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Effect of dietary lysine on performance and expression of electron transport chain genes in the *pectoralis major* muscle of broilers

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*The aim of this study was to evaluate the effect of dietary lysine on performance, protein deposition and respiratory chain gene expression in male broilers. A total of 252 Cobb 500 broilers were distributed, in a completely randomized design, into four treatments with seven replicates of nine birds per experimental unit. Experimental treatments consisted of diets based on corn and soybean meal, with four levels of digestible lysine: 1.016%, 1.099%, 1.182% and 1.265%. The increase in the level of digestible lysine in the diet provided higher weight gains, feed efficiency and body protein deposition. Birds fed the lowest level of dietary lysine (1.016%) showed a lower expression of genes such as NADH dehydrogenase subunit I (*ND1*), cytochrome b (*CYTB*) and cytochrome c oxidase subunits I (*COX I*), II (*COX II*) and III (*COX III*), displaying the worst performance and body protein deposition. This demonstrates the relationship existing between the expression of the evaluated genes and the performance responses. In conclusion, results indicate that broilers fed diets with higher levels of digestible lysine have increased messenger RNA expression of some genes coded in the mitochondrial electron transport chain (*ND1, CYTB, COX I, COX II *and* COX III*). It may be stated that diets with proper levels of digestible lysine, within the 'ideal protein' concept, promote the expression of genes, which increases the mitochondrial energy, thereby fostering body protein deposition and the performance of broilers in the starter phase.*

Keywords: amino acids, genes, protein deposition, respiratory chain

Implications

Broiler nutrition is a strategic area for successful poultry production that has defined the requirements of nutrients essential for performance maximization. However, little is known about the effect of specific nutrients like amino acids on the metabolic events. Knowing the action of dietary lysine on gene expression opens the possibility of manipulating more-economic diets adjusted for meat production.

Introduction

Lysine is one of the most widely studied amino acids in broiler nutrition, mainly because it is the second most limiting amino acid for growth in corn- and soybean meal-based diets, after the first limiting methionine and cysteine (Fakhraei *et al*., 2010; Dozier and Payne, 2012). Because of its broad commercial availability and easy laboratory analysis, lysine has been considered the reference standard for the adjustment of the ideal protein, which has allowed the targeting for body protein deposition and decreased diversion to other metabolic pathways (Garcia *et al*., 2006). Hocquette *et al*. (2007) stated that among the amino acids, lysine probably has the most specific effect on the carcass composition and muscle growth.

Determining the digestible lysine requirement for broilers has been the target of many studies (Dozier *et al*., 2010; Corzo *et al*., 2012) aimed at improvements in performance responses by birds (Kidd *et al*., 1998) and body protein deposition (Fatufe *et al*., 2004; Wijtten *et al*., 2004). However, few studies have addressed the molecular mechanisms affected by the dietary lysine.

The knowledge of the energy requirement for the protein synthesis has been established, and this may require changes in mitochondrial energy production (Buttery and Boorman, 1976). However, little is known about the effect of nutrients, or more specifically, the amino acid lysine on the use or production of energy components. The cell production of high-energy molecules like ATP is a feature of the mitochondrial oxidative phosphorylation (OXPHOS), which is

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composed of two subsystems: the electron transport chain (ETC), which involves complexes I to IV, and ATP synthase, known as complex V (Wallace, 2007).

Given the importance of the mitochondrial DNA (mtDNA) for the encoding of polypeptides that translocate protons via the OXPHOS complex (I, II, III, IV and V), it is of paramount importance to know its regulations in different climatic and/or nutritional conditions, as alterations in these complexes may reduce ATP production, causing a predisposition to the appearance of degenerative and metabolic diseases in humans (Wallace, 2005 and 2007), metabolic syndromes in poultry (Guan *et al*., 2007) and reduction of meat quality in cattle (Mannen *et al*., 2003).

