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Investigation of the Insulin-like Growth Factor System in Breast Muscle during Embryonic and Postnatal Development in Langshan and Arbor Acres Chickens Subjected to Different Feeding Regimens*

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ABSTRACT : Nutrient availability may control muscle growth directly and indirectly through its influence on regulatory factors. We analyzed the effects of nutrient availability on the breast muscle insulin-like growth factor system. Real time RT-PCR was used to quantify the level of transcription in breast muscle from Langshan (LS) layer and Arbor Acres (AA) broiler chickens subjected to different feeding regimens during embryonic and postnatal development. The AA chickens were fed AA diet (AA, control group) while the LS chickens were either fed LS diet (LL) or AA diet (LA). According to our results, insulin-like growth factor (IGF)-II (embryonic day 16 (E16) - postnatal day 42 (P42)), IGF-I receptor (IGF-IR, E18-P42), and IGF binding protein (IGFBP)-2 (E18-P42), -5 (E16-P14), -7 (E12-P0), and -3 (E12-P0) were positively correlated with IGF-I, while IGFBP-3 (P0-P28) was negatively correlated with IGF-I. In comparison, IGF-IR (E18-P42), IGFBP-2 (E18-P42), IGFBP-5 (E14-P0), and IGFBP-3 (E16-P0) were positively correlated with IGF-II, while IGF-IR (E10-E16) and IGFBP-3 (P0-P28) were negatively correlated with IGF-II. Moreover, IGFBP-2 (E16-P42), -7 (E10-E16), and -3 (E10-E16) were positively correlated with IGF-IR, while IGFBP-3 (P0-P28) was negatively correlated with IGF-IR. Finally, IGFBP-7 (E12-P0) was positively correlated with IGFBP-3, while IGFBP-2 (P0-P28) and -7 (P0-P42) were negatively correlated with IGFBP-3. Overall, the AA chickens exhibited higher levels of IGF-I, IGF-IR, and IGFBP-2 mRNA expression than the LL chickens, while the opposite was true for IGFBP-7. No strain differences in IGF-I, IGF-IR, and IGFBP-7 mRNA expression were detected between LA and AA chickens; however, a strain difference was observed for IGFBP-2. LA chickens exhibited higher levels of IGFBP-2 than LL chickens, while the opposite was true for IGFBP-7. Our data show the first evidence that certain genes may be correlated during specific developmental periods and that strain differences in the expression of those genes in LS and AA chickens are due to differential responses to the same diet. (**Key Words :** Gene Expression, Insulin-like Growth Factor, Nutrition, Strain Difference, Breast Muscle)

INTRODUCTION

Insulin-like growth factors (IGF-1 and -2) play important roles in the uptake of amino acids and glucose, DNA synthesis, and the proliferation and differentiation of various cell types during embryogenesis (McMurtry et al., 1997). IGFs also regulate the metabolism of glucose, fat, and muscle protein during posthatch development (Buyse and Decuypere, 1999; Conlon and Kita, 2002). It is evident from many studies that the mechanisms by which IGFs act are complex and involve not only the two IGFs themselves but also two cell membrane receptors and a family of at least six binding proteins (IGFBP-1-6), which are responsible for modulating IGF action (Jones and Clemmons, 1995; Clemmons, 1998).

Several growth factors have been identified as candidate modulators of muscle growth at each stage of development. IGF-1 and IGF-2 exert a general effect on overall body growth (Jones and Clemmons, 1995), and both genes are expressed in muscle together with specific receptors, suggesting a paracrine mode of action. Unlike mammalian cells, chicken cells do not express a second IGF receptor type (type 2 IGF receptor) with a selective specificity for IGF-2 (Duclos et al., 1991). Chicken muscle satellite cells, like the cells in other chicken tissues, express a unique IGF receptor (type 1 IGF receptor, or IGF1R) with similar high affinities for human IGF-1 and IGF-2, and a low affinity for insulin (Duclos et al., 1991). IGFs can stimulate muscle differentiation by up-regulating the transcription of

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Table 1. Ingredients and calculated analysis of the diets[1]

Ingredients (%)	Lanshang diet	Arbor Acres diet
Yellow corn meal	55	56
Soybean meal (dehulled)	11	22.5
Soya bean powder (expanded)	5	10
Fish meal	3	5
Soybean oil	2	2
Dicalcium phosphate	1.7	1.7
Limestone powder	1.5	1.5
Salt	0.3	0.3
Vitamin-mineral premix[1]	1	1
Corn gluten feed	10	0
Wheat middlings	7.5	0
Rapeseed meal	2	0
Total	100	100
Calculated nutrient composition		
Metabolic energy (MJ/kg)	12.09	12.99
Crude protein (%)	17.54	22.23
Animo acids (%)		
Lysine	0.85	1.27
Methionine	0.29	0.36
Methionine+cystine	0.58	0.7
Total P (%)	0.78	0.78
Calcium (%)	1.13	1.22

[1] Supplied (mg/kg diet) niacin, 48; folic acid, 1.2; calcium pantothenate, 12; roxarsone, 50; biotin, 0.06; salinomycin, 90; vitamin A, 5,000 IU; vitamin K_3, 2.1; vitamin B_1, 2.4; vitamin B_2, 9; vitamin B_6, 5.1; vitamin B_{12}, 0.02; vitamin D_3, 3,000 IU; vitamin E, 25.5; Fe, 69, as $FeSO_4 \cdot H_2O$; Mn, 98.6, as $MnSO_4 \cdot H_2O$; Cu, 8, as $CuSO_4 \cdot 5H_2O$; I, 1.14, as KIO_3; Zn, 84, as $ZnSO_4 \cdot H_2O$; Se, 0.30, as $NaSeO_3 \cdot 5H_2O$.

myogenin (Florini et al., 1991). IGF signaling pathways have been elucidated in amphibian, avian, and mammalian species (Rotwein, 1991).

