Arab Univ. J. Agric. Sci. (AUJAS), Ain Shams Univ., Cairo, Egypt
Special Issue, 26(2D), 2377-2384, 2019
Website: http://strategy-plan.asu.edu.eg/AUJASCI/

ESTIMATED GENE EXPRESSION IN MEAT QUALITY TRAIT UNDER STRESS CONDITIONS FOR TWO BROILER STRAINS

[174]

Kareman A. Nasser[1], Lamiaa M. Radwan[1], Mahrous[1] M.Y., Neima K. Alsenosy[2] and Farag[1] A.Z.

1. Poultry Production Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt
2. Genetics Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

*Corresponding author: KaremanAhmed@agr.asu.edu.eg

Received 27 July, 2018 Accepted 18 September, 2018

ABSTRACT

The objective of this study was to estimated gene expression in breast muscles of two broiler strains with used to Myoustatin gene in marketing age (5 weeks of age) under heat stress and compared to control group for 1 hour daily for 7 days in Shalakan farm of Faculty of Agriculture, Ain Shams University, broiler chicks (150 chicks in each strains) of 1 day of age were used in this study. So, the measurements were taken (respiratory rate, rectal temperature and estimated gene expression). The results obtained the gene expression of Myostatin gene in muscle breast was observed highest in Ross strain compared with Indian River strain under heat stress, but no different between strains (Ross and Indian River) in control group. However, respiratory rate of Ross and Indian River under heat at 5 week of age showed that the Indian River strain was high significant at 5 week of age compared to Ross ones. As shown that rectal temperature of Ross and Indian River were a high significant effect of treatment (heat group was higher from control group) and not a significant of strain.The present experiment was conducted to estimated gene expression on meat quality traits of different tropical stress conditions in different broiler strains during 7 days and 5 weeks of age. Introgressions some major genes likes myostatin gene in muscle breast into broiler improve under the heat stress.

Keywords: Indian River, Ross, Myostatin gene, Real-time PCR , Heat stress.

INTRODUCTION

Poultry meat quality is potentially affected by management techniques, weather and rearing conditions, genetics, transportation, and the ability of the birds to respond to the environment, and all the variables that may interact, affecting in the production cycle (**Bertol and Estresse, 2006**). Most of factors influencing poultry meat quality can be controlled during the different production stages, slaughter, and meat processing. Factors include age, gender, nutrition, management, bird density, harvesting method, environmental conditions, handling, etc. (**Mendes et al 2003**)**.** Breeding strategies have recently taken into account meat quality in order to meet consumers needs (**Gaya and Ferraz, 2006**). Different genetic attributes, such as meat quality and carcass yield, were studied by **Garcia et al (2005)**, concluded that some commercial strains had better performance than others, when bird gender is considered. According to **Julian (2006)**, young broilers are very susceptible to variations in environmental temperature, which may lead to critical changes in metabolism due to unbalanced heat exchange. Resistance to heat stress has become an important issue in the last years. Thehigh ambient temperature in tropical climate leads toheat stress to poultry in general and to broilers inparticular. Heat stress results in poor performance ingrowth, feed efficiency and meat yield as well as highermortality. Recent decades have seen significantdevelopments in genetic selection of the meat typefowl, i.e. Broilers **(Havenstein et al 2003a)**. **Ban et al (2013)** mentioned

that Myogenesis process is necessary for muscles proliferation, which controlled by a group of genes such as: MyoD, Myogenin and Myostatin.Myostatin (*MSTN*), as growth and differentiation factor 8 (GDF-8), a member of transforming growth factor β (*TGF-β*) family, is a potent negative regulator of skeletal muscle growth, expressed in the muscle tissues**(Rebbapragada et al 2003 and Kim et al 2006).**

