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Age related changes in PPARγ, COX III mRNA expression, growth performance, carcass quality parameters and gut morphometry in different genotypes of chicken

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ABSTRACT

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Efficient conversion of food into body mass abide associated with altered gene expression of some proteins of the electron transport chain. We evaluated the outcome of age on mRNA expression of Peroxisome proliferator-activated receptor-γ and Cytochrome c oxidase subunit III in breast muscle tissues. Collectively 90 birds each from Nandanam B2, Rhode Island Red, Aseel and White Leghorn, divided into three replicates were accostomed for the study. Production parameters like body weight, body weight gain, cumulative feed consumption, cumulative feed efficiency at two and six weeks were documented along with carcass traits at eight weeks and intestinal morphometry were studied at four and eight weeks of age. Total RNA was drawn out from the breast muscle tissue of male birds and reverse transcribed into cDNA. Real-time PCR analysis was executed using specific primers for the genes. Greatest reduction when comparing second and sixth week old was noted in COX III mRNA expression levels followed by PPARγ. The study revealed phenotypic differences in production traits as well as the difference in expression of mitochondrial gene like COX III and nuclear encoded gene that is involved in fat metabolism like PPARγ gene expression level change with age in chickens.
Keywords: Oxidative damage, Ageing, Mitochondia, PPARγ, COX III

INTRODUCTION

Efficient conversion of food into body mass was reported to be associated with altered gene expression of some proteins of the electron transport chain (Gasparino *et al.*, 2012). All cells need energy to perform their activities. Mitochondria are responsible for producing 90% of the energy needed for cells. Series of studies are conducted to understand relationships of mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers (Bottje *et al.*, 2002, 2004; Iqbal *et al.*, 2004, 2005; Ojano-Dirain *et al.*, 2004, 2005 a,b; Tinsley *et al.*, 2004; Lassiter *et al.*, 2006). These organelles are responsible for transforming chemical energy from metabolites into energy easily accessed by the cell (Schauss *et al.*, 2010).

The aim of this study was to evaluate the mRNA expression of these genes related to energy production and ATP synthesis, where Cytochrome c oxidase subunit III is a mitochondrial encoded subunit in complex IV of the respiratory chain which is responsible for modulating proton pumping and electron transport through the redox centers, and together with subunits I and II, form the functional core of Complex IV (Scheffler, 1999). Therefore, COX III may play a key role in energy production. As age advances oxidative damage occurs, and the mRNA expression of COX III was reported to be reduced (Iqbal *et al.* 2004).

Upsurge production of mitochondrial ROS, which occurs with advancing age, is related to greater oxidative damage in the macromolecules, additionally depletion in the energy production machinery. Birds with lower ATP production due to lower mitochondrial efficiency in producing ATP from substrates show worse efficiency or feed conversion. Therefore, mechanisms that favor a reduced ROS production may be useful in the prevention of age-related issues.

The Peroxisome proliferator-activated receptor-γ an important transcription factor during adipogenesis, which involves the induction and stimulation of fat-specific genes, including adipocyte fatty acid binding protein (aP2), lipoprotein lipase, glycerol-3- phosphate dehydrogenase (GPDH) and fatty acid translocase in mammals. Hence the present study was conducted to evaluate mRNA expression of genes that are associated with mitochondrial energy metabolism cytochrome oxidase III (COX III) and mitochondrial biogenesis (PPAR-γ) in breast muscle of different chicken breeds that are known to have differential phenotypic expression of feed efficiency.

MATERIALS AND METHODS

Experimental design and animals

The experimental protocol was approved by the Institutional Animal Ethics Committee, Tamil Nadu, India.

Collectively 90 birds each from Nandanam B2, Rhode Island Red, Aseel and White Leghorn were

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accustomed for the study. Nandanam B2 is a commercial hybrid dual purpose strain developed by Tamil Nadu Veterinary and Animal Sciences University. The concerned breeds/strain was divided into four treatment groups with three replicates in each group, enclosing 30 birds each. The breeds were selected based on observed high and low feed efficiency over generations in Poultry Research Station, Madhavaram Milk Colony, Chennai-51, with the aim to evaluate mRNA expression of genes that are involved in mitochondrial energy metabolism and mitochondrial biogenesis that are familiar to have differential phenotypic expression.

