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# Expression of Adipocyte Fatty Acid-Binding Protein Gene in Abdominal Adipose Tissue and Its Association with Growth and Fatness Traits in Commercial Meat Type Chickens

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## Abstract

Adipocyte fatty acid-binding protein (A-FABP) gene expression was assessed in abdominal fat tissues of five commercial meat-type chicken hybrids (Aviagen, Arbor Acre, Hubbard, Cobb and Ross) at of 37 days old. Real-time quantitative reverse transcription polymerase-chain reaction was used. The relative A-FABP gene mRNA expression level was calculated with $2^{-\Delta\Delta Ct}$ method using males as calibrators for their target females. The meat type hybrids were diverse in their genetic makeup and response. Aviagen females recorded significant 2.176 more than their males. Hubbard females recorded non-significant 0.6533 fold, same as their males. Arbor acres females are lower than their males by significant 0.1243 fold. Both Cobb and Ross genotypes scored significant 0.3723 and 0.3951, respectively, fold than their males. Hierarchical clustering analysis dendograme method merged Cobb and Ross genotypes to the first closest cluster. Both Arbor Acres and Hubbard had joined into a further cluster. Aviagen genotype was much closer to Arbor Acres and Hubbard nest. Lower abundance of A-FABP gene expression for Arbor Acres was significantly associated with growth and most of carcass parameters retardation. Lower expression of A-FABP gene for Cobb genotype had a unique elevation response for both growth and most of carcass parameters and strong positive association with abdominal fat deposition, especially for males. So, the A-FABP gene could be linked to major gene(s) that influence the abdominal fat content in case of Cobb broilers. FABP4 may provide useful information for further studies on its roles in growth, carcass traits and fatness for both Arbor Acres and Cobb hybrids.

**Keywords:** Gene expression; A-FABP gene; Growth performance; Fat deposition; Meat type chicken

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## Introduction

Excessive adiposity is a problem in modern fast growing broiler industry. Selection in broiler chickens for increased growth rate has resulted in higher body fat deposition, which is considered as a by-product with very low commercial benefit [1]. Although several strategies of selection for leanness in poultry production have been defined, measuring of body fat content, such as abdominal fat (AF), skin fat (SF) and intramuscular fat (IMF), as a major determinant of chicken meat quality, is still difficult because of its tediousness and expensiveness [2,3]. Knowing the molecular mechanisms of growth will add to a more efficient selection process for growth in broiler chickens. Basically, there are two major methods of quantitative trait loci (QTL) determination, the candidate gene approach and the whole-genome scanning [4]. The candidate gene approach is used to detect QTL responsible for genetic variation in the traits of interest. Researchers and producers have paid attention and found several candidate genes or markers for chicken fat traits [5-7].

Fatty acid-binding proteins (FABPs) are a group of carrier-proteins for fatty acids and lipophilic substances like eicosanoids and retinoids [8,9]. These proteins can be divided into two main groups; one was associated with the plasma membrane (F-ABPPM) and the other with the intracellular or cytoplasmic proteins (F-ABPC) [10]. FABPs can reversibly bind to lipids, hydrophobic molecules such as saturated and unsaturated long-chain fatty

acids (FAs) and eicosanoids, with high affinity and selectivity [11]. The overall FABP gene structure is conservative in all family members and consists of four exons separated by three introns [12]. The exon/intron sites are similar, but the length of intron is changeable among the genes [11,13,14]. FABPs have actively facilitated the transport of FAs to the cell for lipid oxidation in the mitochondrion, regulation of lipid-mediated transcription in the nucleus, trafficking, signaling, membrane construction in the endoplasmic reticulum, and regulation of enzyme activity and storage as lipid droplets in the cytoplasm [14]. FABP gene is an important factor that controls intramuscular fat content, which in turn controls meat tenderness and flavor [15]. FABP gene has been shown to be associated with lipid metabolism (lipolysis and lipogenesis), homeostasis in adipocytes, marbling and back fat deposition [9,16]. The FABP4 gene is found to be significantly related to meat tenderness in sheep [17]. The A-FABP was selected as candidate gene for regulating intramuscular fat metabolism in pigs [18] and Carcass weight and marbling [19]. In chicken, the A-FABP gene was used as a marker to identify intramuscular fat acceleration [20]. In cattle, genetic polymorphisms of FABP4 gene were found to be related to meat quality grades [21,22]. In milk, Nafikov et al. [23] showed that some FABP4 haplotypes are correlated with specific fatty acid characters, regardless to differences in milk yield. Also, Liu ZW et al. [24] declared that the overexpression of cattle A-FABP gene in transgenic mice resulted in remarkable increase in TG content. A-FABP promotes the conversion of T4 to T3 in brown adipocytes which increases thermogenesis. In addition, thermo genic stimuli in mice were accompanied by increased levels of A-FABP in both white and brown adipose tissues and the bloodstream [25]. The main aims of this study were to detect the relative expression of A-FABP gene in different commercial meat-type chickens using FQ-RT-PCR and its associations with growth, body composition and fatness traits. It is important to observe the association between transcription levels of the A-FABP and the intramuscular fat (IMF) contents, to provide insights into confirming possible associations and to evaluate the genetic architectures for these genotypes.

# Materials and Methods

## Experimental birds, diets and tissue sampling

This experiment was carried out at Poultry Research Station, Faculty of Agriculture, Cairo University, Egypt. Five broiler genotypes were used in this study and included the following hybrids: Aviagen, Arbor Acre, Hubbard, Cobb and Ross. A total of 5000 chicks, 1000 per each genotype, were used. The chicks were fed a standard starting diet until they reached 14 days old. Then, they received a standard growing diet until 37 days old. Birds were allocated in equal numbers in floor pens and were maintained with a 16-h light and 8-h dark cycle in a temperature-controlled environment with *ad libitum* access to feed and drink. In addition, body weights (BW) of different ages were recorded until the age of 37 days. Ten birds were killed by cervical dislocation at 37 days of age (five for each sex). Carcass traits were measured for the same harvested chickens at the age of 37 days, including carcass weight (CW), breast width (BW), fore half (FHW) and dorsal half (DHW) muscle weight, breast major (MPMW) and small (SPMW) pectoral is muscle weight, thigh muscle weight (TMW), drum muscle weight (DMW), shank length (SL), head weight (HW), neck weight (NW), wing weight (WW), all edible parts (heart, liver, spleen and gizzard) weight, and abdominal fat (AF). Also, body measurements relative to the carcass weight were recorded. Growth efficiency (GE) and specific growth rate (SGR) were calculated according to Gondwe and Wollny [26]. Percentage of total muscular fat (TMF) content was determined according to the international organization for standardization ISO (1973). Both carcass traits and edible part traits were expressed as absolute and percentage of carcass weight at slaughter age (37 days).

