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Comparative Expression Analysis of Ghrelin Hormone in Five Meat-Genotype Broiler Chickens

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Abstract

The present investigation aims to study the Ghrelin gene (GHRL) expression patterns of meat-type hybrids, Aviagen, Arbor Acres, Hubbard, Cobb and Ross at 21 and 37 days of age. There was no association between the level of expression and either phenotypic or carcass parameters for all genotypes at 21 days. The highest transcript abundance of Gherlin mRNA for Ross genotype had a significant negative association with weight gain (WG 3-5 wks), growth efficiency (GE 0-5 and 3-5 wks), specific growth rate (SGR 0-37 and 21-37 day), carcass weight (CW), shank length (SL) and wing weight (WW). Aviagen hybrid, the lowest gene expression of all, had high significant positive association for both abdominal fat and abdominal fat % at slaughter age. Arbor Acre hybrid had significant ($p \leq 0.05$) negative association with fat % of breast muscle meat by. The drumstick muscle fat % of Hubbard hybrid chickens was negatively regressed on the Gherlin mRNA expression. These findings suggested that the differences in transcript level and response among resembles of meat type hybrids might be the result of a different genetic origin for them and further investigation is recommended for Aviagen and Ross hybrids.

Keywords: Gene expression; Ghrelin gene; Growth performance; Meat type chickens

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Abbreviations: GHRL: Ghrelin Gene; GHSR: Secretagogue Receptor; GE: Growth efficiency; RC: Regression coefficient; SGR: Specific Growth Rate

Introduction

Ghrelin first discovery in mammalian species and identified in rat stomach [1]. The proventriculus Ghrelin peptide was isolated for the first time from chicken and 26 amino acids were found compared to 28 in mammals with 54% of similarities to mammal's Ghrelin [2]. Ghrelin has recently been distinguished in birds like Chickens, Ducks, Japanese quail, Emu, Goose, Turkey [3]. Ghrelin is predominantly a product of gastric tissue but its expression has been detected in many tissues including brain, hypothalamus, intestine, cells of the immune system, thyroid gland, kidney, lung, pancreas, pituitary and placenta [4,5]. Chicken Ghrelin gene is primarily present in the proventriculus but absent in the gizzard [6]. Literatures have showed significant functions for Ghrelin as a member of G-protein-coupled receptor family. Main function difference in Ghrelin between birds and mammals reflects its role in feed intake regulation. Several reports showed that the injections of acylated Ghrelin peptide increase plasma corticosterone levels [2,7,8]. The Ghrelin neural network in the paraventricular nucleus (PVN) is complex because of Ghrelin neurons send efferent fibers onto neuropeptide Y (NPY) neurons. As a result, gamma-Amino butyric acid (GABA) release is suppressed, corticotrophin-releasing hormone (CRH)-expressing neurons is stimulated and finally, adrenocorticotrophic hormone (ACTH) and cortisol are released [9]. The acylated Ghrelin peptide injections elevate plasma corticosterone levels [2,7] and suppress feeding behavior in chickens [2,7,10-12]. This finding confirms earlier evidence suggesting that Ghrelin is an anorixegenic hormone, decreases appetite in avian species in chickens [8,10,11,13,14]. Ghrelin was reviewed as an orexigenic in all birds [15,16]. One of the pathways for the anorexigenic effect of high ambient temperature in laying hens might be mediated by its effects on the hypothalamic and gastrointestinal Ghrelin signals [17]. However, Ghrelin may be anti-lipogenic

in birds [11,18] and it significantly increases metabolic rate, lipolysis and weight loss in chickens [19]. Acylated Ghrelin concentrations of garden warblers (Sylvia borin) with larger fat stores were higher than those of birds without fat stores, Further, injections of unacylated Ghrelin decreased food intake and increased migratory restlessness of wild birds [20]. Different dietary energy and protein levels have no effect on Ghrelin gene mRNA expression for broiler chicken [21]. Passive immunization against Ghrelin in turkeys was associated with a significant increase in feed consumption [22]. It is suggested that the loss of anorexigenic Ghrelin is a predatory adaptation that results in increased food-seeking behavior and feeding in falcons [23]. In mammals however, Ghrelin is associated with a positive energy balance and lipogenesis. In addition, high doses of Ghrelin in humans increase ACTH, prolactin, and cortisol levels [24], which may explain the amenorrhea and behavioural changes observed in Anorexia nervosa (AN) patients. The purpose of this study is to figure out and explore the Ghrelin gene expression as a candidate gene responsible for both growth performance and fatness, and compare how its association with both growth traits and fatness among five meat-type hybrids.