All the experimental birds were wing banded and reared upto eighth week of age following standard managemental practices. Aggregate quantity of feed was supplied ad libitum with feed containing 3100 Kcal ME/ kg and 22 per cent dietary crude protein. Clean potable water was supplied ad libitum. The study was carried out during October-December months where the average daily high temperature in the study area (13.1623° N, 80.2433° E) was below 31R"C. Data on phenotypic performance and gene expression studies were recorded.

The day old experimental chicks were weighed with 0.1 g accuracy. Body weight was again recorded at second and sixth week. Based on day old body weight, body weight gain was calculated. Conclusively every two weeks period, left over feed was weighed back and net feed consumption was arrived for each group. Feed efficiency was calculated at 2nd and 6th week.

Carcass characteristics

Conclusively at eighth week of age six birds from individual treatment were taken randomly for carcass study. After bleeding and defeathering, head and shanks were removed. New York dressed weight was calculated. The organ weight of heart, liver and gizzard were noted. Eviscerated carcass with giblets was weighed to actuate ready to cook yield. Each carcass was cut into its component parts namely breast, thighs, drumsticks, wings, back and neck.

Histomorphometry of small intestine

Approximately two cms from each segment of duodenum, jejunum and ileum were cut, washed with PBS (0.1, PH 7.4) and placed separately in a 10 percent formalin solution for further processing. Each sample was cut into 5mm sections and placed into tissue cassettes. The tissues were processed, fixed in paraffin and subsequently cut into 5 µm thick slides with a microtome. Sections were fixed on slides and dyed according to Harris hematoxylin-eosin (HE) technique for light microscopy evaluation. Measurements on villi height, mid villi width, crypt depth and lamina muscularis thickness were recorded.

Gene expression

Mitochondrial mRNA expression of PPARγ and COX III genes were studied in pectoral muscle tissue at second and sixth week by following the protocol below. Two male birds from individual replicate were randomly selected. All animals were killed by cervical dislocation at the same time and breast muscle tissue (pectoralis superficialis) was collected and submerged in RNA later and kept at -80R" C until RNA extraction.

Total RNA was extracted using TRIzol® LS Reagent (Invitrogen, USA) according to the manufactuers instructions (1 ml per 100 mg of tissue). All of the materials used were treated with the ribonuclease (RNase) inhibitor RNase AWAY (Invitrogen, Carlsbad, CA, USA) prior to RNA extraction. The muscle tissue and TRIzol mixture was triturated with a mortar and pestle until it completely dissociated. Next, mixture was then incubated for 5 minutes at room temperature and 200 µl of chloroform was added to the sample, and the mixture was manually homogenised for 1 minute. The samples were then centrifuged for 15 min at 12000rpm at 4°C. The aqueous phase was collected and shifted to a clean tube containing 500 µl of isopropanol was added and mixed by slightly inverting the tube and again homogenized and centrifuged for 10 minutes at 12000rpm at 4°C. The supernatant was discarded. To the RNA pellet obtained, 1ml of 70% Ethanol was added and then stored at -80°C until further use.

The pellet was dried for 1 minute and resuspended in ultrapure RNase-free water, quantified and equal volume of RNA was used across the different samples. The total RNA concentration was measured using a spectrophotometer at a wavelength of 260 nm. RNA integrity was analysed using a 1% agarose gel stained with 10% ethidium bromide and visualised under ultraviolet light.

A High capacity cDNA Reverse Transcriptase kit (Thermo Scientific Revert Aid H Minus First Strand cDNA Synthesis Kit #K1632) kit was used for cDNA synthesis in accordance with manufacturer's instructions. For this reaction, 8 µl of total RNA, 1 µl of Primer (oligo dT) and 3µl of RNAse free water were added to a sterile RNA-free tube. The reaction was then incubated for 5 minutes at 65°C and placed on ice for 1 minute. Subsequently, 4 µl of 5 X Reaction buffer, 2 µl of dNTPs, 1 µl of solution containing reverse transcriptase enzyme and 1 µl RNase inhibitor were mixed to the tubes making it 20 µl. The solution was incubated for 1 hours at 42R"C and finally at 70R"C for 5 minutes for the synthesis of complementary DNA. Next, the reaction was incubated for 5 minutes at 85°C and immediately placed on ice. The samples were stored at "20°C until use.

