EFFECT OF MYOSTATIN GENE POLYMORPHISMS ON SOME QUALITATIVE CHARACTERISTICS OF CARCASS AND IFFERENT BODY MEASUREMENTS FOR BROILER CHICKEN (ROSS 308) RAISED UNDER OF IRAQ CONDITION

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ABSTRACT

Three hundreds of Ross 308 chicks at one day-old were used. The experiment was continuous until sixth week of age. The objective of this study was to identify genotypes Myostatin gene and its relation with some qualitative characteristics of carcass and different body measurements for broiler chicken. Three types of restriction enzymes Aci I (Arthrobacter citreus), Bbv I (Bacillus brevis) and Bbs I (Bacillus brevis), were used in the Restriction fragment length polymorphism (RFLP) analysis.

The results of this study can be summarized as follows:
The percentages of genotypic frequency for the Myostatin gene by using restriction enzyme Aci I (*Arthrobacter citreus*) was 32 %, 54% and 14 % for the (GG, GA and AA), respectively. The differences between genotypes was highly significant, furthermore, the effect of the genotypes of the Myostatin gene on carcass yield percentage was significant (P<0.05) too .Effect of the genotypes of the Myostatin gene on the body measurement was significant (P<0.05) on the breast width during third week and the body length during sixth week. The percentage of genotype distribution for the Myostatin gene by restriction enzyme Bbs I was 6.78%, 86% and 31.36 % for the genotypes. (CC, CT and TT), respectively and the differences between these percentages was highly significant and the effect of the genotypes of the Myostatin gene on the body measurement was significant (P<0.05) on the leg length during third week and leg length, the body length and comb height during sixth week. The percentage of genotype distribution for the Myostatin gene by restriction enzyme Bbv I in samples of studied was 98% and 2 % for the genotypes, (AA and GA) respectively and the differences between these percentages was highly significant and the effect of the genotypes of the Myostatin gene on the body measurement was significant (P<0.01) in the leg length and thigh thickness during sixth week.

Keywords: carcass trait, myostatin, body measurements, restriction enzyme, genotypes.

تأثير طرز جين الميوستاتين (Myostatin) على بعض الصفات النوعية للذبائح وقياسات الجسم المختلفة لفروج اللحم (روز308) المرباة تحت الظروف العراق

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الخلاصة

استخدمت في هذه الدراسة 300 فـــــرخاً من فروج اللحم لهجين روز (Ross – 308) والتي ربيت من عمر يوم واحد لغاية الأسبوع السادس وكانت الدراسة تهدف الى فصل المادة الوراثية وتحديد علاقة التراكيب الوراثية لجين الميوستاتين (Myostatin) مع بعض الصفات النوعية للذبائح وقياسات الجسم المختلفة لفروج اللحم نوع Ross 308 ، و استخدمت ثلاثة أنواع من الانزيمات القاطعة .

Aci I (Arthrobacter citreus) و *BbvI (Bacillus brevis)* و *BbsI (Bacillus brevis)* في الهضم القطعة المضخمة لتحديد التراكيب الوراثية، باستخدام تقنية تعدد المظاهر لأطوال القطع مقيدة الطول(RFLPs).

وكانت نتائج الدراسة كمايلي:

بلغت نسب توزيع التراكيب الوراثية لجين الميوستاتين (Myostatin) باستخدام الانزيم Aci I في العينة المدروسة 32 و54 و14% للتراكيب الوراثية GG وGA وAA على التوالي، وكان التباين بين هذه النسب عالي المعنوية (P≤0.01)، كما كان تأثير

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التراكيب الوراثية لجين الميوستاتين (Myostatin) معنويا (P≤0.05) في نسبة التصافي، أما تأثير التراكيب الوراثية لجين ميوستاتين على ابعاد الجسم فقد كان معنوياً (P≤0.05) في عرض الصدر عند عمر ثلاثة اسابيع،