Ye et al (2007) mentioned that myostatin (*MSTN*) has pleiotropic effects on chicken performance. It plays an essential role in the morphogenesis of chicken intestine, brain, liver, and heart (**Sundaresan et al 2008**). Moreover, it is expressed in the myotome and developing skeletal muscles, and acts to regulate the muscle fiber numbers development (**Liuetal 2016**). Myostatin prevents proliferation of myoblast (**Bass et al 1999**) by inhibiting their cell cycle progression from the G1 to the S phase (**Thomas et al 2000**). **Liu et al (2016)** reported that the *MSTN* gene may be useful in molecular breeding in chicken owing to its negative regulatory effect on muscle mass.In chicken, **Kocamis and Killefer** (**2002)** showed that *MSTN* gene expression is increased in skeletal muscles during the second half of embryonic development and decline on hatching day. Furthermore, it lower expression in muscle of 30-day-old (**Liu et al 2016**). They may contribute to the onset of skeletal muscle development in newly hatched chicks, which is dependent of the fast initiation of neonatal metabolism (**Mott and Ivarie, 2002**). The real-time PCR quantification of mRNA expression is an effective method to quantify nucleic acids from biological samples (**Bustin 2000**). Commonly used detection methods for real-time PCR quantification of an amplicon include fluorescent hybridization probes such as TaqMan probes (**Gibson et al 1996**), molecular beacons (**Tyagi and Kramer, 1996**), and DNA-binding agents like SYBR Green I (**Morrison et al 1998**). The SYBR Green I is a fluorogenic dye that emits a powerful fluorescent signal upon binding to double-stranded DNA while exhibiting few fluorescence when in solution (**Morrison et al 1998**).Therefore, the objective of the proposal was to investigate *MSTN* mRNA expression using real-time PCR with SYBR Green I and to determine its association with body weight and carcass traits, using Ross and Indian River broiler chicken.

Spiers et al (2004) observed that rectal temperature can be considered the best. Isolated criterion to judge heat tolerance and it is an important efficiency indicator in the homoeothermic maintenance facing the thermal environment. Rectal temperature can also be used to assess heat stress impacts.

Sheila, (2017) found that the respiratory functions (tidal volume, ventilation rate, and respiratory rate) varied between days, and were strongly influenced by live weight of the broilers ($P < 0.05$).

MATERIALS AND METHODS

Experimental design

The experiment was designed reared 300 chick of two broiler strain (Ross and Indian River) were divided into equal two groups the first group called to heat group, the heat group was exposed to heat stress on different periods of 7 days and 5 weeks on temperature of 39°c for 1 hour/ 7 days. While, the second groups control group was reared ideal environmental conditions. And, estimated gene expression from breast muscle by Real Time PCR.

Measurements and observations

Heat stress measurements

Heat stress test included (respiratory rate, rectal temperature) were measured at 7 days and 5 weeks of age (10 birds each strain into each treatment).The respiratory rate was measured by counting the panting breaths of the birds for 1 min. While, the rectal temperature was obtained by introducing a digital thermometer into the cloaca of each bird until the reading stabilized.

Breast of weight

Randomly selected 10 birds were taken at 5 weeks of age from each strain and each group (treatment and control) then slaughtered, eviscerated and Weight of breast muscle.

RNA extraction and quantitative real-time PCR

RNA extraction was conceded out in Lab at Genetic Department, Faculty of Agriculture, Ain Shams University, while the gene expression analysis by Real Time PCR was fulfilled at the Department of Poultry Production, Faculty of Agriculture, Ain Shams University. Total RNA was isolated for both two broiler strains (Ross and Indian River) at 5 weeks of age collected twelve samples (3birds\ strain\treatment) from the white muscle samples using Trizol reagent (Molecular Research Center, Inc.) according to the manufacturer's instructions.