Real-time polymerase chain reactions were performed using the fluorescent dye SYBR® Green following the manufacturer's instruction. The real time plates were obtained from Roche, India. The PPARγ and COX III primers were previously described in Ojano-Dirain *et al.* (2007) (Table 1). Two endogenous controls, ß-actin and GAPDH, were tested, and ß actin (accession number L08165) was selected because its amplification was shown to be more efficient.

The Real-time PCR mix was prepared as follows:

2X SYBR Green Mix	10µL
Forward Primer	0.5µL (10pmol/ µL)
Reverse Primer	0.5µL (10pmol/ µL)
Template cDNA	2 µL

The components were mixed gently by vortexing and made total volume upto 20 µL then briefly centrifuged to collect all the components at the bottom of the tube. The reaction was conducted in duplicates for each sample. The cycling protocol was 94°C for 2 minutes followed by 40 cycles of 94°C for 10 seconds and 58°C for 10 seconds with a melting program and finally held at 37°C.

The relative mRNA expression levels of the target genes such as PPARγ and COX III, genes are shown as Ct values in the muscle tissue. The µ-actin Ct value for each sample was subtracted from the Ct value of the target gene to normalize for the host basal levels. Following normalization the mRNA expression levels of the target genes PPARγ and COX III, of each breed are expressed as fold change (2-DDCt) over the respective levels in Nandanam B2.

PPARγ and COX III mRNA expression levels were analyzed in second and sixth week of age and correlated with feed efficiency of the different types of chicken in respective weeks in pectoral muscle tissue.

Statistical analysis

The results were countenance as mean±standard error (S.E). The differences between groups were assessed by applying the Statistical Package for Social Sciences (SPSS) software package for windows as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Effect of different types of chicken on biweekly body weight, body weight gain cumulative feed consumption (g) and feed efficiency were displayed in Table 1-2, respectively.

Body weight and body weight gain

In the present study, highly significant difference observed in biweekly body weight at second and sixth week which was due to different types of chicken. Being broiler type Nandanam B2 attained higher body weight at sixth week of age. Nandanam B2 was followed by RIR, Aseel and lowest in White leghorn.

The finding was in agreement with the earlier results obtained by Sangilimadan et.al. (2013) who studied the performance of Nandanam B2. Few other researchers compared the performance of distinct breeds like Aseel and Kadaknath in their locality like Huanshi et.al (2011) and got similar results. Singh et.al (1999) also reported the same performance of Aseel male and female chicken. Khawaja *et al.* (2012) who compared the growth performance of RIR revealedcomparableresults.

Feed consumption and feed efficiency

Effect of different types of chicken in cumulative feed consumption and feed efficiency at different periods were significantly different and better feed efficiency was seen in Nandanam B2 and then comparable in RIR, White leghorn and Aseel. This could be due to the difference in genetic make up of different types of chicken.

The interpretation of this study coincided with work carried out by Jha and Prasad (2012) who studied the performance of Aseel under deep litter and reported FCR of 5.46 upto 20 week. Gupta *et al.* (2000) concurred that feed consumption of Aseel chicken reared under deep litter at 1st and 2nd month of age were 124g and 300g.

Where as the findings in present study were contrary with the work carried out early by Sangilimadan *et al.* (2013) who studied the performance of Nandanam B2 and reported that feed efficiency at 8th week was 2.49.

Carcass characteristics

Effect of different types of chicken on the carcass traits is presented inTable 3.There was no significant difference was observed in New York dressed weight, eviscerated weight, giblet weight and ready to cook yield in different types of chicken. For New York dressed weight, eviscerated weight, giblet weight and ready to cook yield numerically highest values recorded in Nandanam B2 followed by RIR, Aseel and lowest in WLH.

