Journal of Animal & Plant Sciences, 32(1): 2022, Page: 19-24
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2022.1.0397

ASSOCIATION OF THE PAX7'S 31-BP-INDEL POLYMORPHISM WITH SOME MEAT QUALITY TRAITS IN VIETNAMESE NOI CHICKENS AT 91 DAYS OLD

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ABSTRACT

Pax7 is important for the activation of satellite cells during myogenesis as well as the development of muscle, which can relate to meat quality traits. However, no evidence for this has been reported. In this study, an investigation into the relation between the 31 bp-indel polymorphism of the Pax7 and meat traits was conducted on 355 native Noi broilers. Thus, firstly, three genotypes (EE, EF and FF with frequencies of 0.12, 0.70 and 0.18, respectively) of Noi chickens at this locus were detected based on distinct PCR fragments of agarose gel. Secondly, some traits of meat quality and chemical composition were evaluated on the chicken population. Finally, a correlation analysis among genotypes and the observed traits showed that the 31bp-indel site was significantly associated with (i) the yellowness (b^*) at 3h and 48h post-mortem of beast meat, (ii) the redness (a^*) at 3h and the yellowness (b^*) at 48h post-mortem of thigh meat, and (iii) cooking loss at 48h post-mortem of thigh meat ($p \leq 0.05$). Furthermore, statistically significant differences in the interaction among genotypes and sex for colour traits and pH of breast or thigh meat at different time points as well as the crude protein of breast meat were found ($p \leq 0.05$). In conclusion, the 31 bp-indel of PAX7 gene could be considered as a theoretical basis for further research on the application of the PAX7 gene in Noi chicken breeding programs.

Keywords: genetic variation, meat, Noi chickens, quality traits.

Published first online June 14, 2021 Published final January 07, 2022.

INTRODUCTION

Paired box (PAX) genes encode transcription factors governing the determination of different cell types and even organs in the development of multicellular animals (Vorobyov and Horst, 2006). They are a family of nine regulatory genes (PAX1 to PAX9), playing a crucial role in the formation of skeletal tissues and organs during embryonic development and postnatal muscle growth (Buckingham and Relaix, 2007). While PAX3 is important for the migration of muscle precursors from the somites, PAX7, a paralogue of PAX3, plays a key role in the proliferation and differentiation of muscle fibre as well as regeneration, survival, anti-apoptosis, and self-renewal of satellite cells (Seale *et al.,* 2000; Buckingham and Relaix, 2007, Chang *et al.,* 2011). The absence of both PAX3 and PAX7 will result in the deficit in myogenesis of skeletal muscle, delaying embryonic and fetal development (Relaix *et al.,* 2005).

In chicken, PAX7 is an early marker for satellite cells during the early stages of post-hatch growth (Halevy *et al.,* 2004) and continually expressed during the postnatal development of chickens (Guobin *et al.,* 2011). Zhang *et al.* (2014) showed that a novel 31-bp indel found in intron 3 of the PAX7 gene (located on chromosome 21 and including 10 exons) had a negative effect on chicken growth and carcass traits, and a positive effect on meat quality traits (Zhang *et al.,* 2014). Further findings in male Vietnamese Noi chickens revealed the 31-bp indel in PAX7 that was significantly associated with the feed conversion ratio at the later period (56-84 days of age) (Unpublished). However, the function of PAX7 on the quality of Noi chicken meat has been still missing. The objective of the study was to analyze whether the PAX7 gene's 31bp-indel polymorphisms could be used as a molecular marker for selecting Noi broilers with meat quality traits.

MATERIALS AND METHODS

Animals and sampling: This study was conducted on a resource population of 355 Vietnamese native Noi chickens (164 males and 191 females) raised in cages and fed a commercial diet of 17% crude protein and 3,000 kcal/kg ME from 29-91 days old (Khoa *et al.*, 2019). At 91 days of age, all chickens were slaughtered to evaluate for traits of meat quality. The samples of breast and thigh meat were immediately subjected to chilled storage at 4°C refrigerated conditions for further analysis (Tuoi *et al.*, 2020). Additionally, blood was collected from the wing vein of all chickens at 84 days of age, contained in a 2 mL EDTA tube, and stored at 4°C for DNA extraction later.

