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Effect of CAPN1 gene polymorphism on some physical and sensory traits in broiler meat

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Abstract

This study was conducted in the poultry field, Animal Production Department, College of Agriculture and Marshes, University of Thi-Qar, from 10/11/2021 to 3/2/2022, the period included field and laboratory stud. A total of 100, one day broilers Ross 308 were used. Laboratory analyzes were carried out in the laboratories of the Marshes Research Center, University of Thi-Qar and the laboratories of the College of Agriculture and Marshes, University of Thi-Qar. Three genotypes CC, CT and TT were identified whose frequency was 0.52, 0.34 and 0.14 respectively and the frequency of the C allele was 0.69 and the frequency of the T allele was 0.31. There were significant differences on Water Holding Capacity (WHC) among the genotypes, the CC genotype was superior to the CT and TT genotypes. As for the thawing and cooking loss, there were no significant differences between the genotypes. A significant differences on pH values among the genotypes, the CC genotype was superior to the CT and TT genotypes. Significant differences in flavor between genotypes, the CC genotype was superior to the CT genotype, and the CT genotype was superior to the TT genotype. Highly significant differences in tenderness, the CC genotype was superior to the TT genotype, while the CT genotype did not differ significantly from the CC and TT genotypes. Highly significant differences between the genotypes in juiciness, the CC genotype was superior to the CT and TT genotypes. No significant differences in color and general acceptable.

I. INTRODUCTION

Poultry meat is one of the important sources of animal protein in the human diet, as it has a higher nutritional value than other types of meat (Saxena et al., 2005) as it contains essential amino acids, vitamins and minerals (Wallker et al., 2005). Unsaturated fatty acids compared to beef and sheep, which contain large amounts of saturated fatty acids, which lead to a high level of cholesterol in the blood. About 23 billion chickens are raised in various countries of the world, whose meat production is estimated at about 100 million tons, and eggs at 73 million tons each year. In the past twenty years, production has tripled by FAO (2016). The breast cut is of high nutritional value and is one of the main cuts in poultry and constitutes approximately 50% of the muscle weight, and the process of improving it makes the benefit of the consumed food more, using the relationship between the weight of the breast meat to the total weight (Mcelroy et al., 2002) and the chest muscle meat is closely related Genetically well with a number of traits as evidence of breast shape and other traits (Khalil, 2007) and many studies have indicated the possibility of polymorphisms for many genes with a wide range of specific characteristics of carcass and cut-breasted traits (Rasouli et al., 2013). Which can be used as indicators in selection programs, and the CAPN1 gene is an important marker in this process, as this gene encodes the cysteine protein that breaks down muscle fiber proteins after slaughter. (Geesink& Koohmaraie, 1999). The single nitrogen base polymorphism is a

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crucial guide in discovering new strategies for mapping and filtering the locus of economic quantitative traits in genetic studies. A member of the calpain family, CAPN1 surrounds myofibrillar proteins and regulation of CAPN1 activity has been linked to diversity in meat tenderness and thus CAPN1 is a good candidate and tenderness indicator gene (Geesink Koohmaraie&,1999). The traditional selection methods did not significantly affect the improvement of meat quality, as they were not evaluated at the genetic level (because of the difficulty of obtaining parents), as well as the high cost and length of breeding when using the method of progeny testing, so this study was conducted, which aims to find the relationship The CAPN1 gene with some characteristics (tenderness, juiciness, water carrying capacity, loss during thawing, loss when cooking, etc.)

II. MATERIALS AND METHODS

2-1- The process of drawing blood and the method of extracting the genetic material DNA.

Blood was drawn from the winged vein of birds, where 3 ml of blood was collected from each bird and the samples were kept in test tubes containing EDTA anticoagulant. Genetic DNA was extracted from blood samples according to the instructions of the diagnostic kit