Although studies have demonstrated the relationship between animal performance and the mitochondrial function, not much is known yet about the effect of levels of nutrients on the expression of ETC- and OXPHOS-related genes (Ojano-Dirain *et al*., 2007; Bottje and Carstens, 2009; Wang *et al*., 2012). The increased feed efficiency and protein deposition stimulated by lysine may lead to changes in the gene expression of ETC and OXPHOS, as feed efficiency is related to the mitochondrial function (Bottje *et al*., 2002; Iqbal *et al*., 2004; Wang *et al*., 2012).

Therefore, this study aimed to evaluate the effects of dietary digestible lysine on the messenger RNA (mRNA) expression of OXPHOS-related genes (NADH dehydrogenase subunit I (*ND1*), cytochrome b (*CYTB*), cytochrome c oxidase subunits I (*COX I*), II (*COX II*), III (*COX III*) and ATP synthase F0 subunit 6 (*ATP6*)) by relating it to body protein deposition and performance of broilers in the starter phase (8 to 21 days).

Material and methods

All procedures applied in this experiment were approved by the Committee on Ethics and Animal Protection of Universidade Federal de Sergipe, Sergipe, Brazil.

Experimental design and animals

A total of 252 male broilers of the Cobb 500 commercial line, from 8 to 21 days of age, were used in this experiment. From 1 to 7 days of age, birds were reared in a masonry shed with concrete floor littered with wood shavings, where drinkers and feeders were available for free water and feed consumption. At 8 days of age, with an average initial BW of 177 ± 0.28 g, birds were housed in 0.3 m^2 metabolic cages, distributed, in a completely randomized design, into four treatments with seven replicates of nine birds per experimental unit. Treatments consisted of diets formulated following the nutritional recommendations suggested by Rostagno *et al*. (2011), with four levels of digestible lysine: 1.016%, 1.099%, 1.182% and 1.265%, for the period from 8 to 21 days of age (Table 1). Diets were prepared adopting the dilution technique, in which the experimental diets containing the highest and lowest levels of digestible lysine (1.265% and 1.016%, respectively) were combined with each other at the proportions of 66.67%/33.33% and 33.33%/66.67%, generating diets with intermediate levels (1.099% and 1.182%). Birds and the supplied feed were weighed weekly to determine feed intake, weight gain and feed efficiency. Mortality was checked daily to adjust the performance data. In case of mortality in any experimental unit, the diet reserved to that group would be weighed and considered as intake up to that moment.

Table 1 *Ingredients and calculated nutritional composition of the initial (1 to 7 days) and experimental basal diets (8 to 21 days)*

	Age (days)		
		8 to 21 days	
		Digestible lysine levels (%)	
	1 to 7 days	1.016	1.265
Ingredients			
Corn (7.88%)	53.031	59.207	60.176
Soybean meal (45%)	39.810	29.888	28.637
Meat and bone meal (46%)	–	4.924	4.961
Corn gluten meal (60%)	–	2.200	2.200
Soybean oil	3.030	2.589	2.214
Dicalcium phosphate	1.787	–	–
Limestone	1.217	0.384	0.379
Sodium chloride	0.507	0.435	0.435
DL-Methionine (99%)	0.299	0.177	0.370
L-Lysine HCl (78%)	0.126	0.046	0.325
L-Threonine (98%)	0.043	–	0.154
Vitamin supplement[1]	0.100	0.100	0.100
Mineral supplement[2]	0.050	0.050	0.050
Chemical composition			
CP (%)	22.500	20.800	20.800
Metabolizable energy (kcal/kg)	2980	3050	3050
Fat (%)	5.627	5.934	5.579
Crude fiber (%)	3.027	2.780	2.730
Ash (%)	2.994	2.620	2.560
Calcium (%)	1.000	0.880	0.880
Available phosphorus (%)	0.450	0.393	0.393
Digestible arginine (%)	1.442	1.297	1.262
Digestible glycine + serine (%)	1.899	1.904	1.863
Digestible isoleucine (%)	0.892	0.767	0.746
Digestible lysine (%)	1.250	1.016	1.265
Digestible methionine (%)	0.595	0.456	0.642
Digestible methionine + cysteine (%)	0.900	0.731	0.911
Digestible threonine (%)	0.810	0.680	0.822
Digestible tryptophan (%)	0.257	0.212	0.205
Digestible valine (%)	0.959	0.869	0.848
Potassium (%)	0.882	0.778	0.758
Sodium (%)	0.220	0.218	0.218

