated with progesterone hormone manufactured by (Interval production company) were used to standardize estrus for 14 days and after lifting the sponges and injecting ewes with PMSG at a dose of 500 I.U. per ewe, then subjected to the rams for mating.

Table (2): Components and chemical composition of the standard diet

Ingredients	%	Chemical analysis as dry matter base %	
Barley	60	Dry matter	93.43
Wheat Bran	23	Crud protein	15.75
Soybean meal	7	Crud fiber	8.27
Wheat straw	7.5	Ether extract	3.49
Sodium bicarbonate	0.5	Ash	6.07
Urea	0.75	Nitrogen free extract	66.42
Sodium chloride	0.5	Metabolic energy kcal/kg	2435
Limestone	0.75		

Chemical analysis was laboratory done according to AOAC (2000). Metabolic energy was calculated according (Al-khawaja *et al* 1978).

After the gestational has expired, the number of ewes born from the genotype AA of growth hormone was 17 ewes, and the number of ewes born from the genotype AG was 7 ewes.

Milk measurements

Milk production of each genotype was recorded at two sequence days every month through the period till weaning (3 months) and sample (10%) of milk were taken for estimate milk composition (fat, solids nonfat, protein and lactose) in each period by using the Lactoscac system (Milk - Eko Analyzer) (brand name: Lohan, Model: SP60).

statistical analysis

The data were analyzed statistically using the General Linear Model (GLM) within the ready-made statistical program (SAS 2012) by using complete random design (CRD). The significant differences between the means were determined using Duncan’s multiple range test (Steel and Torrie, 1984). The T test was used at a significant level of 0.05 and 0.01 as in the linear model equation:

$Y_{ij} = \mu + t_i + e_{ij}$, where:

Y_{ij}: the observations of each are the value of (j) observation from (i) genotype.

μ: the overall mean.

T_i: the effect of genotype.

E_{ij}: the random effect of the standard error associated with each observation.

RESULTS AND DISCUSSION

Effect of growth hormone genotype on daily and total milk production

Data in Table (3) showed a significant superiority ($P \leq 0.01$) of the genotype (AA)) in milk yield at the first and second months which was 271 and 331.2 g/day compared to the genotype (AG) 202.0 and 228.6 g respectively, while this increase in milk yield was not significant in the third month of lactation 237.6 compared to 218.6 gm for the AA and AG genotype respectively. Thus, it was noted that there were significant differences in total period of milk production between the two genotypes 25.19 and 19.48 kg for the genetic structures (AA, AG) respectively. As we conclude that it is possible to improve the quantity of milk production in sheep through selection of individuals with the genetic makeup of AA, these results were similar to what was found by (Marques *et al*., 2014) who studying the effect of growth hormone gene in milk yield and found that ewes with a genetic AA structure gave higher milk yield than the genotypes AB and AE, as well as with (Abdel Nour and Micheal, 2011) mentioned that there significant effects of the structure of the growth hormone gene on milk production, (Al-Dabbagh ,2019), reported that the strain has a significant effect on milk production and the reason may be due to its better genetic ability in milk production. But not agree with the result of (Al-Salhi *et al*., 2017) who was found that there are three genetic structures of the growth hormone gene in Awassi ewes (AA, AG. GG), the highest milk yield was in the ewes that have the AG gene, and the ewes that have GG gene structure gave the lowest milk yield (119.24, 127.11 and 73.37) kg respectively.

Effect of growth hormone genotype on milk composition

Table (4) show the components of ewes milk that have differed genotype for growth hormone, I'm found that fat increased in milk of ewes group with genotype AA) as compared ewes that have AG genotype, this increased were significant for the first and third months, while not reach to significant in the second month, the percent of fat were 4.2, 3.7 and 4.5 % for the AA group and were 3.6, 3.4 and 4.1 % for the AG group respectively.

Table (3): Effect of the growth hormone gene on daily and total milk production.

Traits	Genotype	Ewes No.	milk yield
Milk yield at the first month of lactation g/day.	AA	17	271.0 ± 24.2 a
	AG	7	202.0 ± 25.5 b
Milk yield at the second month of lactation g/day.	AA	17	331.2 ± 27.7 a
	AG	7	228.6 ± 8.5 b
Milk yield at the third month of lactation g/day.	AA	17	237.6 ± 26.4
	AG	7	218.6 ± 12.1
Total milk yield during 90 days of lactation. Kg.	AA	17	25.19 ±58.8 a
	AG	7	19.48 ± 29.4 b

** Means within column for each month with different superscripts differ significantly ($p \leq 0.01$).

These results were similar to (Al-Salhi *et al*., 2017), as it was mentioned that the growth hormone gene in the AA genotype GG has a significant effect on milk fat higher than the AG genotype (Al-Rawi, 2000 and Al-Rawi, *et al*, 2011), also we noticed that other milk components (solids non-fat, lactose and protein), were not affected by the differences in growth hormone gene structure, the values were close and ranged 10.04-10.69% for solid non-fat, 4.59-4.79% of lactose and 4.80-5.05% of protein. These results were similar to what was found by (Al-Salhi *et al*., 2017), so (Al-Jawari, 2011 and Molic *et al*., 2008) they stated that the genetic did not significantly affect the percentage of protein and lactose in milk.

Table (4): Effect of the growth hormone gene on milk composition.