The first, add 1ml traizol on 100 mg tissues powder then incubate 5 min., add 200 ml chloroform (vortex), incubate 2-15 min at room temperature. Centrifuge 12000xg/15min.Trizol Extraction Cont. (Chloroform is added for phase separation allowing collection of the aqueous phase containing RNA which is precipitated with addition of Isopropyl, RNA precipitate is often invisible before centrifugation but may forms a gel-like pelleton the side and bottom of the tube, Final wash with ethanol). Second, transfer phase add 0.5 ml iso-propanol 5-10 min at room temperature, centrifuge 12000xg /10min. Thirdly, wash pellet with 75% ethanol 1ml centrifuge 7500 xg/5 min air dry.Fourthly, 50 ml DEPC water/10-15 min at 55° -60°C. Five micrograms of total RNA, prepared using Trizol reagent, was reverse transcribed using a mixture of oligo(dT)12–18 and random primers, and Moloney murine leukemia virus reverse transcriptase (M-MLV), reverse transcription (**RT**; Invitrogen), were used to produce cDNA. Two steps quantitative real-time polymerase chain reaction (qPCR) analysis was performed for *Myostatin*gene and the housekeeping gene *β-actin*, which was selected as a reference gene. qPCR was performed using specific primers **(Table 1)** and following SYBR Green PCR Master Mix.

All reactions were performed in triple in a total volume of 25 µL, containing 12.5 µL of RealQ-PCR 2× Master Mix, 0.4 µL of each primer (10 pmol), 1 µLcDNA, and 10.7 µLRNase-free water. A two-step PCR kit was used, with an initial activation step for 15 minutes at 95°C, followed by 35 cycles of denaturation at 95°C for 20 seconds, and annealing and extension at 60°C for 60 seconds. The threshold cycle (Ct) was determined for *Myostatin*and*β-actin* in each sample, the sequence of primers used are listed in **Table (1).** Gene expression levels were calculated using the following equation:Relative copy number =2−ΔCt (ΔCt = $Ct_{Myostatin}$− $Ct_{\beta\text{-}actin}$).

Table 1. The Sequence of primers used in this study

Primer	Sequence 5′→3
Myostatin	5-GAGGTCAGAGTTACAGACACA -3
gene	5-TCATGAGCACCCGCAACGATC-3
â-actin	F- GGAAGTTACTCGCCTCTG
	R-AAGACACTTGTTGGGTTAC

Statistical analysis

Data were subjected to two -way analysis of variance between strains and treatment. Their interaction using the General Linear Models (GLM) procedure of SAS User's Guide, Ver.8.2, 2001. Duncan's multiple range tests were used to test differences among means according to **Duncun (1955)** separate means when separation was relevant.

$$Y_{ijk}= \mu + S_i + T_j+(S^* T)_{ij} +e_{ijk}$$

Where:

µ= Ovellall mean,
S_i=Strain effect,
T_j= Treatment effect,
$(S^* T)_{ij}$ = Interaction between Strain and Treatment
e_{ijk}= Experimental error.

Gene expression analysis

Statistical analysis was performed using SPSS 18 for Windows, Multiple comparisons were assessed with one-way ANOVA.

Statistical significance was defined as *P*, 0.05.

RESULTS AND DISCUSSION

Heat stress measurements

As shown **Table (2)** the rectal temperature were slightly differ among Ross and Indian River under heat at 5 week of age. But, the rectal temperature degree was a high significant for heat treatment compared to control. On the other hand, the respiratory rate of Ross and Indian River under heat at 5 week of age appear that the Indian River strain was high significant at 5 week of age compared to Ross ones. Also, the heat treatment was significant differences compared to control treatment. That rectal temperature can be considered the best Isolated criterion to judge heat tolerance and it is an important efficiency indicator in the homoeothermic maintenance facing the thermal environment. Rectal temperature can also be used to assess heat stress impacts **Spiers et al (2004)**.

On the other hand, **Sheila, (2017)** observed that the respiratory functions (tidal volume, ventilation rate, and respiratory rate) varied between days, and were strongly influenced by live weight of the broilers ($P < 0.05$). Previous results indicate that there are no significant differences in thermal tolerance potential between Ross and Indian River broiler strain.

