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Association of neuropeptide-Y gene polymorphic variants with quantitative traits in Jabalpur colour and Kadaknath chicken

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ABSTRACT

The present study included 60 birds of Kadaknath and Jabalpur colour to explore polymorphism of NPY gene and its association with quantitative traits. PCR-RFLP assay of NPY/*Dra1* gene (240 bp) locus revealed three genotypes, viz. AA (240 bp/240 bp), AB (240/161/79 bp) and BB (161/79 bp) in Kadaknath and Jabalpur colour chicken. Genotype AA (240 bp/240 bp) was predominately found in both the chicken population. The population of Kadaknath was in Hardy Weinberg equilibrium, while Jabalpur colour was not in HWE at NPY gene locus. Analysis of variance showed highly significant differences between breeds, whereas the effect of genotypes and breed × genotype interaction was non-significant. The means of body weight at sexual maturity, body weight at 20, 30 and 40 weeks, egg weight and egg production at 40 week and shape index were significantly higher in Jabalpur colour than Kadaknath. Although the effect of genotypes was non-significant but comparatively higher adult body weight was recorded for AA genotype in Jabalpur colour and AB genotype in Kadaknath at 20, 30 and 40 weeks of age. Similarly, AA genotype was marginally superior in Jabalpur colour, while genotype BB exhibited marginal superiority in Kadaknath for egg production at 40 weeks of age.

Keywords: Chicken, Jabalpul colour, Kadaknath, NPY gene, PCR-RFLP, Polymorphism, Quantitative traits

The native chicken breeds play a crucial role in economy and socio-cultural life of the rural community. They are the good source of cash income to the farmers. The native chicken has an ability to tolerate the harsh environmental condition and poor husbandry practices without much loss in production. In chicken, quantitative traits like egg production and growth are considered to be the most valued economic traits. These traits are affected by the many environment factors, endocrine factors and different feeding. The use of multi traits selection along with marker assisted selection will be more effective for improvement of reproductive and productive traits. Thus, selecting individual bird with additional information on their genotype for markers associated with quantitative trait loci for reproduction, production and fitness is more preferred. Neuropeptide Y (NPY) gene influences the release of gonadotropin releasing hormone that plays a critical role in controlling food intake in birds, timing of puberty, ovulation time and also influence egg production rate (Nguyen *et al.* 2015). It is thought that many of the variations in growth and production traits such as body weight, egg production rate and egg quality are directly or indirectly influenced by some candidate genes such as NPY genes in a variety of poultry species (Xu *et al*. 2011). Thus, in view of the above, the NPY gene has been chosen as a candidate gene to explore the association of NPY gene polymorphic variants with quantitative traits in Jabalpur colour and Kadaknath chicken.

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MATERIALS AND METHODS

Birds (120) comprising 60 each of Jabalpur colour and Kadaknath birds were randomly selected from the flocks of two genetic groups maintained at All India Coordinated Research Project (AICRP) on Poultry Breeding, Department of Poultry Science, N.D.V.S.U., Adhartal, Jabalpur. Blood (2 ml) sample was collected from each bird along with records of quantitative traits (i.e., age at first egg, body weight at sexual maturity, body weight at 20, 30 and 40 weeks, egg production up to 40 weeks of age and egg weight at 40 weeks of age). The egg quality traits of both the genetic groups were studied considering three consecutive day eggs per birds. The shell thickness, shape Index, Haugh unit, egg quality traits were recorded at 40 weeks of age. The genomic DNA was extracted using the method as described by John *et al.* (1991). DNA quality was assessed through 0.8% horizontal submarine agarose gel electrophoresis (Sambrook and Russell 2001). The gene specific primer (Forward primer- 5-TCTCAGAGCTCCAACGTATGA-3; Reverse primer: 5-ATATTTCTGTGCTGAACAACA-3) was used to amplify the region of interest in the NPY gene (Li *et al*. 2009). PCR was carried out in a final volume of

25µl containing 90-100ng DNA templates, 12.5µl 2× PCR Master mix (Fermentas), 1.25 µl of each primer and remaining nuclease free water. The PCR cycling condition were as follows: an initial denaturation at 95ºC for 5 min followed by 36 cycles of denaturation at 95ºC for 50 sec, annealing at 60.5ºC for 50 sec and extension at 72ºC for 1 min and final extension at 72ºC for 10 min. Amplicons were visualized by electrophoresis in 2% agarose gel. The amplified PCR product (240 bp) of each sample was digested with 1 µl of *Dra1* restriction endonuclease in manufacturer's recommended assay buffers in a final reaction volume of 30 µl. It was incubated at 37°C for overnight digestion in water bath. The digested products were resolved on 2.5% agarose gel along with 100 bp DNA ladder as a molecular size marker and then genotyped using Gel documentation system (Gel-Doc, Bio-Rad, USA).

Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version (Yeh *et al.* 1999). The standard method and formulae were used for measurements of different egg quality traits (Singh 1985). The associations of polymorphic variants with quantitative traits were analyzed by following linear model (Harvey 1990).

$$Y_{ijk} = \mu + G_i + B_j + GB_{ij} + e_{ijk}$$

where, Y_{ijk}, economic traits of k^{th} birds of j^{th} breed for i^{th} genotype; µ, overall mean of the trait; G_i, fixed effect associated with the genotypes; B_j, fixed effect of breeds; $(GB)_{ij}$, interaction effects between i^{th} genotype with j^{th} breed and e_{ijk}, residual error.

RESULTS AND DISCUSSION

Genetic polymorphism of NPY gene in chicken: The 240 bp amplicon of NPY gene on digestion with *Dra1* revealed three different patterns viz. AA (240 bp), AB (240, 161 and 79 bp) and BB (161 and 79 bp) in Kadaknath and Jabalpur colour chicken. AA genotype was predominantly found in both the chicken population. Similarly, frequency A allele was estimated to be higher in Jabalpur colour and Kadaknath chicken than B allele (Table 1). The population of Kadaknath was in Hardy Weinberg equilibrium, while Jabalpur colour was not in HWE at NPY gene locus. The RFLP patterns of genotypes obtained in the present study are in accordance with the findings of Alameri *et al*. (2019) in Iraqi local Brown chicken and Chau and Nguyen (2016) Noi native chicken. Similar result of HWE was also reported by Thakur *et al.* (2009) in Kadaknath chicken.

Association of NPY gene polymorphic variants with quantitative traits: The breed-wise least square means of different quantitative traits has been depicted in Table 2.

Age at first egg (AFE): The results of least square analysis of variance showed highly significant (p<0.01) differences among breeds, whereas the effect of genotype and breed × genotype interaction was non-significant. Significantly lower mean AFE (days) was recorded in Jabalpur colour as compared to Kadaknath chicken (Table 2). The present findings indicate that Jabalpur colour birds started laying eggs earlier than Kadaknath chicken with highly significant difference among each other. These results agree with the findings of Xu *et al*. (2011) in Ningdu Sanhuang chicken but disagree with the findings of Chau and Nguyen (2016) in Noi chicken.

Table 1. Frequencies of genotypes and alleles at NPY gene locus

Genotype	Breed	
	Jabalpur Colour	Kadaknath
AA	0.60 (18)	0.47 (14)
AB	0.17 (5)	0.37 (11)
BB	0.23 (7)	0.16 (5)
Allele		
A	0.68	0.65
B	0.32	0.35
Chi-square value	9.80**	4.19 NS

**Significant (p<0.01), NS-Non significant, Figure in parenthesis denotes number of birds

Body weight at sexual maturity (BWSM): Significantly higher mean BWSM (g) was recorded in Jabalpur colour as compared to Kadaknath chicken. The effect of genotype was found to be non-significant, however, AA genotype in Jabalpur colour and AB genotype in Kadaknath chicken revealed affinity towards higher gain in body weight at sexual maturity (Table 2). The present findings are in agreement with the findings of Alameri *et al*. (2019) in Iraqi local Brown chicken.

Adult body weight at 20, 30 and 40 weeks (BW20, BW30, BW40): The highly significant (p<0.01) differences were observed between Jabalpur colour and Kadaknath for mean body weight at 20, 30 and 40 weeks of age, whereas the difference in mean weight of AA, AB and BB genotypes in Jabalpur colour and Kadaknath chicken were found to be non-significant (Table 2). The present findings are in agreement with the findings of Alameri *et al*. (2019) in Iraqi local Brown chicken.

Egg weight (EW40) and egg production (EP40) at 40 weeks of age: Highly significant (p<0.01) differences were observed between least square means in Jabalpur colour and Kadaknath chicken for egg weight and egg production at 40 weeks of age, however the least square means for different genotypes for egg weight at 40 weeks were found to be non-significant. The genotypic least square means egg weights (g) and egg production (Nos.) at 40 weeks were found to be significantly higher in Jabalpur colour than Kadaknath chicken. Although the differences between least square means among various genotypes within breeds were non-significant but comparatively higher egg production was recorded for BB genotype in Kadaknath birds up to 40 weeks of age, while it was almost equal among all the genotypes of Jabalpur colour (Table 2). The present findings are in agreement with the findings of Alameri *et al*. (2019) in Iraqi local Brown chicken and Nguyen *et al*. (2015) in Noi chickens.