[1]The vitamin supplement contained per kg of diet: vitamin A = 7000 IU, vitamin D_3 = 2500 IU, vitamin E = 18 IU, vitamin K_3 = 1 mg, vitamin B_1 = 1.5 mg, vitamin B_2 = 5.5 mg, vitamin B_6 = 1.6 mg, vitamin B_{12} = 12 μg, pantothenic acid = 10 mg, biotin = 0.05 mg, folic acid = 0.9 mg, nicotinic acid = 32.5 mg.
[2]The mineral supplement contained per kg of diet: iron = 30 mg, manganese = 60 mg, zinc = 50 mg, iodine = 2.5 mg, selenium = 0.25 mg.

Birds were maintained under a continuous light period (24 h/day), with feed and water available *ad libitum*. During the entire experimental period, the temperature and humidity of the broiler house were recorded for every 15 min using Thermo-Hygrometers (model ITR 157; Instrutherm Company, São Paulo, Brazil) placed at three different points in the shed (front, middle and back). The temperature ranged from 22.7°C in the morning to 28.5°C in the afternoon. Relative humidity in the rearing facility ranged from 81.0% in the morning to 68% in the afternoon.

Protein deposition

To determine the body protein at time 0 (8 days), 10 chicks were selected and slaughtered by cervical dislocation. To determine the body protein deposition over the experimental period, seven birds from each treatment with a weight close to the average of the group (±10%) were selected at 21 days. Of the selected birds, three were fasted for ~8 h to empty the digestive tract, and slaughtered by cervical dislocation. The remaining four birds were slaughtered by the same method, without previous feed deprivation, and samples of the *pectoralis major* muscle were collected from these animals. These four birds had their intestinal tract washed with distilled water for removing the intestinal content and were regrouped into the respective treatment for analysis of protein deposition.

Selected birds were slaughtered by cervical dislocation and ground individually with feathers and entrails in a cutter machine for 10 min. After the material was homogenized, a 300-g sample was collected and stored in a freezer at −10°C until analyses. The Kjeldahl method (AOAC, 2000) was employed to determine the protein. Daily body protein deposition values were calculated, considering the difference in the amount of protein deposited in the 8- to 21-day period.

Gene expression

For the evaluation of gene expression, four birds were used per treatment, at 21 days of age. Immediately after the slaughter, a sample of the *pectoralis major* muscle was collected from the center of the left-lateral position and immersed in an RNAholder solution (BioAgency Biotecnologia, São Paulo, SP, Brazil) to stabilize and preserve the mRNA expression of the tissue at the time of collection, kept at 4°C for 24 h, and stored at −20°C until the RNA extraction.

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer recommendations. The RNA concentration was determined in a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was checked in 1% agarose gel stained with 10% ethidium bromide and visualized under UV light. Total RNA was stored at −70°C until its use. All materials were treated by spraying with an RNaseExterminator decontaminant solution (BioAgency Biotecnologia). The complementary DNA was synthesized from the samples of the *pectoralis major* muscle using a SuperScript™ III First-Strand Synthesis SuperMix Kit (Invitrogen, Carlsbad, CA, USA), following the procedures recommended by the manufacturer. Quantitative real-time (qRT) PCR reactions were performed by SYBR Green detection (SYBR Green PCR Master Mix; Applied Biosystems, Carlsbad, CA, USA).