من جانب أخر، بلغت نسبة توزيع التراكيب الوراثية لجين الميوستاتين (Myostatin) باستخدام الانزيمBbs I في العينة المدروسة 6.78 و61.86 و31.36% للتراكيب الوراثية CC وCT وTTعلى التوالي، وكان التباين بين هذه النسب عالي المعنوية (P≤0.01) ، كما كان تأثير التراكيب الوراثية لجين الميوستاتين (Myostatin) معنويا في ابعاد الجسم فقد كانت لها تأثيرات معنوية (P≤0.05) في طول الارجل عند عمر الأسبوع الثالث، في حين نجد ان التراكيب الوراثية لها تأثيرات معنوية (P≤0.05) في طول الارجل وطول الطير وارتفاع العرف خلال أسبوع السادس.

بلغت نسب توزيع التراكيب الوراثية لجين الميوستاتين (Myostatin) باستخدام الانزيم Bbv I في العينة المدروسة 98 و2% للتراكيب الوراثية AA وGAعلى التوالي، وكان التباين بين هذه النسب عالي المعنوية، كما كان تأثير التراكيب الوراثية لجين الميوستاتين (Myostatin) معنويا (P≤0.05) في طول الارجل وعالية المعنوية (P≤0.01) في سمك الفخذ خلال الأسبوع السادس.

الكلمات المفتاحية: صفات الذبيحة، الميوستاتين، قياسات الجسم، الانزيمات القاطعة، تراكيب الوراثية.

INTRODUCTION

Poultry meat is one of major source of high-quality protein in food of human. Skeletal muscles are main tissue productive for meat. Muscle growth rely on increase in the number and size of cells after hatching (1). There are many genes that enhance or retard the growth of muscle cells, one of most important genes that have an effect on growth and some traits of productivity is Myostatin gene because it has a role in regulating muscle growth and body mass determination and that association of this gene with body weight is higher than correlations with specific qualities for meat (2), polymorphisms for gene Myostatin have several effects on important traits such as body weight and carcass characteristics, such as abdominal fat percentage and increase in dressing percentage resulting from increase of amount for meat produced at the expense of bones (3).

Recent developments in molecular biology and biostatistics have created revolution in molecular genetics, particularly in identifying genes which impact various traits that can help in selection, as well as detecting and identifying many of polymorphisms within DNA that have been used as genetic markers to improve livestock by dependence on genotype in programs of improvement (4),Several polymorphisms have been identified in gene sequence as Myostatin possess a relationship with traits growth and carcass (5).

Researcher (6) find on presence of polymorphisms in first exon of correlated with body weight for ages of different, too (7) on existence of polymorphisms in first exon, which have correlations with proportion of breast muscle and proportion of abdominal fat, increased interest by biotechnology as one of solutions for problems facing humanity for shrinking of gap between production and consumption. Molecular genetics gives better results than dependence on external appearance in electoral programs. Which allows for early selection, which reduce the range of the generation and thus reduce the costs of breeding, which are expensive.

Materials and Methods

This study was conducted at the Poultry Farm of Animal Production Department College of Agriculture, University of Qadisiyah the molecular analysis were performed in laboratory for College of Agriculture, University of Baghdad and Almusayab Bridge Company. Three hundred birds (Ross 308) were used in this study and marked with wing tags. Birds were reared on sawdust litter for period 42 days. The blood were collected from the brachial vein of all chicks under the study. These samples were placed in EDTA tubes and kept in freezer (-18 ºC) for DNA isolation. DNA was e

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xtracted from the blood according to the instructions manufacture (Genial of Taiwan) and after the extraction for DNA was used Sambrook method (8) to make sure availability DNA, Primers were supplied from company (Integrated DNA Technologies), the sequence of primers for myostatin gene as follows ,Which has been obtained from company Geneaid.

F: 5'- TGGCATATATAAGGCACACCA-3'
R: 5' - GGGAGAGCCTGAGAAGGAGT-3'

PCR reactions program was modified to be suitable for Myostatin gene and performed at PCR machine according to the cycles as shown in table (1).