(Kit supplied by the Korean Geneaid Company) 30 µl of bird blood was added to the 1.5 ml tube, and 200 µl of PBS (Phosphate buffered saline) was added to the blood capping, then 20 µl of Proteinase K solution was added at a concentration of (20 mg/ml) the samples were agitated by (Vortex) the tube was incubated for 2 minutes at room temperature and 200 µl of GSB (Genomic lysis/Binding buffer) was added. At a temperature of (60°C), then the Elution Buffer substance was heated at a rate of 100 microliters for each sample at a temperature of 60°C, and 200 microliters of Ethanol Absolute were added at a concentration of 100%. From the mixture after removing the formed blood clot using a Micropipette, it is placed in a filter tube, then the samples are placed in a centrifuge 14000 r/min, and then the filter is disposed of and new filter tubes are used, and then 400 mikes are added Roller of Wash Buffer solution 1), then the samples are placed in the centrifuge 14000 cycles / minute for a minute, then the filtrate is disposed of, then the filtration membrane is returned to the collection tubes, and 600 microliters of Wash Buffer solution were added 2), then the samples are placed in the centrifuge 14000 cycles / minute for 3 minutes, then the filtrate is disposed of, then the filtration membrane is returned to the collection tubes, and then a new Eppendorf tube with a capacity of 1.5 ml is placed in it. The filter tube is then added 100 microliters of the previously prepared Elution Buffer. Centrifuge at 14,000 rpm for 1.5 minutes for the purpose of extracting the purified DNA, so that the samples were ready for electrophoresis to verify the presence of the genetic material using 1% acarose gel.

2-2- Gel Electrophoresis

The migration process was carried out to separate the DNA segments through agarose gel to ensure the presence of the DNA. TAE buffer (Tris Acetate EDTA) solution of 50 X concentration was used, prepared by Bioneer Company. For every 10 ml of TAE buffer, 490 ml of distilled water was added. It was used to prepare solution 1. X To prepare the agarose gel and also to fill the tank of the electrophoresis device. Clean 30 ml of 1 X concentration of (TAE) solution into a glass conical flask (flask) that can withstand high temperature and add 0.3 g of agarose (1% agarose) and close the mouth of the

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flask with a piece of of tin and put it on a hot plate device to heat the mixture to a boiling point and wait until the agarose minutes melt and get a transparent solution. Leave the solution to cool for several minutes and prepare the mold into which the agarose is poured by placing the special comb to make the pits of the agarose gel in the place designated for it at the end of the basin. We add the agarose mixture to cover the entire area of the basin and in order not to cause bubbles, leave the mixture until the agarose hardens and remove the comb carefully without creating A tear in the gel. Put the gel based on the plate in its designated place inside the electrophoresis device. Fill the electrophoresis basin with buffer solution to the extent that fills the loading holes and to the extent that the level of the buffer solution rises from the agarose gel by about 3 mm.

2-3- Loading samples of DNA

Mixed 5 µl of DNA samples with 2 µl of Loading Dye dye prepared from Promeca in addition to 1.5 µl of Diamond™ Nucleic Acid Dye dye also prepared from Promeca and diluted by TE at a dilution ratio (1 µl dye to 100 µl). of TE solution) and mix the mixture well using a micropipette on a plastic tape (Laboratory Film). Then the samples were carried into the pits of the prepared agarose gel. After all the samples were loaded into the pits, the transparent plastic cap was placed and the electrodes were connected to the Power Splay using 70 volts for a period of 85 and Milliampere for half an hour and the agarose gel was examined after the end of the migration time using a UV Gel Documentation device, and transfer pictures were taken using the installed camera designated for this purpose.

2-4- Primers

Table (1) shows the Primers **used in the experiment**

Gene	**Primers**	Size
CAPN1	**TCACCTCACGTGCCTCTCTCA**	217bp
	CGGAACACTTACGTCGAT	

2-5- Technique PCR

Table (2): Program for PCR technique of CAPN1 gene using Touchdown method**.**

No.Cycle	**time**	**Temperature**	**Stages**
1	**5**	95C °	**Initial Denaturation**
30	**30**	95C °	**Denaturation**
	30	60C °	**Annealing**
	1	72C °	**Extension**

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1	**10**	72C °	**Final Extension**
1		4C °	**Cooling**

2-6- Sequencing

After confirming the size of the specialized PCR product for the studied gene by comparing it with the standard DNA strand DNA Ladder (with a size of 100 base pairs), 20 microliters were taken for each sample of the PCR product and sent to the Korean Macrogene Company, where the samples were purified and then the base sequence analysis process using sanger sequencing technology and received The results of base sequencing were analyzed using BLAST tools at the NCBI International Gene Bank website with the use of some bioinformatics programs.