The IGFBP family, a crucial component of the IGF system, consists of high-affinity (IGFBP-1 to -6) and low-affinity (IGFBP-7 to -10) binding proteins (Hwa and Rosenfeld, 1999). The low-affinity IGFBPs, such as IGFBPrP1, may have other biological properties independent of their IGF binding capacity. Western ligand blotting experiments suggest that additional, as yet uncharacterized, IGFBP species exist in birds (Beccavin et al., 1999, 2001), which may play a role in modulating muscle growth.

The ability to regulate growth is one of the most important features of life. Nutrient availability controls overall body and muscle growth directly or indirectly through its influence on regulatory factors. Numerous studies have demonstrated that the plasma IGF-I concentration and IGFBPs are altered by nutritional status (Thissen et al., 1994); however, the response of the IGF system to diet varies with the stage of development (Fliesen et al., 1989; Oster et al., 1996).

In this study, we used real-time quantitative PCR to assess IGF-I, IGF-II, IGF-IR, IGFBP-2, IGFBP-5, IGFBP-7, and IGFBP-3 mRNA expression in the breast muscle of slow-growing Langshan (LS) layer and rapidly-growing Arbor Acres (AA) broiler chickens. AA chickens were fed AA diet (AA, control group), whereas LS chickens were fed either LS (LL) or AA diet (LA) under the same environmental conditions. Based on a comparison of the experimental and control groups, we discussed whether diet has an effect on strain differences in breast muscle weight (BMW) and IGF system genes expression during postnatal development. Our goals were: i) to reveal ontogenic changes in IGF system genes expression during embryonic and postnatal development; and ii) to understand the effect of diet on strain differences in BMW and IGF system genes expression during postnatal development.

MATERIALS AND METHODS

Animals and tissues sampling

Fertile LS and AA chicken eggs were incubated in a standard commercial incubator. On incubation days 8, 10, 12, 14, 16 and 18, 8 eggs were removed, embryos were weighed and samples of the breast muscle were collected. LS and AA poults were reared from hatching to 7 weeks of age under normal conditions in the Nanjing Agricultural University Animal Farm. AA chickens were fed AA standard diet and LS chickens were divided into two groups fed LS standard diet and AA standard diet respectively. The differences in nutritional values between the Langshan and Arbor Acres diets are shown in Table 1. Diets and water were available *ad libitum*. On postnatal days 0, 14, 28 and 42, 8 chickens, randomly selected from each group, were weighed, and sacrificed for tissue sampling. The period of LL and AA groups includes embryonic days 8, 10, 12, 14, 16, 18 and postnatal days 0, 14, 28 and 42, while the period of LA group includes postnatal days 14, 28 and 42. All tissue samples were snap frozen in liquid nitrogen and stored at -80°C prior to RNA isolation.

RNA extraction and cDNA synthesis

Total RNA was isolated from breast muscle using TRIzoL reagent® (Invitrogen, 15596-026) according to the manufacturer's protocol. RNA integrity was electrophoretcally verified by ethidium bromide staining and its purity was examined by UV spectrophotometry (OD260/OD280). The reverse transcription (RT) was carried out following the Promega protocol. The first mix was prepared with a final volume of 10 μl by using 2 μg total RNA, 1 μg Oligo (dT15) and 0.1% Diethyl-pyrocarbonate (DEPC) solution. This mix was heat-denatured at 70°C for 5 min. Samples were then placed on ice and briefly centrifuged. The second mix was prepared with a final volume of 15 μl by using 0.5 mM dNTP, 25 U RNAse inhibitor (Promega, N2511), 200 U M-MLV transcriptase (Promega, M1701), 5×RT buffer and 0.1% DEPC solution. Then the second mix was added to each

Table 2. Primer sequences and PCR conditions[1]

Item	Accession no.	Primer sequence (5'→3')	Size (bp)	A.T.[2] (°C)
β-Actin	NM_205518	F[3] 5'-TGCGTGACATCAAGGAGAAG-3'	300	56
		R[3] 5'-TGCCAGGGTACATTGTGGTA-3'		
IGF-I	NM_001004384	F 5'-GTATGTGGAGACAGAGGCTTC-3'	200	59
		R 5'-TTTGGCATATCAGTGTGGCGC-3'		
IGF-II	XM_421026	F 5'-TGTGGAGGAGTGCTGCTTTC-3'	98	66
		R 5'-GGGAGGTGGCGGAGAGGTCA-3'		
IGF-IR	AJ223164	F 5'-CAAGCATGCGTGAGAGGATA-3'	404	56.1
		R 5'-CAAACCTTTCCCTCCTTTCC-3'		
IGFBP-2	NM_205359	F 5`-GGGAAGGATGCCTGTGTAGA-3'	299	58
		R 5`-CACGTCCAGTGCAAAACATC-3`		
IGFBP-5	AF293839	F 5'-CCTCTCTGACCTCAAAGCTGA-3'	198	58
		R 5'-ACCATCCTCTGGCTGCTCT-3'		
IGFBP-7	CB018442	F 5'-CCA GAG AAT GGA GCT TCT GC-3'	243	58
		R 5'-CAT CAC AGC TCT GCA CCA TC-3'		
IGFBP-3	EF624351	F 5'-CTC TTC GGA ATC CAA GCA AG-3'	400	58
		R 5'-CCA CCA CAG GAG ACA GAC AG-3'		

[1] IGF = Insulin-like growth factor; IGFBP = IGF binding protein.
[2] A. T. = Annealing temperature. [3] F = Forward; R = Reverse.

sample to form a 25 μl reaction mix. The mixtures were incubated at 42°C for 1 h and then, 95°C for 5 min. The cDNAs (RT products) were obtained and stored at -20°C for quantification by real-time quantitative PCR (Q-PCR).