Egg quality traits in Kadaknath and Jabalpur colour chicken: The means for various egg quality traits at 40 weeks

Table 2. Least square means for various economics traits at NPY gene locus in Kadaknath and Jabalpur colour chicken

Factor	Age at first egg (days)	Body weight at sexual maturity (g)	Adult body weight at (20 weeks) (g)	Adult body weight at (30 weeks) (g)	Adult body weight at (40 weeks) (g)	Egg weight at 40 weeks (g)	Egg production at 40 weeks (Nos)
Jabalpur colour	153.47^{b}± 0.63	1654.19^{a}± 18.00	1613.13^{a}± 17.26	1825.27^{a}± 16.78	2035.32^{a}± 16.82	58.36^{a}± 0.09	90.50^{a}± 0.69
Kadaknath	163.43^{a}± 0.60	1166.67^{b}± 17.16	1098.93^{b}± 16.46	1313.16^{b}± 16.01	1538.44^{b}± 16.04	47.18^{b}± 0.08	60.47^{b}± 0.66
Genotype							
AA	157.91± 0.53	1414.01± 15.24	1359.13± 14.62	1572.89± 14.21	1784.13± 14.25	52.85± 0.08	74.83± 0.58
AB	159.18± 0.80	1420.73± 23.07	1368.82± 22.13	1580.45± 21.51	1810.09± 21.56	52.88± 0.18	74.61± 0.88
BB	158.00± 0.87	1396.57± 25.05	1340.14± 24.02	1554.29± 23.36	1766.43± 23.41	52.57± 0.13	77.01± 0.96
Breed × Genotype							
Jabalpur colour × AA	153.39^{b}± 0.70	1669.44^{a}± 20.16	1631.11^{a}± 19.33	1837.22^{a}± 18.80	2042.00^{a}± 18.84	58.32^{a}± 0.10	90.67^{a}± 0.77
Jabalpur colour × AB	153.00^{b}± 1.31	1656.00^{a}± 38.26	1614.00^{a}± 36.69	1830.00^{a}± 35.67	2041.11^{a}± 35.75	58.38^{a}± 0.19	90.40^{a}± 1.47
Jabalpur colour × BB	154.00^{b}± 1.13	1637.14^{a}± 32.33	1594.29^{a}± 31.01	1808.57^{a}± 30.15	2022.86^{a}± 30.22	58.39^{a}± 0.16	90.43^{a}± 1.24
Kadaknath × AA	162.43^{a}± 0.79	1158.57^{b}± 22.86	1087.14^{b}± 21.92	1308.57^{b}± 21.32	1527.14^{b}± 21.37	47.38^{b}± 0.11	59.00^{b}± 0.88
Kadaknath × AB	165.36^{a}± 0.90	1185.45^{b}± 25.79	1123.64^{b}± 24.73	1330.91^{b}± 24.05	1578.18^{b}± 24.11	47.39^{b}± 0.13	58.82^{b}± 0.99
Kadaknath × BB	162.00^{a}± 1.33	1156.00^{b}± 38.26	1086.00^{b}± 36.69	1300.00^{b}± 35.67	1510.00^{b}± 35.75	46.76^{b}± 0.19	63.60^{b}± 1.47

Values with different superscript in columns differed significantly ($p<0.05$).

Table 3. Least square means of egg quality traits at NPY gene locus in Kadaknath and Jabalpur colour chicken