Phenotyping: As described by Tuoi *et al*. (2020), (i) the quality traits of breast and thigh meat including pH value (Cömert *et al*., 2016), parameters of surface colour (L^*, a^* and b^*) (C.E.I., 1978), cooking loss (Bertram *et al*., 2003), and drip loss (Guan *et al.*, 2013) were evaluated at three different time points of 3, 24, and 48 h post-mortem. Chemical compositions of breast meat such as dry matter (DM), ether extract (EE) and crude protein (CP) were analyzed (AOAC, 2005).

Genotyping: Genomic DNA was extracted from the collected blood samples by a standard procedure using Proteinase K digestion followed by a phenol-chloroform extraction and a precipitation with ethanol (Ausubel *et al*., 1995).

The primer pair of PAX7.F: 5'-CTTTTTCTCTCTCCCCTTCC-3' and PAX7.R: 5'-CAGACCCTCAGCACAACTCA-3' (Zhang *et al.*, 2014) was used for PCR amplification. PCR was performed in a 20 µl reaction containing 1x PCR Buffer, 1.5 mM $MgCl_2$, 1.25 mM for each dNTPs, 5 pM for each primer, 1U *Taq-polymerase* (Thermo Fisher Scientific), and 100 ng genomic DNA. A thermal cycle was set as follows: an initial denaturation at 94℃ for three minutes followed by 35 cycles of denaturation at 94℃ for 45 seconds, annealing at 57℃ for 45 seconds and an extension at 72℃ for 45 seconds, and an additional extension of 72℃ for 10 minutes. A PCR reaction was carried out on the Veriti™ 96-Well Thermal Cycler (Applied Biosystems). Genotype of 31 bp-indel was determined according to the size of PCR fragment generated (the presence or absence of 31 bp-indel) on 2% agarose gel electrophoresis. The expected lengths of amplicon with and without 31 bp-indel of PAX7 gene corresponding to E and F alleles were 588 bp and 557 bp, respectively.

Analysis: In this study, (i) genotypic and allelic frequencies were determined in Hardy Weinberg equilibrium by using Chi-square test; and (ii) the General Linear Model (Minitab ver. 16.0) was used to analyze differences among the PAX7 genotypes and phenotypic traits of meat quality as follows: $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha*\beta)_{ij} + \varepsilon_{ijk}$, whereas y_{ijk} is the dependent variable, μ is the overall population mean, α_I is the fixed effect of sexes (i=1-2), β_j is the fixed effect of genotypes (i=1-3), $(\alpha*\beta)_{ij}$ is the fixed effect of sex and genotype interaction, and ε_{ijk} is the random error.

RESULTS AND DISCUSSION

Genotypic and allelic frequency: The obtained data in Table 1 indicated that all genotypes EE, EF and FF were detected with different frequencies in the Noi population as well as within either males or females. The heterozygous genotypes EF (0.70) had the highest frequency, while the homozygous ones EE (0.12) was lowest. The allelic frequencies E and F were 0.47 and 0.53 in population, 0.45 and 0.55 in males, as well as 0.49 and 0.51 in females, respectively. These frequencies significantly deviated from Hardy-Weinberg equilibrium ($p \leq 0.05$). Zhang *et al*. (2014) reported that the EF genotype was the dominant genotype and the E allelic frequency (0.51-0.55) was a little higher than the F allelic frequency (0.45-0.49) in an F2 Gushi ×Anka broiler.

