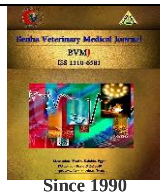
Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/



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Original Paper

Growth performance and some related growth genes expression for some native layer and hybrid broiler chicken breeds

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[2]**ARTICLE INFO**

Keywords

Cobb

Fayoumi

Genetic expression

Mitochondrial genes

Received 09/09/2021
Accepted 21/09/2021
Available On-Line 01/01/2022

ABSTRACT

This research was carried out to evaluate the difference in the mRNA genetic expression of the liver genes as adenine nucleotide translocase (ANT), cytochrome oxidase subunit III (COX III), Insulin like growth factor binding protein 2 (IGFBp2) and Avian uncoupling protein (Av UCP), as well as growth performance include (body weight and body weight gain) between Cobb broilers and Fayoumi native breeds. 120 females of one-day-old Cobb and Fayoumi chicks had been used. Chicks were distributed equally in three replicates. the results showed that, expression of adenine nucleotide translocase (ANT) had non-significant difference between cobb and Fayoumi ($P > 0.05$) but there was significant difference in COX III, IGFBp2 , Av UCP gene expressions and body weight, body weight gain between cobb and Fayoumi breeds.($P<.05$). From the obtained results it could be concluded that, variations in the genetic expression levels of the mitochondrial genes (ANT, COX III, Av UCP) and growth-related gene (IGFBp2) related to differences in growth performance of chickens are good biomarkers for selection and improvement of growth traits in chickens.

1. INTRODUCTION

Growth efficiency is the important trait in broiler meat production chicken which depends on several genes' expression Anh et al. (2015). Progresses in growth rate depend on complicated processes that involve the regulation by nervous, hormonal and genetic pathways (Zhang et al., 2008). However, growth as polygenic trait, can be refined by the marker-assisted selection that is more precise in judging the animal's hereditary importance (Dekkers, 2004). The activity of the insulin-like growth factors (IGFs) is controlled by a family of structurally well-maintained insulin-like growth factor binding proteins (IGF-binding proteins; which bind to IGF-1 and IGF-2 proteins but do not attach to insulin (Russo, 2005). Adenine nucleotide translocase (ANT), uncoupling protein (avUCP) and cytochrome c oxidase III (COX III) are essential proteins implicated in mitochondrial production of energy. In poultry, there is a relationship between the mRNA expression of these genes and growth efficiency (Bottje et al., 2009; Ojano-Dirain et al., 2007 and Del Vesco et al. 2013). ANT is concerned with displacement of ADP from the cytoplasm to the mitochondria also translocation of ATP across the internal mitochondrial layer (Ojano-Dirain et al., 2007). ANT increases the releasing of energy through the activity of ATP synthase. The mitochondrial function may be defaulted by the incapacity of ADP/ATP. It is likely to present some relations between ANT expression and the expression of the growth performance parameters (Bottje et al., 2006). AvUCPs are transporters located within the internal membrane of mitochondria, converting the energy produced to heat, which is processed by protons leakage via the membrane (Ledesma et al., 2002). In poultry, avUCP is involved in lowering the reactive oxygen species production (ROS) by establishing a slight uncoupling in ATP production (Abe et al., 2006). Also, COX III has a function in the electron-transport process. It had role in efficacy of oxidative phosphorylation. COX III is a part of the protein complex IV of the mitochondrion, and it is accountable for proton generation and electron transport. COX III is very relevant for mitochondrial energy performance (Scheffler, 1999). Fayoumi is a unique layer breed with genetic characteristics differs from other broilers (Baloza *et al.* 2014). Cobb chicken categorized as higher growing breed (Antar et al. 2020). Therefore, the present research investigated the link between variation of mitochondrial genes expression and growth rate differences between Fayoumi and Cobb.

