Journal of Animal and Veterinary Advances 7 (12): 1624-1627, 2008
ISSN: 1680-5593

Mutation in Exon 4 of apoVLDL-II Gene is a Candidate for Meat Tenderness in Chicken

[1]Wu Sheng-Long, [2]H.H. Musa, [1]Bao Wen-Bin, [3]Wang Ke-Hua,
[2]Zhu Guo-Qian and [1]Chen Guo-Hong
[1]College of Animal Science and Technology,
[2]College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
[3]Poultry Institute, Chinese Academy of Agricultural Science, Yangzhou 225003, China

Abstract: The aim of this experiment was to investigate the effect of mutation in apoVLDL-II (apo very low density lipoprotein II) gene on meat quality traits of genetically fat (Anka) and lean (Rugao) chicken breeds. Meat quality traits such as Water Holding Capacity (WHC), pH, Color Density (CD) and tenderness (Shear value (kg cm^{-2})) were analyzed from breast muscle. Polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) technique was developed to analyze a 280 bp region of the apoVLDL-II gene (exon 4). The average gene frequency of two breeds estimated was found to be 0.076±0.008 for allele A and 0.932±0.008 for allele B. The effective number of allele, gene diversity and Shannon's Information index were 1.1642±0.0195, 0.1408±0.0144 and 0.2691±0.0212, respectively. Mutation in the apoVLDL-II gene was found to significantly ($p<0.05$) affect meat tenderness, whereas no significant effect was observed on OD, pH and WHC.

Key words: Mutation, apoVLDL-II gene, meat tenderness

INTRODUCTION

Marker-assisted selection is the process of using the results of DNA testing to assist in the selection of individuals to become parents in the next generation. Marker-assisted selection is a combined product of traditional genetics and molecular biology. MAS allows for the selection of genes that control traits of interest. Combined with traditional selection techniques, MAS has become a valuable tool in selecting organisms for traits of interest, such as color, meat quality, or disease resistance. There are two types of molecular markers to find the genes of interest: Linked and Direct.

Important traits for meat quality that may benefit from MAS are meat pH, marbling and tenderness and many SNPs have been described for each of these. WHC, pH, CD and tenderness, usually determined in breast muscles, are crucial for the culinary value and technological properties of chicken meat and have been previously investigated. According to McIlveen and Buchanan (2001), flavor, tenderness, appearance and juiciness appear to be the most important determinants of sensory enjoyment for the UK consumer. Many factors affect the quality of meat, including the way animals are fed, managed, slaughtered and carcass handling, both pre- and post-slaughter (Mullen *et al*., 2006). Enfalt *et al*. (1997) suggested that the lower pH found in outdoor reared pigs could be the consequence of better capacity to utilize substrates other than glycogen during transport to the slaughter house. It could be suggested that genetic strain has a role in the improvement of customer appraisal of poultry meat (Abeni and Bergogoglio, 2001). Nishimura *et al*. (1999) reported that intramuscular fat in Longissimus muscle may physically alter the connective tissue structure and thereby reduce toughness of the meat. Although, intramuscular fat plays an important role in broiler meat quality flavor and juiciness (Chizzolini *et al*., 1999). In addition, Touraille *et al*. (1991) observed that tenderness was high in all cases but decreased with age which is in contrast to Sonaiya *et al*. (1990), who found no difference due to age. There are several approaches to identifying markers for MAS. One of them is a candidate gene approach which begins with an examination of the physiological pathways underlying the trait of interest. Recently, we used apoVLDL-II as a candidate gene for meat quality; this gene is involved in lipid transportation in chicken. Therefore, our aim was to investigate the effect of mutation in the apoVLDL-II gene on meat quality traits in chicken.

MATERIALS AND METHODS

Experimental animals: Anka as fat chicken and Rugao as lean chicken provided by the Jiangsu Poultry Institute, Yangzhou, China were used in this study. Chickens were reared in the same management system, namely 118 of the

Corresponding Author: G.H. Chen, College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China

birds were killed and carcasses were dissected manually. Meat quality traits such as Water Holding Capacity (WHC), pH, Colour Density (CD) and tenderness Shear value (kg cm^{-2}) were analyzed from breast muscle according to Castellini *et al*. (2002).