The primers utilized for the amplification reactions for the target genes (*ND1*, *CYTB*, *COX I*, *COX II*, *COX III* and *ATP6*) were obtained from the nucleotide sequences of mRNA of *Gallus gallus* from the Ensembl database (http://www.ensembl.org/Gallus_gallus). These sequences were used to design the primers using PrimerQuest software, available at http://www.idtdna.com/SciTools/Applications/primerQuest, provided by Integrated DNA Technologies Inc. (Coralville, IA, USA). The mRNA levels of these target genes (Table 2) were normalized to the average hydroxymethyl-bilane synthase and hypoxanthine phosphoribosyltransferase 1 levels (ΔC_t), in accordance with Nascimento *et al*. (2015).

Statistical analyses

Performance and protein deposition data were analyzed using the GLM procedure of SAS software (SAS Institute Inc., Cary, NC, USA). When significant effects were detected ($P < 0.05$), means were compared using the Student–Newman–Keuls test. Results are expressed as means and standard errors. Cq data were analyzed statistically using the %QPCR_MIXED SAS® macro statement (https://msu.edu/~steibelj/JP_files/QPCR.html) developed by commands on SAS PROC MIXED, which considers the independent random effects for target and reference genes in each biological replicate (Steibel *et al*., 2009).

Results

Performance and protein deposition

In the evaluation period from 8 to 21 days of age, increasing the level of inclusion of digestible lysine in the diets improved weight gain, feed efficiency and protein deposition ($P < 0.05$) (Table 3). The use of 1.016% digestible lysine provided lower weight gains and feed efficiency when compared with the increasing levels of lysine in the diets. Similar responses were found for feed efficiency and body protein deposition.

Gene expression

Results of relative expression were interpreted by contrasting with pairwise comparison between treatments. The results of the qRT-PCR analysis in the *pectoralis major* muscle of broilers at 21 days of age showed significant differences in gene expression between the dietary treatments. Birds fed diets formulated with 1.099% digestible lysine showed a 3.6 and 3.04 times higher expression of *COX II* ($P < 0.05$) and *COX III* ($P < 0.10$) genes, respectively, than those fed 1.016% digestible lysine (Figure 1). Similarly, birds receiving diets with 1.265% digestible lysine displayed a greater *ND1* gene expression than birds fed 1.099% ($P < 0.01$) and 1.182% ($P < 0.10$) lysine. For the gene from OXPHOS complexes III (*CYTB*) and IV (*COX I*), a significant expression was observed when the diet containing 1.265% lysine was used compared with other dietary lysine levels (Figure 1).

Table 2 *Primer pairs used to analyze gene expression*

Gene names	Gene symbol	Identification	Primer sequence	Annealing temperature (°C)	Amplicon (bp)
NADH dehydrogenase subunit I	*ND1*	ENSGALT00000029102	5′-AGAAGGAGAGTCAGAGCTAGTC 3′-CTTGGGTTCAGGAATAGGACG	62	135
Cytochrome b	*CYTB*	ENSGALT00000029080	5′-TCCTCATCCTCTTCCTAATCCC 3′-TGTTCTACTGGTTGGCTTCC	62	142
Cytochrome c oxidase subunit I	*COX I*	ENSGALT00000029093	5′-GTCCTCATTACTGCCATCCTAC 3′-GGTGTTGGTATAGGATTGGGTC	62	139
Cytochrome c oxidase subunit II	*COX II*	ENSGALT00000029090	5′-GAACCATCCTACCCGCTATTG 3′-TGGTGTCCGATGGCTTTTAG	62	115
Cytochrome c oxidase subunit III	*COX III*	ENSGALT00000029087	5′-AGGATTCTATTTCACAGCCCTACAAG 3′-AGACGCTGTCAGCGATTGAGA	60	71
ATP synthase F0 subunit 6	*ATP6*	ENSGALT00000029088	5′-ACAACCAGCCTACTTATTCGG 3′--GTTAGGGCGGAGATTGATGG	62	140
Hydroxymethyl-bilane synthase	*HMBS*	ENSGALE00000001922	5′-TGACCTGGTAGTTCACTCCTT 3′-TTGCAAATAGCACCAATGGTAAAG	60	75
Hypoxanthine phosphoribosyltransferase 1	*HPRT1*	AJ132697	5′-GCACTATGACTCTACCGACTATTG 3′-CAGTTCTGGGTTGATGAGGTT	60	112