Table (1). The conditions of PCR amplification steps.

Steps	Temperature(^{o}C)	Time	No. of Cycles
Initial denaturation	94	5 min.	1
Denaturation	94	45 sec.	30
Annealing	56	45 sec.	
Extension	72	45 sec.	
Final extension	72	5 min.	1

The step listed below in table were used to amplify the target segment of a gene myostatin. After PCR reaction, detection from mutations in region exon 1 G1601A, G1784A and C1874T, was performed by utilizing of Aci I, Bbv I and Bbs I enzymes from Biolabs, (PCR-RFLP), according to Table (2). The reaction mixture was incubated at 37 ° C for 4 hours for each enzyme individually.

Table (2) Component for PCR-RFLP.

Reaction size 10 reactions	Component
0.5 µl	ScaI (20000 units\ml)
5 µl	PCR Product
1.5 µl	1X TBE Buffer Solution
3 µl	DNase Free Water

Electrophoresis

3µl of the DNA ladder was loaded with µl5 of PCR-RFLP products in agarose gel 3% concentration (1X TBE Buffer). The Electrophoresis was carried out with a voltage of 70 V and with a current of 40 ampere for 1.5 hours. The beams were seen by UV transiluminater and photographed using the photo documentation system, the results were as shown in the pictures.

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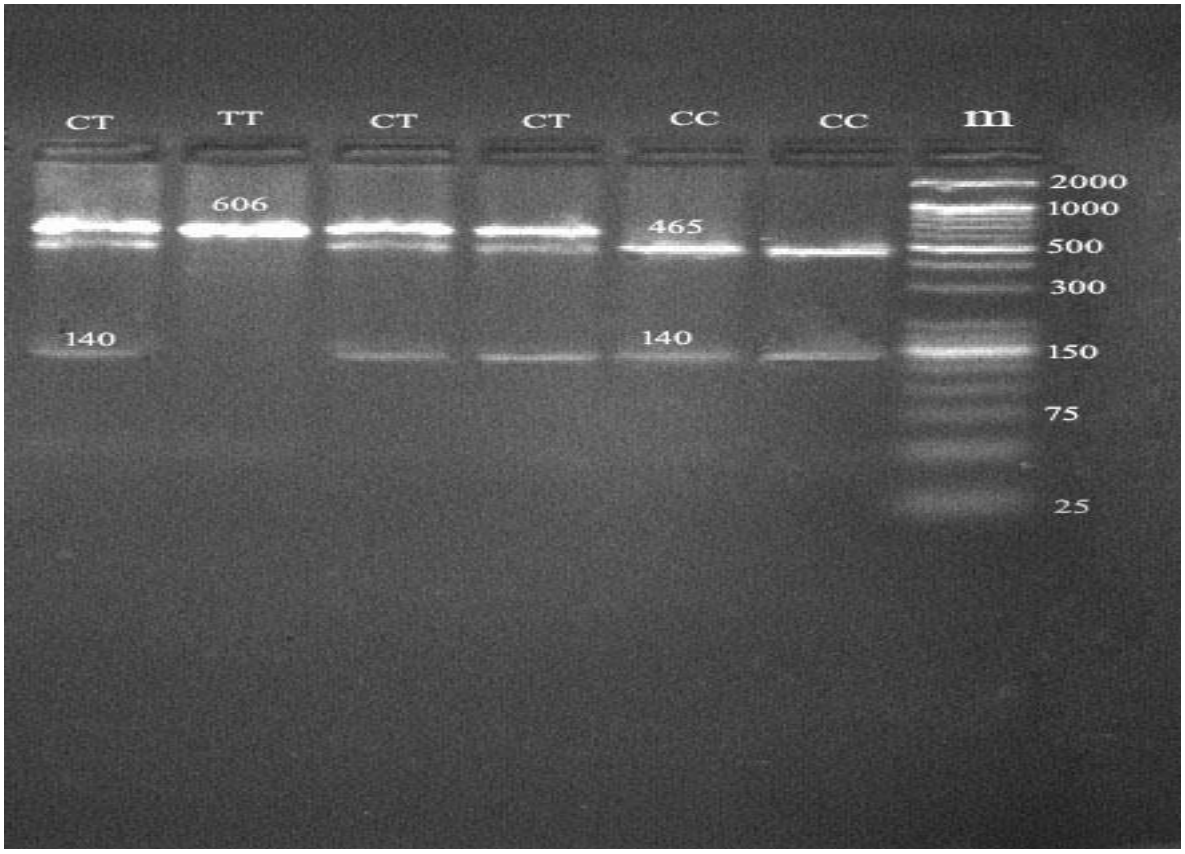