Table 2. Means ± SE of effect of strains and treatment and their interaction on of heat stress measurement for Ross and Indian Riverstrains

Strains (S)	Treatment(T)		Overall	Prob.		
	Control	Heat		T	S	T*S
Rectal temperature						
Ross	40.56±0.06	41.32±0.12	**40.94**			
Indian River (IR)	40.36±0.12	41.36±0.17	**40.86**	0.0001	NS	NS
Overall	**40.46^{B}**	**41.34^{A}**				
Respiratory rate						
Ross	88.20±2.61	79.80±5.41	**84^{B}**			
Indian River (IR)	87.00±6.36	99.00±9.04	**93^{A}**	0.001	0.001	0.0001
Overall	**87.6^{B}**	**89.4^{A}**				

T=Treatment, S=strain. a, b, c values in the same row for each parameter with different letters are significantly different (P <0.0001). NS, not significant.

Breast muscle weight

The heat stress was significant decrease breast muscle weight **(Table 3)**. On the other hand, Ross broiler was significant heaver breast muscle weight compared to Indian River ones. These results were agreed with **Hristakieva et al (2014). Radwan et al (2018)** had observed that carcass and carcass parts heaver weighted for strain increase myogenin gene expression when compared between Ross and Cobb strains.

Table 3. Means ± SE of effect of strains, treatment and their interaction on of breast weight for Ross and Indian River strains (5 weeks)

Strains (S)	Treatment(T)		Overall	Prob.		
	Control	Heat		T	S	T*S
Weight of breast, gm						
Ross	186.02±15.50	165.60±10.40	**175.81^{A}**			
Indian River (IR)	171.38±14.89	166.86±11.54	**169.12^{B}**	0.001	0.001	0.001
Overall	**178.7^{A}**	**166.23^{B}**				

Gene expression

In the present study, two strains of broiler chicks (Ross and Indian River) were used and effects of heat stress on Myostatin gene expression in breast muscle tissue were evaluated by Real-time PCR technique, as shown in **Fig. (1)**.

There were no significantly differences in Myostatin expression levels between Ross strain and Indian River strain in control, In contrast, the birds under heat stress showed decrease Myostatin expression levels compared with control, whereas Ross strain exhibited significantly higher gene expression ($p < 0.05$) than Indian River strain **(Table 2 and Fig. 1).** Myostatin (*MSTN*) is a regulator of growth and differentiation of skeletal muscle in many species (**Morissette et al 2006**). Moreover**,** *MSTN* was regulated development of muscle fiber **(Liu et al 2016)**.

Liu et al (2016) exhibited that mRNA level of *MSTN* in breast muscle was lower in Wuding chicken than in broilers before 30 day. In contrast, after 30day, themRNA level was higher in breast muscle of Wuding chicken more than in broilers. They suggest that *MSTN* was negative effect of the chicken *MSTN* gene on the regulation of growth performance and carcass traits was observed.