Discussion

In this study, the diets with increasing levels of digestible lysine provided significant increases in weight gain, feed efficiency and body protein deposition, corroborating other studies (Leclercq, 1998; Fatufe *et al.*, 2004; Wijtten *et al.*, 2004). The observed weight gain has the direct participation of muscle growth, which is influenced by the balance of dietary amino acids, more specifically lysine. Another important aspect that increases weight gain in birds is the tissue hydration, as 3 g of water is retained per gram of protein deposited (Leenstra, 1986). In this study, there was an average increase of 19% body water gain in birds fed diets with higher lysine levels (data not shown).

In the experimental period, the body protein deposition of 14.7 g/bird per day observed with the digestible lysine level of 1.265% was 14.3% higher than the lowest level utilized (1.016%). Rostagno *et al.* (2011) recommended 1.174% digestible lysine in the diet for broilers in the period from 8 to 21 days of age, which can explain why lysine deficiency compromises protein deposition, as was observed by other authors (Fatufe *et al.*, 2004; Wijtten *et al.*, 2004). It should be noted that protein deposition depends on the dynamics of the synthesis and degradation processes. In this context, studies suggest that the increased protein deposition with increasing lysine levels in the diet may be a result of the increased synthesis, reduced degradation or changes in both components of the protein turnover (Buttery and Lindsay, 1980; Urdaneta-Rincon and Leeson, 2004). Within the 'ideal protein' concept, lysine undoubtedly promotes muscle growth; however, other amino acids such as leucine, glycine and arginine are essentially important for the protein synthesis, especially given their influence on the expression of mechanistic target of rapamycin, an important signaling pathway that regulates the protein synthesis (Wu *et al.*, 2014).

Broilers, more efficient in feed conversion and weight gain, may show changes in the expression of ETC genes that cause alterations in the use of nutrients (Iqbal *et al.*, 2004 and 2005; Ojano-Dirain *et al.*, 2007). These studies demonstrate that problems in ETC with inefficiency in mitochondrial ATP production are associated with birds with low efficiency in converting nutrients into body components, resulting in low performance. The weight gain and protein deposition observed in this study were related to the alteration in the expression of ETC genes in the *pectoralis major* muscle of broilers. This observation is supported by the lower expression of ETC genes on the reduced level of digestible lysine in the diet, which probably reduced the availability of ATP in the cells.

The *pectoralis major* muscle is made up of glycolytic fibers and has few mitochondria compared with muscles with oxidative fibers; however, it has an ~60% higher ATP content (Pearson and Young, 1989). This ATP is important for both rapid contraction metabolism of the glycolytic fibers and because of the need for protein deposition. Protein deposition is a process that demands a large amount of energy.

Table 3 *Effects of the digestible lysine levels on performance and body protein deposition of male broilers*

	Digestible lysine (%)								
	1.016		1.099		1.182		1.265		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*-value
BWG (g)	863[b]	11.561	873[ab]	14.910	916[a]	16.728	905[ab]	13.633	0.0439
FE (g : g)	0.724[b]	0.007	0.746[ab]	0.008	0.762[ab]	0.013	0.778[a]	0.011	0.0078
PD (g/bird per day)	12.86[b]	0.179	13.49[ab]	0.264	14.07[ab]	0.609	14.70[a]	0.237	0.0093

BWG = BW gain; FE = feed efficiency; PD = protein deposition.
[a,b]Values within a row with different superscript letters differ significantly at $P < 0.05$.

