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RESEARCH ARTICLE

Prolactin receptor gene expression of primary feather length in two broiler chicken strains under two management systems

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ABSTRACT

The study evaluated the prolactin receptor (PRLR)gene expression of primary feather length in Arbor Acres Plus and Marshall chicken strains which were reared under two management systems (without access or with access to pasture) in Nasarawa State, Nigeria in an 8-week trial. Data on weekly primary feather length were collected on selected birds which were tagged for subsequent sex identification. On the last day of the experiment, sixteen muscle tissue samples of both sexes [8 from birds without access to pasture (4 Arbor Acres Plus + 4 Marshall) and 8 birds with access to pasture (4Arbor Acres Plus + 4 Marshall)] were collected for the gene expression analysis. The PRLR gene expression data were subjected to analysis of variance to test the effects of strain, management system and sex as well as their interaction. Although, there were no significant fixed and interaction effects, PRLR was up-regulated in both strains, management systems and sexes due to the positive log2 fold changes (9.75±1.71 to 13.48±1.71). The present findings on PRLR gene expression may guide subsequent studies and management and breeding decisions to improve the performance of broiler chickens.

Keywords: Chicken, housing, feathers, PRLR gene, interaction.

INTRODUCTION

The growth and structure including the moultingpatterns of feathers are very important in the poultry industry (Leeson and Walsh, 2004). This is because of the role of feathers in the physical protection, thermalcontrol, flight and display in courtship of birds (Cangar *et al.*, 2008; Khosravinia, 2009; Moghbeli Damane, *et al.*, 2018; Terrill and Shultz, 2023). Feathers also have influence on economic traits of importancein birds as a result of their contribution to the regulation of the body temperature, thus ensuring thermal comfort (Fotsa *et al.*, 2001; Khosravinia, 2009; Kondo *et al.*, 2018). The primary feathers in broiler chickens, also known as "Primary remiges", are the large and long outermost stiff flight feathers attached to the wings of birds. The evolution of flight feather is key to the evolution of bird with asymmetric shapes of the vanes along the rachis (Kondo *et al.*, 2018).The growth of the feathers begins at around the fifth day of incubation while the keratinisation process is normally completed 2 to 3 days before hatching takes place (Leeson and Walsh, 2004). Feather development occurs rapidly between 3 and 6 weeks of age (Moran, 1981).The phenotype of feather can be influenced by genetics, nutrition, production system, environment (light, temperature, humidity, ventilation, among others), age and sex of birds (Chen *et al.*, 2015; Wecke *et al.*, 2017; Weimer*et al.*, 2018; Vargas *et al.*, 2020; Noubandiguim *et al.*, 2021).

In the poultry industry, the use of primary and secondary feathers' relative length is a reliable cost-effective method to determine the sex of day-old chicks immediately they are hatched (Xie *et al.*, 2013; Derks *et al.*, 2018). In the light of this, there is an increasing interest in early feathering trait due to its association with an increased growth rate (Fotsa *et al.*, 2001; Mahmoud *et al.*, 2018). There are many genes of chickens that are associated with traits of economic importance (Liu *et al.*, 2020). One of such genes is the prolactin receptor (PRLR) (Yakubu and Salako, 2016).

The PRLR is a type I cytokine receptor, that binds prolactin in order to act on target cells, thereby mediating various physiological functions including growth (Liang *et al.*, 2019) .It has been reported that the PRLR expression level is 1.78 times higher in late feathering than in early feathering

chickens (Luo *et al*., 2012). However, Zhao *et al.* (2016) did not find significant difference between the expression levels of PRLR as regards early feathering and late feathering birds. This calls for more information on the association between the PRLR gene and primary feathers in poultry. Therefore, the present study aimed to determine the PRLR gene expression of primary feather length of broiler chickens.

MATERIALS AND METHODS

Study Location

The experiment took place at the Livestock Unit of the Teaching and Research Farm of the Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi (NSUK), Nasarawa State, North Central Nigeria.