2-7-physical tests

2-7-1-water holding capacity

The water carrying capacity was estimated according to the mentioned method from Babji and colleagues (1982) by homogenizing 20 g of meat with 60 ml of distilled water at 4 °C for 1 minute, then centrifuging the mixture for 10 minutes at a speed of 3000 rpm at 5 °C and then removing the liquid The tubes were turned downwards and left for 5 minutes, then the WHC was weighed and estimated according to the following equation:

WHC=4 * weight of deposit- Weight of the homogenized mixture/ **Weight of the homogenized mixture *100**

2-7-2- thawing loss

It was estimated according to the method of Nam et al. (2000) and the meat samples were weighed after being frozen for 24 hours, then the samples were left until completely thawing and the exuded water was removed and weighed again, and the percentage of loss was extracted according to the following equation:

TL%= Weight of frozen sample- Sample weight after thawing/ Weight of frozen sample*100

2-7-3- Cooking loss

It was calculated according to the method of Rasmusseiin and Mast (1989) and samples were weighed before grilling and then grilled by placing them on a laboratory hot plate for 6 minutes with the samples being turned on each side for 3 minutes to ensure that the samples reached a temperature of 70 °C and then weighed after grilling and the percentage of loss was calculated according to the equation:

CL%= weight before cooking- weight after cooking/ weight before cooking*100

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2-8- Sensory tests

Samples were evaluated according to Levie (1970) method. Cooked meat samples were presented for the purpose of sensory evaluation by a number of experienced arbitrators in the Department of Animal Production / College of Agriculture and Marshes to evaluate the samples in terms of color, tenderness, flavor and juiciness (Juiciness) and general acceptance according to the sensory assessment scale of 9 degrees.

III. RESULTS AND DISCUSSION

3-1- Amplification of the CAPN1 gene segment

Through the results obtained, which showed the success of the amplification process for the studied segment of the CAPN1 gene after performing the electrophoresis process, as its size reached (217 base pairs) as shown in the figure below **(mean ± standard error).**

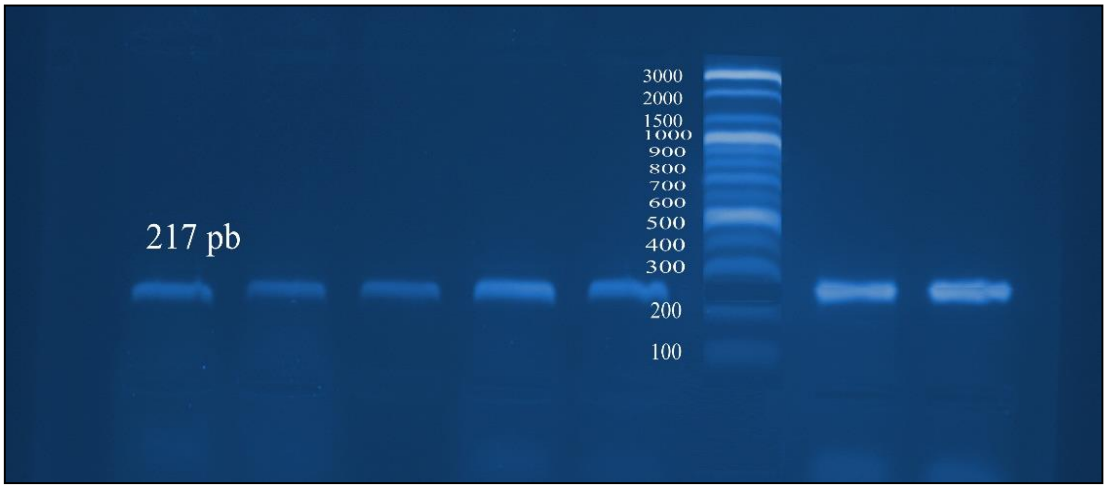

Figure (1) PCR product for CAPN 1 gene with a size of 217 base pairs carried over on agarose gel at a concentration of 1.5%.