Month of lactation	genotype	ewes No.	Milk components %			
			fat	solid non-fat	Lactose	Protein
first month	AA	17	4.2± 0.4 a**	10.04± 0.3	4.79± 0.2	4.80± 0.3
	AG	7	3.6± 0.4 b	10.24± 0.3	4.59± 0.3	4.91± 0.3
second month	AA	17	3.7± 0.5	10.66± 0.2	4.78± 0.3	5.05± 0.3
	AG	7	3.4± 0.4	10.69± 0.2	4.79± 0.2	5.00± 0.3
third month	AA	17	4.5± 0.5 a*	10.42± 0.3	4.69± 0.3	4.96± 0.3
	AG	7	4.1± 0.3 b	10.47± 0.4	4.73± 0.2	4.92± 0.3

Means within column for each month with different superscripts differ significantly **(p ≤ 0.01), *(P ≤ 0.05).

Results of milk components yield are showed in Table (5). during all the period of lactation (three months) fat yield was significantly higher in the ewes group with AA genotype (11.53, 12.42 and 10.67 g/day) as compared that have AG gene (7.16, 7.71 and 8.87 g/day). with exception third month we found that milk protein, lactose and solid non-fat yield significantly (P ≤ 0.01) higher in the group of ewes with AA genotype as compared AG, and these differences were disappeared in the third month and the values were close between two groups.

Table (5): Effect of the growth hormone gene on milk composition yield.

Months	Genotype	Milk components yield g/day			
		Fat %	Solids non-fat %	Lactose %	Protein %
first month	AA	11.53 ±1.72 a **	27.21 ±2.56 a**	12.34 ±1.33 a**	13.00 ±1.35 a**
	AG	7.16 ±1.04 b	20.68 ± 2.69 B	9.30 ±1.53 b	9.94±1.59 b
second month	AA	12.42 ±2.43 a**	35.27±2.77 a**	15.79±1.29 a**	16.68±1.37 a**
	AG	7.71±0.72 b	24.47±1.05 B	10.95±0.62 b	11.42±0.69 b
third month	AA	10.67±1.83 a*	24.72±2.64 n.s	11.14±1.42 n.s	11.80±1.52 n.s
	AG	8.87±0.96 b	22.88±1.34 n.s	10.34±0.87 n.s	10.75±0.95 n.s

Means within column for each month with different superscripts differ significantly **(p≤ 0.01), *(P ≤ 0.05).

This increase is important for new born lambs and provide most requirements of protein and energy for growth. According to these results, the ewes that have the genotype AA are expected to have higher weights for their lambs at weaning, and also, related to weight at later ages.

CONCLUSIONS

One of the most important methods of increasing animal production and their numbers, is application genetic improvement programs. Molecular genetics programs and detection of genes related to productive performance have provided an important opportunity to improve local animals. The results of this study showed that the genotype AA of growth hormone is closely related to milk production and its fat content in local Awassi ewes, therefore, these ewes are expected to produce a greater number of lambs at weaning age because of their ability to survive due to the possibility of equipping them with a greater amount of nutrients.

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CONFLICT OF INTEREST

There are no conflicts of interest, according to the authors.

تأثير التراكيب الوراثية لجين هرمون النمو في النعاج العواسية على إنتاج الحليب ومكوناته

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الخلاصة

أجريت هذه الدراسة في قسم الإنتاج الحيواني/ كلية الزراعة والغابات/ جامعة الموصل للمدة من 2022/4/3 الى 2023/2/3 استخدم فيها 40 نعجة من اغنام العواسي تراوحت أعمارهم بين (3 – 5) سنة وبمتوسط وزن 42 كغم. تم توزيع النعاج على مجموعتين حسب تركيب جين هرمون النمو (AA وAG) لدراسة تأثير التركيب الوراثي لجين هرمون النمو على إنتاج الحليب ومكوناته باستخدام تقنية PCR – RFLP. وتم الكشف عن جين هرمون النمو عن طريق تضخيم الجين المعتمد على بادئ الجينوم. بلغ عدد النعاج التي لها تركيب وراثي AA هو 17 نعجة، بينما التي لديها التركيب الوراثي AG بلغت 7 نعاج فقط. أظهرت النتائج أن النعاج التي تحتوي على التركيب الوراثي AA من جين هرمون النمو اعطت كمية أكبر من الحليب. (P <0.01) وكان إجمالي إنتاج الحليب خلال 90 يومًا 25.19 كغم مقارنة مع تلك التي تحتوي على التركيب الوراثي لهرمون النمو والذي كان 19.48 كغم. ترافقت هذه الزيادة في إنتاج الحليب مع زيادة في دهون الحليب معنوياً في الشهرين الأول والثالث من فترة التربية، بينما لم تتأثر مكونات الحليب الأخرى من البروتين واللاكتوز والمواد الصلبة اللادهنية باختلاف التركيب الوراثي لهرمون النمو. نستنتج من نتائج هذه الدراسة أن التركيب الوراثي لهرمون النمو له دور مهم في إحداث تغيرات في كمية الحليب المنتج ومحتواه الدهني في النعاج العواسية المحلية، وهذا له تأثير على نمو الحملان ونموها والقدرة على البقاء على قيد الحياة حتى فطام الحملان.

الكلمات المفتاحية: النعاج العواسية، التركيب الوراثي، تحليل الحليب، تقنية البلمرة المتسلسل.

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