Establishment of standards of chicken genes for real-time Q-PCR

Recombinant plasmids containing a specific fragment of chicken each target gene and the endogenous reference gene (β-actin) were constructed to establish the real-time Q-PCR standards. Briefly, specific fragments of respective genes were amplified using the same primers as in subsequent real-time Q-PCR (Table 2). PCR products were excised after being confirmed by electrophoresis on 2% TAE agarose gel and purified by V-gene DNA Purification Kit (V-gene Biothechnology Ltd., China) following the supplier's manual, then cloned into the pMD19-T vector (TakaRa, Dalian, China) and subsequently transformed into competent *Escherichia coli* DH5alpha cells. According to the manufacturer's instructions, the recombinant plasmids were purified with a plasmid Maxi Kit (Generay Biotech Co. Ltd, Shanghai, China) and solubilized in sterile water as stocks. The concentrations of the stocks were determined by measuring OD260 using spectrophotometer (WFZ800-D3B, Beijing, Rarleigh Analytical Instrument Corp.). For a standard curve, serial ten-fold dilutions of these stocks were made to obtain batches of standards in the range of 10^1-10^6 copies/μl for real time Q-PCR.

Real-time Q-PCR assay

Quantitative analysis of PCR was performed in the DNA Engine Opticon (MJ Research) using SYBR Green I (Generay, RS0976). Primer sequences, annealing temperature and the approximate sizes of the amplified fragments were shown in Table 2. For each amplification, 2 μl standards or test RT products or water was added to a premix of 18 μl, which contains 10 μl SYBR® Premix Ex TaqTM (2×)(TaKaRa, DRR041A), 1 μl primers mix (Table 2, 0.2 μM final concentration each), 7 μl sterile water. The optimized thermal cycling programs were 95°C for 1 min followed by 45 cycles of 95°C for 10 sec, annealing temperature (Table 2) for 20 sec and 72°C for 20 sec. Specificities of amplification products were confirmed by melting curve analysis (60 to 95°C in 0.2°C/s increments).

Real-time Q-PCR data calculation

The method used for quantification of expression was the relative standard curve method. The quantification was normalized to an endogenous control (β-actin), and standard curves were prepared for each target gene and the endogenous reference. For each experimental sample, the amount of mRNA of each target gene and β-actin was determined from their respective standard curve. The exported Excel files contained the values of threshold cycle (Ct, which was defined as the cycle at which fluorescence rose above background for each sample during the log-linear phase of the reaction) and the copy concentrations of the respective gene in test samples. The mRNA relative expression level of each target gene in test samples was expressed as the copy concentration of each target gene divided by that of β-actin gene (target gene/β-actin).

Statistical analysis

All data were analyzed using statistical software SPSS (Version13.0, SPSS Inc., USA). Data of ontogenic changes of gene expression were subjected to a one-way ANOVA followed by Duncan's multiple range tests for comparison