# Gene expression analysis

## RNA extraction and reverse transcription-PCR assay for A-FABP gene expression

About 0.5 g tissue from abdominal fat was aseptically removed after slaughter and placed in RNA *later* solution and kept at -80°C until the time of analysis. Total RNA was isolated from five chicken abdominal fat tissues per sex (5♂ + 5♀) per genotype, using Qiagen's RNeasy Lipid Tissue Mini Kit and Qiazol lysis reagent procedure according to the manufacturer's protocol Qiagen, (Germany). The quantity and integrity of isolated RNA were determined for each sample by using NanoDrop™ 2000 Spectrophotometer-Thermo Scientific Inc (Wilmington, Delware- USA). RNA samples were stored at -80°C until use. Reverse Transcription (RT) Polymerase Chain Reaction (PCR) was performed using a High Capasity cDNA reverse transcription kit containing RNA (1 µg), 20 pmol gene-specific primer, 9700 GeneAmp PCR-Applied Biosystems (California, USA). The mixture was incubated at 25°C for 10 min for enzyme activation, 37°C for 120 min, 85°C to deactivate the enzyme, and then stored at -20°C. A chicken A-FABP fragment (138 bp) was amplified with a sense primer (5'-AAGACTGCTACCTGGCCTGA-3') and an anti-sense primer (5'-TCCCTTCCCCAGACACAATA-3'). The primers were designed according to the sequences of A-FABP gene in *Gallus gallus*, which was used as target gene. Chicken ribosomal 18S RNA was chosen as a reference gene. The fragment size was 148 bp, the sense primer was (5'-CGCGTGCATTTATCAGACCA-3'), and the anti-sense primer was (5'-ACCCGTGGTCACCATGGTA-3'), (Primer- Invetrogen, USA).

## Real-time PCR testing on mRNA level in abdominal fat tissue

A-FABP mRNA gene quantitation from abdominal fat tissue was assessed by real-time RT-PCR using a master mix containing SYBR™ Green PCR Master Mix- Life Technologies (California, US). Ten pmol forward primer, 10 pmol reverse primer, cDNA, and water to perform real-time PCR. The following PCR protocol was used on the 500 Real-Time PCR System-Applied BiosystemsTM (California USA). Initial steps include 2 min at 50°C and 10 min at 95°C, followed by two-step amplification program (15 sec at 95°C followed by 1 min at 61°C) and repeated 45 times. Runs were performed in three technical replicates per sample.

## Statistical analysis

Expression levels of mRNA, as cycle threshold values, for each gene (A-FABP and 18S) were deviated from its cycle threshold

values for ribosomal 18S RNA (housekeeping gene). The relative quantification method was conducted following the equations: $\Delta C_t = C_t^{A\text{-}FABP} - C_t^{18S}$. After all the $\Delta C_t$ values were obtained for all biological and technical replicates, the mean $\Delta C_t$ values for each female genotype were compared to the mean $\Delta C_t$ for its male (calibrator). Thus, all the five genotypes data for the A-FABP gene were expressed as the fold-change relative to male genotype. The amount of target molecules relative to the calibrator males were calculated by $2^{-\Delta\Delta CT}$ method.

Data was analyzed using SAS 9.1. The model included genotype and sex as main fixed effects; the individual bird was the experimental unit for gene expression analysis. 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: $Y=\mu+G+S+e$, where $Y$=an observation on the trait, $\mu$=the overall population mean, $G$=the fixed effect of genotype, $S$=the fixed effect of sex 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 neighbor hierarchical method by computer program SAS 9.1.

# Results

## Expression levels of the fat deposition gene among genotype groups

### Least squares analysis of variance means

Different comparisons between genotypes had been observed in **Tables 1-3**. For combined sexes as presented in **Table 1**, difference in expression level among genotypes showed that Ross genotype recorded the highest $\Delta_{CT}$ mean (16.15) (lowest expression) of all. Least squares means of $\Delta_{CT}$ for Hubbard group recorded the lowest (highest expression) value (14.03) but is not significantly different from Aviagene genotype. Both Cobb and Arbor Acre recorded intermediate values and are significantly the same. As shown in **Table 2**, least squares mean of $\Delta_{CT}$ for Ross males was significantly the highest of all genotypes (15.74) (the lowest expression). Meanwhile, Hubbard males scored significantly the lowest least squares mean at all (13.89) (the highest expression). Least squares means of females at **Table 3.** Revealed that Aviagene females scored the lowest least squares mean at all (14.00), (the highest expression) but it does not significantly differ from Hubbard females. Ross females recorded highest least squares mean of all (16.55), (the lowest expression) but not significantly differed from both Cobb and Hubbard females.

### Linear contrasts in two-way analysis of variance

Contrasts facilitate comparisons among groups widely and observe difference between specific pairs of groups [27-29]. In general for both sexes, only Aviagene genotype recorded lowest $\Delta_{CT}$ least squares means over the rest (+ 0.044) and become not significantly superior over the rest ($p$ 0.8428). In the same time,

**Table 1** Least squares means of ΔCT ± standard errors for different genotypes (both sexes) and linear pair genotype contrasts and contrast versus the rest (linear function ± SE).

| Versus the rest | | | | p-Value | F-Value | Contrast SS | Estimate | Versus | ΔCT1 | Genotype{5*(3rep-n)} |
|---|---|---|---|---|---|---|---|---|---|---|
| p-Value | F-Value | Contrast SS | Estimate | | | | | | | |
| 0.8428 | 0.04 | 0.04722718 | 0.044 ± 0.225 | 0.0099 | 6.85 | 8.19146257 | 0.739 ± 0.284 | Arbor Acre | $14.28^{c}$ ± 0.201 | Aviagen-30 |
| | | | | 0.0001 | 15.8 | 18.90277404 | -1.122 ± 0.282 | Hubb | | |
| | | | | 0.1362 | 2.5 | 2.68780879 | -0.423 ± 0.282 | Cobb | | |
| | | | | 0.0007 | 12.15 | 14.53388355 | 0.984 ± 0.282 | Ross | | |
| 0.0001 | 15.51 | 18.5590087 | -0.879 ± 0.225 | 0.0099 | 6.85 | 8.19146257 | 0.738 ± 0.284 | Aviagen | $15.02^{b}$ ± 0.201 | Arbor Acre-30 |
| | | | | <0001 | 43.44 | 51.98129077 | -1.861 ± 0.284 | Hubb | | |
| | | | | <0001 | 16.93 | 20.26374474 | -1.162 ± 0.282 | Cobb | | |
| | | | | 0.3865 | 0.75 | 0.90299399 | 0.245 ± 0.282 | Ross | | |
| <.0001 | 42.03 | 50.2920122 | 1.447 ± 0.228 | 0.0001 | 15.8 | 18.90277404 | -1.122 ± 0.283 | Aviagen | $14.03^{c}$ ± 0.201 | Hub-30 |
| | | | | <0001 | 43.44 | 51.98129077 | 1.861 ± 0.283 | Arbor Acre | | |
| | | | | 0.0145 | 6.13 | 7.33477107 | 0.699 ± 0.282 | Cobb | | |
| | | | | <0001 | 55.65 | 66.58666313 | 2.106 ± 0.282 | Ross | | |
| 0.0113 | 6.6 | 7.89341517 | 0.573 ± 0.228 | 0.1362 | 2.25 | 2.68780879 | -0.423 ± 0.283 | Aviagen | $15.45^{b}$ ± 0.204 | Cobb-29 |
| | | | | <0001 | 16.93 | 20.26374474 | 1.162 ± 0.287 | Arbor Acre | | |
| | | | | 0.0145 | 6.13 | 7.33477107 | -0.699 ± 0.284 | Hubb | | |
| | | | | <0001 | 24.84 | 29.72198034 | 1.407 ± 0.282 | Ross | | |
| <0001 | 28.21 | 33.7620232 | -1.186 ± 0.225 | 0.0007 | 12.15 | 14.53388355 | 0.984 ± 0.284 | Aviagen | $16.15^{a}$ ± 204 | Ross-29 |
| | | | | 0.3865 | 0.75 | 0.90299399 | -0.245 ± 0.284 | Arbor Acre | | |
| | | | | <0001 | 55.65 | 66.58666313 | -2.106 ± 0.284 | Hubb | | |
| | | | | <0001 | 24.84 | 29.72198034 | -1.407 ± 0.282 | Cobb | | |