Cut up parts

Effect of different types of chicken on the cut up parts (%) is presented inTable4.There was no significant difference observed inpercentagesofcutupparts in different types of chicken. Quite differently numerical difference was evident between the different types of chicken having highest in Nandanam B2 and lowest inWLH.

Intestinalmorphometry

The analysed data are presented as morphometry study of duodenum, jejunum and ileum at 4th week in Table 5, 6 and 7 and at 6th week in Table 8, 9 and 10 respectively. There was significant ($P<0.05$) difference observed in different types of chicken with highest mid villi width and highly significant ($P<0.01$) difference was seen in lamina muscularis thickness in Nandanam B2 followed by RIR, Aseel and lowest in WLH at fourth week in duodenum. But there was significant ($P<0.05$) difference observed in different types of chicken with highest mid villi width in Nandanam B2 followed by RIR, Aseel and lowest in WLH at 6th week old. No significant difference was observed in jejunum morphometry in both the periods in different types of chicken. In ileum morphometry study, there was significant ($P<0.05$)

Table 1: Biweekly mean (±SE) body weight (g) and cumulative mean (±SE) body weight gain between 0 and 6 weeks of age in different types of chicken

Breeds/ Strain	Duration (weeks)			
	0-2		0-6	
	Body Weight (Grams)	Body Weight Gain (Grams)	Body Weight (Grams)	Body Weight Gain (Grams)
Nandanam B2	152.52^{b}±2.87(90)	119.47^{b}±2.78(90)	605.50^{c}±14.6(78)	572.63^{c}±14.5(78)
RIR	89.03^{a}±1.53(85)	53.65^{a}±1.47(85)	367.90^{b}±7.07(73)	332.60^{b}±6.97(73)
Aseel	86.72^{a}±1.48(73)	55.19^{a}±1.41(73)	358.57^{b}±7.56(61)	327.14^{b}±7.53(61)
WLH	82.35^{a}±1.25(89)	51.55^{a}±1.23(89)	325.93^{a}±5.71(74)	295.15^{a}±5.73(74)
F value	305.72**	320.24**	182.05**	183.47**

The figures in parentheses indicate number of observations.
Means bearing different superscript within a column differs significantly
******–Highly significant (p<0.01)

Table 2: Cumulative mean (±SE) feed consumption (g) and feed efficiency in between 0 and 6 weeks of age in different types of chicken

Type	Duration (weeks)			
	0-2		0-6	
	Feed consumption	Feed efficiency	Feed consumption	Feed efficiency
Nandanam B2	195.47^{b}±0.68	1.28^{a}±0.03	1111.48±55.5	1.72^{a}±0.09
RIR	140.14^{a}±1.68	1.41^{ab}±0.02	1092.34±32.8	2.13^{b}±0.12
Aseel	132.78^{a}±7.80	1.52^{b}±0.06	1061.53±38.9	2.45^{b}±0.13
WLH	133.35^{a}±5.59	1.89^{c}±0.07	1059.25±24.9	3.60^{c}±0.12
F value	11.204*	24.950**	7.856^{NS}	44.934**

Means bearing different superscript within a column differs significantly
NS- Non significant; *****– Significant(p<0.05); ******–Highly significant(p<0.01)

Table 3: Mean (±SE)carcass characteristics(%) at eighth week of age in different types of chicken.

Type	Carcass characteristics(percent)			
	New York dressed weight	Eviscerated weight	Giblet weight	Ready-to-cook weight
Nandanam B2	93.03±0.67	57.50±3.74	5.55±0.37	63.05±3.82
RIR	91.79±0.29	54.09±2.04	5.42±0.30	59.51±1.93
Aseel	90.99±0.59	54.10±0.76	5.41±0.38	59.21±1.04
WLH	90.93±0.64	50.68±0.44	5.13±0.18	55.82±0.43
Fvalue	2.912^{NS}	1.635^{NS}	0.292^{NS}	1.791^{NS}

NS-Non-significant(P>0.05)

Table 4: Mean (±SE) cutup parts (%) at eighth week of age in different types of chicken