3-2- Sequencing analysis of the studied segment of the 1CAPN gene

After the success of the amplification process of the studied piece of the CAPN1 gene, 50 samples were sent to the Korean company (Macrogen) for the purpose of obtaining the sequence of the nitrogenous bases of the studied piece and then it is molecularly analyzed and the variations in that piece of the 1CAPN gene are determined, as in the figure below

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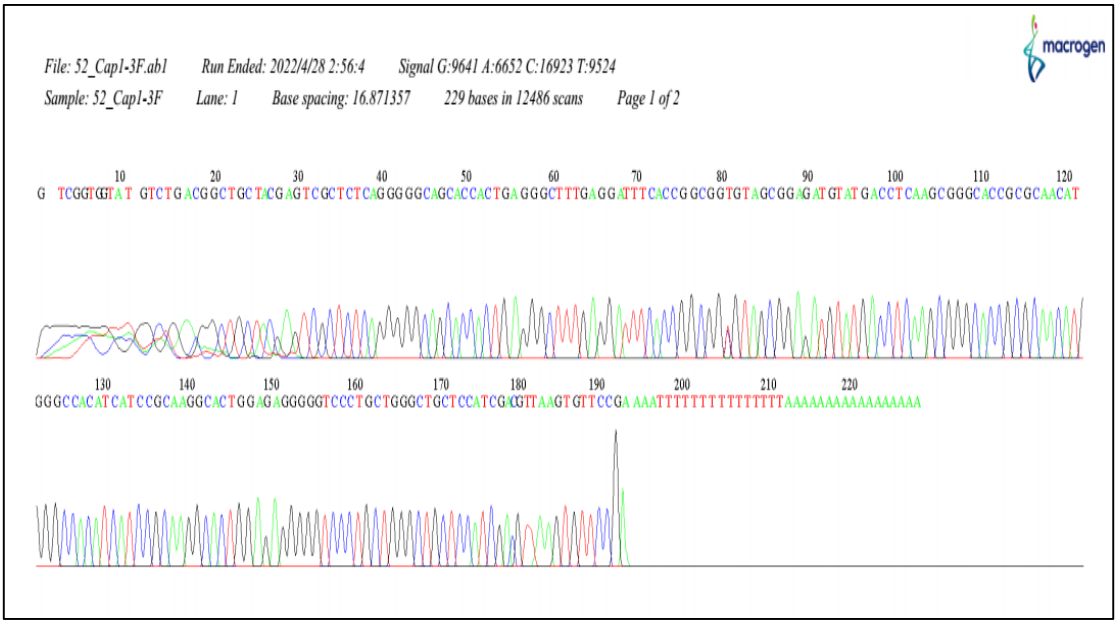

Figure (2) Nitrogenous base sequence analysis of the studied region of the CAPN-1 gene by the Korean company Macrogen.

3-3- Ratios of genotypes and allele frequency

Table (3) shows the frequency of the genotypes and the frequency of the C and T alleles of the CAPN-1 gene for the variable locus 82.C>T

The frequency of the genotypes and the frequency of the Tand C of the CAPN-1 gene for the variable locus 82.C>T

chi-square value	frequency of the allelic	Allelic	frequency of the genotypes	genotypes
2.11	**0.69**	**C**	**0.52**	**CC**
			0.34	**CT**
	0.31	**T**	**0.14**	**TT**

3-4- Relationship of CAPN1 gene genotype with meat physical traits

The results in Table No. (4) indicate that there are highly significant differences (P<0.01) in the water carrying capacity between the genotypes, as the CC genotype was superior to the CT and TT genotypes, Darwish and colleagues (2012) & Al-Husseiny & Khrebish (2019) indicated Water carrying capacity is one of the indicators of meat quality, as it affects the quality of the product, cooking characteristics, juiciness and tenderness.The results showed that there were no significant differences between the genotypes in the loss during thawing, and these results were in agreement with Molee and

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colleagues (2018), while they did not agree with Shu and colleagues (2015). Also, the results showed that there were no significant differences in the loss during cooking between the genotypes. These results were in agreement with Molee et al. (2018) while not consistent with Abtehal and colleagues (2016). The reason for the high water carrying capacity may be due to high pH values, or due to the increased ability of meat protein to bind water due to ionic strength (Al-Alwani et al., 2002) or it may be due to nutritional factors (Sahib and colleagues, 2009).