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

**Table 2** Least squares mea of $\Delta_{CT}$ ± standard errors for different males' genotypes and linear pair genotype contrasts and contrasts versus the rest (linear function ± SE).

| Versus the rest | | | | p-Value | F-Value | Contrast SS | Estimate | Versus | ΔCT | Genotype {5* (3rep-n)} |
|---|---|---|---|---|---|---|---|---|---|---|
| p-Value | F-Value | Contrast SS | Estimate | | | | | | | |
| 0.0207 | 5.6 | 3.48103454 | -0.538 ± 0.227 | 0.3115 | 1.04 | 0.64575489 | -0.293 ± 0.287 | Arbor Acre | $14.56^{bc}$ ± 0.284 | Aviagen-15 |
| | | | | <0001 | 26.22 | 16.2912115 | -1.473 ± 0.287 | Hubb | | |
| | | | | 0.009 | 7.21 | 4.48296292 | -0.773 ± 0.287 | Cobb | | |
| | | | | 0.1842 | 1.8 | 1.11745545 | 0.385 ± 0.287 | Ross | | |
| 0.4528 | 0.57 | 0.35422457 | -0.171 ± 0.227 | 0.3115 | 1.04 | 0.64575489 | -0.293 ± 0.287 | Aviagen | $14.27^{cd}$ ± 0.284 | Arbor Acre-15 |
| | | | | 0.0001 | 16.82 | 10.45001663 | -1.180 ± 0.287 | Hubb | | |
| | | | | 0.1001 | 2.78 | 1.72583948 | -0.479 ± 0.287 | Cobb | | |
| | | | | 0.021 | 5.57 | 3.46215392 | 0.679 ± 0.287 | Ross | | |
| <0001 | 32.82 | 20.395145 | 1.303 ± 0.227 | <0001 | 26.22 | 16.29121159 | -1.473 ± 0.287 | Aviagen | $13.89^{d}$ ± 0.284 | Hubb-15 |
| | | | | 0.0001 | 16.82 | 10.45001663 | 1.180 ± 0.287 | Arbor Acre | | |
| | | | | 0.0175 | 5.93 | 3.6823171 | 0.700 ± 0.287 | Cobb | | |
| | | | | <.0001 | 41.75 | 25.94206101 | 1.859 ± 0.287 | Ross | | |
| 0.0643 | 3.53 | 2.19630481 | 0.427 ± 0.227 | 0.009 | 7.21 | 4.48296292 | -0.773 ± 0.287 | Aviagen | $15.04^{b}$ ± 0.284 | Cobb-15 |
| | | | | 0.1001 | 2.78 | 1.72583948 | 0.479 ± 0.287 | Arbor Acre | | |
| | | | | 0.0175 | 5.93 | 3.6823171 | -0.700 ± 0.287 | Hubb | | |
| | | | | <.0001 | 16.22 | 10.0768059 | 1.159 ± 0.287 | Ross | | |
| <0001 | 20.14 | 12.51158 | -1.021 ± 0.227 | 0.1842 | 1.8 | 1.11745545 | 0.385 ± 0.287 | Aviagen | $15.74^{a}$ ± 0.284 | Ross-15 |
| | | | | 0.021 | 5.57 | 3.46215392 | -0.679 ± 0.287 | Arbor Acre | | |
| | | | | <.0001 | 41.75 | 25.94206101 | -1.859 ± 0.287 | Hubb | | |
| | | | | 0.0001 | 16.22 | 10.0768059 | -1.159 ± 0.287 | Cobb | | |

n: Number of missed replicate; ΔCT[1]: It represent significance within column with different super alphabetic.

**Table 3** Least squares mea of $\Delta_{CT}$ ± standard errors for different females' genotypes and linear pair genotype contrasts and contrast versus the rest (linear function ± SE).

| Versus the rest | | | | p-Value | F-Value | Contrast SS | Estimate | Versus | ΔCT | Genotype {5* (3rep-n)} |
|---|---|---|---|---|---|---|---|---|---|---|
| p-Value | F-Value | Contrast SS | Estimate | | | | | | | |
| 0.1071 | 2.67 | 4.72230866 | 0.627 ± 0.384 | 0.0005 | 13.28 | 23.5338638 | 1.771 ± 0.486 | Arbor Acre | $14.00^{b}$ ± 0.284 | Aviagen-15 |
| | | | | 0.117 | 2.52 | 4.46216758 | -0.771 ± 0.486 | Hubb | | |
| | | | | 0.8803 | 0.02 | 0.04049631 | -0.073± 0.486 | Cobb | | |
| | | | | 0.0017 | 10.6 | 18.78663761 | 1.582 ± 0.486 | Ross | | |
| <.0001 | 17.06 | 30.2201764 | -1.586 ± 0.384 | 0.0005 | 13.28 | 23.53386384 | 1.771 ± 0.486 | Aviagen | $15.77^{a}$ ± 0.284 | Arbor Acre-15 |
| | | | | <.0001 | 27.37 | 48.49110831 | -2.542 ± 0.486 | Hubb | | |
| | | | | 0.0003 | 14.41 | 25.52683008 | -1.844 ± 0.486 | Cobb | | |
| | | | | 0.699 | 0.15 | 0.26709866 | -0.188 ± 0.486 | Ross | | |
| 0.0001 | 17.15 | 30.3938333 | 1.591 ± 0.384 | 0.117 | 2.52 | 4.46216758 | -0.771 ± 0.486 | Aviagen | $14.19^{b}$ ± 0.284 | Hubb-15 |
| | | | | <.0001 | 27.37 | 48.49110831 | 2.542 ± 0.486 | Arbor Acre | | |
| | | | | 0.1555 | 2.06 | 3.6524843 | 0.697 ± 0.486 | Cobb | | |
| | | | | <.0001 | 23.46 | 41.5604545 | 2.354 ± 0.486 | Ross | | |
| 0.0654 | 3.5 | 6.2064308 | 0.719 ± 0.384 | 0.8803 | 0.02 | 0.04049631 | -0.073 ± 0.486 | Aviagen | $15.85^{a}$ ± 0.294 | Cobb-14 |
| | | | | 0.0003 | 14.41 | 25.52683008 | 1.844 ± 0.486 | Arbor Acre | | |
| | | | | 0.1555 | 2.06 | 3.6524843 | -0.697 ± 0.486 | Hubb | | |
| | | | | 0.0011 | 11.61 | 20.57159893 | 1.656 ± 0.486 | Ross | | |
| 0.0008 | 12.36 | 21.9036208 | -1.351 ± 0.384 | 0.0017 | 10.6 | 18.78663761 | 1.582 ± 0.486 | Aviagen | $16.55^{a}$ ± 0.294 | Ross-4 |
| | | | | 0.699 | 0.15 | 0.26709866 | 0.188 ± 0.486 | Arbor Acre | | |
| | | | | <.0001 | 23.46 | 41.5604545 | -2.354 ± 0.486 | Hubb | | |
| | | | | 0.0011 | 11.61 | 20.57159893 | -1.656 ± 0.486 | Cobb | | |

n: Number of missed replicate; ΔCT[1]:It epresents significance within column with different super alphabetic (P<0.05).