Type	Cutup parts (percent)					
	Breast	Back	Thigh	Drumstick	Neck	Wing
Nandanam B2	23.01±1.53	19.47±2.08	16.77±0.83	17.51±0.37	6.46±0.39	11.23±0.15
RIR	22.46±2.10	21.36±1.43	15.93±0.69	17.29±0.96	6.78±0.48	10.76±0.76
Aseel	22.13±1.55	23.12±1.99	15.34±0.63	17.40±0.35	5.47±0.57	11.12±0.45
WLH	22.18±2.07	19.86±1.34	16.30±0.63	17.69±0.68	7.13±0.38	11.72±0.49
Fvalue	0.291^{NS}	1.041^{NS}	2.141^{NS}	1.329^{NS}	1.849^{NS}	1.996^{NS}

NS–Non-significant(P>0.05)

difference observed in different types of chicken with highest mid villi width and lamina muscularis thickness in Nandanam B2 followed by RIR, Aseel and lowest in WLH in 4th week of age but there was negligible difference observed in 6th week of age.

PPARγ Gene Expression

These results clearly indicated positive attributes of this candidate gene for better feed efficiency and weight gain in broiler type of chicken. Among different type of chicken, mRNA expression was highest in

Table 5: Duodenum (Mean±S.E) morphometry (µm) at fourth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	1493.54±74.65	175.30[b]±8.11	130.77±16.63	264.71[b]±21.88
RIR	1125.67±21.16	156.64[ab]±2.05	118.98±7.15	203.99[a]±8.47
Aseel	1202.42±60.76	134.82[a]±14.70	98.97±12.18	150.51[a]±20.54
WLH	1311.94±36.76	132.95[a]±10.29	149.86±19.72	172.97[a]±16.13
Fvalue	2.380[NS]	4.097*	2.103[NS]	7.967**

a,b,–meanswithincoloumnbearingdifferentsuperscriptsdiffersignificantly(P<0.05)
*****–Significant(P<0.05); ******–Highly Significant (P<0.01)NS-Non Significant

Table 6: Jejunum (Mean±S.E) morphometry(µm) at fourth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	955.07±68.92	142.28±6.64	140.29±17.70	189.97±23.26
RIR	1055.11±38.43	133.02±7.46	131.64±14.26	164.00±17.39
Aseel	882.81±84.16	131.38±16.28	121.44±4.77	161.50±21.14
WLH	823.31±65.06	127.85±16.12	113.20±10.54	168.29±17.05
Fvalue	2.271[NS]	0.266[NS]	0.858[NS]	0.417[NS]

NS-NonSignificant

Table7: Ileum (Mean±S.E) morphometry(µm) at fourth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	1107.79±68.92	148.87[b]±7.54	118.19±13.20	239.23[b]±29.53
RIR	996.90±68.73	98.55[a]±14.55	122.60±4.20	161.23[a]±17.39
Aseel	1073.95±84.16	109.83[a]±12.54	97.79±19.33	156.84[a]±13.43
WLH	829.07±42.56	133.56[ab]±11.49	113.20±13.44	182.87[ab]±15.63
Fvalue	1.452[NS]	3.704*	1.221[NS]	3.586*

a,b,–meanswithincoloumnbearingdifferentsuperscriptsdiffersignificantly(P<0.05)
*****–Significant (P<0.05)NS-NonSignificant

Table 8: Duodenum (Mean±S.E) morphometry (µm) at sixth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	1423.32±74.65	180.87[b]±4.99	137.60±16.63	197.38[b]±16.09
RIR	1380.51±21.16	139.90[ab]±13.68	127.30±7.15	207.24[a]±16.49
Aseel	1263.90±60.76	142.52[a]±14.62	142.52±12.18	221.47[a]±24.54
WLH	1355.15±36.76	177.74[a]±13.07	177.74±19.72	160.69[a]±15.56
Fvalue	0.295[NS]	3.325*	0.435[NS]	1.960[NS]

a,b,–meanswithincoloumnbearingdifferentsuperscriptsdiffersignificantly(P<0.05)
*****–Significant (P<0.05) NS-Non Significant