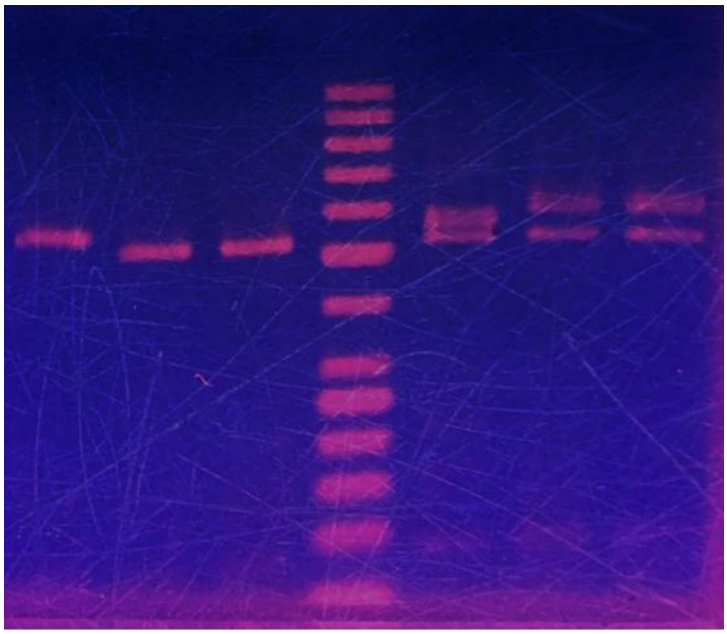

Figure 1: A representative pattern of various PAX7 genotypes (from the left side, well 1: EE genotype, 31bp inserted; well 2-3: FF genotype, 31bp-deleted; well 4: DNA ladder; well 5-7: EF genotypes)

Table 1: Genotypic and allelic frequency.

Locus	Observed population					Expected population			HWE χ^2
	Genotype			Allele		Genotype			
Population									
PAX7-31bp	EE (43)	EF (247)	FF (65)	E	F	EE	EF	FF	55.93
(n=355)	0.12	0.70	0.18	0.47	0.53	0.22	0.50	0.28	≤0.00001[S]
Male									
PAX7-31bp	EE (19)	EF (109)	FF (36)	E	F	EE	EF	FF	19.37
(n=164)	0.12	0.66	0.22	0.45	0.55	0.20	0.49	0.30	≤0.0001[S]
Female									
PAX7-31bp	EE (24)	EF (138)	FF (29)	E	F	EE	EF	FF	37.99
(n=191)	0.13	0.72	0.15	0.49	0.51	0.24	0.50	0.26	≤0.0001[S]

[s]: *The results are significantly different from HWE at p≤0.05.*

Association study: The results in Table 2 showed no significant association between the 31-bp-idel polymorphism and the values of pH or drip loss (p>0.05). However, the significant differences of this polymorphism on some colour traits such as the yellowness (b^*) at 3 h and 48 h post-mortem for of beast meat samples (p≤0.05), the redness (a^*), and the yellowness (b^*) of thigh meat after slaughtering at 3 h and 48 h time points, respectively were found. At the same time points, the lightness (L^*) of breast meat was always higher than that of thigh meat in three different genotypes, while the redness (a^*) were in contrast to two other kinds of meat. It was probably due to the higher content of heme pigment and a higher proportion of red muscle fibres which were known to be high in myoglobin concentrations, while the breast meat was almost composed of white fibres (Barbut, 2001; Wideman *et al.,* 2016).

Especially, the yellowness (b^*) had many fluctuations in the same genotypes at various observed time points. Furthermore, the 31bp-idel showed a significant association with the cooking loss of thigh meat at the 48-h post-mortem where the chickens with the genotype EE (31.72%) had the highest value followed by the genotype EE (31.33%) and the FF (31.21%). Compared with the homozygous genotypes, chickens with the EE genotype displayed the observed values higher than those with the FF one, mostly and generally.

Table 2: Effects of genotypes on the quality traits of breast and thigh meat.

Traits	Genotypes			SEM	P
	EE (n=43)	**EF** (n=247)	**FF** (n=65)		
Breast meat					
3h post-mortem					
pH value	5.65	5.62	5.64	0.02	0.490
L^*	56.64	57.61	57.11	0.52	0.374
a^*	1.22	1.05	1.12	0.24	0.883
b^*	12.25[a]	12.56[ab]	11.47[b]	0.36	0.049
Cooking loss (%)	26.14	25.42	25.08	0.81	0.748
Drip loss (%)	2.73	2.66	2.85	0.12	0.437
24h post-mortem					
pH value	5.56	5.56	5.58	0.02	0.517
L^*	57.14	57.21	57.45	0.51	0.913
a^*	1.64	1.45	1.55	0.26	0.855
b^*	11.50	11.54	10.79	0.38	0.281
Cooking loss (%)	29.60	30.48	31.34	0.99	0.590
Drip loss (%)	2.12	2.06	2.01	0.10	0.827
48h post-mortem					
pH value	5.55	5.54	5.56	0.02	0.746
L^*	56.24	56.70	57.34	0.46	0.360
a^*	2.20	1.82	1.37	0.29	0.241
b^*	12.28[a]	12.32[ab]	11.24[b]	0.36	0.050
Cooking loss (%)	28.54	30.78	30.69	0.94	0.256
Drip loss (%)	1.44	1.63	1.69	0.10	0.345