Materials and Methods

Experimental birds, diets and tissue sampling

This experiment was conducted at the Poultry Research Station, Faculty of Agriculture, Cairo University, Egypt. In this study, five broiler genotypes including Aviagen, Arbor Acre, Hubbard, Cobb and Ross were used. A total of 5000 broiler chicks (1000 per genotype) were tested. They were fed ad-libitum on the standard starter diets for 14 days and then received standard growing diet up to 37 days of age. Birds were allocated in equal numbers in individual cages and daily exposed to 16 h light and 8h dark cycle under the same environmental conditions with access to feed and fresh tap water all over the day during the experimental period. The body weights (BW) were repeatedly recorded until the age of 37 days of age. Total of 50 birds (6 individuals × 5 genotypes) were slaughtered by cervical dislocation at 37 days of age and (4 individuals × 5 genotypes) for 21 days of age. Proventriculus was aseptically removed and placed in RNA later and kept at -80°C until analysis. Carcass traits were measured for the same slaughtered chickens at 37 days of age, which including carcass weight (CW/g), fore half (FH), major and small pectoral muscle weight (MMP/g) and (SMP/g), thigh muscle weight (TMW/g), drumstick muscle weight (DMW/g), shank length (SL/cm), head weight (HW/g), neck weight (NW/g), edible parts weights/g (heart, liver, spleen and gizzard), abdominal fat (AF/g) and wing weight (WW/g). Relative measures according to the carcass weight were also recorded. Growth efficiency and specific growth rate were calculated [25]. Feed conversion ratio (FCR) was obtained by dividing the total feed intake of each bird by the total grams of live body weight of bird at slaughter age.

Gene expression analyses

RNA extraction and reverse transcription-PCR assay for Ghrelin gene expression: Total of 0.5 g from chicken proventriculus tissues was removed after slaughter and placed in RNA later solution and kept at -80°C until the time of analysis. Total mRNA was isolated by using Qiazol (Rneasy tissue mini kit) reagent procedure, Qiagen according to the manufacturer's instructions (Qiagen, Germany). The quantity and integrity of isolated RNA were determined for each sample by using using NanoDropTM 2000 Spectrophotometer-Thermo Scientific Inc (Wilmington, Delware- USA). Then RNA samples were stored at -80°C until use. Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed by using a HIGH CAPACITY cDNA reverse transcription kit containing RNA (1 µg) and 20 pmol gene-specific primer, 9700 GeneAmp PCR-Applied Biosystems (California, USA). The mixture was incubated at 25°C for 10 minutes for enzyme activation, 37°C for 120 minutes, 85°C for deactivation for the enzyme, and then stored at -20°C. A chicken Ghrelin fragment (203 bp) was amplified with a sense primer (′5-CCT TGG GAC AGA AAC TGC TC-3′) and an anti-sense primer (′5-CAC CAA TTT CAA AAG GAA CG-3′) [16]. Chicken ribosomal 18S RNA was chosen as a reference gene. (Fragment size: 148 bp): Sense primer (′5-CGC GTG CAT TTA TCA GAC CA-3′) and an anti-sense primer (′5-ACC CGT GGT CAC CAT GGT A-3′), (Primer- Invetrogen, USA).

Real-time PCR testing on mRNA level in proventriculus: Ghrelin mRNA quantitation in proventriculus tissue by real-time RT-PCR using a master mix containing SYBRTM Green PCR Master Mix-Life Technologies (California, US). Ten pmol forward primer, 10 pmol reverse primer, cDNA, water was prepared to perform real-time PCR. The following PCR protocol was used on the 500 Real-Time PCR System-Applied Biosystems TM (California USA). Initial steps contain 2 minutes at 50°C and 10 minutes at 95°C, followed by two-step amplification program (15 seconds at 95°C followed by 1 minute at 61°C) repeated 45 times. Runs were performed in three technical replicates per sample.

Statistical analysis: Expression levels of mRNA were measured as cycle threshold values for each gene relative to its cycle threshold values for ribosomal 18S RNA (housekeeping gene). The relative quantification was calculated with the following equations: ΔCt=CtGhrelin-Ct18S; After all the ΔCt values were obtained for all biological and technical replicates, the mean ΔCt values for each genotype five weeks was compared to the mean ΔCt for the three weeks as a calibrator using the 2-ΔΔCT method. Thus, all the five genotypes data five weeks for Ghrelin is expressed as the fold-difference relative to calibrator three weeks. The amount of target molecules relative to the calibrator was calculated by 2-ΔΔCT method. Data was analyzed using SAS (2004). The model included genotype and age as main fixed effects; the individual bird was the experimental unit for gene expression analysis. Gene expression-trait association analysis was performed by SAS GLM procedure.