Figure (1) digestion for products Myostatin gene by using enzyme Bbs I on Agarose gel, M;2000.

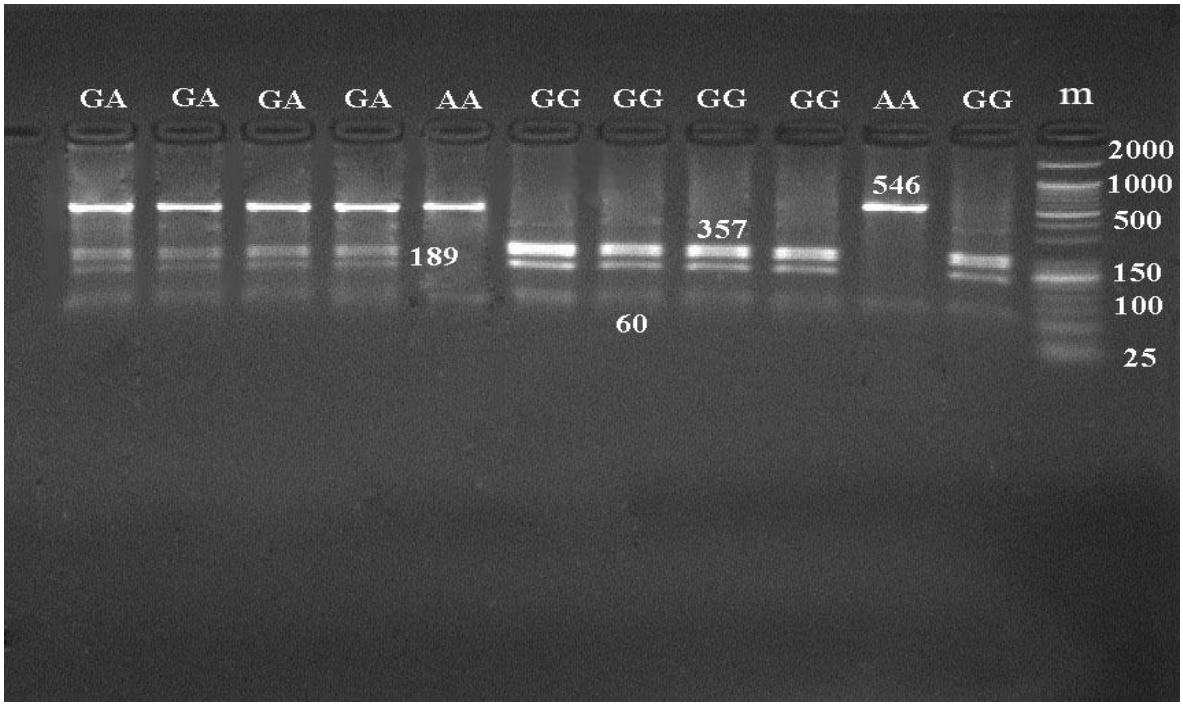

Figure (2) digestion for products of Myostatin gene by using enzyme Aci I on Agarose gel, M;2000.