DNA extraction and primers design: DNA was isolated from whole blood collected using the saturated salt method previously described Sambrook *et al*. (2001). The following forward primer: 5' ACT GCC TAT TCC TGC CTT CT 3' and Reverse primer: 5' CAC CGA CTT TTC TTC CAA CT 3', were designed using the Oligo 6.0 program to amplify a 280 bp fragment of the apoVLDL-II gene according to the chicken genomic sequence in GenBank (Accession number: J00810).

PCR-SSCP: PCR-SSCP analysis was carried out in total volume 20 μL using 100 ng of template DNA as follows: 13.3 μL of sterilized distilled water, 0.5 μL (5 pmol) each of forward and reverse primer, 1.5 μL 10×PCR Buffer (Mg^{2+}), 2.5 μL of 2.5 mM dNTP mixture and (5 U uL^{-1}) of Taq polymerase (TakaRa Biotechnology Dalian Co., Ltd.). The PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec and a final extension of 72°C for 8 min. The PCR product was mixed with 5 μL of loading buffer, denatured at 98°C for 10 min and then quickly place in an ice box and incubated for 5 min at -20°C. Exactly 10 μL of PCR product was loaded onto a 12% (39:1) polyacrylamide gel and run at 150 V for 9 h and silver stained.

Statistical analysis: The relationship between meat quality and the apoVLDL-II genotype (Exon 4) was determined using the General Linear Model (GLM) performed by the SPSS version 11.5 software and significant difference obtained ($p<0.05$). The genetic parameters of gene frequency, effective number of alleles, gene diversity and Shannon's information index were estimated using the POPGEN program, version 1.31.

RESULTS

A 280 bp region of the apoVLDL-II gene (exon 4) was analyzed by PCR-SSCP. Two genotypes were observed (homozygous AA and heterozygous AB) in genetically fat and lean chickens (Fig. 1). The distribution of apoVLDL-II genotypes for each breed was estimated by direct account, the B allele was found to be highly predominant compared to the A allele. In addition, gene frequency, effective number of alleles, gene diversity and Shannon's information index were estimated (Table 1). The average gene frequency of the 2 breeds is 0.076±0.008 for the A allele and 0.932±0.008 for the B allele. The effective number of allele, gene diversity and Shannon's Information index were 1.1642±0.0195, 0.1408±0.0144 and 0.2691±0.0212, respectively (Table 1). The apoVLDL-II gene was found highly diverse in fat chicken (Anka) when compared to the lean chicken (Rugao).

Table 1: Genetic parameters at the exon 4 region of the apoVLDL-II gene in chicken breeds

Populations	No.	A[1]	B[2]	na[3]	ne[4]	h[5]	I[6]
Anka	59	0.0847	0.9153	2	1.1836	0.1551	0.2902
Rugao	59	0.0678	0.9322	2	1.1447	0.1264	0.2479
Mean	118	0.0763	0.9238		1.1642	0.1408	0.2691
S.D.		±0.0080	±0.0080		±0.0195	±0.0144	±0.0212

[1,2]Gene frequency; [3]Observed number of alleles; [4]Effective number of alleles; [5]Nei's (1973) gene diversity; [6]Shannon's Information index

Table 2: The effect of polymorphism in the exon4 region of the apoVLDL-II gene on meat quality

Parameters	No.	OD[1]	pH	WHC (%)[2]	SHV (kg cm^{-2})[3]
Anka breed					
AA	5	0.62±0.23	5.68±0.10	0.29±0.03	3.67±0.69
AB	54	0.65±0.35	5.72±0.11	0.33±0.04	3.25±0.68
Rugao breed					
AA	4	0.44±0.21	5.75±0.05	0.36±0.04	3.22±0.64
AB	55	0.65±0.28	5.67±0.08	0.32±0.05	2.59±0.63
All breeds					
AA	9	0.54±0.23	5.71±0.08	0.32±0.05	3.47±0.67[a]
AB	109	0.75±0.33	5.69±0.96	0.32±0.05	2.92±0.73[b]

[1]Colour density; [2]Water holding capacity (%); [3]Shear value (kg cm^{-2}); Means in a column that are followed by the different letter are significant at ($p<0.05$)

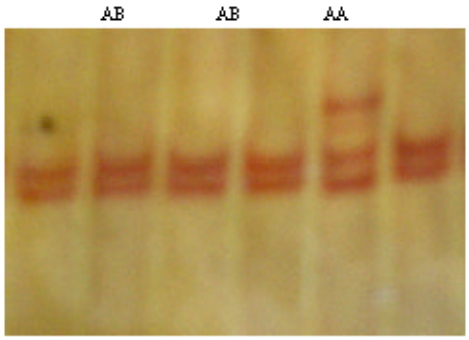

Fig. 1: PCR-SSCP analysis of the mutation in the exon 4 region of the apoVLDL-II gene in genetically fat and lean chicken

This study also aims to investigate the effect of the apoVLDL-II gene on carcass quality of the genetically fat and lean chicken (Table 2). The results indicate that the breed has no significant effect on meat quality. However, mutation in the apoVLDL-II genotype significantly ($p<0.05$) affects meat tenderness, but does not significantly affect CD, pH and WHC (Table 2).