Table 9: Jejunum (Mean±S.E)morphometry (µm) at sixth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	1069.41±76.16	155.51±15.99	94.41±12.13	186.73±24.47
RIR	1121.53±52.26	134.46±5.26	119.48±13.16	193.20±22.79
Aseel	1163.60±58.19	151.70±15.51	116.50±12.20	212.07±15.57
WLH	1107.80±16.65	135.85±3.58	119.66±12.63	207.651±35.40
F value	0.496[NS]	0.865[NS]	0.939[NS]	0.217[NS]

NS-Non Significant

Nandanam B2 followed by Rhode Island Red, Aseel and White leghorn. White leghorn being layer type had negative trend in expression level. PPARγ gene can be utilized as a significant molecular tool for genotype based selection of feed efficiency traits as one of the important upregulating factor.

Relationship between different type of chicken and mRNA expression levels of PPARγ gene in pectoral

Table 10: Ileum (Mean±S.E) morphometry (µm) at sixth week of age in different types of chicken

Type	Villi Height	Mid Villi Width	Crypt Depth	Lamina Muscularis
Nandanam B2	1176.54±41.67	151.92±18.60	103.72±9.62	194.58±13.96
RIR	1081.36±68.73	132.64±14.04	104.97±9.48	185.61±6.89
Aseel	1122.49±71.72	126.75±6.20	119.21±9.99	189.70±16.06
WLH	1041.00±8.81	111.84±4.11	108.08±7.50	193.75±23.71
F value	0.534^{NS}	1.838^{NS}	0.594^{NS}	0.064^{NS}

NS-Non Significant

Table 11: mRNA expression of PPAR gene in breast muscle tissue at second week of age in different types of chicken

Type	µ-actin Average	PPAR Average	ΔCt	ΔCt (Average)	ΔΔCt	Fold change	% Fold change	Mean±S.E
Nandanam B2	22.05	28.44	6.39	7.10	1	1.00	100.00	_
	21.61	27.88	6.26			1.00	100.00	
	20.00	27.32	7.32			1.00	100.00	
	20.07	27.73	7.66			1.00	100.00	
	27.10	32.37	5.27			1.00	100.00	
	21.84	31.56	9.71			1.00	100.00	
RIR	17.27	25.10	7.83		0.72	0.60	60.42	69.92±39.67
	19.99	28.50	8.51		1.41	0.38	37.58	
	18.48	28.75	10.26		3.16	0.11	11.17	
	18.84	28.61	9.77		2.66	0.16	15.75	
	26.70	32.64	5.93		-1.16	2.25	224.70	
Aseel	19.26	28.86	9.60		2.50	0.18	17.65	48.92±13.02
	20.68	29.24	8.55		1.45	0.37	36.55	
	20.35	27.86	7.51		0.41	0.75	75.16	
	20.83	28.18	7.35		0.24	0.84	84.26	
	22.24	31.04	8.79		1.69	0.31	30.95	
WLH	19.27	28.33	9.06		1.95	0.26	25.76	35.92±5.77
	24.42	33.46	9.04		1.93	0.26	26.12	
	19.93	27.85	7.92		0.82	0.57	56.57	
	21.67	30.09	8.42		1.32	0.40	40.00	
	20.78	29.57	8.78		1.68	0.31	31.17	

muscle tissue at second and sixth week age as expressed by percent fold change recorded. Comparison of Nandanam B2 with other type of birds showed down regulation of Rhode Island Red, Aseel and White leghorn respectively. Lowest percent fold change and mRNA expression was observed in White leghorn type of chicken, clearly reflecting the layer type of birds necessitating lower body weight and better egg production. The expression levels of this PPARγ mitochondrial gene was increased at sixth week in Rhode Island Red showed upregulation in dual purpose type of chicken in contrast to second week. This may be with advancement of age there is more fatty acid uptake and metabolism.