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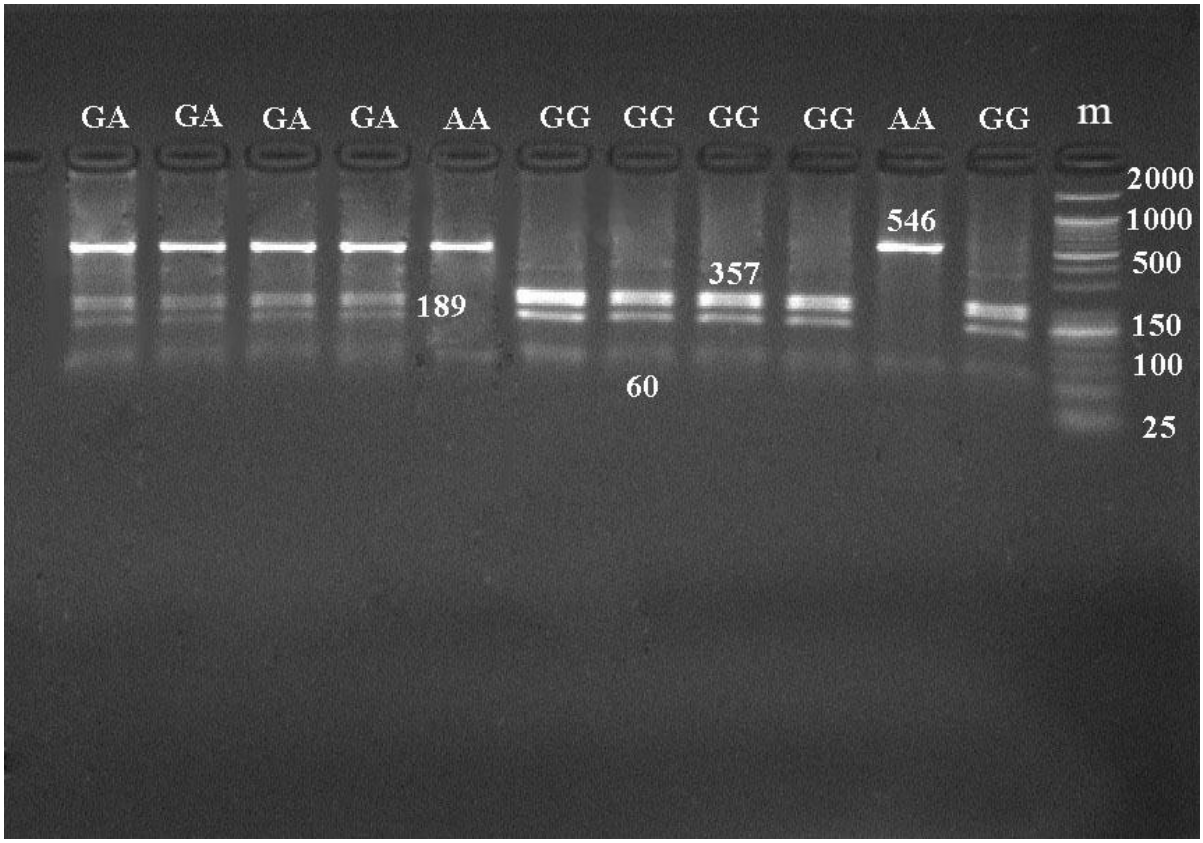

Figure (3) digestion for products of Myostatin gene by using enzyme on agarose gel Bbv I, . M;2000

Data were statistically analyzed using the program Statistical Analysis System -SAS 2012 (9)**.**

Results and Discussion

Carcass weight and dressing

Effect of genotypes different for Myostatin gene resulting from using enzyme Aci I

Results of the statistical analysis (Table 3) showed no significant differences in weight of carcass among the genotypes resulting from digestion of Myostatin gene by Aci I. The carcass weight was 1447.21, 1497.86 and 1459.37 g for AA, GA and GG, respectively, this result was consistent with (10), which pointed to absence significant differences in weight of carcass among the genotypes different in exon one. Results of study showed that there were significant differences in percentage for dressing according to genotype of gene Myostatin, broiler which carry genotype GA was highest percentage (72.06%) compare with other genotypes GG lower 71.45% followed by genotype AA with 71.33% (3) result was consistent with results(7) , which indicate a significant correlation between the different genotypes and carcass traits in exon one .