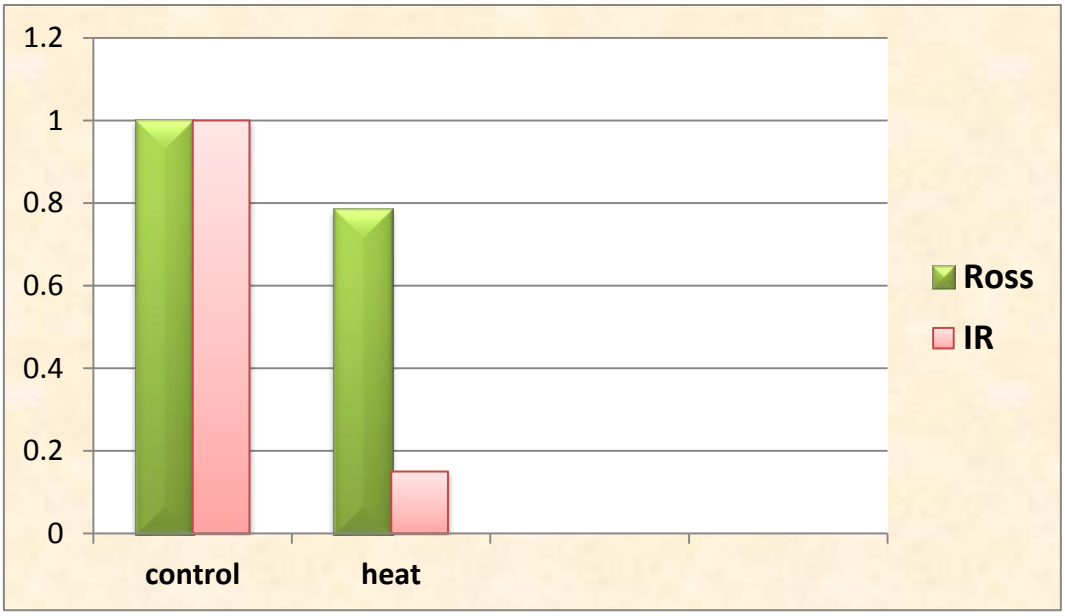

Fig.1. Myostatin gene in breast muscle of Ross and Indian River strain at 5 week of age significantly (*P* ≤0.05). ** p≤0.01.* p≤0.05 and NS, not significant

In humans, a loss-of-function mutation in the myostatin gene in children high muscle size and strength (**Schuelke et al 2004**). In addition, Myostatin is a negative regulator of skeletal muscle growth and a loss of functional myostatin protein increases muscle hypertrophy and hyperplasia in cattle (**Moon et al 2005**). In chickens, the suppression ofmyostatin during embryonic improvement or postnatal growth will be an effective strategy to improve skeletal muscle growth and carcass composition in farm poultry (**Kocamis et al 1999a**).

Several studies have reported that myostatin negatively regulates skeletal muscle growth (**McPherron et al 1979**). Myostatin gene polymorphisms have effects on multiple traits. Mutations in myostatin regulatory regions have been shown to be associated with abdominalfat weight, abdominal fat percentage, birth weight, breast muscle percentage and breast muscle weight in chickens (**Gu et al 2004**). Additionally, **Casas et al (2004)** stated that calves with two copies of the inactive allele of myostatin were more likely to die before weaning, were heavier at birth and leaner, and had more muscle mass than animals with zero or one copy; heterozygous calves had the heaviest bodyweight at weaning and the highest live weight; calves with zero copy were the highest in fat content.

CONCLUSION

Differences in body weight among Ross and Indian River strains under heat stress due to Myostatin gene was high level gene expression for Ross Strain than River for groups subjected to heat stress in breast muscle tissue.

REFERENCE

Ban Q., Liang Y., Zhao Z., Liu and Li Q. 2013. Differential expression levels of genes related to myogenesis during embryogenesis of quail and chicken. **Pak. Vet. J., 33, 317-320.**

Bass J, Oldham J., Sharma M. and Kambadur, R. 1999. Growth factors controlling muscle development**. Domest. Anim. Endocrinol. 17, 191-197.**

Bertol, T.M. and Estressepré-abate, 2006. Conseqüênciaspara a sobrevivênciaCiência **Rural; 36(1), 349-356.**

Blokhuis H.J., 2004. Recent developments in european and internationalda carne emfrangos. **Ciência Rural; 36(1), 349-356.**

Bustin S.A. 2000. Absolute quantification of mRNA using realtime reverse transcription polymerase chain reaction assays. **J. Mol. Endocrinol. 25, 169–193.**

Casas E., Bennett G.L., Smith T.P.L. and Cundiff L.V. 2005. Association of myostatin on early calf mortality, growth, and carcass composition traits in crossbred cattle. **J. Anim. Sci. 82, 2913–2918.**

Castelhano-Barbosa E.C., Gabriel J.E., Alvares L.E. and Monteiro-Vitorello C.B. 2005. Temporal and spatial expression of the myostatin gene during chicken embryo development *growth.* **Dev. Aging 69, 3-12.**