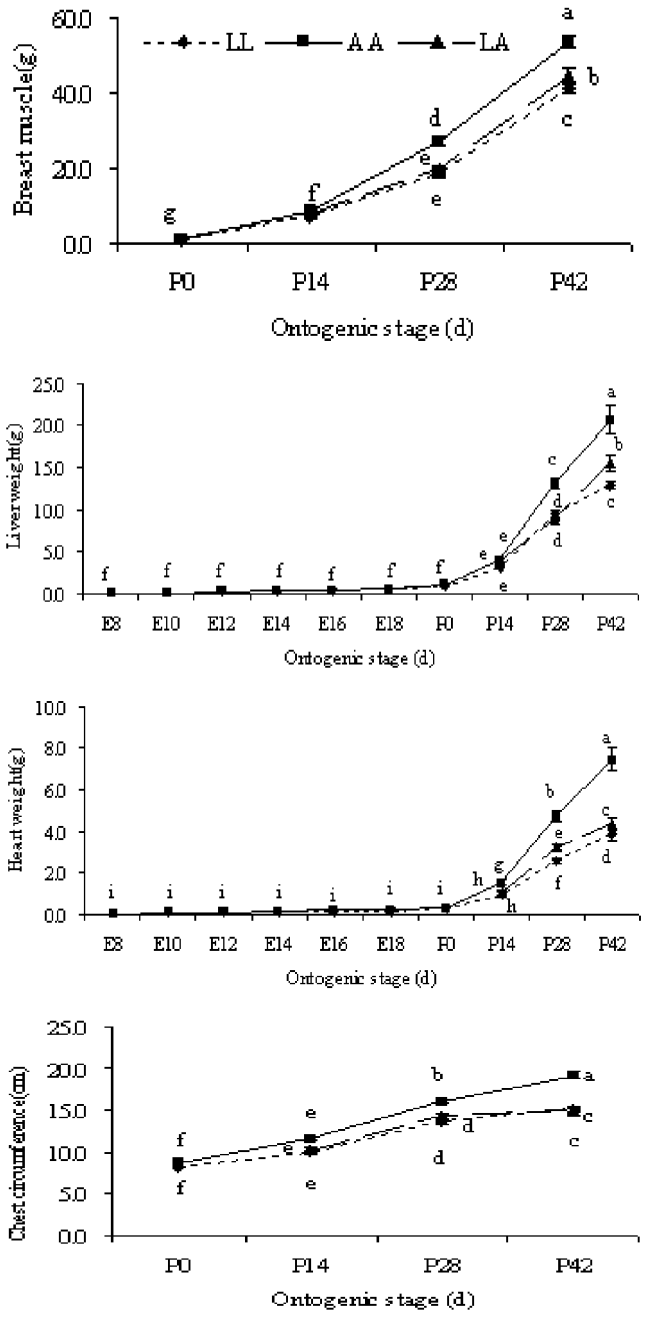

Figure 1. Developmental changes in breast muscle weight (BMW), liver weight (LW), heart weight (HW), and chest circumference (CC) of Langshan (LS) and Arbor Acres (AA) chickens. Values represent the means±SEM. Different letters denote statistically significant ($p<0.05$) differences for mean comparisons. LL: LS chickens fed with standard LS diet, LA: LS chickens fed with standard AA diet, AA: AA chickens fed with standard LS diet.

of the means. For evaluating the effects of the diet on gene expression in general at the postnatal days 14, 28 and 42, the General Linear Models (GLM) procedure (with developmental age and strain as fixed factors) of SPSS software was used, in which the Duncan's multiple range test option was selected. Results are expressed as the means ±SEM. Significance for mean differences was set at $p\leq0.05$. Pearson correlation coefficient was used to characterize the relationship of BMW and genes mRNA expression.

RESULTS

Developmental changes of general body growth in chickens

The general features of body growth, including BMW, liver weight (LW), heart weight (HW), and chest circumference (CC), in LS and AA chickens during the ontogenic stage are shown in Figure 1. A significant strain difference in BMW was observed on postnatal day 28 (P28) and P42 between AA and LL chickens. Diet significantly influenced body growth in LS chickens. On P42, the average BMW of LA chickens was significantly higher than that of LL chickens. Notwithstanding the influence of diet, strain differences in BMW existed between LS and AA chickens on P42, even when they were fed the same diet ($p<0.05$). As shown in Figure 1, LW, HW, CC also increased rapidly as the growth period progressed in LS, LA, and AA chickens.

IGF system genes expression in the breast muscle of chickens

Except for a decrease on embryonic day 12 (E12), strong mRNA expression of IGF-I was observed up to E14, which then decreased until E18, increased sharply on P0, and decreased progressively from P0 to P42 (Figure 2). IGF-II expression was comparatively high during embryonic development and then decreased progressively from P0 to P42. IGF-IR expression increased from E18 to P0 and then declined during postnatal development. IGFBP-2 expression was weak in both strains from E8 to E16; however, it subsequently increased between E16 and P0 then dropped to a relatively low level during postnatal development. IGFBP-5 expression was relatively constant throughout development. Except for an increase on E14, IGFBP-7 expression was weak during embryonic development, and then increased through P0 and decreased on P28. The level of IGFBP-3 expression in each strain was low prior to E18; however, it gradually increased from E18 to P28 then remained strong through P42.

Effect of diet on strain differences in body growth and IGF system genes expression

As presented in Table 3, general linear model (GLM) analysis revealed general strain differences when the data from P14, P28, and P42 in LL and AA chickens were pooled: $F(1, 42) = 55.47$ (LW), 123.94 (HW), 163.49 (CC), 102.56 (BMW); $p<0.05$ (Duncan's multiple range test). AA chickens exhibited greater LW, HW, CC, and BMW than LL