Results of linear contrasts given in **Table 2** for males revealed that $\Delta_{CT}$ least squares means for both Arbore Acre and Hubbard genotypes is not significantly superior over the rest. ($p$=0.4528 and $p$=0.0643, respectively). Results of linear contrasts for females given in **Table 3** revealed that $\Delta_{CT}$ least squares means for both Aviagene and Cobb is significantly the same($p$=0.8803). They had $\Delta_{CT}$ mean 0.627 and 0.719, respectively over the rest and both are not significantly superior over the rest. ($p$=0.1071 and

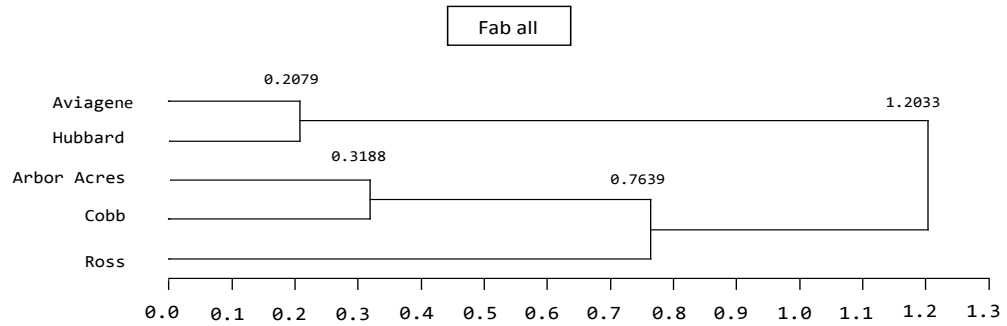


**Figure 1** Dendogram trees between both males and females of five genotypes using nearest neighbor hierarchical cluster method. The number at the nodes indicate the showing the average distance of cluster.

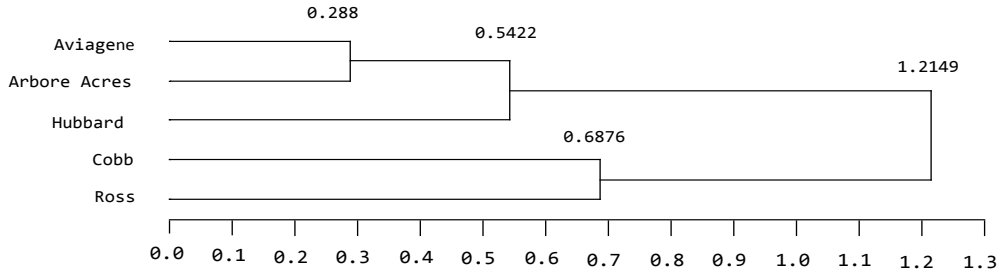


**Figure 2** Dendogram trees between males of five genotypes using nearest neighbor hierarchical cluster method. The number at the nodes indicate the showing the average distance of cluster.

*p*=0.0654, respectively) and their least square mean superiority over the rest was extremely the same (*p*=0.627 and *p*=0.719 respectively). Arbor Acres and Ross had significantly the same $\Delta_{CT}$ but they both had significantly lower $\Delta_{CT}$ from the rest (-1.586 and -1.351 respectively).

**Genotypes allocation to clusters and cluster distance**

***Combined sexes:*** As shown in **Figures 1 and 2,** phylogenetic tree for five independent genotype populations by the nearest neighbor method shows three distinct clusters. The first one aggregates the, Aviagene and Hubbard populations. Their least square means are significantly the same **(Table 1)**, they seems to be the most homologous groups. Their inter cluster distance was lowest among all clusters (0.2079). The second cluster compresses Arbor Acre and Cobb genotypes at 0.3118 point of distance. Similar genotypes possess the existence of high gene flow [30]. The third one, Ross genotype formed its own branch (cluster) to the closest cluster to it, Arbor Acre and Cobb at 0.7639 degree of distance. Finally, the dendogram distances obtained by the nearest-neighbor method for the five genotype populations was (1.2033).

***Phylogenetic tree for the five males:*** The dendogram clustering procedures splits the genetic divergence between the five genotypes males in a three distinct clusters. The first cluster grouped Aviagene and Arbor Acre males as the nearest homologous group confirming having a significant similar pattern for both as revealed in **Table 2**. Also they both recorded a smallest non-significant $\Delta_{CT}$ least squares means estimate (-0.293). Their inter cluster distance was lowest among all clusters (0.288). Hubbard males, the second one, had merged to the nearest one Aviagene and Arbor Acres recording node distance at (0.5422). Cobb and Ross males groups are merged in the third cluster although their $\Delta_{CT}$ least squares means are not significantly the same. The intra cluster distance was found to be (0.6876) reflects a high variation exists and low gene flow among them [30]. Finally, maximum divergence between five male's genotypes was shown by the dendogram clustering at degree of distance (1.2149).

***Phylogenetic tree for the five females:*** As shown in **Figure 3**, three distinct subgroups had been aggregated the five population groups. The Arbor Acre females had joined to Cobb at minimum divergence distance of (0.0136). Aviagen and Hubbard had compressed in the second one in an inter class distance of (0.1242). Ross, the third one, had merged to the nearest one, Arbor Acres and Cobb females at (0.4449) degree of distance. Finally, maximum divergence between five female's genotypes was shown by the dendogram clustering at degree of distance (1.2642).

**Significance of sex difference**

***Analysis of variance and contrasts:*** Aviagene, the unique genotype that their females possess significantly lower $\Delta_{CT}$ least squares means over than males (14.00 vs. 14.56) (higher expression over males). Other genotypes showed significantly higher $\Delta_{CT}$ least squares means for females over males. Hubbard genotype showed the lowest contrast estimate coefficient of females than males of all sex comparisons (0.153). It is the only genotype that their females observe non-significant (*p*=0.4079) superiority over males as shown in **Table 4**.