Gene expression-phenotype association analysis and contrast were performed by SAS GLM procedure. The genetic effects were analyzed by fixed procedure according to the following model: Y=µ+G+A+e, where Y=an observation on the trait, µ=the overall population mean, G= the fixed effect of genotype, A=the fixed effect of age and e=the residual random error. The significant associations were calculated using simple linear regression as the following model: $Y=b_0+b_1X+e$ where Y=the dependent phenotypic variable, X=the independent target gene expression variable deviated from its housekeeping gene, b_0=the intercept and b_1=the association of gene effect and e=the residual random

error. Clustering procedures used to calculate nearest neighbour hierarchical method by computer program SAS 9.1.

Results and Discussion

Expression of Ghrelin mRNA level in chicken proventriculus by real-time quantitative PCR

Least squares analysis of variance

Three weeks of age: RT-PCR was performed to detect the Ghrelin gene expression patterns between individuals from five meat chickens genotypes, Aviagen, Arbor Acre, Hubbard, Cobb and Ross. As presented in **Table 1**, expression levels among genotypes showed that Ross genotype recorded the lowest ΔCT mean (12.22) (highest expression) of all. Least squares means of ΔCT for other genotypes, Aviagen, Arbore Acres, Hubbard and Cobb are significantly the same.

Five weeks of age: RT-PCR for Gherlin mRNA assay showed that as presented in **Table 2** both Aviagen and Arbore Acres showed significantly the highest least squares means of ΔCT expression among genotypes (lowest expression), (17.71 and 17.27, respectively). Ross genotype recorded the lowest ΔCT mean (14.87), (highest expression) of all. Meanwhile, both Hubbard and Cobb recorded intermediate significant different scores of all.

Linear contrasts in two-way analysis of variance

At 3-weeks of age: non-significance pair's differences between Aviagen, Arbor Acres, Hubbard and Cobb were shown as revealed by linear contrasts coefficients **(Table 1)**. Only Ross genotype had significant pair linear coefficient difference with others. Over the rest contrast, only Hubbard genotype recorded highest ΔCT least squares means over the rest (+ 2.98) and become significantly superior over the rest (p 0.0424). Ross genotype recorded lowest ΔCT least squares means than the rest (-5.308) and become significantly lower than the rest (p 0.0004).

At 5-weeks of age: Ross, the lowest significant ΔCT mean of all genotypes seemed to be not significant from Hubbard genotype (p=0.2525) although they are significantly separated by analysis of variance. Also contrast showed a non- significance difference between both Arbor Acres and Cobb (p=0.1424), although least square means by analysis of variance observe a significant difference as shown in **Table 2**. The same relationship was observed between both Aviagen and Cobb. Contrast analysis confirms the non-significance difference between Aviagen and Arbor Acres. Contrast over the rest revealed that Aviagen and Arbore Acres genotypes recorded highest ΔCT least squares means over the rest (+1.043 and +1.593), respectively and become significantly superior over the rest (p 0.0423 and 0.0034), respectively. Hubbard and Ross genotype recorded lowest ΔCT least squares means against the rest (-1.047 and -1.965), respectively, and become significantly retarded than the rest (p 0.0415 and 0.0002), respectively. Cobb genotype, the unique that represent non- significant ΔCT mean (0.376) over the rest. As reported by the authors [26,27] contrasts observe comparisons among groups and show difference between specific pairs of groups obviously.

Genotypes allocation to clusters and cluster distance

Three weeks: Phylogenetic tree for five independent genotype populations by the nearest neighbour method shows three distinct clusters. The first one aggregates the, Aviagen and

Table 1 Least squares mean of Gherlin Δ_{CT} ± standard errors for different genotypes pairs and genotype contrasts versus the rest (linear function ± SE) at 3-weeks of age.

n=Number of missed replicate; ΔCT1=It represent significance within column with different super alphabetic.