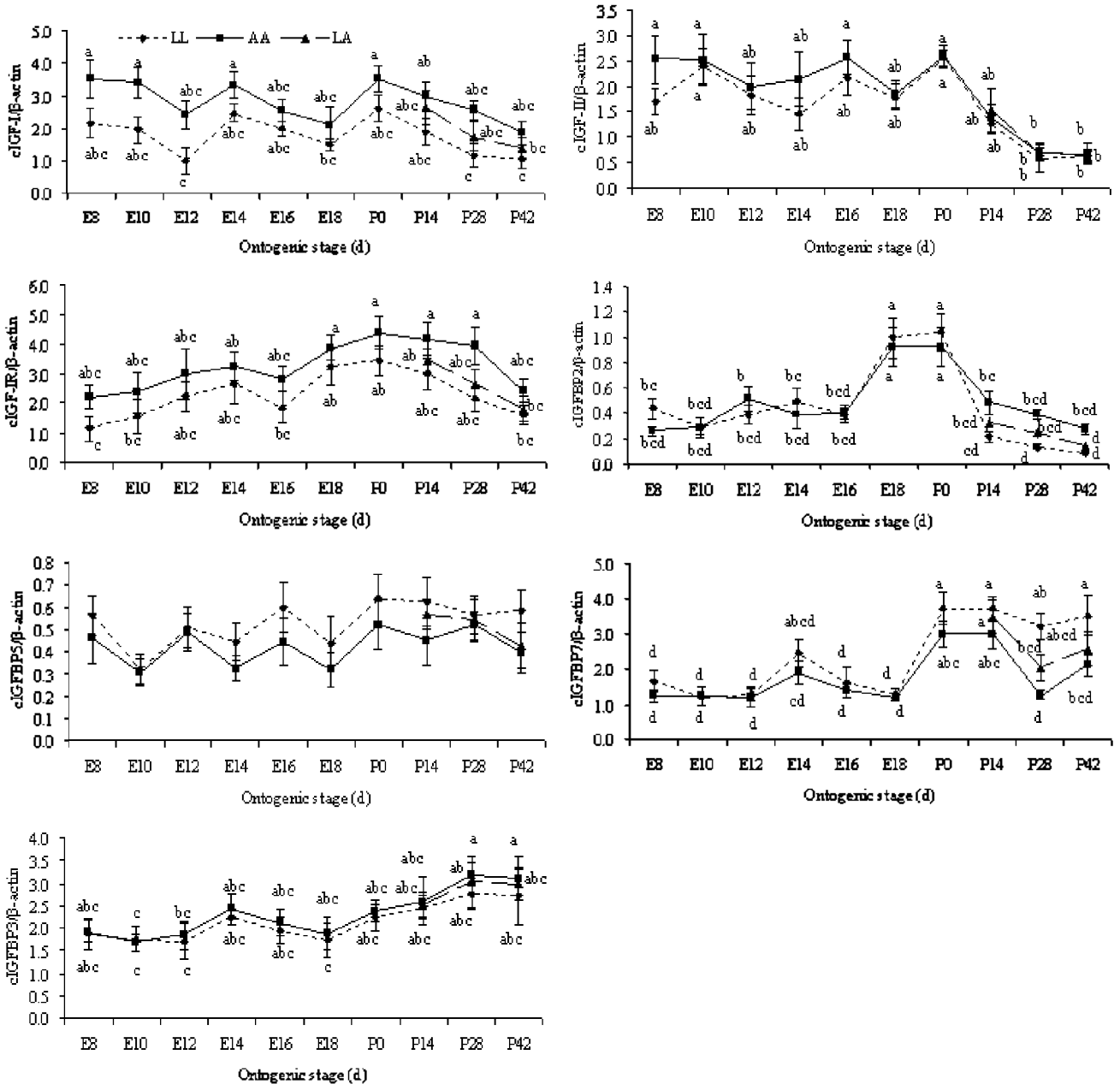

Figure 2. Developmental changes of relative expression levels of insulin-like growth factor (IGF)-I, IGF-II, IGF-IR, IGF binding protein (IGFBP)-2, -5, -7 and -3 mRNA in Langshan (LS) and Arbor Acres (AA) chickens. Values represent the means±SEM. Different letters denote statistically significant ($p<0.05$) differences for mean comparisons. LL: LS chickens fed with standard LS diet, LA: LS chickens fed with standard AA diet, AA: AA chickens fed with standard LS diet.

chickens, although the strain differences were not significant in some age groups. Likewise, strain differences in several body growth indices (F (1, 42) = 26.54 (LW), 74.52 (HW), 98.64 (CC), 35.20 (BMW); $p<0.05$) existed between LA and AA chickens. In comparison, LA chickens exhibited greater LW, HW, and BMW (F (1, 42) = 5.29 (LW), 9.78 (HW), 4.08 (BMW); $p<0.05$) than LL chickens.

Additional strain differences were revealed by GLM analysis when the P14, P28, and P42 data from LL and AA chickens were pooled: F (1, 42) = 9.21 (IGF-I), 8.72 (IGF-IR), 30.04 (IGFBP-2), 13.34 (IGFBP-7); $p<0.05$ (Duncan's multiple range test) (Table 4). IGF-I, IGF-IR, and IGFBP-2 mRNA expression was higher in AA chickens than in LL chickens, while the opposite was true for IGFBP-7, although the strain differences in some age groups were not significant. No strain differences in IGF-I, IGF-IR, and IGFBP-7 transcription existed between LA and AA chickens (F (1, 42) = 2.34 (IGF-I), 3.02 (IGF-IR), 2.18 (IGFBP-7); $p>0.05$); however, a strain difference was observed in IGFBP-2 expression (F (1, 42) = 9.64; $p<0.05$).

Table 3. General linear models (GLM) analysis of strain differences in body growth of postnatal Langshan (LS) and Arbor Acres (AA) chickens[1]

ITEM	LL/AA		LA/AA		LL/LA	
	F (1,42)	P	F (1,42)	P	F (1,42)	P
LW	55.47	*	26.54	*	5.29	*
HW	123.94	*	74.52	*	9.78	*
CC	163.49	*	98.64	*	0.58	0.45
BMW	102.56	*	35.20	*	4.08	*

* $p<0.05$.
[1] LL = LS chickens fed LS diet; LA = LS chickens fed AA diet; AA = AA chickens fed AA diet.
LW = Liver weight; HW = Heart weight; CC = Chest circumference; BMW = Breast muscle weight.

LA chickens expressed more IGFBP-2 ($F(1, 42) = 10.35$; $p<0.05$) than LL chickens, while the opposite was true for IGFBP-7 ($F(1, 42) = 4.95$; $p<0.05$).

Correlation between BMW and IGF system genes expression in chickens

To investigate the relationship between BMW and IGFs expression, a correlation analysis of LS and AA chicken development was performed (Table 5). IGFBP-3 (P0-P28) was positively correlated with BMW, while IGF-I (P0-P42), IGF-II (P0-P42), IGF-IR (P0-P42), and IGFBP-2 (P0-P42) were negatively correlated with BMW.

Correlation coefficients were also calculated for the various IGF genes during LS and AA chicken development (Table 6). IGF-II (E16-P42), IGF-IR (E18-P42), IGFBP-2 (E18-P42), IGFBP-5 (E16-P14), IGFBP-7 (E12-P0), and IGFBP-3 (E12-P0) were positively correlated with IGF-I, while IGFBP-3 (P0-P28) was negatively correlated with IGF-I. In comparison, IGF-IR (E18-P42), IGFBP-2 (E18-P42), IGFBP-5 (E14-P0), and IGFBP-3 (E16-P0) were positively correlated with IGF-II, while IGF-IR (E10-E16) and IGFBP-3 (P0-P28) were negatively correlated with IGF-II. IGFBP-2 (E16-P42), -7 (E10-E16), and -3 (E10-E16) were also positively correlated with IGF-IR, while IGFBP-3 (P0-P28) was negatively correlated with IGF-IR. Finally, IGFBP-7 (E12-P0) was positively correlated with IGFBP-3, while IGFBP-2 (P0-P28) and IGFBP-7 (P0-P42) were negatively correlated with IGFBP-3.