On comparing the mRNA expression levels between weeks behaved differently where upregulation was observed in Aseel and White leghorn at second week and downregulation was observed in Aseel and White leghorn at sixth week. The result coincides with the Sato (2004) who studied chicken PPARγ mRNA expression in abdominal adipose tissue tended to increase with age, as shown by higher expression levels at 6 week than at 1 and 2 week of age. It also coincides with the work of Rosen *et al.* (1999) who stated that PPAR-γ is activated by fatty acids that control adipocyte differentiation along with fatty acid uptake and metabolism.

The result contradicts with Ojano-Dirain *et al.* (2007) who reported that there were negligible differences in breast muscle PPAR mRNA expression. Also dissimilar with Matsubara *et al.* (2005) who reported that PPARγ is possibly a key regulator in the early stages of chicken preadipocyte differentiation.

COX III Gene Expression

Effect of type of chicken like the broiler, layer and dual purpose on the mRNA expression of COX III mitochondrial gene in breast muscle at the sixth week

Table 12: mRNA expression of PPAR gene in breast muscle tissue at sixth week of age in different types of chicken

Type	μ-actin Average	PPAR Average	ΔCt	ΔCt (Average)	ΔΔCt	Fold change	% Fold change	Mean ±S.E
Nandanam B2	24.00	34.86	10.86	7.42	1	1.00	100.00	_
	23.50	32.19	8.69			1.00	100.00	
	27.09	34.86	7.76			1.00	100.00	
	33.85	35.33	1.47			1.00	100.00	
	28.87	35.31	6.43			1.00	100.00	
	23.72	33.02	9.30			1.00	100.00	
RIR	26.24	35.12	8.88		1.45	0.36	36.37	93.31±51.22
	29.84	35.12	8.88		6.55	0.01	1.06	
	28.02	43.82	13.97		-1.37	2.59	258.65	
	26.93	34.07	6.05		0.37	0.77	77.16	
	29.60	34.72	7.79		1.3	0.40	40.44	
Aseel	21.54	33.92	12.37		4.95	0.03	3.23	37.85±13.59
	26.98	35.40	8.41		0.99	0.50	50.21	
	28.67	36.83	8.15		0.73	0.60	60.15	
	40.19	36.43	8.32		0.81	0.57	36.98	
	31.81	34.84	8.20		0.75	0.53	38.77	
WLH	27.99	34.47	8.53		1.21	0.48	26.58	26.55±8.73
	20.85	29.52	8.67		1.25	0.42	41.93	
	25.62	37.75	12.13		4.70	0.04	3.82	
	33.18	43.18	10.00		2.57	0.17	16.74	
	23.58	32.19	8.61		1.19	0.44	43.71	

Table 13: mRNA expression of COX III gene in breast muscle tissue at second week of age in different types of chicken

Type	μ-actin Average	COX III Average	ΔCt	ΔCt (Average)	ΔΔCt	Fold change	% Fold change	Mean ±S.E
Nandanam B2	22.05	14.65	-7.40	-6.78	1	1.00	100.00	
	21.61	13.94	-7.67			1.00	100.00	
	20.00	13.71	-6.29			1.00	100.00	
	20.07	14.03	-6.04			1.00	100.00	
	27.10	17.97	-9.13			1.00	100.00	
	21.84	17.69	-4.15			1.00	100.00	
RIR	17.27	10.38	-6.89		-0.10	1.10	108.22	73.15±12.45
	19.99	13.30	-6.69		0.10	0.90	93.89	
	18.48	12.24	-6.24		0.50	0.70	68.73	
	18.84	12.93	-5.90		0.90	0.50	54.49	
	26.70	21.23	-5.47		1.30	0.40	40.44	
Aseel	19.26	14.04	-5.21		1.60	0.30	33.77	65.01±24.00
	20.68	16.27	-4.41		2.40	0.20	19.33	
	20.35	13.23	-7.12		-0.30	1.30	126.49	
	20.83	13.78	-7.05		-0.30	1.20	120.50	
	22.24	17.46	-4.78		2.00	0.20	24.98	
WLH	19.27	14.18	-5.09		1.70	0.30	31.08	43.86±9.85
	24.42	20.37	-4.04		2.70	0.20	15.01	
	19.93	13.87	-6.05		0.70	0.60	60.46	
	21.67	15.41	-6.26		0.50	0.70	69.69	
	20.78	15.22	-5.56		1.20	0.40	43.05	