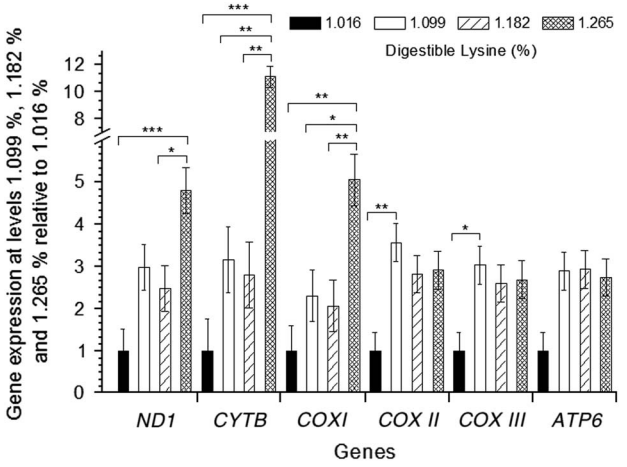

Figure 1 Effect of digestible lysine levels on the relative expression of mitochondrial genes from the *pectoralis major* muscle of broilers in the starter phase. Results of the three highest levels of digestible lysine (1.099%, 1.182% and 1.265%) are presented as a quantification relative to the lowest level (1.016%). Data on the six genes are presented as mean ± *Sy.x*. Significant differences between treatments are represented by asterisks (*$P < 0.10$, **$P < 0.05$, ***$P < 0.01$). *ND1* = NADH dehydrogenase subunit I; *CYTB* = cytochrome b; *COX I* = cytochrome c oxidase subunit I; *COX II* = cytochrome c oxidase subunit II; *COX III* = cytochrome c oxidase subunit III; *ATP6* = ATP synthase F0 subunit 6.

However, protein deposition is not equal to the protein synthesis, but the final result of the synthesis and degradation processes. Considering that four ATPs are required to form a peptide bond, the synthesis of 1 g of protein requires the caloric expenditure of 0.7 kcal (Buttery and Boorman, 1976). This value is lower than the 1.28 kcal/g of protein of synthesis proposed by Aoyagi *et al*. (1988) for broiler chickens, corresponding to 7.52 ATPs per synthesized peptide bond on a molar basis.

The ATP is necessary for the coding and activation of the amino acids as well as for the synthesis of transporter mRNA (Watson *et al*., 2013).

Broilers fed diets containing 1.016% digestible lysine showed reduced expression of genes such as *ND1*, CTYB, *COX I*, *COX II* and *COX III*. In addition, the birds from these groups had the lowest weight gain, feed efficiency and protein deposition, which may demonstrate the relationship existing between gene expression and performance responses. The NADH dehydrogenases are part of complex I, responsible for pumping protons into the inner mitochondrial membrane. The proteins *COX I*, *COX II* and *COX III* that are part of ETC complex IV are responsible for the electron transfer and proton pump, relevant to mitochondrial efficiency. Bottje and Carstens (2009) and Ojano-Dirain *et al*. (2007) observed lower mRNA expression of *COX III* in the pectoral muscle of birds with low feed efficiency compared with more-efficient birds. *CYTB*, as a subunit in the functional center of ETC complex III, may present an expression modified molecularly in broilers with different feed efficiencies. Testing this hypothesis, Tinsley *et al*. (2010) found that *CYTB* was less expressed in the cardiac muscle of the most efficient birds, whereas Iqbal *et al*. (2005) did not observe differences in the expression of this protein in the liver. It can thus be noted that birds with different efficiencies have mitochondrial genes with distinct expressions in different tissues (Bottje *et al*., 2006; Ojano-Dirain *et al*., 2007). ATP synthase consists of two well-defined functional domains – F1 and F0 – which play a direct role in the translocation of protons across the membrane for ATP formation. In our study, no difference was observed in the expression of *ATP6* in the different treatments; however, birds that were fed diets with higher lysine levels had the gene expression increased approximately threefold as compared with those fed 1.016% digestible lysine. According to Jonckheere *et al*. (2012), the mtDNA *ATP6* gene is responsible for encoding the subunit A of the F0 functional domain, which, together with subunit A6L, provides the stabilization of holocomplex V. These authors also stated that the subunit A forms the most important basis for the dimerization of ATP synthases. These observations can explain part of the low performance shown by birds that consumed lower lysine levels.