Versus the rest				p-Value	F-Value	Contrast SS	Estimate	Versus	ΔCT1	Genotype {4(3rep-n)}
p-Value	F-Value	Contrast SS	Estimate							
0.9057	0.01	0.2365568	0.170 ± 1.43	0.811	0.06	0.9636581	-0.428 ± 1.784	Arbor Acre	17.03 ± 1.291	Aviagen (11)
				0.2229	1.52	25.4190466	-2.254 ± 1.826	Hubb		
				0.5713	0.32	5.4181214	-1.017 ± 1.78	Cobb		
				0.0177	6.04	100.6648373	4.383 ± 1.784	Ross		
0.6117	0.26	4.3542556	0.706 ± 1.38	0.811	0.06	0.9636581	-0.428 ± 1.784	Aviagen	16.60 ± 1.291	Arbor Acre (10)
				0.3113	1.05	17.4616573	-1.825 ± 1.784	Hubb		
				0.737	0.11	1.9023723	-0.588 ± 1.741	Cobb		
				0.0081	7.64	127.3932134	4.812 ± 1.741	Ross		
0.0424	4.35	72.470515	2.98 ± 1.43	0.2229	1.52	25.4190466	-2.254 ± 1.826	Aviagen	17.62 ± 1.231	Hubb (11)
				0.3113	1.05	17.4616573	1.825 ± 1.784	Arbor Acre		
				0.4912	0.48	8.0241318	1.237 ± 1.784	Cobb		
				0.0005	13.85	230.8443412	6.638 ± 1.784	Ross		
0.3024	1.09	18.1192568	1.442 ± 1.38	0.5713	0.32	5.4181214	-1.017 ± 1.784	Aviagen	18.86 ± 1.291	Cobb (11)
				0.737	0.11	1.9023723	0.588 ± 1.741	Arbor Acre		
				0.4912	0.48	8.0241318	-1.237 ± 1.784	Hubb		
				0.0032	9.62	160.4307311	5.400 ± 1.741	Ross		
0.0004	14.73	245.5752531	-5.308 ± 1.38	0.0177	6.04	100.6648373	4.383 ± 1.784	Aviagen	12.22 ± 1.231	Ross (11)
				0.0081	7.64	127.3932134	-4.812 ± 1.741	Arbor Acre		
				0.0005	13.85	230.8443412	-6.638 ± 1.784	Hubb		
				0.0032	9.62	160.4307311	-5.400 ± 1.74	Cobb		

Table 2 Least squares mean of Gherlin Δ_{CT} ± standard errors for different genotypes pairs and genotype contrasts versus the rest (linear function ± SE) at 5-weeks of age.

Versus the rest				p-Value	F-Value	Contrast SS	Estimate	Versus	ΔCT1	Genotype {6 (3rep-n)
p-Value	F-Value	Contrast SS	Estimate							
0.0423	4.25	15.5768514	1.043 ± 0.507	0.5051	0.45	1.64019271	-0.440 ± 0.657	Arbor Acre	17.71 ± 0.478	Aviagen (16)
				0.0104	6.88	25.17246141	1.672 ± 0.637	Hubb		
				0.4053	0.7	2.56143161	0.533 ± 0.637	Cobb		
				0.0003	14.25	52.15900068	2.407 ± 0.637	Ross		
0.0034	9.08	33.2348859	1.593 ± 0.507	0.5051	0.45	1.64019271	-0.440 ± 0.657	Aviagen	17.27 ± 0.450	Arbor Acre (18)
				0.0019	10.32	37.79931862	2.112 ± 0.657	Hubb		
				0.1424	2.19	8.0279405068.67	0.973 ± 0.657	Cobb		
				<.0001	18.76	742734	2.847 ± 0.657	Ross		
0.0415	4.29	15.6933955	-1.047 ± 0.507	0.0104	6.88	37.79931862	1.672 ± 0.637	Aviagen	16.74 ± 0.450	Hubb (18)
				0.0019	10.32	25.17246141	2.112 ± 0.657	Arbor Acre		
				0.0778	3.19	11.674311654.86	-1.138 ± 0.637	Cobb		
				0.2525	1.33	159437	0.734 ± 0.637	Ross		
0.4588	0.55	2.0280427	0.376 ± 0.507	0.4053	0.7	2.56143161	0.533 ± 0.637	Aviagen	15.60 ± 0.450	Cobb (18)
				0.1424	2.19	8.0279405	-0.973 ± 0.657	Arbor Acre		
				0.0778	3.19	11.67431165	1.138 ± 0.637	Hubb		
				0.0043	8.63	31.60319083	0.734 ± 0.637	Ross		
0.0002	15.11	55.3075118	-1.965 ± 0.507	0.0003	14.25	52.15900068	2.407 ± 0.637	Aviagen	14.87 ± 450	Ross (18)
				<.0001	18.76	68.67742734	-2.847 ± 657	Arbor Acre		
				0.2525	1.33	4.86159437	-0.734 ± 0.637	Hubb		
				0.0043	8.63	31.60319083	-1.873 ± 0.637	Cobb		

n=Number of missed replicate; ΔCT1=It represent significance within column with different super alphabetic.