2. MATERIAL AND METHODS

2.1. Chickens, Management and housing

This study was carried out in the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University following guidelines of institutional Animal Care and Use Committee Research Ethics Broad (BUFVTM 01-09-21). The 120 one-day-old Cobb broiler and fayoumi native chicks had been used 60 chicks of Cobb and 60 chicks of Fayoumi. They were bought from El-Dakahlia Company. Each group contained three replicates of 20 chicks. The house was clean, disinfected and well-ventilated space with proper environmental temperature. Light program was continued for 24 hr. throughout the experiment. The litter was fresh wood shaving. Feed and water presented all the time. Chicks

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were provided with the basal diet (Table 1) for eight weeks as recommended by NRC (1994)

Table (1): The ingredients and nutrient makeup of the used Basal diet

Ingredients	Starter	Grower	Finisher1	Finisher2
Yellow corn	53.97	57.17	58.66	62.38
Soya bean meal-44	33.40	32.70	31.50	22.40
Corn gluten meal	5.70	2.60	1.80	5.70
Vegetable oil	2.30	3.40	4.40	4.20
Lime stone	1.45	1.35	1.23	2.70
Di calcium phosphate	1.43	1.23	1.00	1.05
L-lysine	0.39	0.29	0.21	0.37
DL-methionine	0.31	0.31	0.29	0.24
Vit&min premix	0.30	0.30	0.30	0.30
Sodium chloride	0.29	0.29	0.29	0.29
Sodium bicarbonate	0.16	0.12	0.13	0.14
L-therionine	0.15	0.10	0.05	0.08
Anticolestrdia	0.05	0.05	0.05	0.05
Antimycotoxin	0.05	0.05	0.05	0.05
Choline chloride	0.05	0.05	0.05	0.05
Energy enzyme	0.02	-	-	-
Phytase enzyme	0.01	0.01	0.01	0.01
Nutrients				
MEn (Kcal/kg)	22.02	20.02	19.06	17.97
Linoliec Acid	3976.22	3027.19	3101.28	3150.54
Crude fat	2.13	2.62	3.04	2.96
Crude fiber	4.98	6.04	7.03	6.99
Lysine Dig	3.47	3.46	3.40	2.87
Methionine Dig	1.22	1.12	1.03	0.97
Methionine+ cysteine	0.62	0.59	0.55	0.51
Dig				
Threonine	0.91	0.85	0.8	0.76
Threonine Dig	0.96	0.85	0.77	0.74
Calcium	0.83	0.73	0.66	0.63
Available phosphorus	0.99	0.90	0.80	1.35
Chloride	0.47	0.43	0.39	0.38
Sodium	0.23	0.23	0.23	0.23
Acid Base Balance (mg/kg)	0.17	0.16	0.16	0.16

2.2. Samples collection, RNA isolation and Real-time PCR for gene expression

Samples of liver, (N = five chicken per group) were gathered, kept in sterile RNase-free tubes and then preserved at -80˚C until use. Extraction of overall RNA was performed by using the manufacturer's procedure with Trizol Reagent (Invitrogen, Korea). Concentration and purity of RNA was tested by Spectro Star Nanodrop (BMG Lab Tec.GmbH, Germany) at 260/280 nm absorbance. Then total RNA (about 2 µg) had been reverted to complementary DNA by using 2X Reverse Transcriptase Master Mix (Applied Bio system, USA) as stated by the manufacturer guidelines. With the support of NCBI Primer-BLAST software, primers had been planned as shown in table 2.

Table (2): Specific oligonucleotide primer sequence used in this study

Primer name	Sequence (5´ - 3´)	Amplicom (bp)
β-actin	F-ACCCCAAAGCCAACAGA R-CCAGAGTCCATCACAATACC	136
Av Ucp	F-GCCCGCAACTCCATCATTA R-TTCATGTACCGCGTCTTCAC	41
COX III	F-AGGATTCTATTTCACAGCCCTACAAG R-AGACGCTGTCAGCGATTGAGA	71
ANT	F-TATCAGCTGGATGATTGCACAGA R-ACATGATATCAGCTCCTTTGCGT	67
IGFBp2	F-CACAACCACGAG-GACTCAAA R-CATTCACCGACATCTTGCAC	299

The primers were designed based on retrieved sequence from GenBank access number for β-actin (L08165), Av Ucp (AB088685), COX III (NP_006921), ANT (AB088686), IGFBp2 (NM_205359.1). the annealing temperature for all primers was 60 °C