Table (4) shows the physical characteristics of meat ± standard error between the different genotypes of the CAPN-1 gene

Significant	standard error ± Value	No	genotypes	Adjective
**	**a 0.25 ± 19.88**	26	**CC**	**water holding capacity**
	b 0.43 ± 18.52	17	**CT**	
	b 0.71 ± 18.42	7	**TT**	
	0.23 ± 19.22	50	**Total**	
N.S	**0.16 ± 2.01**	26	**CC**	**thawing loss**
	0.19 ± 2.17	17	**CT**	
	0.40 ± 2.11	7	**TT**	
	0.12 ± 2.07	50	**Total**	
N.S	**0.54 ± 24.49**	26	**CC**	**Cooking loss**
	0.62 ± 24.94	17	**CT**	
	1.16 ± 24.63	7	**TT**	
	0.38 ± 24.67	50	**Total**	

3-5- Relationship of the CAPN1 gene genotype with sensory traits

The results in Table No. (6) indicate that there are no significant differences in general acceptance and color between the CC, CT and TT genotypes, and the results showed that there were significant differences in flavor between the genotypes, as the CC genotype was superior to the CT genotype and the CT genotype was superior to the TT genotype, and between Al-Fayyadh and Naji (1989) that the source of flavor in cooked poultry meat results from the interaction between sugars and amino acids, which is called Maillard reaction, which generates a specific taste that can be distinguished by the tongue. The genotype CC on the TT genotype, but the CT genotype did not differ significantly from the two genotypes CC and TT, and these results were in agreement with Shu and colleagues (2015). On the genotypes CT and TT, the values reached (7.15, 6.58, and 6.85), respectively. The reason for the increase in tenderness and juiciness may be due to the high water holding capacity and the collagen quantities at these ages are few and the diameter is short Muscle fiber, Saeed (2004) stated that the degree of general acceptance is the outcome of the common gustatory sense of flavor, juiciness and tenderness.

It should be noted that the study of sensory evaluation of the CAPN1 gene on poultry is almost non-existent, except for the tenderness, which was measured mechanically using the shear force.

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Table (6) shows the sensory characteristics of meat ± standard error between the different genotypes of the CAPN-1 gene

Significant	standard error ± Value	No	genotypes	Adjective
N.S	0.22 ± 6.42	26	**CC**	general acceptance
	0.34 ± 6.35	17	**CT**	
	0.43 ± 6.00	7	**TT**	
	0.17± 6.34	50	**ألمجموع**	
N.S	0.15 ± 7.26	26	**CC**	Color
	0.22 ± 6.58	17	**CT**	
	0.42 ± 6.28	7	**TT**	
	0.13 ± 6.90	50	**ألمجموع**	
*	**a** 0.15 ± 7.26	26	**CC**	Flavor
	b 0.25 ± 5.58	17	**CT**	
	c 0.42 ± 5.28	7	**TT**	
	0.18 ±6.40	50	**ألمجموع**	
**	a 0.15 ± 6.96	26	**CC**	Tenderness
	ab 0.24 ± 6.41	17	**CT**	
	b 0.42 ± 6.28	7	**TT**	
	0.13 ± 6.68	50	**ألمجموع**	
**	a 0.16 ± 7.15	26	**CC**	Juiciness
	b 0.22 ± 6.58	17	**CT**	
	b 0.26 ± 6.85	7	**TT**	
	0.12 ± 6.92	50	**ألمجموع**	

IV. REFERENCES

1. **Al-Alwani, Mahmoud Ahmad Hammadi..(2002**) Evaluation of Meat of Aged Layering Chickens. Master's Thesis, Department of Livestock Resources, College of Agriculture, University of Anbar.
2. **Al-Fayyad, Hamdi Abdel Aziz and Saad Abdel Hussein Naji. (1989).** Poultry products technology. The first edition - Directorate of Higher Education Printing Press - Baghdad **.**
3. **Al-Husseiny, K. S. J., & Khrebish, M. T. (2019).** Determination of chemical content and some physical properties and meat pigments (myoglobin, meta myoglobin and oxymyoglobin) in different parts of slaughtered animals. *Basrah Journal of Agricultural Sciences*, *32*(Spec. Issue 2), 302-319. https://doi.org/10.37077/25200860.2019.250
4. **Abtahal Y.Anaas,Mohd.Nazmi Bin Abd.Manap.(2016).** Association between single Nucleotide polymorphism of Calpain1 Gene and Meat Tenderness Traits in Different Genotypes of Chicken: Malaysian Native and Commercial Broiler Line.International Scholarly and Scientific Research &Innovation 10(2) 2016.