DISCUSSION

Expression of IGF-I, IGF-II and IGF-IR genes in the breast muscle tissue during development

Except for a decrease on E12, IGF-I expression was

Table 4. General linear models (GLM) analysis of strain differences in insulin-like growth factor system genes of postnatal Langshan (LS) and Arbor Acres (AA) chickens[1]

ITEM	LL/AA		LA/AA		LL/LA	
	F (1,42)	P	F (1,42)	P	F (1,42)	P
IGF-I	9.21	*	2.34	0.13	2.34	0.13
IGF-II	0.25	0.62	0.06	0.81	0.50	0.48
IGF-IR	8.72	*	3.02	0.09	0.80	0.38
IGFBP-2	30.04	*	9.64	*	10.35	*
IGFBP-5	2.77	0.10	0.54	0.47	1.06	0.31
IGFBP-7	13.34	*	2.18	0.15	4.95	*
IGFBP-3	0.68	0.42	0.11	0.74	0.30	0.59

* $p<0.05$.
[1] LL = LS chickens fed LS diet; LA = LS chickens fed AA diet; AA = AA chickens fed AA diet.
IGF = Insulin-like growth factor; IGFBP = IGF binding protein.

Table 5. Correlation coefficients between breast muscle weight (BMW) and insulin-like growth factor genes mRNA expression during corresponding development period of Langshan (LS) and Arbor Acres (AA) chickens[1]

ITEM1	ITEM2	Pearson correlation			Period
		LL	AA	LA	
BMW	IGF-I	-0.865	-0.980(*)	-0.903	P0-P42
	IGF-II	-0.76	-0.808	-0.816	P0-P42
	IGF-IR	-0.956(*)	-0.960(*)	-0.983	P0-P42
	IGFBP-2	-0.678	-0.819	-0.987	P0-P42
	IGFBP-3	0.993	0.999(*)	1.000(**)	P0-P28

* $p<0.05$; ** $p<0.01$.
[1] LL = LS chickens fed LS diet; LA = LS chickens fed AA diet; AA = AA chickens fed AA diet.
BMW = Breast muscle weight; GF = Insulin-like growth factor; IGFBP = IGF binding protein.

strong prior to E14, and then decreased until E18. It then increased sharply on P0 and decreased progressively from P0 to P42 (Figure 2). IGF-I, which is believed to stimulate embryonic growth (Girbau et al., 1987) and the differentiation of myoblasts (Schmid et al., 1983) and lens fiber cells (Beebe et al., 1987), has been detected in the circulation in chick embryos, with maximum levels on E14-17 (Kikuchi et al., 1991). Other studies have reported negative correlations between the IGF-I concentration and body growth (Huybrechts et al., 1985; Pym et al., 1991; Bacon et al., 1993). In growing cattle, a strong correlation has been detected between the accretion rate of the longissimus muscle and the serum IGF-I level (r = 0.82; Vestergaard et al., 2003). Kita et al. (2002) suggested that muscle protein degradation would be increased by IGF-I infusion as well as protein synthesis in fasted chicks. Our results show that IGF-I (P0-P42) is negatively correlated with BMW (Table 5).

The breast muscle IGF-1 mRNA level is high in high-growth chickens (Guernec et al., 2001). We found that AA chickens had higher levels of IGF-I mRNA ($F(1, 42) = 9.21$; $p<0.05$) than LL chickens, although the strain differences in some age groups were not significant (Table 4).

Several previous studies have reported that fasting (Lowe et al., 1989) or protein restriction (Sánchez-Gómez et al., 1999) significantly reduces muscle IGF-1 mRNA expression, although the magnitude of this effect may differ between species (Hua et al., 1993) or muscles. Sun et al. (2007) indicated that the effects of the limiting amino acids on nutrition of animals were likely intermediated via their effects on plasma IGF-I and liver IGF-I mRNA level. In our study, no strain differences in IGF-I expression ($F(1, 42) = 2.34$ (IGF-I); $p>0.05$) were detected between LA and AA chickens, which suggests that a component of the AA diet resulted in the up-regulation of IGF-I mRNA expression in the breast muscle of LS and AA chickens (Table 4).

Fetal muscle IGF-II mRNA expression is strong during early development in domestic animals and then progressively decreases with gestational age (Gerrard and Grant, 1994; Listrat et al., 1994; Gerrard et al., 1998). In our study, the mRNA expression of IGF-II was relatively high during embryonic development and then decreased progressively from P0 to P42 (Figure 2). Based on a correlation analysis, IGF-II (P0-P42) was negatively correlated with BMW (Table 5). Tesseraud et al. (2003) suggested that IGF-II may be less important than IGF-I in controlling body and muscle growth in chickens.

Chickens selected for high growth rates exhibit higher plasma IGF-II concentrations than chickens selected for low growth rates (Beccavin et al., 2001). In a model of broiler strains divergently selected for high or low growth potential (Scanes et al., 1989), the level of IGF-II did not differ between the two genotypes. Our results show no difference in the breast muscle transcription of IGF-II ($p>0.05$) between LL, LA, and AA chickens (Table 4).

The mRNA level of IGF-II in muscle is significantly altered by the nutritional state of hatching chicks (Beccavin et al., 2001). In contrast, the effect of diet-related changes on the concentration of IGF-II in birds is uncertain (Kita et al., 1996; Leili et al., 1997). Our results indicate no difference in the level of IGF-II expression ($F(1, 42) = 0.50$; $p>0.05$) between LL and LA chickens (Table 4), which suggests that AA diet has no significant effect on IGF-II expression in the breast muscle of LS and AA chickens.