Effect of genotypes different for Myostatin gene resulting from using enzyme Bbs I

Table (3) showed no significant differences in weight of carcass among genotypes resulting from digestion of Myostatin gene by Bbs I. The carcass weight was (1557.67, 1458.89 and 1503.39) g for (CC, CT and TT) respectively. This result was consistent with (11). Results of statistical analysis (Table 3) showed no significant difference between different genotypes for CC, TT and CT respectively in percentage for dressing. This result came compatible with (10) showed no significant differences in weight of carcass among genotypes in exon one.

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Effect of genotypes different for Myostatin gene resulting from using enzyme Bbv I

Table (3) showed no significant differences in weight of carcass among genotypes resulting from digestion of Myostatin gene by Bbv I. The carcass weight was1435.50 and 1480.12 g for GA and GG respectively. This result was consistent with (10) refer to mutations in the Myostatin gene not associated with carcass characteristics. (Table 3) showed no significant difference between different genotypes for GA and GG respectively in percentage for dressing. This result came compatible with (12).

The majority of qualities not possess significant correlations with genotypes different resulting from lack differences in body weights for broiler at the age of marketing (week six) , and body weight has impact in most productivity feature, so there is no significant differences between genotypes different in weight carcass and percentage for dressing.

Table (3) Effect of gene Myostatin polymorphism on Carcass weight and dressing.

Enzyme	Genotypes	Carcass weight(g) ±SE	Dressing(%) ±SE
Aci I	AA	1447.21±55.21	71.33± 0.48b
	GA	1497.86± 26.89	72.06± 0.19a
	GG	1459.37±27.67	71.45±0.12
		N.S	*
Bbs I	CC	1557.67±45.2	72.47±0.46
	CT	1458.89±23.20	71.63±0.16
	TT	1503.39±31.63	71.88±0.25
Bbv I		N.S	N.S
	AA	1480.12±19.34	71.79±0.13
	GA	1435.50±55.60	70.98±0.06
		N.S	N.S

Means with different letters within each column for each enzyme indicate significant differences, N.S (no significant), *($p<0.05$) significant.

Carcass main, secondary cuts and abdominal fat

Results of current study (Table 4) showed that carcass main, secondary cuts and abdominal fat was not be influenced by various genotypes for myostatin gene, and this result was congruent with (12), indicating there is no correlation between genotypes different for Myostatin gene with carcass main and secondary cuts for broiler.

Results of current study showed (Table 4) there was no significant difference between different Genotypes for Myostatin gene in carcass main, secondary cuts and abdominal fat , And this result agree with what was indicated with (12) the presence of different genotypes in exon one gene myostatin non associated with carcass main and secondary cuts

Table (4) showed that different genotypes effect of Myostatin gene in carcass main, secondary cuts and abdominal fat was not significant between different genotypes. Agree with what he found (12).

It may be due to the absence of significant differences in percentage of cuttings may be due to the absence of differences in weight of carcass significantly at age of marketing

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Table (4) Effect of gene Myostatin polymorphism on carcass main, secondary cuts and abdominal fat.