Breed/Genotype		Parameter					
		Wight of egg (g)	Shape index	Egg shell thickness (mm)	Albumin index	Yolk index	Haugh unit (HU)
Jabalpur colour genotype	AA	54.64± 0.65 (53.33-55.96)	75.91±0.65 (74.59-77.23)	0.33±0.004 (0.33-0.34)	11.85±0.39 (11.06-12.65)	43.44±0.45 (42.53-44.35)	74.06±0.73 (72.59-75.52)
	AB	55.86±1.24 (53.37-58.36)	74.77±1.24 (72.28-77.27)	0.33±0.007 (0.32-0.34)	11.73±0.75 (10.22-13.24)	43.59±0.86 (41.85-45.32)	75.72±1.38 (72.94-78.49)
	BB	54.17±1.05 (52.06-56.28)	76.00±1.05 (73.89-78.11)	0.33±0.006 (0.32-0.34)	11.04±0.63 (9.77-12.32)	42.15±0.73 (40.69-43.61)	72.78±1.17 (70.43-75.13)
Kadaknath genotype	AA	44.03±0.74 (42.54-45.52)	73.79±0.74 (72.30-75.29)	0.34±0.004 (0.33-0.35)	11.13±0.44 (10.23-12.03)	43.19±0.58 (42.02-44.36)	64.64±0.82 (62.98-66.33)
	AB	44.80±0.83 (43.12-46.49)	74.24±0.84 (72.55-75.92)	0.34±0.005 (0.33-0.35)	11.85±0.51 (10.84-12.87)	43.47±0.52 (42.44-44.50)	65.58±0.93 (63.71-67.46)
	BB	41.52±1.24 (39.02-44.01)	73.56±1.24 (71.07-76.06)	0.34±0.007 (0.32-0.35)	12.60±0.75 (11.09-14.11)	41.63±0.86 (39.89-43.36)	62.47±1.38 (59.69-65.25)
Jabalpur colour		54.89^{a}± 0.58 (53.72–56.07)	75.56^{a}±0.58 (74.39-76.74)	0.33±0.003 (0.32-0.34)	11.54±0.35 (10.83-12.25)	43.06±0.41 (42.24-43.87)	74.19^{a}±0.65 (72.88-75.49)
Kadaknath		43.45^{b}±0.55 (42.33–44.57)	73.86^{b}±0.56 (72.75-74.98)	0.34±0.003 (0.33-0.34)	11.86±0.34 (11.19-12.54)	42.76±0.38 (41.99-43.54)	64.23^{b}±0.62 (62.98-65.48)
Overall genotype	AA	49.34±0.49 (48.34-50.33)	74.85±0.49 (73.86-75.85)	0.34±0.003 (0.33-0.34)	11.49±0.30 (10.89-12.09)	43.45±0.34 (42.76-44.14)	69.34±0.55 (68.24-70.45)
	AB	50.33±0.75 (48.83-51.84)	74.51±0.75 (73.00-76.01)	0.33±0.004 (0.33-0.34)	11.79±0.45 (10.88-12.70)	43.39±0.52 (42.34-44.43)	70.65±0.83 (68.97-72.33)
	BB	47.84±0.81 (46.21-49.48)	74.78±0.81 (73.15-76.42)	0.33±0.004 (0.32-0.34)	11.82±0.49 (10.84-12.81)	41.89±0.56 (40.76-43.02)	67.63±91 (65.81-69.45)

Values with different superscript in column differed significantly ($p<0.05$).

among the two breeds is given in Table 3. Significantly higher egg weight, shape index and Haugh unit were recorded in Jabalpur colour as compared to Kadaknath chicken (Table 3). Although the differences between least square means for egg quality traits among various genotypes within breeds were non-significant but comparatively higher yolk index and Haugh unit were recorded in AB genotype of Jabalpur colour and Kadaknath chicken, while albumin index was found higher in AA genotype of Jabalpur colour and BB genotype of Kadaknath chicken. Similarly, higher shape index was found in BB genotype of Jabalpur colour and AB genotype of Kadaknath chicken (Table 3). In this study, estimates of internal egg quality traits were optimum in Jabalpur colour and Kadaknath chicken. Higher values of Haugh unit indicate that the quality of albumin was good in Jabalpur colour and Kadaknath chicken. The present findings are in agreement as reported by Parmar *et al*. (2006) in Kadaknath chicken. However, Kumar *et al*. (2014) reported higher shape index and egg shell thickness (mm) in RIR (77.28±3.21) and Bovans White (78.43±2.88) chicken under intensive system of management and Rath *et al*. (2015) reported lower mean albumen index in White Leghorn layer (9.98±0.05) under intensive system of management.

On the basis of present findings, it is concluded that, the population of Kadaknath chicken was in HWE, whereas Jabalpur colour population was not in HWE at NPY locus. The mean of AFE, BWSM, BW20, BW30, BW40, EW40, EP40 and shape index were found significantly superior in Jabalpur colour than Kadaknath. Comparatively higher adult body weights at 20, 30 and 40 weeks were recorded for AA genotype in Jabalpur colour and AB genotype in Kadaknath. Similarly, egg production up to 40 weeks was found marginally superior for AA genotype of Jabalpur colour and BB genotype of Kadaknath.

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