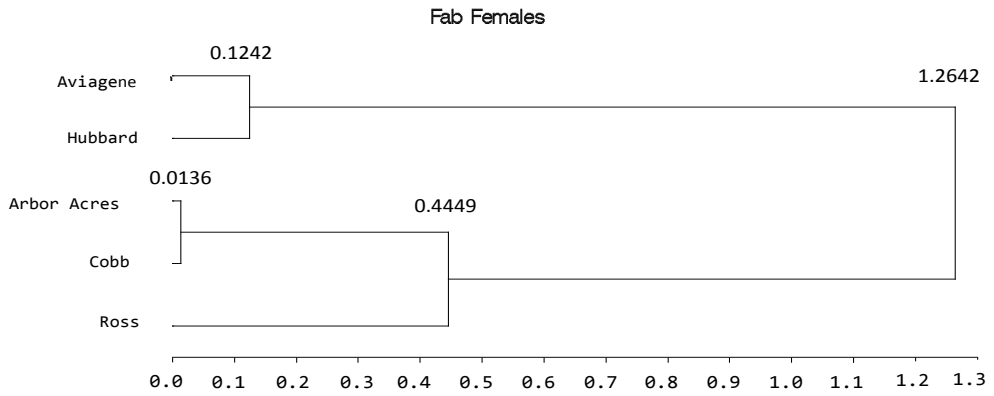


**Figure 3** Dendogram trees between females five genotypes using nearest neighbor hierarchical cluster method. The number at the nodes indicate the showing the average distance of cluster.

**Table 4** Females contrast versus males for AFBP mRNA $\Delta_{CT}$ (linear function ± SE) for five genotypes.

| Females vs. the rest | | | | *p*-Value | *F*-Value | Contrast SS | Estimate | $\Delta_{CT}$ | Genotype |
|---|---|---|---|---|---|---|---|---|---|
| *p*-Value | *F*-Value | Contrast SS | Estimate | | | | | | |
| 0.0493 | 4.22 | 2.3611518 | -0.280 ± 0.1365 | 0.0493 | 4.22 | 2.36115184 | -0.561 ± 0.273 | 14.00 ± 0.193 | Aviagen |
| | | | | | | | | 14.56 ± 0.193 | |
| 0.0291 | 5.29 | 16.95921 | 0.751 ± 0.326 | 0.0291 | 5.29 | 16.9592101 | 1.503 ± 0.653 | 15.77 ± 0.462 | Arbor Acre |
| | | | | | | | | 14.27 ± 0.462 | |
| 0.4079 | 0.71 | 0.70711 | 0.153 ± 0.182 | 0.4079 | 0.71 | 0.70710996 | 0.307 ± 0.365 | 14.19 ± 0.258 | Hubbard |
| | | | | | | | | 13.89 ± 0.258 | |
| 0.0019 | 11.86 | 4.6805514 | 0.401 ± 0.116 | 0.0019 | 11.86 | 4.68055142 | 0.803 ± 0.233 | 15.85 ± 0.167 | Cobb |
| | | | | | | | | 15.04 ± 0.162 | |
| 0.0286 | 5.35 | 4.6457138 | 0.400 ± 0.173 | 0.0286 | 5.35 | 4.64571383 | 0.800 ± 0.346 | 16.55 ± 0.249 | Ross |
| | | | | | | | | 16.74 ± 0.241 | |

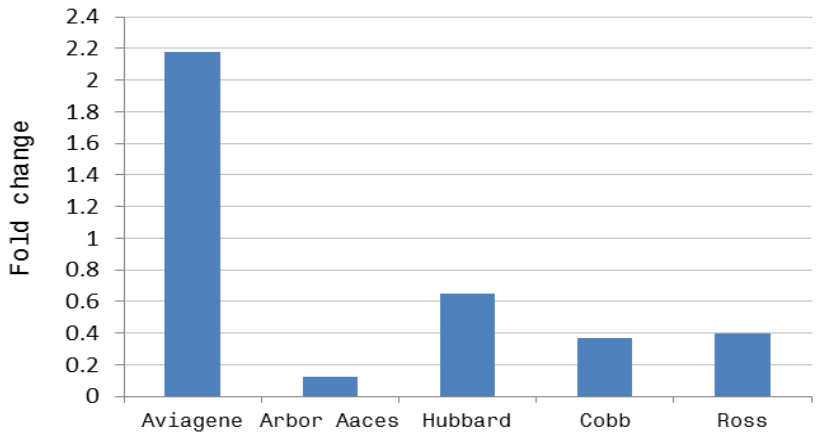


**Figure 4** Mean ± STDEV for Quantitative real-time PCR method (qPCR) for the five genotypes.

**Fold change profile of fab gene for females over males**

For each genotype, the gene expression profile of male genotype was used as the calibrator and fold change analyses is shown in **Figure 4**. Depending on **Table 5**, the expression of the A-FABP, only females of Aviagene genotype that express 2.176 fold more than males. Otherwise, Hubbard population, females possess 0.653 fold as males. Females of Ross, Cobb possess 0.395, 0.374, respectively. Arbor Acres females had the lowest 0.123 fold as their males. Consequently, both Aviagene and Hubbard females are nested in one cluster as shown in **Figure 3**.

**A-FABP and association with genotype, growth performance and carcass parameters**

The A-FABP mRNA levels were significantly associated by genotype is consistent with that obtained by Li et al., (2008). Regarding to

**Table 5** Gherlin mRNA $\Delta_{CT}$ ± STDEV for five genotypes and their Fold change expression calculated by $\Delta\Delta C_T$ method.

| Genotype | ΔΔCT<br>ΔCT Females-ΔCT Males | Average ΔΔCT | Fold Change |
|---|---|---|---|
| Aviagen | -0.5612 ± 3.942 | -1.1222<br>(-4.503 to 3.381) | 2.176751765 |
| Arbor Acres | 1.5037 ± 3.655 | 3.007<br>(-2.152 to 5.159) | 0.124353927 |
| Hubbard | 0.3071 ± 4.126 | 0.614<br>(-3.819 to 4.433) | 0.653334942 |
| Cobb | 0.7125 ± 4.569 | 1.425<br>(-3.857 to 5.282) | 0.372431722 |
| Ross | 0.6697 ± 3.819 | 1.339<br>(-3.149 to 4.489) | 0.395187062 |

**Table 6** Association between A-FABP gene expression and phenotypic traits in both sexes of the commercial meat type chicken at 37-days old.