Garcia R.G., Mendes A.A., Costa C., Paz I.C.L.A., Takahashi S.E., Pelícia K.P., Komiyama C.M. and Quinteiro R.R. 2005. Desempenho e qualidade da carne de frangos de cortealimentados com diferentesníveis de sorgo emsubstituiçãoaomilho.ArquivoBrasileiro de MedicinaVeterinária **e Zootecnia 57(5), 634-643.**

Gaya L.G. and Ferraz J.B.S., 2006. Aspectosgenético-quantitativos da qualidadeDesempenho e qualidade da carne de frangos de cortealimentados com diferentesníveis de sorgo emsubstituiçãoaomilho. **ArquivoBrasileiro de Medicina Veterinária e Zootecnia 57(5), 634-643.**

Gibson U.E., Heid C.A. and Williams P.M. 1996. A novel method for real time quantitative RT-PCR. Genome **Res. 6, 995–1001.**

Gu Z., Zhu D., Li N., Li H., Deng X. and Wu C. 2004. The single nucleotide polymorphisms of the chicken myostatin gene are associated with skeletal muscle and adipose growth, Science in China **Ser. C Life Sciences 47, 26–31.**

Havenstein G.B., Ferket P.R. and Qureshi M. A. 2003 a. Growth, livability and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets**. Poultry.Sci., 82, 1500-1508.**

Hristakieva P., Mincheva N., Oblakova M., Lalev M. and Ivanova I. 2014. Effect of genotype on production traits in broiler chickens. **Slovak J. Anim. Sci., 47, 19-24.**

Julian R.J. 2006. Production and growth related disorders and other metabolic diseases of poultry: a review. The Veterinary Journal (in press). **[Accessed Dec. 10, Available from: URL: http:// www.sciencedirect.com.**

Kim Y.S., Bobbili N.K., Paek K.S. and Jin H.J. 2006. Production of a monoclonal anti-myostatin antibody and the effects of in ovo administration of the antibody on posthatch broiler growth and muscle mass. **Poult Sci., 85(6), 1062-1071.**

Kocamis H. and Killefer J., 2002. Myostatin expression and possible functions in animal muscle growth. **Domest. Anim. Endocrinol. 23, 447-454.**

Liu L.X., Dou T.F., Li Q.H., Rong H., Tong H.Q., Xu Z.Q., Huang Y., Gu D.H., Chen X.B., Ge C.R. and Jia J.J. 2016. Myostatin mRNA expression and its association with body weight and carcass traits in Yunnan Wuding chicken Genet. **Mol. Res. 15(4), 60-69.**

McPherron A.C., Lawler, A.M. and Lee S.J. 1997. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. **Nature 387, 83-90.**

Mendes A.A., Rendimento, E. Qualidade D., Carcaça D. Frangos and Corte D. 2003. In: ConferênciaApinco de Ciência e TecnologiaAvícolas; Campinas, São Paulo. **Brasil. pp. 79-99.**

Moon Y.S., Leea A.H.G., Yin Y.H., Jin X., Hong Z.S., Cho J.S., Kim S.C., You S.K., Jin D.I., Han J.Y. and Choi Y.J. 2005. Effect of Maternal Passive Autoimmunization against Myostatin on Growth Performance in Chickens. **Asian-Aust. J. Anim. Sci., 18(7), 1017-1021.**

Morissette M.R., Cook S.A., Foo S. and McKoy G. 2006. Myostatin regulates cardiomyocyte growth through modulation of Akt signaling. **Circ. Res. 99, 15-24.**

Morrison T.B., Weis J.J. and Wittwer C.T. 1998. Quantification of low-copy transcripts by continuous SYBR Green Imonitoring during amplification. **Biotechniques 24, 954–959.**

Radwan L.M., Mahrou M.Y., Alsenosy N.K. and El-Sabry M.I. 2018. Interaction between heat stress and early age on muscle development and related to gene expression in two strains of broilers chickens. Asian J. Anim. Vet. Adv., 2018. ISSN 1683-9919. DOI: 10.3923/ ajava.2018.