enzyme	genotypes	(%) Breast ±SE	(%)Thigh ±SE	(%) Neck ±SE	Back (%) ±SE	Wing (%) $\pm SE$	Abdominal fat (%) ±SE
Aci I	AA	± 34.37 0.73	± 27.13 0.45	± 5.47 0.16	0.81 ± 19.0	± 10.4 0.30	0.14 ± 2.24
	GA	34.82 0.37±	± 27.49 0.32	± 5.61 0.09	0.41± 19.2	± 10.3 0.15	0.07 ± 2.38
	GG	± 34.08 0.58	± 27.35 0.73	± 5.77 0.16	0.60 ± 19.7	± 10.2 0.12	0.08 ± 2.41
		N.S	N.S	N.S	N.S	N.S	N.S
Bbs I	CC	± 35.20 0.81	± 28.32 1.57	± 5.87 0.24	1.27 ± 18.2	0.21 ± 9.9	0.14 ± 2.30
	CT	± 34.98 0.37	27.40± 0.40	± 5.67 0.07	0.38 ± 19.3	0.11± 10.4	0.06 ± 2.38
	TT	33.88± 0.53	27.97±0.33	± 5.52 0.19	0.61± 19.7	0.21± 10.3	0.10± 2.37
Bbv I		N.S	N.S	N.S	N.S	N.S	N.S
	AA	± 34.67 0.31	± 27.36 0.30	0.08 ± 5.6	19.4± 0.32	± 10.3 0.10	0.05 ± 2.37
	GA	± 34.64 1.60	± 28.00 1.31	0.25± 5.6	19.1±0.78	± 10.1 0.52	0.51 ± 2.46
		N.S	N.S	N.S	N.S	N.S	N.S

Means with different letters within each column for each enzyme indicate significant differences, N.S (no significant).

Body measurement during three week for rearing

Table (5) showed non-significant differences between the various genotypes(AA ,GA and GG) for Myostatin gene resulting from using enzyme Aci I in body measurement during three week except Breast width showed significant differences between the different genotypes (AA ,GA and GG) . The genotype AA was superior on genotypes GA in Breast width. There were no-significant differences between genotypes AA and GG in Breast width.

Results of Table (5) showed non-significant differences between of various genotypes(CC, CT, TT) in body measurement during three week for rearing various genotypes to Myostatin gene resulting from digested by Bbs I, but were significant differences between of various genotypes shank length showed significant differences between the different genotypes (CC,CT,TT) . The genotype CC and TT was superior on genotypes CT in breast width.

Appear from results which obtained from a table (5) non-significant differences between the various genotypes for Myostatin gene resulting from using enzyme Bbv I in body measurement during three weeks for rearing between various genotypes to Myostatin gene. Absence of significant differences in body measurement during three week for rearing may be due to absence of differences in weight live during age three week from rearing. The environmental factors may be affecting directly or indirectly the gene myostatin expression which affects the

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effectiveness and impact on the body or tissue, led to the difference between the results of this experiment and other results in a different environment.

Table (5) Effect of polymorphism for gene Myostatin on body organs measurement during three week for rearing.

enzyme		body measurements				
Aci I	genotypes	Breast Width ±SE	Shank Length $\pm SE$	thigh Thickness ±SE	length breastbone ±SE	body length $\pm SE$
	AA	0.61 ± 10.9 a	± 51.6 1.14	0.56 ± 20.0	0.24 ± 8.90	0.60 ± 35.2
	GA	0.20 ± 9.82 b	± 51.4 0.58	± 20.41 0.29	0.09 ± 8.59	0.80 ± 34.0
	GG	0.27 ± 10.2 ab	± 50.9 0.78	0.45 ± 20.4	0.15 ± 8.67	0.38 ± 34.4
		*	N.S	N.S	N.S	N.S
Bbs I	CC	0.68 ± 10.6	± 52.2 2.61 a	1.46 ± 20.5	0.39 ± 8.85	1.19 ± 34.9
	CT	0.22 ± 10.1	± 50.6 0.50 b	0.28 ± 20.2	0.09 ± 8.57	0.68 ± 33.9
	TT	0.25 ± 9.91	± 52.4 0.69 a	0.33 ± 20.6	0.13 ± 8.80	0.41 ± 35.0
Bbv I		N.S	*	N.S	N.S	N.S
	AA	17. ± 10.1	± 51.2 0.43	0.24 ± 20.3	0.07 ± 8.67	0.47 ± 34.3
	GA	0.73 ± 9.20	± 52.0 2.49	0.41 ± 20.6	0.52 ± 8.57	0.91 ± 34.4
		N.S	N.S	N.S	N.S	N.S

Means with different letters within each column for each enzyme indicate significant differences, N.S (no significant), *($p<0.05$) significant

Body measurement during sixth week for rearing

Table (6) showed non-significant differences between the various genotypes (AA ,GAand GG)for Myostatin gene resulting from using enzyme Aci I in body measurement during sixth week except body length showed significant differences between the different genotypes (AA ,GA and GG) . The genotype AA was superior on genotypes GG in body length. There were no-significant differences between genotypes AA and GA in Breast width.