| Trait | Aviagen | P-Value | Arbor Acre | P-Value | Hubbard | P-Value | Cobb | P-Value | Ross | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| LBW 37-d | 24.16 ± 71.88 | 0.2869 | -83.03 ± 32.07 | 0.1071 | -32.64 ± 58.03 | 0.5913 | 112.84 ± 148.02 | 0.5637 | -61.05 ± 68.96 | 0.4054 |
| FH | -41.52 ± 37.46 | 0.3102 | -35.35 ± 12.35 | 0.0210 | -2.49 ± 18.27 | 0.8954 | 66.94 ± 55.73 | 0.2835 | -5.12 ± 33.48 | 0.8827 |
| FH% | -0.04 ± 0.014 | 0.0241 | 0.001 ± 0.007 | 0.255 | 0.008 ± 0.004 | 0.0571 | 0.04 ± 0.014 | 0.047 | 0.00 ± 0.00 | 0.4587 |
| DH | 8.87 ± 23.93 | 0.7237 | -35.32 ± 11.65 | 0.0163 | -7.98 ± 19.04 | 0.6878 | 13.75 ± 44.92 | 0.7719 | -24.00 ± 23.33 | 0.3377 |
| Bw | -0.04 ± 0.31 | 0.9112 | -0.33 ± 0.11 | 0.0151 | 0.19 ± 0.18 | 0.3269 | 0.58 ± 0.28 | 0.0916 | 0.056 ± 0.34 | 0.8718 |
| Cw | 11.45 ± 52.61 | 0.8349 | -91.53 ± 33.27 | 0.0250 | -26.065 ± 39.94 | 0.5348 | 55.06 ± 101.92 | 0.6122 | -21.23 ± 56.71 | 0.7192 |
| DMW | 3.86 ± 2.49 | 0.1727 | -3.72 ± 1.48 | 0.0363 | -0.45 ± 2.36 | 0.8536 | 2.56 ± 6.15 | 0.6943 | -1.58 ± 2.97 | 0.3714 |
| TMW | -1.41 ± 7.36 | 0.8541 | -7.12 ± 2.31 | 0.0151 | 0.07 ± 4.75 | 0.9875 | 3.14 ± 14.09 | 0.8326 | -4.15 ± 4.34 | 0.6112 |
| MPMW | 0.43 ± 1.74 | 0.812 | -1.97 ± 0.58 | 0.0094 | -1.04 ± 1.04 | 0.4151 | 2.61 ± 2.78 | 0.3893 | -0.22 ± 1.21 | 0.9822 |
| SPMW | -1.83 ± 6.92 | 0.8001 | -8.29 ± 3.33 | 0.0375 | -3.39 ± 3.86 | 0.1879 | 9.14 ± 12.98 | 0.5127 | -0.24 ± 1.53 | 0.8635 |
| Lw% | 0.004 ± 0.003 | 0.2016 | 0.00 ± 0.001 | 0.576 | -0.001 ± 0.003 | 0.7153 | -.008 ± 0.003 | 0.0275 | -0.001 ± 0.002 | 0.5401 |
| SpW% | -0.00 ± 0.00 | 0.6921 | 0.0003 ± 0.0001 | 0.0221 | 0.000 ± 0.000 | 0.5135 | 0.00 ± 0.000 | 0.8812 | -0.000 ± 0.000 | 0.2488 |
| LW | -1.83 ± 4.79 | 0.7161 | -1.52 ± 0.98 | 0.1607 | -0.84 ± 0.75 | 0.0447 | 8.08 ± 3.91 | 0.0936 | -1.27 ± 3.53 | 0.7295 |
| LW% | -0.001 ± 0.003 | 0.6738 | 0.002 ± 0.001 | 0.0381 | -0.000 ± 0.000 | 0.4057 | 0.006 ± 0.004 | 0.2485 | -0.000 ± 0.002 | 0.8240 |
| HW | 1.17 ± 0.41 | 0.0305 | -0.32 ± 0.21 | 0.1569 | -.077 ± 0.41 | 0.1009 | -0.14 ± 0.88 | 0.8830 | -0.098 ± 0.54 | 0.8593 |
| HW% | 0.0009 ± 0.0003 | 0.0139 | 0.00 ± 0.00 | 0.2635 | -0.000 ± 0.000 | 0.1221 | -0.000 ± 0.000 | 0.5258 | 0.000 ± .0002 | 0.824 |
| GW% | 0.00 ± 0.00 | 0.4434 | 0.00 ± 0.00 | 0.0536 | 0.000 ± 0.000 | 0.7114 | -0.002 ± 0.000 | 0.0431 | -0.000 ± 0.001 | 0.9463 |
| HEW | 3.76 ± 2.61 | 0.1995 | 0.002 ± 0.001 | 0.4127 | 0.85 ± 1.69 | 0.6285 | -1.95 ± 4.02 | 0.6476 | -1.74 ± 3.79 | 0.6592 |
| HE&N% | 0.003 ± 0.002 | 0.3744 | 0.004 ± 0.001 | 0.003 | 0.000 ± 0.002 | 0.9141 | 0.001 ± 0.009 | 0.8807 | -0.001 ± 0.003 | 0.6227 |
| AF | -3.84 ± 4.53 | 0.4294 | -2.51 ± 1.19 | 0.0681 | -0.93 ± 3.42 | 0.7938 | 17.07 ± 4.34 | 0.011 | -1.01 ± 2.77 | 0.7265 |
| AF% | -0.004 ± 0.004 | 0.3428 | 0.00 ± 0.001 | 0.86 | -0.000 ± 0.003 | 0.9326 | 0.014 ± 0.003 | 0.0062 | -000 ± 0.002 | 0.6974 |
| BRF% | -0.03 ± 0.04 | 0.4769 | 0.013 ± 0.04 | 0.7149 | 0.05 ± 0.60 | 0.4475 | -0.19 ± 0.10 | 0.1056 | 0.02 ± 0.06 | 0.6784 |
| THF% | -0.18 ± 0.13 | 0.2368 | 0.07 ± 0.08 | 0.3853 | -0.04 ± 0.33 | 0.9108 | -1.72 ± 0.80 | 0.0840 | -0.12 ± 0.24 | 0.6234 |
| DRF% | 2.95 ± 1.38 | 0.0764 | -0.11 ± 0.40 | 0.7980 | -0.08 ± 0.90 | 0.2661 | -0.30 ± 0.64 | 0.7743 | 0.086 ± 0.14 | 0.5651 |

Values represent regression coefficient ±S.E. Values within a column significantly (P<0.05); LBW 37-d:Live Body Weight at 37- days; FH%:Fore Half%; DH:Dorsal Half; BW:Breast Width; CW:Carcass Weight; DMW:Drum Muscle Weight; TMW:Thigh Muscle Weight; MPMW:Major Pectoralis Muscle Weight; SPMW: Small Pectoralis Muscle Weight; LW: Liver Weight; HW: Heart Weight; GW: Gizzard Weight; HEW: Head Weight; HE&N: Head & neck; AF: Abdominal Fat; BRF: Breast Fat; THF: Thigh Fat; DRF:Drum Fat.

other FABP4 $\boldsymbol{\Delta_{CT}}$ genotypes means, higher FABP4 $\boldsymbol{\Delta_{CT}}$ mean (low expression) for Arbor Acres genotype appears to depress the development of growth performance and general carcass traits in Arbor Acre hybrid as given in **Table 6**. FABP4 $\boldsymbol{\Delta_{CT}}$ displayed significant negative association with live body weight of 37-day old (-83.03), fore muscle half weight (-35.35) and dorsal muscle half weight (-35.32), breast width (-0.33) carcass weight (-91.53), thigh muscle weight (-7.12), drum muscle weight (-3.72), major pectoralis muscles weight (-1.97) and small pectoralis muscles weight (-8.29).