IGF-IR expression in breast muscle is strong during the early postnatal period and then decreases (Yun et al., 2005). In our study, the mRNA level of IGF-IR increased from E18 to P0 and then declined during postnatal development (Figure 2). These peculiar patterns of expression suggest that IGF-IR mRNA expression may differ in chickens depending on the strain. In growing cattle, a positive correlation has been reported between the accretion rate of the longissimus muscle and the number of type I IGF receptors (r = 0.52) (Vestergaard et al., 2003). We found a negative correlation between IGF-IR (P0-P42) and BMW (Table 5).

In our study, AA chickens exhibited higher levels of IGF-IR mRNA expression ($F(1, 42) = 8.72$; $p<0.05$) than LL chickens (Table 4). Trouten-Radford et al. (1991) also suggested a difference in IGF-IR density in breast muscle between fast- and slow-growing chicken strains.

Matsumura et al. (1996) found that starvation of 4-week-old chickens for 5 days caused a 1.7- to 2.2-fold increase in IGF-IR mRNA expression in kidney, liver, and muscle; however, the mRNA levels were reduced to the control level by refeeding of the starved chickens for 24 h. In our study, no strain differences in IGF-IR expression were detected ($F(1, 42) = 3.02$ (IGF-IR); $p>0.05$) between LA and AA chickens (Table 4), which suggests that a specific component of the AA diet induced the up-regulation of IGF-IR in the breast muscle of LL chickens.

Expression of IGFBP-2, IGFBP-5, IGFBP-7 and IGFBP-3 genes in the breast muscle tissue during development

IGFBP-2 expression in skeletal muscle decreases ($p<0.05$) with increasing age in fetuses and neonatal pigs (Gerrard et al., 1999). Our results revealed that IGFBP-2 expression in the breast muscle of both chicken strains was weak between E8 and E16, then rose between E16 and P0, and decreased to a relatively low level during postnatal development (Figure 2). The muscle IGFBP-2 mRNA expression profile might be similar between neonatal pigs and chickens.

In turkey muscle satellite cells, IGFBP-2 expression coincides with IGF-II expression during myoblast differentiation (Ernst et al., 1996). Orlowski et al. (1990) reported the coexpression of IGFBP-2 and IGF-II in fetal rat muscle and hypothesized that the relative ratio of IGFBP-2 to IGF-II may regulate the activity of IGF-II. It was also suggested that IGF-II binds to IGFBP-2 with greater affinity than IGF-I (Baxter and Martin, 1989). In our study, IGFBP-2 (E18-P42) was positively correlated with IGF-II (Table 6), which suggests the parallel expression of these genes from E18-P42. Our results also indicated that IGFBP-2 was negatively correlated with BMW (P0-P42; Table 5), which supports the idea that IGFBP-2 plays an important role in modulating the growth-promoting effect of circulating IGFs by promoting the formation of an IGFIGFBP complex in ruminants and chickens (Kita et al., 2002; Lee et al., 2005).

IGFBP-2 is sensitive to the dietary protein level (Kita et al., 2002; Lee et al., 2005). The deficiency of essential amino acids stimulated chickens to increase gizzard IGFBP-2 gene expression (Kita et al., 2005). In our study, the level of IGFBP-2 was higher in LA than in LL chickens ($F(1, 42) = 10.35$; $p<0.05$; Table 4), which suggests that a protein component of the AA diet resulted in the up-regulated expression of IGFBP-2 in the breast muscle of LL chickens.

IGFBP-5 is the most conserved of the IGFBPs (James et al., 1993). IGFBP-5 mRNA has been detected as early as E10.5 in rat embryos, especially in muscle progenitors, and it is present in the muscle cells of the embryo in all regions throughout fetal development (Green et al., 1994). IGFBP-5 is associated with myoblast differentiation and is highly expressed in developing embryonic muscles (Cerro et al., 1993; Tollefsen et al., 1989). In our study, IGFBP-5 expression was relatively constant throughout the experimental period (Figure 2). The constant expression profile of breast muscle IGFBP-5 mRNA suggests the conservation of important functions during evolution, particularly in muscle differentiation.

Our results showed no difference in IGFBP-5 mRNA expression ($p>0.05$) among LL, LA, and AA chickens (Table 4). IGFBP-5 expression increased in high-growth strain versus low-growth strain mice, suggesting positive effects for the protein on muscle growth, at least in high-growth strain mice (Hoeflich et al., 2004). A decrease in IGFBP-5 expression was detected after muscle starvation in rainbow trout (Gabillard et al., 2006). These conflicting results suggest that the strain differences in IGFBP-5 mRNA expression among poultry are different from those in mammals and fish.