Thigh meat					
3h post-mortem					
pH value	5.93	5.95	5.97	0.02	0.547
L^*	56.41	56.49	55.59	0.50	0.350
a^*	4.86^{ab}	5.31^{a}	4.29^{b}	0.30	0.021
b^*	11.58	10.94	10.27	0.48	0.275
Cooking loss (%)	28.08	28.07	28.73	0.81	0.802
Drip loss (%)	2.74	2.63	2.75	0.17	0.799
24h post-mortem					
pH value	5.82	5.83	5.82	0.03	0.879
L^*	55.94	56.30	55.73	0.45	0.566
a^*	5.27	5.46	4.80	0.34	0.296
b^*	11.07	10.71	11.21	0.49	0.676
Cooking loss (%)	30.88	32.45	30.45	1.02	0.214
Drip loss (%)	2.46	2.36	2.21	0.11	0.418
48h post-mortem					
pH value	5.80	5.82	5.79	0.03	0.499
L^*	55.16	56.15	55.53	0.47	0.259
a^*	5.94	6.76	5.66	0.49	0.125
b^*	13.26^{a}	12.65^{a}	10.39^{b}	0.62	0.006
Cooking loss (%)	31.72	31.33	31.21	0.91	0.006
Drip loss (%)	1.86	2.09	1.95	0.11	0.289

Mean values in the same row having different superscripts are significantly different ($p \leq 0.05$). L^*= lightness, a^* = redness, b^* = yellowness.

In Table 3, (i) for breast meat, there was a statistically significant interaction between the genotype and sex on L^* and b^* at 3h, pH at 24h and 48h, b^* at 24h and 48h post-mortem ($p \leq 0.05$); (ii) for thigh meat, the interaction between the genotype and sex on b^* at 48h post-mortem ($p \leq 0.05$) was found. The level of mRNA for PAX7 was dependent on the type and size of the muscle, which was particularly higher in breast muscle (Ropka-Molik *et al.,* 2011; Zhang *et al.,* 2014). The interaction results between the genotype and sex of breast meat in the present study confirmed this view. However, these differences were not transparently identified between genders. According to (Wideman *et al.,* 2016), the colour of breast and thigh seemed to be independent of the chicken's sex and might be associated with their age.

Table 3: Effects of sex and genotype interaction on the quality traits of breast and thigh meat.

Traits	Genotypes						SEM	P
	EE		EF		FF			
	Male (n=19)	Female (n=24)	Male (n=109)	Female (n=138)	Male (n=36)	Female (n=29)		
Breast meat								
3h post-mortem								
pH value	5.68	5.61	5.64	5.60	5.63	5.65	0.03	0.211
L^*	57.41^{ab}	56.03^{ab}	58.40^{a}	56.99^{b}	56.08^{a}	58.35^{ab}	0.73	0.027
a^*	1.28	1.17	0.84	1.22	1.58	0.57	0.34	0.310
b^*	11.32^{ab}	12.98^{ab}	11.72^{a}	13.22^{b}	11.09^{a}	11.91^{ab}	0.50	0.000
Cooking loss (%)	27.95	24.70	25.12	25.66	26.80	23.00	1.14	0.170
Drip loss (%)	2.90	2.59	2.68	2.64	2.67	3.07	0.18	0.455
24h post-mortem								
pH value	5.60^{ab}	5.53^{ab}	5.60^{a}	5.52^{b}	5.60^{ab}	5.56^{ab}	0.02	0.004
L^*	58.33	56.20	57.78	56.75	56.89	58.15	0.72	0.211
a^*	1.27	1.92	1.31	1.55	1.87	1.16	0.37	0.637
b^*	10.33^{ab}	12.43^{ab}	10.80^{a}	12.12^{b}	10.18^{a}	11.56^{ab}	0.53	0.002
Cooking loss (%)	29.22	29.90	30.56	30.41	31.62	30.98	1.41	0.943
Drip loss (%)	2.24	2.02	2.05	2.06	1.87	2.17	0.15	0.742
48h post-mortem								