At young slaughter age (37days old) and growing ration, Cobb males seemed to be sensitive at low gene expression as presented in **Table 7.** It was denoted that many growth performance and carcass traits were significantly (p<0.05) had positive association higher FABP4 $\boldsymbol{\Delta_{CT}}$ mean (low expression) such as live body weight of 21 days old (166.41), live body weight of 28 days old (397.40), live body weight of 35 days (605.61), weight gain of 0-21 days (162.81), specific growth rate of 0-21 days (0.01), specific growth rate of 0-37 days (0.01), growth efficiency of 0-21 days (3.07), and growth efficiency 0-37 days (15.20). High abdominal fat accumulation may responsible for elevation for live body weight parameters in male Cobb hybrids. The study of Chen et al. observed that the A-FABP transcript levels are increased rapidly with the body weight in pigs until 60-70 kg and lasted at high levels in both breeds studied and this may be due to the elevated

IMF (intramuscular fat content) and high marbling in pigs, which are responsible for increasing body weight, and not due to the muscular growth itself [18]. Emphasizing this hypothesis, Nafikov et al. reported that certain FABP4 haplotypes have an association with particular fatty acid profiles in milk without differences in milk yield in cattle [23].

Ross females showed significant positive association of FABP4 $\Delta_{CT}$ on specific growth rate and growth efficiency at 21-37 days as in **Table 8**. Other fluctuated effects were shown among other genotypes. FABP4 $\Delta_{CT}$ mean for Hubbard genotype observed significant ($p<0.05$) negative association with liver weight (-0.84) **(Table 6)** and negative association with heart weight (-0.93) for males **(Table 7).** Aviagene genotype, both sexes **(Table 6)**, a significant negative correlation (-0.04) of FABP4 $\Delta_{CT}$ mean with half percentage of the fore muscle weight and positive association with the heart weight (1.17) was found. Difficult ability to detect significant associations between FABP4 and quantitative nature of growth parameters for commercial broilers is not only controlled by many genes and environmental factors but also broad variety of populations of different origins and breeding history [31,32].

**Association of A-FABP gene expression with fat accumulation**

Fat deposition in chickens was basically occurred in visceral adipose tissue and muscles, particularly the intramuscular fat content (IMF). The results demonstrated that FABP4 $\Delta_{CT}$ mean displayed positive significant association with abdominal fat (17.07) and abdominal fat percentage (0.014 g) only in Cobb genotype **(Table 6)** and significant positive correlation with abdominal fat in their males (34 g) **(Table 7)**. As previously noticed at **Table 4 and Figure 1**, they had significantly higher mRNA expression than females. Negative association of FABP4 $\Delta_{CT}$ mean with abdominal fat weight (-3.40). **Table 8** was notice for Ross females although they had FABP4 mRNA expression lower than males **(Table 4 and Figure 1)**. As reported by Li et al. [33], The A-FABP gene expression is affected by gender. Only Aviagen females that had significantly

**Table 7** Association between A-FABP gene expression and phenotypic traits in the commercial meat type male chicken at 37-days old.

| Trait | Aviagen | P-Value | Arbor Acre | p-value | Hubbard | p-value | Cobb | p-value | Ross | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| LBW 21-d | -29.74 ± 52.45 | 0.6279 | 36.70 ± 33.06 | 0.3479 | -21.90 ± 39.97 | 0.6218 | 166.41 ± 36.94 | 0.0459 | 40.87 ± 55.79 | 0.5169 |
| LBW 28-d | -25.04 ± 93.35 | 0.8137 | 38.63 ± 60.71 | 0.5698 | -51.19 ± 59.88 | 0.4555 | 397.40 ± 8.79 | 0.0465 | 81.01 ± 67.56 | 0.3676 |
| LBW 35-d | -149.14 ± 123.36 | 0.3502 | 34.93 ± 30.97 | 0.3414 | -57.44 ± 73.75 | 0.4929 | 605.61 ± 23.59 | 0.0392 | 96.23 ± 91.42 | 0.3698 |
| WG 0-21 | 27.99 ± 51.55 | 0.6416 | 34.84 ± 32.91 | 0.3674 | -17.49 ± 39.88 | 0.6907 | 162.81 ± 33.72 | 0.0403 | 39.66 ± 55.60 | 0.5272 |
| SGR 0-21 | 0.00 ± 0.004 | 0.9739 | 0.001 ± 0.002 | 0.6947 | 0.003 ± 0.004 | 0.5274 | 0.01 ± 0.002 | 0.0426 | 0.002 ± 0.005 | 0.7123 |
| SGR 0-37 | -0.005 ± 0.001 | 0.0096 | -0.00 ± 0.00 | 0.7309 | 0.00 ± 0.001 | 0.5048 | 0.01 ± 0.002 | 0.0429 | 0.00 ± 0.003 | 0.9875 |
| GE 021 | 0.05 ± 1.28 | 0.9737 | 0.29 ± 0.65 | 0.6876 | 0.69 ± 0.91 | 0.5048 | 3.07 ± 0.66 | 0.0435 | 0.52 ± 1.19 | 0.691 |
| GE037 | -6.48 ± 0.87 | 0.0176 | -0.45 ± 1.23 | 0.7407 | 2.66 ± 1.99 | 0.2725 | 15.20 ± 3.39 | 0.0463 | 0.06 ± 3.54 | 0.9867 |
| SpW | -1.22 ± 0.35 | 0.0717 | 0.12 ± 0.33 | 0.7362 | 0.27 ± 0.29 | 0.419 | 1.89 ± 0.18 | 0.0087 | -0.31 ± 0.20 | 0.2232 |
| HW | 0.59 ± 0.41 | 0.2904 | 0.40 ± 0.27 | 0.2329 | -0.93 ± 0.24 | 0.0298 | 3.09 ± 1.21 | 0.1263 | -0.18 ± 1.12 | 0.882 |
| AFW | -8.66 ± 7.37 | 0.3608 | 1.28 ± 2.92 | 0.6905 | -3.65 ± 2.43 | 0.2310 | 34.03 ± 4.05 | 0.0139 | 7.07 ± 5.30 | 0.9867 |
| BMF% | -0.14 ± 0.03 | 0.0522 | -0.04 ± 0.05 | 0.4992 | 0.04 ± 0.11 | 0.7319 | -0.11 ± 0.30 | 0.7444 | -0.12 ± 0.10 | 0.3189 |
| TMF% | -0.07 ± 0.21 | 0.7782 | 0.27 ± 0.32 | 0.4555 | 0.37 ± 0.58 | 0.5728 | -3.82 ± 1.20 | 0.0857 | -0.35 ± 0.37 | 0.4146 |
| DMF% | 5.10 ± 2.71 | 0.2002 | 1.33 ± 1.23 | 0.3588 | -0.74 ± 0.90 | 0.0549 | 1.59 ± 1.65 | 0.4361 | 0.10 ± 0.33 | 0.7894 |

Values represent regression coefficient ±S.E. Values within a column significantly ($P<0.05$); LBW 21, 28, 35-d:Live Body Weight at 21, 28, 35-days; WG 0-21:Weight Gain 0-21 day old; SGR 0-21, 0-37:Specific Growth Rate 0-21, 0-37 days old; SpW:Spleen Weight; HW: Heart Weight; AFW: Abdominal Fat Weight; BMF: Breast Muscle Fat; TMF: Thigh Muscle Fat; DMF%:Drum Muscle Fat%.