Experimental Design

A total of 100 randomly selected day-old chicks comprising equal number of Arbor Acres Plus (Amo Brand) and Marshall Strains were kept indoors without access to pasture. Also, a total of 100 birds of both broiler strains were kept indoor but had access to pasture (*Mucuna pruriens*) from week 5 to week 8. The experiment was a 2x2 factorial arranged in a completely randomized design. Each treatment group was replicated two times with 25 birds per replicate.

Experimental Birds' Management

Each bird was individually tagged with an identification number for subsequent sex identification. The initial weight of each bird that was housed on deep litter was taken. From week 1 to week 4, the birds were raised on starter ration while from week 5 to week 8, they were fed commercially produced broiler finisher ration. The trial lasted eight weeks.

Data Collection

The primary feather length of each selected bird was taken on a weekly basis using a measuring tape.

Birds' Tissue Collection

At the end of the 8-week experiment, a total of sixteen randomly selected birds were slaughtered. They comprised two males and two females of each chicken strain in each management system. Muscle tissue was extracted from each bird. The muscle was then preserved to prevent autolysis and putrefaction before laboratory analysis.

Isolation of Total RNA and Synthesis of cDNA of Prolactin Receptor

Trizol reagent (invitrogen, USA) was used to extract total RNA of PRLR from the muscle tissue. DNase 1 was added to the RNA sample to remove genomic DNA contamination. NanoDrop Spectrophotometer (Thermo fisher scientific, Waltham, MA, USA) was used to determine the quality of RNA. The visualization of the 28S/18S rRNA ratio, after electrophoresis on 1.5% agarose gels was used to assess the integrity of the RNA. The high-capacity cDNA reverse transcription kit (Invitrogen, USA) was used to synthesize cDNA from 1 ug RNA using following the procedure of Hu *et al*. (2017).

Real time qPCR Primers

Primers for the real time qPCR (RT-qPCR) of PRLR including the control (18S rRNA) were obtained from published sequences as indicated in Table 1.

Quantitative PCR (qPCR) Analysis

The reactions of the RT-qPCR were performed on the CFX384TM real-time PCR detection system (BIO-RAD, USA) using SYBR Green master mix. The thermal conditions were: pre-denaturation at 95℃ (5min), 40 cycles of denaturation at 95℃ (15 s), annealing/extension at corresponding temperature (Table 1) of each primer set for 30s (Hu *et al*., 2017) and elongation at 72 °C for 16 s (Zhou *et al*., 1996). The no template and negative controls without reverse transcriptase were also included in all qPCR runs. Melting curve analyses were used to validate specific target for each primer set. Also, the amplicons' identity was verified using sequencing. In order to determine the amplification efficiency of PCR reactions, standard curves were generated using 5-fold serial dilutions of cDNA. The 18S rRNA gene (housekeeping gene) was used as the internal control. The relative levels of mRNA of PRLR were calculated using $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001) and normalized to 18S rRNA. All the results of the qPCR appeared as logarithmic (Log2) fold-differences when compared to an appropriate experimental control.

Statistical analysis

Descriptive Statistics (Mean+S.E.) were computed for the gene expression data using IBM SPSS (2020). The effects of strain, management and sex including their interaction on PRLR gene expression were assessed using analysis of variance (ANOVA) of R (2021) software.

RESULTS

The effects of strain, management system and sex on PRLR expression of feathers in chickens are presented in Figure 1. There were no significant (P>0.05) fixed effects. Also, there were no significant (P>0.05) interaction effects on the log2 fold change values of PRLR gene (Table 2).