5. **Babji, A.S., G.W. Froning and D.A. Ngoka . 1982** . The effect of preslaughter environmental temperature in the presence of electrolyte treatment on turkey meat quality. *Poult. Sci.,* 61: 2385-2389.
6. **Darwish, O. M. I., El-Geddawy, M. A. H., Khalifa, R. M. B., & Mohamed. R. A. A. (2012).** Physico-chemical changes of frozen chicken burger formulated with some spices and herbs. *Frontiers in Science, 2*(6), 192-199. http://doi.org/10.5923/j.fs.20120206.10
7. **FAO . 2016** . Food and Agriculture: Key to Achieving the 2030 Agenda for Sustainable Development. FAO, Rome.
8. **Geesink, G.H., Koohmaraie, M., 1999**. Effect of calpastatin on degradation of myofibrillar proteins by μ-calpain under postmortem conditions. J. Anim. Sci. 77, 2685–2692.
9. **J. T. Shu, M. Zhang, Y. J. Shan et al., 2015.** "Analysis of the genetic effects of CAPN1 gene polymorphisms on chickenmeat tenderness," *Genetics and Molecular Research*, vol. 14, no. 1, pp. 1393–1403.
10. **Khalil, Maher Hassab Al-Nabi. (2007).** Fundamentals of animal genetics and breeding. Qassim University, College of Agriculture and Veterinary Medicine, Department of Animal Production and Breeding, Qassim University Press.
11. **Levie, A. (1970).** The meat hands book 1st ed. The AVI Publishing Co.
12. Connecticut, West Port..
13. **Mcelroy, J.P., D.E. Harry, J.C.M. Dekkers and S.J. Lamont. 2002.** Molecular Markers Associated with Growth and Carcass Traits in Meat-type Chickens. 7th World Congress on Genetics Applied to Livestock Production, August pp:19-23.
14. **Molee, A., P. Kuadsantia, and P. Kaewnakian. 2018**. Gene effects on body weight, carcass yield, and meat quality of Thai indigenouschicken. Jpn. Poult. Sci. 55:94–102.
15. **Nam , J. ; Park , H.; Songa, C.K.; Kim , D. G.; Moon, Y.H. and Jung,I.C.(2000)** Effect of freezing and re-freezing treatment on Chicken meat quality. Food Sci., 20: 222-229 .
16. **Rasouli Z., S. Zerehdaran, M.A. Azari, and M.S. Shargh. 2013.** Genetic polymorphism of the CAPN1 gene is associated with meat quality traits in Japanese quail. British poultry Science, vol. 54, No. 2, pp:171-175.
17. **Rasmussein, A. L. and Mast, M. G. (1989).** Effect of Feed with Drawal on Composition and Quality of Broiler Meat. Poultry Sci.68:1109-1113.
18. **Saeed, Bakar Ali Haj. (2004).** The use of electrical stimulation technology in tenderizing the meat of aged laying hens. PhD thesis - Department of Livestock - College of Agriculture - University of Baghdad.
19. **Saxena, V.K., P. Singh., A.B. Pramod., K.A. Ahmed., M. Saxena and R.V. Singh 2005**. Taq I PCR-RRFLP anlysis of myostatin gene revealed species sites in turkey and chicken. European Symposium on the Quality of Poultry Meat Doorwerth. Central Avian Research Institute, Izatnagar, INDIA-243 122.

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 11, Issue 2 (2022) PP 102-111
https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/UTJagr.Vo11.N2/12*

20. **Sahib, Maha, Salem Saleh Al-Tamimi, Mona Turki Al-Mousawi. (2009).** A study of the physical characteristics of imported frozen chicken thighs, the Iraqi Journal of Market Research and Consumer Protection, Volume 1, No. 2.
21. **Walker,** P.; **Rhubart-Berg, P.; McKenzie, S.; Kelling, K. and Lawrence,R.S. (2005).** Public Health Implications of Meat Production and Consumption. Public Health Nutr., 8: 348-356.