Results of table (6) showed non-significant differences between of various genotypes(CC , CT , TT) in body measurement during sixth week for rearing various genotypes to Myostatin gene resulting from digested by

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Bbs I, but were significant differences between of various genotypes shank length , body length and comb , showed genotype TT was superior on genotypes CC and CT.

Results which obtained from a table (6) appeared non-significant differences between the various genotypes for Myostatin gene resulting from using enzyme Bbv I in body measurement during sixth week for rearing between various genotypes to Myostatin gene except shank length and thigh thickness showed significant differences between the different genotypes (AA and GA) . The genotype AA was superior on genotypes GA in shank length and thigh thickness.

It may be due to the absence of significant differences in body measurement during three week for rearing may be due to absence of differences in weight live during six week for rearing.

Table (6) Effect polymorphism for gene Myostatin on body measurement during six week for rearing.

Enzyme		Body organs measurements					
Aci I	Genotypes	Breast Width ±SE	Shank Length ±SE	Thigh Thickness ±SE	Length Breast bone $\pm SE$	Body length ±SE	Comb Height ±SE
	AA	±17.4 0.53	1.56 ±79.5	0.90 ± 35.1	0.27 ± 14.8	± 54.0 0.55 a	0.90 ± 12.2
	GA	17.9 0.29±	1.14± 78.6	0.64± 35.3	0.13 ± 14.6	± 53.8 0.33 ab	0.70 ± 13.5
	GG	17.9 0.31±	± 78.5 1.02	0.57 ± 35.5	0.19 ± 14.4	± 52.6 0.47 b	0.67 ± 11.6
		N.S	N.S	N.S	N.S	*	N.S
Bbs I	CC	18.1 1.02±	± 78.1 0.64 b	1.78 ± 36.3	0.34 ± 14.8	± 53.1 0.78 b	0.26 ± 10.1 b
	CT	17.7 0.23±	± 77.7 0.83 b	0.54 ± 35.1	0.13 ± 14.5	± 53.0 0.32 b	0.55 ± 12.3 ab
	TT	18.1 0.42±	± 81.3 1.66 a	0.62 ± 35.7	0.16 ± 14.6	± 54.5 0.35 a	0.94 ± 14.3 a
Bbv I		N.S	*	N.S	N.S	*	*
	AA	17.9 0.20±	± 78.9 0.70 a	0.35 ± 35.6 a	0.10 ± 14.5	± 53.5 0.25	0.48 ±12.7
	GA	16.6 0.50±	± 73.9 6.46 b	4.57± 29.7 b	0.51 ± 14.8	± 52.4 1.53	1.05 ± 14.0
			*	**	N.S	N.S	N.S

Means with different letters within each column for each enzyme indicate significant differences, N.S (no significant), *($p<0.05$) significant. * *($p<0.01$) significant.

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CONCLUSIONS

The results of the current study of Myostatin gene in broiler chicken (Ross 308) showed the presence of several mutations in exon 1 region, G1601A, G1784A and C1874T respectively and along the studied area, They are detected using an enzyme by Aci I (*Arthrobacter citreus*), Bbv I (Bacillus brevis) and Bbs I (Bacillus brevis).

- The different genotypes for Myostatin gene resulting from mutation C1874T have a correlation with body weight during the first weeks, fifth week and along the legs during third week and length of legs, length of bird and height comb during the sixth week.
- The different genotypes for Myostatin gene resulting from mutation G1784A mutation have been associated with length of legs and thigh thickness during the third week.

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