Arbore Acres genotypes as in **Figure 1**. Their least square mean are significantly the same **(Table 1)**, they seems to be the most homologous groups. Their inter cluster distance was lowest among all clusters (0.1079). The second cluster compresses Hubbard and Cobb genotypes at 0.147 point of distance. The third one, aggregate the first and the second clusters in a nearest third nest at 0.4364 degree of distance. Finally, Ross is far away from the four genotype by 1.0806 point. Dissimilarity between genotypes reflects the existence of low gene flow between them [28].

Five weeks: The dendogram clustering procedures at five weeks of age splits the genetic divergence between the five genotypes in three distinct clusters as in **Figure 2**. The first cluster grouped Aviagen and Arbor Acre as the nearest homologous group confirming having a significant similar pattern for non-significant ΔCT least squares means as revealed in **Table 2**. Also they both recorded a highest significant ΔCT least squares means over the rest genotypes (+1.043) and (+1.593), respectively. Their inter cluster distance was lowest among all clusters (0.1659). The second one, Hubbard, is much close to the first nest and had joined to it at (0.423) points. Cobb and Ross genotypes had merged in the third cluster. Their ΔCT least squares means are significantly the same **Table 2**. The intra cluster distance was found to be (0.4571) reflects a high variation exists among them [28]. Finally, the three clusters, aggregates the five genotypes at 1.252 point confirming the existence variation between them.

Fold change profile of Ghrelin mRNA level in proventriculus for five weeks over three weeks

For each genotype, the gene expression profile of three weeks of age was used as control (calibrator) for 5 weeks of age and fold change analyses is shown in **Figure 3**. Depending on **Table 3**, the expression of the Cobb Ghrelin mRNA 5-weeks of age had significantly elevated 7 folds than that of three weeks where ΔCT was significantly reduced by age (-3.254). Hubbard genotype Ghrelin mRNA elevated by 2.289 at older age, but not significant by age. Other genotypes their Ghrelin mRNA expression scored non-significant retardation by age, the lowest retardation was for Ross genotype (0.1986).

Association between the mRNA expression and phenotypic traits at 21 days of age

There was no association between the level of Ghrelin mRNA expression and all studied phenotypic growth parameters at 21 days of age for all hybrid genotypes.

Association between the mRNA expression and both growth and carcass traits at slaughter

As listed in **Tables 4 and 5**, there were no significant association between GHRL mRNA expression levels and most of the studied growth and carcass traits for Aviagen, Arbor Acre, Hubbard and Cobb hybrids except the Ross genotype, the significantly had the lowest ΔCT mean of all (highest expression). The endogenous Ghrelin seems to inhibit chicken growth for most of its recorded growth parameter traits **(Table 4)**. The Ross hybrid, the highest gene expression, observed a highly significant ($p \leq 0.01$) negative association between the level of expression and weight gain 21-37 day by -83.952, significant negative ($p \leq 0.05$) slop for growth efficiency 0-37 days (-2.406), growth efficiency 21-37 days (-0.457), specific growth rate 0 - 37 days (-0.011), specific growth rate 21-37 days (-0.026) as in **(Table 3)**. Also as presented

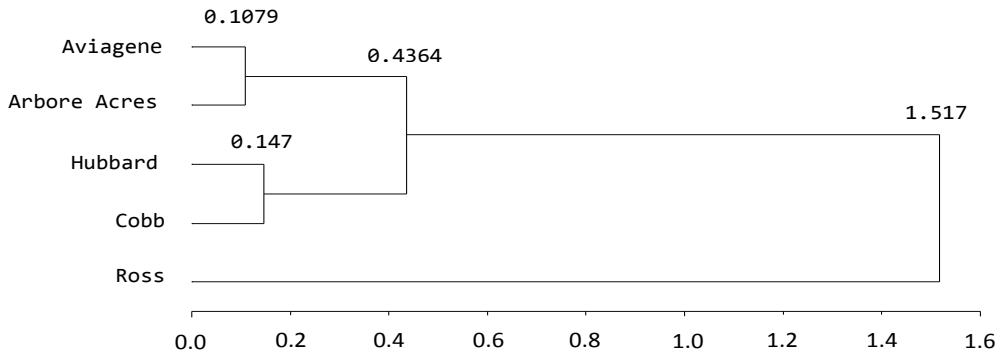

Figure 1 Dendogram trees between five genotypes using nearest neighbor hierarchical cluster method at 3-weeks of age. Nodes numbers indicate the average distance of cluster.