Rebbapragada H.A., Wrana J.L., Celeste A.J. and Attisano L. 2003. Myostatin Signals through a Transforming Growth Factor β-Like Signaling Pathway To Block Adipogenesis. **Mol. Cell Biol. 23(20), 7230–7242.**

Schuelke M., Wagner K.R., Stolz L.E., Hübner C., Riebel T., Kömen W., Braun T., Tobin J.F. and Lee S.J. 2004. Myostatin mutation associated with gross muscle hypertrophy in a child, New Engl. **J. Med. 350, 2682–2688.**

Sundaresan N.R., Saxena V.K., Singh R. and Jain P. 2008. Expression profile of myostatin mRNA during the embryonic organogenesis of domestic chicken (*Gallus gallusdomesticus*). **Res. Vet. Sci. 85, 86-91.**

Thomas M., Langley B., Berry C. and Sharma M. 2000. Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. **J. Biol. Chem. 275, 40235-40243.**

Tyagi S. and Kramer F.R. 1996. Molecular beacons: Probes that fluoresce upon hybridization. **Nat. Biotechnol. 14, 303–308.**

Ye X., Brown S.R., Nones K., Coutinho L.L., Dekkers J.C. and Lamont S.J. 2007. Associations of myostatin gene polymorphisms with performance and mortality traits in broiler chickens. **Genet Sel. Environmental 39(1), 73-89.**

مجلة اتحاد الجامعات العربية للعلوم الزراعية ، جامعة عين شمس ، القاهرة ، مصر
مجلد(26)، عدد(2D)، عدد خاص ، 2377-2384، 2019
Website: http://strategy-plan.asu.edu.eg/AUJASCI/

تقدير التعبير الجينى فى صفة جودة اللحم تحت ظروف الاجهاد الحرارى لسلالاتين التسمين [174]

كريمان احمد ناصر[1] – لمياء مصطفى رضوان[1] – محمود يوسف محروس[1] –
نعمه قطب السنوسى[2] – على زين الدين فراج[1]

1- قسم انتاج الدواجن – كلية الزراعه – جامعه عين شمس – ص.ب 68- حدائق شبرا 11241 – القاهرة – مصر
2- قســـم الوراثـــه – كلية الزراعه – جامعه عين شمس – ص.ب 68- حدائق شبرا 11241 – القاهـــرة – مصر

*Corresponding author: KaremanAhmed@agr.asu.edu.eg

Received 27 July, 2018 Accepted 18 September, 2018

الموجـــــــز

الهدف من هذه الدراسة كانت لتقييم التعبير الجينى فى سلالتين تسمين فى عضله الصدر باستخدام جين الميوستاتين عند عمر التسويق (5 اسابيع) تحت الاجهاد الحرارى لمده ساعه/7 ايام فى مزرعه شلقان التابعه لكلية الزراعة جامعه عين شمس. حيث استخدم عدد 300 كتكوت تسمين عمر يوم تم تقسيمهم الى 150 كتكوت في كل معامله. ثم تم اخذ المقاييس الاتيه (مقاييس الاجهاد الحرارى، وزن عضله الصدر، تقدير التعبير الجينى) فى عمر 5 اسابيع. وكانت النتائج المتحصل عليها في التعبير الجينى حيث ظهر ان سلاله الروص كانت اعلى معنويا بالمقارنه بسلاله النهر الهندى تحت الاجهاد الحرارى لكن ظهر اختلاف غير معنوي فى مجموعه الكنترول، وايضا ظهر ان في مقاييس الاجهاد الحرارى ان سلالة النهر الهندى كانت اعلى معنويا فى معدل التنفس ودرجه حرارة المستقيم بالمقارنه بسلاله الروص تحت الاجهاد الحرارى لكن ادى الى اختلاف غير معنوي فى مجموعه الكنترول.

الكلمات الداله: سلاله الروص ،سلالة النهر الهندى، الاجهاد الحرارى، PCR

تحكيم: ا.د أحمد جلال السيد
ا.د مدحت الدينـارى