IGFBP-5 preferentially binds IGF-II (Clemmons et al., 1992). We found that the mRNA expression of IGFBP-5 and IGF-II was positively correlated (E14-P0; Table 6). IGFBP-5 protein may have potential modulatory roles in type I fiber-dominated muscles, aging, and regrowth from atrophy in rats (Spangenburg et al., 2003). So we suggested that the coordinated regulation of IGFBP-5 and IGF-II

Table 6. Correlation coefficients between insulin-like growth factor genes mRNA expression during corresponding development period of Langshan (LS) and Arbor Acres (AA) chickens[1]

Gene1	Gene2	Pearson correlation			Period
		LL	AA	LA	
IGF-I	IGF-II	0.887(*)	0.528	0.985	E16-P42
	IGF-IR	0.835	0.795	0.966	E18-P42
	IGFBP-2	0.655	0.422	0.961	E18-P42
	IGFBP-5	0.781	0.942	ND	E16-P14
	IGFBP-7	0.852	0.889(*)	ND	E12-P0
	IGFBP-3	0.969(**)	0.942(*)	ND	E12-P0
		-0.998(*)	-0.944	-1.000(**)	P0-P28
IGF-II	IGF-IR	-1.000(**)	-0.695	ND	E10-E16
		0.902(*)	0.648	0.907	E18-P42
	IGFBP-2	0.922(*)	0.931(*)	0.899	E18-P42
	IGFBP-5	0.932	0.913	ND	E14-P0
	IGFBP-3	0.997(*)	0.861	ND	E16-P0
		-0.972	-0.909	-1.000(**)	P0-P28
IGF-IR	IGFBP-2	0.782	0.655	1.000(*)	E16-P42
	IGFBP-7	0.792	0.68	ND	E10-E16
	IGFBP-3	0.663	0.822	ND	E10-E16
		-0.994	-0.973	-1.000(**)	P0-P28
IGFBP-3	IGFBP-2	-0.874	-0.812	-1.000(**)	P0-P28
	IGFBP-7	0.87	0.79	ND	E12-P0
		-0.822	-0.917	-0.964	P0-P42

* $p<0.05$; ** $p<0.01$.

[1] LL = LS chickens fed LS diet; LA = LS chickens fed AA diet; AA = AA chickens fed AA diet.

IGF = Insulin-like growth factor; IGFBP = IGF binding protein; ND = Not done.

expression may signal the resumption of myogenic activity.

IGFBP-7 is widely distributed in normal tissues (Akaogi et al., 1996). To our knowledge, ours is the first report to show a difference in IGFBP-7 mRNA expression between chicken strains. In our study, IGFBP-7 expression was weak during embryonic development, except for an increase on E14, but it increased through P0 and then decreased on P28 (Figure 2). LL chickens exhibited higher levels of IGFBP-7 expression than AA chickens ($F(1, 42) = 13.34$ (IGFBP-7); $p<0.05$), and no strain differences in IGFBP-7 expression were detected ($F(1, 42) = 2.18$ (IGFBP-7); $p>0.05$) between LA and AA chickens (Table 4). Based on our results, IGFBP-7 expression might be correlated with growth in chickens, and the observed strain differences in IGFBP-7 expression in breast muscle may be the result of nutritional effects on transcription.

The cysteine-rich IGFBP-7 protein family, which is structurally similar to the IGFBP superfamily (Hwa et al., 1999), mediates various biological functions. In BALB/c3T3 mouse fibroblasts, IGFBP-7 proteins stimulate cell growth and enhance the mitogenic effects of IGF-I, whereas in selected osteosarcoma cells they inhibit cell growth (Akaogi et al., 1996; Kato et al., 1996). Also, IGFBP-7 specifically binds IGF-I (Oh et al., 1996), and IGF-I up-regulates IGFBP-7 expression in the P69 prostate cancer cell line (Hwa et al., 1998). We also found that IGFBP-7 (E12-P0) was positively correlated with IGF-I (Table 6), which suggests the parallel expression of these genes from E12-P0.

According to our results, the expression of IGFBP-3 in either strain was weak up to E18; however, it subsequently increased (E18-P28) and remained at a high level up to P42 (Figure 2). In BN/F344 rat muscle, IGFBP-3 mRNA expression increases with age, although the increase is not significant (Severgnini et al., 1999). We found that IGFBP-3 was positively correlated with BMW (P0-P28; Table 5).

In contrast, no clear pattern of serum IGFBP-3 expression was detected in response to divergent selection for high and low body growth in mice (Hoeflich et al., 2004). Our results revealed no difference in breast muscle IGFBP-3 mRNA expression ($p>0.05$) among LL, LA, and AA chickens (Table 4).

Brain IGFBP-3 expression in rats is markedly increased by a ketogenic diet, but it is not altered by calorie restriction alone (Cheng et al., 2003). Food restriction was shown to decrease serum IGFBP-3 expression on day 50 in rats (Gautsch et al., 1998). Our results indicated no difference in the level of IGFBP-3 mRNA expression ($F(1, 42) = 0.30$; $p>0.05$) between LL and LA chickens (Table 4), which suggests that diet did not alter the abundance of IGFBP-3 mRNA in the breast muscle and that nutrient availability is not a key regulator of IGFBP-3 expression in chicken breast muscle.

A 16-kDa proteolytic fragment of IGFBP-3, which has no affinity for IGFs, inhibits the mitogenic effects of IGF-I on chick embryo fibroblasts (Zadeh et al., 1997). It was also strongly suggested that IGFBP-3 plays a role in IGF-I-mediated postnatal growth in mice (Ning et al., 2006). Our results indicated that IGFBP-3 was positively correlated with IGF-I (E12-P0) and BMW (P0-P28), whereas a negative correlation was detected between IGFBP-3 (P0-P28) and IGF-I (Tables 5 and 6), which suggests that these two genes may be regulated in a coordinated fashion during development.

CONCLUSIONS

In conclusion, our data indicate clear ontogenic changes in IGF system gene expression in breast muscle during embryonic and postnatal development, which suggests that these genes may be coordinately regulated during growth and development in LS and AA chickens. In addition, by comparing the expression of these genes in both strains under their respective standard diets and under the same (AA) diet, we found that the observed strain differences in gene expression were due to a differential response to diet at the level of transcription. The data presented in this study may be used to elucidate the possible regulatory mechanism of IGF system genes in breast muscle and the possible effects of nutrition on growth and endocrine status in poultry.

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