The mRNA quantification was performed by using Real time PCR, Applied Biosystem 7500 Fast, USA. SYBER Green Master Mix was used for the quantitative PCR in 20 µL reaction mix (TOPreal ™ qPCR 2X PreMIX). For qPCR the thermal conditions were first pre-incubation at 95 °C for 10 min after that 40 cycles of 95 °C for 15 sec and 60 °C for 1min. The $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001) was used to determine the comparative quantification of gene expression

2.3. Growth parameters

2.3.1. Body Weight (BW)

The chicks were weighed individually (in gram) at day one, and then the life body weight was recorded every week untill 8week (Omar, 2014).

2.3.2. Body weight gain (BWG)

The chicks were weighed individually (in gram) at day one, and then the life body weight was recorded every week until 8week (Omar, 2014).

2.4. Statistical analysis

The SPSS statistical software (version 16; SPSS Inc., Chicago, IL, USA) was used for Data analysis. The results achieved were found by the independent sample T-test study to be mean ± SE. Meaningful significance (P<0.05).

3. RESULTS

The obtained results were showed in Table 3. Females Cobb had significantly increased body weight and significantly increased (P<0.05) body gain than in females Fayoumi native breed.

Table (3): *Average weekly weight and body gain (g) of Cobb chickens and Fayoumi*

Groups	Species	Body weight	Body gain
First week	Cobb	49.0887^{a} ±0.62	85.3256^{a} ±3.66
	Fayoumi	28.05^{b} ±0.03	36.0981^{b} ±0.18
Second week	Cobb	134.41^{a} ± 4.27	231.57^{a} ±2.96
	Fayoumi	64.15^{b} ±0.22	41.0881^{b} ±0.67
Third week	Cobb	365.99^{a} ±7.23	323.98^{a} ±1.85
	Fayoumi	105.24^{b} ±0.86	80.0592^{b} ±1.003
Fourth week	Cobb	689.97^{a} ±8.84	452.59^{a} ±7.8
	Fayoumi	185.29^{b} ±1.86	74.8761^{b} ±0.53
Fifth week	Cobb	1142.6 ^{a} ±4.57	56.6340^{a} ±13.33
	Fayoumi	260.17^{b} ±2.38	42.8709^{b} ±0.98
Sixth week	Cobb	1199.2^{a} ±14.88	213.57^{a} ±24.03
	Fayoumi	303.04^{b} ±1.41	53.31^{b} ±1.5
Seventh week	Cobb	1412.8^{a} ±18.86	575.099^{a} ±28.5
	Fayoumi	356.35^{b} ±2.89	125.61^{b} ±4.27
Eighth week	Cobb	1988.8^{a} ±19.8	260.22^{a} ±7.49
	Fayoumi	481.96^{b} ±7.13	76.6694^{b} ±0.46

Values are means ± standard error. Mean values with different letters within the same row significantly varied P<0.05 and non-significant when P>0.05.

Results of gene expression in liver found a significant higher expression of IGFBp2 and AvUCP in Fayoumi than Cobb; Moreover, a significant decrease in the gene expression of COXIII gene expression in Fayoumi than Cobb. There is no significant change in ANT gene expression in both breeds as showed in table (4)

4. DISCUSSION

Results of the growth performance of the current study showed that the females Cobb had significantly (p<0.05) high body weight and body gain than observed in females Fayoumi native breeds. This is in harmony with that attained by (Kebede, 2017, Jia et al., 2018 and Antar et al., 2020) who found that Cobb breeds had more levels of growth hormones and IGF-1 gene expression more than Fayoumi breeds.