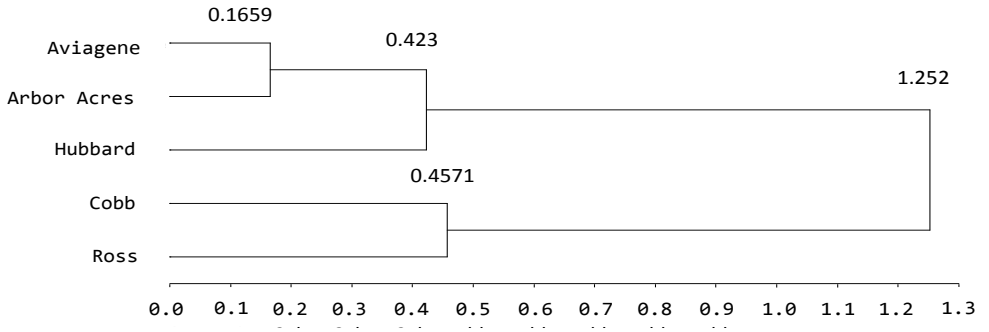

Figure 2 Dendogram trees between five genotypes using nearest neighbor hierarchical cluster method at 5-weeks of age. Nodes numbers indicate the average distance of cluster.

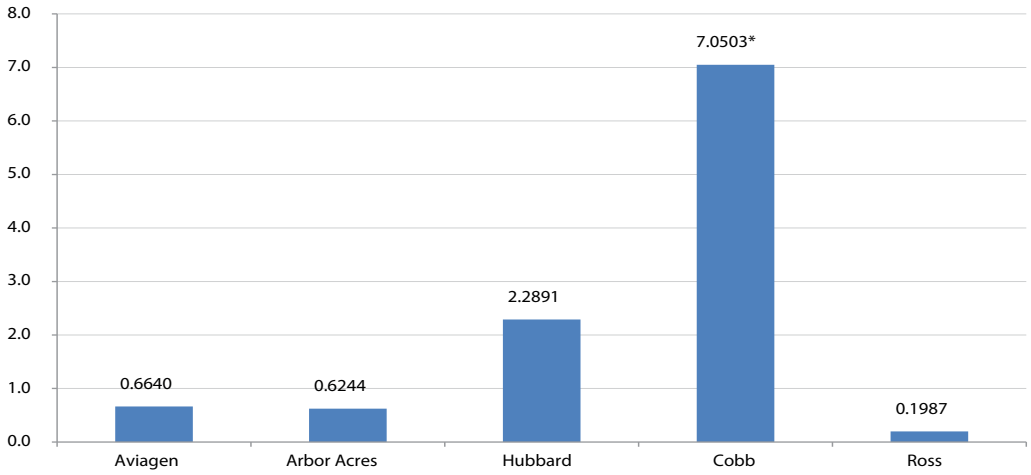

Figure 3 Mean ± STDEV for Quantitative real-time PCR method (qPCR) for the five genotypes.

Table 3 Five weeks contrast versus three weeks for Gherlin mRNA Δ_{CT} (linear function ± SE) for five genotypes and its Fold change expression calculated by $\Delta\Delta C_T$ method.

Genotype	Estimate	ΔΔCT	Average ΔΔCT	Fold Change
		ΔCT 5 wks-ΔCT 3 wks		
Aviagen	0.683 ± 1.134NS	0.591 ± 1.874	0.591 (-1.284 to 2.466)	0.6639507
Arbor Acres	0.672 ± 0.333NS	0.679 ± 1.146	0.679 (-0.467 to 1.825)	0.62444169
Hubbard	-0.878 ± 1.011NS	-1.194 ± 2.282	-1.194 (-3.476 to 1.087)	2.28908328
Cobb	-3.254 ± 1.328	-2.818 ± 10.638	-2.818(-13.46 to 7.820)	7.05031524
Ross	1.619 ± 1.283 NS	2.332 ± 1.519	2.332(-0.187 to 4.850)	0.19866653

Table 4 Regression coefficient (RC ± SE) differences for gene expression on growth traits between chickens with different genotypes at 37-days of age.

Genotype	Avian	Arbor Acre	Hubbard	Cobb	Ross
BW 14-d	7.72 ± 4.84	-13.60 ± 35.35	-4.940 ± 13.73	13.13 ± 54.85	-2.44 ± 90.32
BW 28-d	123.85 ± 38.40	-23.51 ± 75.56	-29.22 ± 32.37	-61.87 ± 19.08	-29.94 ± 193.28
WG 21-37d	48.32 ± 42.17	-15.68 ± 100.95	21.42 ± 20.48	0.81 ± 119.09	-83.95 ± 60.68
GE 0-37d	0.40 ± 0.56	-1.07 ± 0.50	0.245 ± 0.29	-1.162 ± 1.03	-2.41 ± 0.56
GE 21-37d	0.062 ± 0.04	-0.13 ± 0.16	0.07 ± 0.06	0.11 ± 0.13	-0.46 ± 0.13
SGR 0-37d	0.002 ± 0.002	-0.005 ± 0.001	0.002 ± 0.001	-0.007 ± 0.004	-0.011 ± 0.00
SGR 21-37d	0.004 ± 0.002	-0.009 ± 0.01	0.005 ± 0.003	0.000 ± 0.005	-0.026 ± 0.01
FCR	0.018 ± 0.004	0.013 ± 0.011NS	-0.004 ± 0.003NS	-0.006 ± 0.012NS	-0.067 ± 0.022