Table 14: mRNA expression of COX III gene in breast muscle tissue at sixth week of age in different types of chicken

Type	â-actin Average	COX III Average	ÄCt	ÄCt (Average)	ÄÄCt	Fold change	Mean ±S.E
Nandanam B2	24.00	15.59	-8.41	-10.80	1	1.00	_
	23.50	14.14	-9.36			1.00	
	27.09	14.48	-12.61			1.00	
	33.85	18.26	-15.59			1.00	
	28.87	18.28	-10.59			1.00	
	23.72	15.47	-8.25			1.00	
RIR	26.24	18.29	-7.94		2.90	0.10	4.46±2.32
	29.84	15.29	-14.55		-3.70	13.40	
	28.02	15.80	-12.22		-1.40	2.70	
	26.93	15.01	-11.91		-1.10	2.20	
	29.60	16.84	-12.76		-2.00	3.90	
Aseel	21.54	13.61	-7.93		2.90	0.10	10.19±4.79
	26.98	13.99	-12.99		-2.20	4.60	
	28.67	14.12	-14.55		-3.70	13.40	
	40.19	24.62	-15.57		-4.80	27.30	
	31.81	18.55	-13.26		-2.50	5.50	
WLH	27.99	14.73	-13.26		-2.50	5.50	3.53±1.99
	20.85	16.17	-4.67		6.10	0.00	
	25.62	14.17	-11.44		-0.60	1.60	
	33.18	18.99	-14.18		-3.40	10.40	
	23.58	15.34	-8.24		2.60	0.20	

shows a reduction in gene expression levels in contrast to the second week. Expression levels of mRNA as reflected by fold change positively link with metabolic regulating factor for energy production of AMP and ATP at mitochondria, subsequently for the body weight gain and feed efficiency traits.

Percent fold change was lowest in white leghorn and highest in Nandanam B2 in between in Rhode Island Red and Aseel. Variation within group was lowest in white leghorn, followed by Rhode Island Red and Aseel, indicating greater variability in gene pool level. The mRNA expression was much higher in Aseel group of birds. COX III mitochondrial gene can be considered as singular important genotype biomarker tool for future selection for better feed efficiency. On comparing the mRNA expression levels between weeks behaved differently where downregulation was observed in RIR, Aseel and white leghorn at sixth week. This could be due to greater ROS production and greater protein oxidation that are consistently found in birds with lower feed efficiency thereby decreasing the cellular efficiency.

This study coincides with the work done by Iqbal *et al.* (2004) who discussed that COX III mRNA levels in pectoral muscle were beneath in poor feed efficiency contrast with the better feed efficiency birds. Similarly, Ojano-Dirain *et al.* (2007) suggested that a greater ROS production and greater protein oxidation are consistently found in birds with lower feed efficiency, indicating that this factor may alter the expression of mitochondrial genes.

The findings concurred with Barazzoni *et al.* (2000) who verified a reduction in COX III mRNA expression related to altered oxidative capacity of mitochondria in older animals. This would indicate that maintaining transcription levels may be essential to mitochondrial oxidative capacity and the continuance of efficient use of nutrients. Also similar to Bottje *et al.* (2002) who stated that increased oxidative stress and protein oxidation in the low-FE phenotype is likely caused by increased mitochondrial reactive oxygen species.

The finding was in agreement with Kemp *et al.* (2003) who opined that COX III plays an important role in mitochondrial energy efficiency. Also similar to Scheffler (1999) who reported that COX III may play a key role in energy production. Zhang *et al.* (2010) reported that ROS production and expression of proteins of the respiratory chain complexes involved in metabolism, with the feed efficiency of animals.

Bottje and Carstens (2009) reported that the low-FE phenotype generated more mitochondrial ROS than the high-FE phenotype.The low-FE broiler phenotype revealed site-specific defects in electron transport, developing in increased mitochondrial ROS production and increased protein oxidation in several tissues.

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