Table (3): *Average weekly weight and body gain (g) of Cobb chickens and Fayoumi*

Gene	groups	N	0 week	2 week	4 weeks	6 weeks	8 weeks
ANT	Cobb	5	2.0420^{a} ±0.19	2.23^{a} ±.14	3.33^{a} ±0.18	1.24^{a} ±0.14	1.24^{a} ±0.14
	Fayomi	5	1.7460^{a} ±0.08	2.66^{a} ±.20	2.8^{a} ±0.17	1.17^{a} ±.06	1.17^{a} ±0.06
COX III	Cobb	5	2.2920^{a} ±0.19	15.17^{a} ±.49	31.03^{a} ±1.43	17.01^{a} ±.98	15.01^{a} ±0.98
	Fayomi	5	0.38^{b} ±0.03	1.25^{b} ±.220	1.15^{b} ±0.13	8.36^{b} ±0.46	6.36^{b} ±0.46
IGFBP2	Cobb	5	0.42^{b} ±0.05	1.69^{a} ±.26	.098^{b} ±0.03	0.17^{b} ±.01	0.17^{b} ±0.01
	Fayomi	5	3.4^{a} ±0.3	.17^{b} ±.064	1.5^{a} ±0.2	0.998^{a} ±0.2	0.99^{a} ±0.21
Av UCP	Cobb	5	0.9420^{a} ±0.123	2.45^{b}.13	1.56^{b} ±0.12	1.2^{b} ±0.19	1.2^{b} ±0.19
	Fayomi	5	0.5200^{b} ±0.098	.41019.93^{a}±	16.81^{a} ±0.93	27.16^{a} ±0.56	27.16^{a} ±0.56

AvUCP: Avian uncoupling protein, COX III: cytochrome oxidase subunit III, ANT: adenine nucleotide translocase, IGFBp2: Insulin like growth factor binding protein 2. Values are means ± standard error. Mean values with different letters within the same row significantly varied P<0.05 and non-significant when P>0.05.

Also Cobb chicken had higher insulin level than Fayoumi chicken throughout the life as insulin had anabolic effect so high level of insulin increase growth rate. Insulin plays important role in regulating metabolism, while it increases growth by improving protein synthesis and controlling many growth-related genes (Taniguchi et al., 2006). The Avian broilers had highly increased feed intake. the little weight gain found for Fayoumi chick from hatch to eight weeks was equal to 48.71 g, while the broilers showed the highest BW gains and had more growth rate (daily BW gain) (Jia et al. 2018). The current study showed that there is non-significant change between Cobb and Fayoumi native breeds (P>0.05) in the expression of Avian ANT gene. ANT function is to increase the amount of ADP then transformed into ATP by ATP synthase enzyme. According to Ojano-Dirain et al. (2007), broilers had a change in ANT expression when existence of worse in feed conversion ratio as a function of their lower efficiency in energy manufacture. There was a significant decrease in the gene expressions of COXIII in Fayoumi native breeds than Cobb p<0.05. COX III is an important for ATP generation efficiency (Scheffler, 1999). The lower level of this gene may be due to extreme damage caused by the release of ROS (Kemp et al., 2003). Ojano-Dirain et al. (2007) observed that low down feed efficiency accompanied with high ROS release and protein oxidation in poultry. Moreover, this study recorded a significant upregulation of AvUCP gene expression in Fayoumi than Cobb breed which are in agreement with Evock-Clover et al., 2002 and Toyomizu et al., 2006 who found that AvUCP is activated during feed deprivation which is thought to has a function in thermogenesis, controlling the production of reactive oxygen species (ROS) (Criscuolo et al., 2005). This current study showed a significant increase in IGFBP2 gene expression in Fayoumi native breeds than Cobb p<0.05, this results are consistent with those obtained by Mao et al (1998) who found that various broilers had alteration in hepatic GH binding activities, but similar unchanging levels of GH receptor (GHR) mRNA. Because GH levels were intensely related with GHBP levels reversely, the higher plasma GH levels in Cobb chickens (Antar et al., 2020) might be due to their lower plasma GHBP concentration. Although unclear biological function of GHBP still remains, it can inhibit the activity of GH pathway (Frystyk, 2008).

5. CONCLUSION

The obtained results in this study intensify the concept that; the selection for growth performance and body weight is related to modifications in the expression of the mitochondrial genes (ANT, COXIII, Av UCP) and growth-related gene (IGFBp2). Therefore, those genes (ANT, COXIII, UCP and IGFBp2) may be considered good genetic markers that might be used in selection program for the improvement of growth trait in chickens.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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