In conclusion, our findings indicate that broilers fed diets with higher levels of digestible lysine increased the mRNA expression of some genes coded in the mitochondrial ETC (*ND1*, *CYTB*, *COX I*, *COX II* and *COX III*). It may be stated that diets with proper levels of digestible lysine, within the 'ideal protein' concept, promote the expression of genes, which increases the mitochondrial energy, thereby fostering body protein deposition and the performance of broilers in the starter phase.

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References

Aoyagi Y, Tasaki I, Okumura J and Muramatsu T 1988. Energy cost of whole-body protein synthesis measured in vivo in chicks. Comparative Biochemistry and Physiology. A, Comparative Physiology 91, 765–776.

Association of Official Analytical Chemists (AOAC) 2000. Official methods of analysis, vol. 2, 17th edition. AOAC, Arlington, VA, USA.

Bottje WG and Carstens GE 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. Journal of Animal Science 87, 48–63.

Bottje WG, Iqbal M, Tang ZX, Cawthon D, Okimoto R, Wing T and Cooper M 2002. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. Poultry Science 81, 546–555.

Bottje WG, Pumford NR, Ojano-Dirain C, Iqbal M and Lassiter K 2006. Feed efficiency and mitochondrial function. Poultry Science 85, 8–14.

Buttery PJ and Boorman KN 1976. Energetic efficiency of amino acid metabolism. In Protein nutrition and metabolism (ed. DJA Cole, KN Boorman, PJ Buttery, D Lewis, RJ Neale and H Swan), pp. 197–206. Publication European Association Animal Production, London, UK.

Buttery PJ and Lindsay DB 1980. Protein deposition in animals. Butterworth Publishers, Woburn, MA, USA.

Corzo A, Mejia L, McDaniel CD and Moritz JS 2012. Interactive effects of feed form and dietary lysine on growth responses of commercial broiler chicks. Journal of Applied Poultry Research 21, 70–78.

Dozier WA, Corzo A, Kidd MT, Tillman PB, McMurtry JP and Branton SL 2010. Digestible lysine requirements of male broilers from 28 to 42 days of age. Poultry Science 89, 2173–2182.

Dozier WA and Payne RL 2012. Digestible lysine requirements of female broilers from 1 to 15 days of age. Journal of Applied Poultry Research 21, 348–357.

Fakhraei J, Loutfollahian H, Shivazad M, Chamani M and Hoseini SA 2010. Reevaluation of lysine requirement based on performance responses in broiler breeder hens. African Journal of Agricultural Research 5, 2137–2142.

Fatufe AA, Timmler R and Rodehutscord M 2004. Response to lysine intake in composition of body weight gain and efficiency of lysine utilization of growing male chickens from two genotypes. Poultry Science 83, 1314–1324.

Garcia AR, Batal AB and Baker DH 2006. Variations in the digestible lysine requirement of broiler chickens due to sex, performance parameters, rearing environment, and processing yield characteristics. Poultry Science 85, 498–504.

Guan X, Geng T, Silva P and Smith EJ 2007. Mitochondrial DNA sequence and haplotype variation analysis in the chicken (*Gallus gallus*). Journal of Heredity 98, 723–726.