**Table 8**. Association between A-FABP gene expression and phenotypic traits in the commercial meat type female chicken at 37-days old.

| Trait | Aviagen | P-Value | Arbor acre | P-Value | Hubbard | P-Value | Cobb | P-Value | Ross | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| SGR21-37 | -0.005 ± 0.007 | 0.5347 | -0.001 ± 0.001 | 0.3769 | -0.006 ± 0.005 | 0.3494 | 0.01 ± 0.01 | 0.4645 | 0.003 ± 0.0003 | 0.0148 |
| GE 21-37 | -0.17 ± 0.30 | 0.5711 | -0.05 ± 0.04 | 0.3680 | -0.24 ± 0.19 | 0.3283 | 0.31 ± 0.29 | 0.4795 | 0.10 ± 0.01 | 0.0118 |
| MPMW% | -0.003 ± 0.003 | 0.4260 | -0.001 ± 0.0002 | 0.0130 | 0.001 ± 0.006 | 0.9229 | -0.005 ± 0.01 | 0.4815 | 0.006 ± 0.009 | 0.5241 |
| L L | -0.03 ± 0.21 | 0.9093 | -0.052 ± 0.010 | 0.6520 | 0.21 ± 0.08 | 0.1107 | -0.75 ± 0.20 | 0.1644 | 0.11 ± 0.003 | 0.001 |
| L W % | 0.001 ±0.001 | 0.603 | 0.001 ± 0.001 | 0.1653 | -0.001 ± 0.002 | 0.5583 | -0.01 ± 0.001 | 0.0489 | -0.001 ± 0.001 | 0.4335 |
| WW % | -0.01 ± 0.01 | 0.2288 | 0.004 ± 0.002 | 0.1443 | 0.003 ± 0.001 | 0.0217 | -0.01 ± 0.001 | 0.1851 | 0.00 ± 0.004 | 0.9368 |
| SpW | 0.54 ± 0.11 | 0.0379 | 0.20 ± 0.15 | 0.2745 | -0.012 ± 0.23 | 0.9502 | -0.52 ± 0.09 | 0.1065 | 0.002 ± 0.21 | 0.9924 |
| H W | 0.85 ± 0.09 | 0.0108 | 0.0001 ± 0.0001 | 0.3723 | -0.001 ± 0.001 | 0.5744 | -0.00 ± 0.00 | 0.8935 | 0.0003 ± 0.0001 | 0.2823 |
| H W% | 0.00 ± 0.001 | 0.072 | 0.002 ± 0.001 | 0.3048 | 0.001 ± 0.004 | 0.4903 | -0.001 ± 0.003 | 0.9194 | -0.001 ± 0.002 | 0.2121 |
| HE & N% | 0.005 ± 0.01 | 0.5321 | 0.004 ± 0.001 | 0.011 | 0.001 ± 0.002 | 0.6701 | 0.002 ± 0.001 | 0.5925 | -0.003 ± 0.004 | 0.5732 |
| AFW | 6.48 ± 3.63 | 0.2165 | -3.51 ± 1.59 | 0.1145 | -1.01 ± 3.47 | 0.7977 | 14.88 ± 4.42 | 0.1837 | -3.40 ± 0.60 | 0.0294 |
| B M F% | 0.04 ± 0.05 | 0.5009 | 0.04 ± 0.05 | 0.4909 | 0.04 ± 0.09 | 0.6657 | -0.10 ± 0.05 | 0.2709 | 0.06 ± 0.05 | 0.3451 |
| T M F% | -0.07 ± 0.17 | 0.7292 | 0.08 ± 0.04 | 0.1145 | -0.19 ± 0.31 | 0.5980 | 0.31 ± 0.30 | 0.4904 | -0.07 ± 0.46 | 0.8964 |
| D M F% | 0.38 ± 0.95 | 0.7276 | 0.002 ± 0.26 | 0.9939 | -0.48 ± 0.30 | 0.2515 | -0.30 ± 0.10 | 0.2034 | -0.01 ± 0.12 | 0.9458 |

Values represent regression coefficient ±S.E. Values within a Column Significantly ($P<0.05$); SGR 21-37:Specific Growth Rate 21-37days; GE 21-37:Growth Efficiency 21-37 days; MPMW: Major Pectoralis Muscle Weight; LL: Leg Length; LW: leg Weight; WW: Wing Weight; HW: Heart Weight; HE&N: Head&neck; AFW: Abdominal Fat Weight; BMF: Breast Muscle Fat; TMF: Thigh Muscle Fat; DMF%:Drum Muscle Fat%.

higher A-FABP gene expression than males **(Table 4 and Figure 1).** Results herein showed that there was no association with fat accumulation in abdomen or in muscle for all other hybrids. Nevertheless, the non-significance effect of the A-FABP gene transcription on the intramuscular fat content percentage (IMF) in breast, thigh and drum muscles of all of the five genotypes is consistent with the findings of Ye et al. [20] who found that the A-FABP gene mRNA expression level is positively correlated significantly with abdominal fat but not with IMF content in Rugao and Luyuan chickens. Finally, the non-specificity of the A-FABP gene for abdomen fat and IMF content for these hybrids has many reasons. Firstly, it may be due to the same moderate calorie content of ration for these hybrids (growing ration), where feeding strategy is necessary to alter intramuscular fat profile in meat through manipulating gene expression of enzymes related to fat accumulation [34]. The finding of Saez et al. [35] who found that the A-FABP protein content in pectoralis major (PM) muscle of ducks was not significantly affected by dietary level for each nutritional condition, have to be emphasized. Secondly, the young slaughter age (37 day) as a broiler where age is a strong factor for fat accumulation. Third, broilers are selected for high growth traits not fatness. In marbled pork production, it is known that production is characterized by largely elevated IMF with higher A-FABP transcript levels in muscle of fatty pig breed compared to the leaner ones where Fatty acids are transported in fewer quantities through intracellular trafficking in leaner breeds resulting in less IMF deposits than fatty ones [18,21,36]. Therefore, an association between DNA polymorphisms in the A-FABP gene and fat accumulation in chickens is reported [37] and pigs [38,39]. In exon 1, a substitution mutation is significantly associated with abdominal fat, subcutaneous fat and intramuscular fat content of chicken [40]. Also, a new G/A polymorphism in exon 3 of the chicken A-FABP gene is associated with abdominal fat percentage [41,42].

## Conclusion

Genetic divergence for A-FABP gene quantity among five commercial hybrids was achieved using clustering analysis hierarchical method. Cobb and Ross genotypes were much closer to each other and the same for both Arbor Acres and Hubbard at further distance. Aviagen genotype is unique but much closer to Arbor Acres and Hubbard genotype. Little abundance of mRNA A-FABP gene is responsible for growth performance depression in Arbor Acre genotype and responsible for strongly positive association with growth performance for the Cobb males. A-FABP gene has no effect on total muscle fat% content for all genotypes and can manifest a potential use in advanced molecular research to heal the excess of abdominal fat in Cobb genotype.

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