BW: Body Weight; WG: Weight Gain; GE: Growth Efficiency; SGR: Specific Growth Rate

Table 5 Regression coefficient (RC ± SE) differences for gene expression on carcass traits between chickens with different genotypes at 37-days of age.

Traits	Genotype				
	Avian	Arbor Acre	Hubbard	Cobb	Ross
CW/g	83.57 ± 84.29	-05.14 ± 164.88	47.30 ± 38.34	-114.57 ± 205.80	-603.01 ± 215.6
LW/g	-3.41 ± 8.14	-18.97 ± 15.85	8.71 ± 3.17	-1.04 ± 19.48	-37.07 ± 15.32
SL/cm	-0.07 ± 0.33	-0.678 ± 0.65	0.298 ± 0.21	0.213 ± 0.71	-1.40 ± 0.43
WW/g	1.640 ± 7.42	-13.03 ± 23.66	4.54 ± 3.36	-5.06 ± 28.72	-58.75 ± 14.95
DW/g	-0.73 ± 4.70	-16.92 ± 5.84	1.21 ± 2.93	-12.88 ± 10.96	-22.48 ± 14.46
DW %	-0.004 ± 0.004	-0.01 ± 0.002	-0.001 ± 0.002	-0.01 ± 0.003	0.004 ± 0.01
FH/g	0.049 ± 0.01	-82.57 ± 63.31	-0.002 ± 0.004	-0.048 ± 0.04	-0.023 ± 0.19
SP%	0.001 ± 0.001	0.001 ± 0.00	-0.001 ± 0.00	-0.002 ± 0.0005	0.001 ± 0.002
Heart/g	-0.64 ± 0.54	-2.48 ± 1.20	0.781 ± 0.17	0.121 ± 0.07	-4.73 ± 1.71
Head%	-0.01 ± 0.001	0.000 ± 0.003	0.000 ± 0.002	-0.007 ± 0.02	-0.008 ± 0.01
Neck%	0.005 ± 0.005	0.005 ± 0.006	0.000 ± 0.003	0.006 ± 0.02	0.021 ± 0.005
AF/g	15.01 ± 2.31	-2.92 ± 3.24	-6.99 ± 3.22	-25.41 ± 15.26	-22.15 ± 9.86
AF %	0.012 ± 0.001	0.003 ± 0.01	-0.01 ± 0.003	-0.021 ± 0.01	-0.002 ± 0.01
BF %	0.05 ± 0.09	-0.39 ± 0.11	0.008 ± 0.07	0.087 ± 0.36	-0.044 ± 0.25
DF %	0.83 ± 0.62	-2.65 ± 2.34	-2.01 ± 0.31	1.01 ± 1.45	0.87 ± 0.74

CW: Carcass Weight; LW: Leg Weight; SHL: Shank Length; WW: Wing Weight; DW: Drumstick Weight (g); FH: Fore Half; SP: Small Pectorals; AF: Abdominal Fat; BF: Brest Fat, DF: Drumstick Fat; RC: Regression Coefficient; SE: Standard Error.