pH value	5.59^{ab}	5.51^{ab}	5.57^{a}	5.52^{b}	5.59^{ab}	5.52^{ab}	0.02	0.008
L*	57.36	55.35	56.74	56.66	56.46	58.42	0.66	0.141
a*	1.62	2.66	1.82	1.81	1.74	0.91	0.42	0.285
b*	11.51^{ab}	12.88^{ab}	11.56^{a}	12.92^{b}	10.30^{a}	12.39^{ab}	0.50	0.000
Cooking loss (%)	29.44	27.83	30.38	31.09	31.38	29.84	1.33	0.534
Drip loss (%)	1.51	1.38	1.53	1.70	1.50	1.94	0.15	0.150
Thigh meat								
3h post-mortem								
pH value	5.91	5.95	5.95	5.94	6.01	5.92	0.03	0.388
L*	57.40	55.60	56.82	56.24	54.92	56.49	0.72	0.231
a*	5.38	4.43	5.24	5.37	4.25	4.34	0.43	0.103
b*	11.24	11.86	10.54	11.25	9.92	10.73	0.68	0.406
Cooking loss (%)	28.77	27.51	27.62	28.43	29.92	27.14	1.15	0.555
Drip loss (%)	2.76	2.73	2.63	2.63	2.54	3.04	0.24	0.818
24h post-mortem								
pH value	5.82	5.83	5.85	5.82	5.78	5.86	0.04	0.755
L*	56.81	55.27	56.82	55.92	55.62	55.86	0.64	0.330
a*	4.88	5.57	5.34	5.55	4.38	5.26	0.48	0.459
b*	10.65	11.39	10.63	10.77	10.36	12.12	0.69	0.459
Cooking loss (%)	28.58	32.66	32.43	32.47	28.47	32.59	1.44	0.120
Drip loss (%)	2.52	2.41	2.32	2.40	2.35	2.07	0.16	0.637
48h post-mortem								
pH value	5.85	5.76	5.86	5.79	5.81	5.77	5.81	0.146
L*	56.34	54.48	56.23	56.08	55.17	55.97	55.71	0.377
a*	5.65	6.10	6.90	6.66	5.52	5.84	6.11	0.475
b*	11.91^{ab}	14.05^{a}	12.66^{a}	12.64^{a}	9.00^{a}	12.09^{b}	12.06^{ab}	0.005
Cooking loss (%)	32.64	31.19	30.10	32.27	31.29	31.10	31.43	0.428
Drip loss (%)	1.99	1.79	2.00	2.16	1.79	2.15	1.98	0.267

Mean values in the same row having different superscripts are significantly different (p≤0.05). L*= lightness, a* = redness, b* = yellowness.

The dominance effect of 31bp-indel of the PAX7 gene was unclear for the chemical compositions (CM, CP and EE) of breast meat (p>0.05), although chickens carrying the EE genotype showed the higher values of these traits (Table 4). Results in Table 4 showed that the female chickens carrying the EE genotype showed a higher value of CP content than the male ones (24.53 and 23.22, respectively).

Table 4: Effects of genotypes on the chemical composition of breast meat.

Traits	Genotypes			SEM	P
	EE (n=43)	**EF** (n=247)	**FF** (n=65)		
DM (%)	25.22	25.13	25.03	0.13	0.682
CP (%)	23.95	23.86	23.77	0.13	0.695
EE (%)	0.54	0.52	0.53	0.03	0.858

Table 5: Effects of sex and genotype interaction on the chemical composition of breast meat.

Traits	Genotypes						SEM	P
	EE		EF		FF			
	Male (n=19)	**Female** (n=24)	**Male** (n=109)	**Female** (n=138)	**Male** (n=36)	**Female** (n=29)		
DM (%)	24.70	25.63	25.10	25.16	25.07	24.98	0.18	0.139
CP (%)	23.22^{b}	24.53^{a}	23.92^{ab}	23.82^{b}	23.62^{b}	23.95^{ab}	0.18	0.004
EE (%)	0.51	0.56	0.53	0.51	0.54	0.52	0.04	0.946

^{ab}*Mean values in the same row having different superscripts are significantly different (p≤0.05).*

In conclusion, significant differences of the 31bp-indel of the PAX7 gene with some colour traits of meat, especially breast meat, were found in the Vietnamese Noi chicken population. These results could be a foundation for further studies on molecular markers in poultry breeding programs, especially Noi chickens as well as other Vietnamese local chicken breeds for traits of meat quality.

Acknowledgements: This study is funded in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan.

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