Hocquette JF, Tesseraud S, Cassar-Malek I, Chilliard Y and Ortigues-Marty I 2007. Responses to nutrients in farm animals: implications for production and quality. Animal 1297–1313.

Iqbal M, Pumford NR, Tang ZX, Lassiter K, Wing T, Cooper M and Bottje W 2004. Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex activity. Poultry Science 83, 474–484.

Iqbal M, Pumford NR, Tang ZX, Lassiter K, Ojano-Dirain C, Wing T, Cooper M and Bottje W 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. Poultry Science 84, 933–941.

Jonckheere AI, Smeitink JA and Rodenburg RJ 2012. Mitochondrial ATP synthase: architecture, function and pathology. Journal of Inherited Metabolic Disease 35, 211–225.

Kidd MT, Kerr BJ, Halpin KM, McWard GW and Quarles CL 1998. Lysine levels in starter and grower-finisher diets affect broiler performance and carcass traits. Journal of Applied Poultry Research 7, 351–358.

Leclercq B 1998. Lysine: specific effects of lysine on broiler production: comparison with threonine and valine. Poultry Science 77, 118–123.

Leenstra FR 1986. Effect of age, sex, genotype and environment on fat deposition in broiler chickens – a review. World's Poultry Science Journal 42, 12–25.

Mannen H, Morimoto M, Oyama K, Mukai F and Tsuji S 2003. Identification of mitochondrial DNA substitutions related to meat quality in Japanese black cattle. Journal of Animal Science 81, 68–73.

Nascimento CS, Barbosa LT, Brito CO, Fernandes RPM, Mann RS, Pinto APG, Oliveira HC, Dodson MV, Guimarães SEF and Duarte MS 2015. Identification of suitable reference genes for real time quantitative polymerase chain reaction assays on pectoralis major muscle in chicken. PLoS ONE 10, e0127935.

Ojano-Dirain C, Toyomizu M, Wing T, Cooper M and Bottje WG 2007. Gene expression in breast muscle and duodenum from low and high feed efficient broilers. Poultry Science 86, 372–381.

Pearson AM and Young RB 1989. Muscle and meat biochemistry. Academic Press, San Diego, CA, USA.

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira Rd, Lopes DC, Ferreira AS, Barreto S and Euclides RF 2011. Tabelas brasileiras para aves e suínos. Universidade Federal de Viçosa, DZO, Viçosa, MG, Brasil.

Steibel JP, Poletto R, Coussens PM and Rosa GJM 2009. A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics 94, 146–152.

Tinsley N, Iqbal M, Pumford NR, Lassiter K, Ojano-Dirain C, Wing T and Bottje W 2010. Investigation of mitochondrial protein expression and oxidation in heart muscle in low and high feed efficient male broilers in a single genetic line. Poultry Science 89, 349–352.

Urdaneta-Rincon M and Leeson S 2004. Muscle (pectoralis major) protein turnover in young broiler chickens fed graded levels of lysine and crude protein. Poultry Science 83, 1897–1903.

Wallace DC 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annual Review of Genetics 39, 359–407.

Wallace DC 2007. Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. Annual Review of Biochemistry 76, 781–821.

Wang JW, Chen W, Kang XT, Huang YQ, Tian YD and Wang YB 2012. Identification of differentially expressed genes induced by energy restriction using annealing control primer system from the liver and adipose tissues of broilers. Poultry Science 91, 972–978.

Watson JD, Baker TA, Bell SP, Gann A, Levine M and Losick M 2013. Molecular biology of the gene. Benjamin-Cummings Publishing Company, New York, NY, USA.

Wijtten PJA, Prak R, Lemme A and Langhout DJ 2004. Effect of different dietary ideal protein concentrations on broiler performance. British Poultry Science 45, 504–511.

Wu G, Bazer FW, Dai Z, Li D, Wang J and Wu Z 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annual Review of Animal Biosciences 2, 387–417.