in **Table 4**, carcass weight (-603.009), shank length (-1.402) and wing weight (-58.748). This is suggested that Ghrelin plays negative effect on chicken growth for that hybrid. Only for the Arbor Acre hybrid, the Specific Growth rate 0-37 (-0.005) **(Table 3)**, and the drumstick weight traits had a negative significant regression on the Ghrelin expression as expressed by the negative slop of (-16.92) **(Table 5)**. On the contrary, it seems that the lower expression in Aviagen hybrid as in **(Table 4)**, had significant ($p \leq 0.05$) positive association with the weights at early ages 14 days and 28 days by about 7.727 and 123.856, respectively. Genotype with the lowest Ghrelin mRNA was the predominant for chicken growth [29]. Growth performance and carcass traits varied among the different five meat type hybrids according to their different Ghrelin mRNA expression. Similar studies showed significant differences between genotypes of Ghrelin gene on some growth traits of TC and RW chicken breeds [30-32]. The growth and body composition traits are important for livestock production. However, these traits are controlled by poly-genes and according to the major gene model; a few genes may be responsible for a relatively large proportion of the genetic variation. Furthermore, these genes may be linked to some quantitative trait loci that may be associated with growth and body composition traits of animals. Most of the variations in the gene are single nucleotide polymorphisms (SNPs). A single SNP can greatly affect the performance traits. For example, the sex-linked dwarf allele in chickens is a single nucleotide mutation at an exon-intron junction of the GH receptor gene GHR [33]. A QTL for muscle growth in pigs was caused by a nucleotide substitution in intron 3 of the insulin-like growth factor 2 gene [34]. In chickens, a total of 37 SNPs and one 6 bp indel were detected in the full length of the GHSR gene. Some of these SNPs were associated with chicken fatness and muscle fiber traits [35]. Similarly, the presence or absence of specific allele's formation leads to potential correlations between the gene polymorphisms and economic traits in chickens [29]. In chickens and ducks, the several polymorphisms were found in GHRL gene and some of them were scientifically associated with abdominal fat weight, crude protein content of leg muscle and subcutaneous fat thickness [36]. In pigs, the mutation located in the promoter region affected expression of the GHRL gene that leads to a significant ($p \leq 0.05$) different expression level between three genotypes [37]. Perhaps, the existences of SNPs influence on splicing and express of Ghrelin gene and its function in chickens.

Association between the Ghrelin mRNA expression and fatness traits

Interestingly, for fatness, in Aviagen hybrid, the highest ΔCT mean (lowest gene expression) of all **(Table 2),** had high significant

positive association for both abdominal fat and abdominal fat % by about 15.0117 and 0.0118, respectively **(Table 5)** at slaughter age. Meanwhile, Arbor Acre, the lowest ΔCT mean (the highest) expression of the gene in **Table 2** had significant negative association with fat % of breast muscle meat by (-0.395) **(Table 5)**. The drumstick muscle fat % of Hubbard hybrid chickens was negatively regressed on the ΔCT mean GHRL mRNA expression by (-2.013) as in **Table 5**. These findings are consistent with other researches whom concluded that Ghrelin hormone may be anti-lipogenic in birds and with the Ghrelin inhibits fat build-up in birds by decreasing lipogenic enzyme fatty acid synthase (FAS) in the liver, the major site for building up fat stores in birds and significant increase in metabolic rate [11,18,19].

Association between the Ghrelin mRNA expression and feed conversion ratio

There is significant difference among the five hybrids for their feed conversion ratios (FCR) at 37 days of age **(Table 5)**. Although there is neither significant difference among them for live body weight nor total feed consumption per bird up to the same age (Ghaly, M. Unpublished data). It is noticed that the reduction of Ghrelin mRNA expression for Aviagen genotype (highest ΔCT mean) was accompanied by a significant positive regression on FCR ratio (0.018). It is supposed that the shortage presence of the Ghrelin mRNA expression for Aviagen genotype lead to an elevation of muscle growth itself but its genetic potential did not respond enough according to the previous results **(Table 4)**, recording finally higher (inefficient) FCR values. On the contrary, the significant negative regression of FCR ratio (-0.067) **(Table 4)** due to the excess of Ghrelin mRNA expression (lowest ΔCT mean) for Ross genotype may explain the decrease in its feed consumption, followed by a significant decrease in growth parameters. This finding confirms that Ghrelin is an anorixegenic hormone, that decreases appetite in Aviagen species and inhibits feeding behaviour [2,7,8,10-13,38-41] in chickens and as anorexigenic in all birds [15].

Conclusion

Under the experiment conditions, it can be concluded that even the five commercial hybrids that are recognized for only meat-type purpose with a similar growth rate performance, a difference in transcript level of Ghrelin mRNA among them was noticed. Gherlin hormone expression dose not correlated with growth parameters at early ages in broilers. At marketing age, the Ross hybrid had the highest hormone expression and it was associated with inhibition of its growth parameters. The Aviagen hybrid had the lowest hormone expression and this was associated with increase abdominal fat accumulation. The higher Gherlin hormone expression was responsible for decreasing muscles breast fat % for Arbor Acre broilers and muscles drumstick fat % in Hubbard broilers. The different transcription level of Ghrelin mRNA might be the result of different genetic origin of these hybrids, which could be explained by the existence of polymorphisms specific for their ancestor breeds that have not been identified so far. As a result, Ghrelin appears to correspond to growth-related quantitative traits differently, even among meat-type genotypes.

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