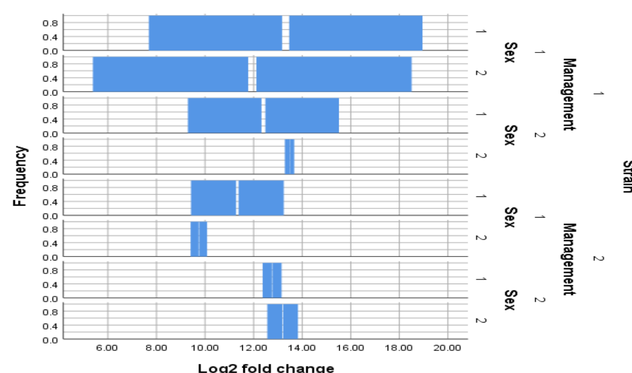

Figure 1. PRLR relative gene expression (Ct values in Log2 fold change) in broiler chickens based on strain, management system and sex. *Strain: 1 (Marshall), 2 (Arbor Acres Plus), Management system: 1 (Without access to pasture), 2 (With access to pasture); Sex: 1 (Male), 2 (Female).*

Table 1. RT-qPCR primer sequences of PRLR gene and control 18S rRNA

Gene	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')	Tm (℃)	Gen Bank Accession No.
PRLR	CCTTCCACCAGTGCTTCAA	AGGAGGCTGACTGTTAGGT	56.4	NM_204854
18S rRNA	TTAAGTCCCTGCCCTTTCTACAC	CGATCCGAGGAACCTCAATAAAC	60.0	AF173612

Source: Hu *et al*. (2017).

Table 2. Effects of strain, management system and sex interaction for PRLR relative gene expression (Log2 fold change values±S.E.M) in broiler chickens

Gene	Marshall				Arbor Acres Plus				*P*- value
	Without Access to Pasture		With Access to Pasture		Without Access to Pasture		With Access to Pasture		
	Male	Female	Male	Female	Female	Female	Male	Female	
PRLR	13.32±1.71	11.95±1.71	12.41±1.71	13.48±1.71	11.33±1.71	9.75±1.71	12.76±1.71	13.20±1.71	0.931

Within a row, means are not significantly (P>0.05) different; S.E.M: standard error of mean

DISCUSSION

PRLR is an important regulator gene for cell growth and differentiation including immune response of early and late feathering chickens (Wilkanowska *et al*., 2014, Mo *et al*., 2022 a and b; Farrar *et al*., 2022). Therefore, its characterization helps to provide insights into the regulatory mechanisms of PRLR expression conserved in birds (Wilkanowska *et al*., 2014). The rate at which the feather grows in chicks, including early-feathering and late-feathering phenotypes is important in the poultry industry as it is widely exploited as a means of sex determination (Derks *et al*., 2018; Liu *et al*., 2020). The PRLR expression, which is widely found in all embryonic and somatic tissues, is higher in late-feathering than in early- feathering chicks (Luo *et al*., 2012). In the present study, PRLR was upregulated in both strains, management systems and sexes due to the positive log2 fold changes. Sex differences in the expression of PRLR gene were not observed in the present study despite the fact that sex-linked phenotypes of late-feathering and early-feathering are controlled by a pair of alleles of K and k^+ in Chromosome Z (Shen *et al*., 2023). It has been reported that feathers on male chicks were shorter when the birds were 10 days old; however, the feathers grew faster than those on female chicks; and after 31 days of age, the feathers of the males were longer feathers than those of their female counterparts (McDougal and Keshavarz, 1984). This was buttressed by the findings of Noubandiguim *et al*. (2021), where the females had longer primary feathers from hatch to 14 days of age, whereas at six weeks of age, the primary feather length was greater in males. Contrastingly, Farrar *et al*. (2022), reported differences in prolactin (PRL) and PRLR expression in the sexes which indicated that gene expression might allow males to compensate for lower prolactin levels by upregulating PRLR in all the tissues. In a related study, Zhao *et al*. (2016) found no significant difference between the expression levels of PRLR in the early feathering and late early feathering chicks.

It was difficult to compare the present findings with the results of earlier researchers due to dearth of information in literature. However, the current observations are in tandem with the report that PRLR regulates feather growth after hatching (Derks *et al*., 2018; Okamura *et al*., 2019).

CONCLUSION

PRLR was upregulated in both strains, management systems and sexes due to the positive log2 fold changes**.** However, fixed effects of strain, management system and sex and their interaction on PRLR gene expression of feather length were not significant. Future studies on the PRLR gene expression of feather length could guide appropriate management and breeding decisions especially during the cold season, where birds that feather faster stand to survive better.

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