DISCUSSION

The difference in allele frequency in this study may be due to differences in breeding regime associated with meat quality (Zhang *et al*., 2002). Genetic diversity is the important measure of genetic variation in any population, in this study apoVLDL-II gene was highly diverse in fat compared with lean chicken. This is in comparison to other methods to measure genetic variability such as using microsatellites. According to Romanov and Weigend (2001), they reported heterozygosity above 0.6 in chickens using microsatellite techniques which was higher than our result generated by using PCR-SSCP techniques.

Poultry meat is one of the leanest meats and it is important to keep up with consumer demand. Therefore, different methods have been used to reduce fatness in poultry. In this study, we indicate that breed has no significant effect on breast muscle quality. Touraille *et al*. (1991) report that age is the most significant factor of variation in the thigh and breast muscles. Chembers *et al*. (1989) report that tenderness and juiciness decreased between 9 and 16 weeks whereas the flavor increased. In France, older birds had a higher overall acceptability mainly explained by their greater flavor (Touraille *et al*., 1991). In contrast, Yamashita *et al*. (1976) reported that Japanese consumers preferred meat from young chickens because they were tenderer and judged to have more flavor. Juiciness, flavor and selection against fatness of the meat, were associated with decreased tenderness. In addition, Chambers *et al*. (1989) found that carcass fatness had a significant and positive effect on flavor, juiciness and tenderness of dark meat.

In this study, mutation in the apoVLDL-II genotype has significantly ($p<0.05$) affected meat tenderness but does not significantly affect CD, pH and WHC. These results are consistent with Grey *et al*. (1986), who compared lines of turkeys with different growth rates; they concluded that some of them had variations in tenderness that might result from genetic differences among birds. However, Latif *et al*. (1998) indicated that it was due to interaction between genotype and rearing condition. On the other hand, tenderness was observed positively correlated with CD (Barbut, 1996; Le *et al*., 1999).

CONCLUSION

In conclusion, the apoVLDL-II gene was found highly diverse in fat chicken (Anka) when compared to the lean chicken (Rugao) the breed has no significant effect on meat quality. However, mutation in the apoVLDL-II genotype significantly affects meat tenderness, but does not significantly affect CD, pH and WHC.

ACKNOWLEDGEMENT

This study was supported by Chinese National Programs for High Technology Research and Development (863 Project) (No.2006AA10Z1E7).

REFERENCES

Abeni, F. and G. Bergogoglio, 2001. Characterization of different strains of broiler chicken by carcass measurements, chemical and physical parameters and NIRS on breast muscle. Meat Sci., 57: 133-137.

Barbut, S., 1966. Estimates and detection of the PSE problem in young turkey breast meat. Can. J. Anim. Sci., 76: 455-457.

Castellini, C., C. Mugnai and A. DalBosco, 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci., 60: 219-225.

Chambers, J.R., A. Fortin, D.A. Mackie and E. Larmond, 1989. Comparison of sensory properties of meat from broilers of modern stocks and experimental strains differing in growth and fatness. Can. Inst. Food Sci. Technol. J., 22: 353-358.

Chizzolini, R.E., V. Zanardi, G. Dorigoni and S. Ghidini, 1999. Calorific value and cholesterol content of normal and low fat meat and meat products. Trends Food Sci. Technol., 10: 119-128.

Enfalt, A.C., K. Lundstrom, I. Hansson, N. Lundheim and P.E. Nystrom, 1997. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. Meat Sci., 45: 1-15.

Grey, T.C., N.M. Griffiths, J.M. Jones and D.A. Robinson, 1986. Study of some factors influencing the tenderness of turkey breast muscle. Lebensmittel-Wissenschaften und Technologie, 13: 412-414.

Latif, S., E. Dworschak, A. Lugasi, E. Barna, A. Gergely, P. Czuczy, J. Hovari, M. Kontraszti, K. Neslenyi and I. Bodo, 1998. Influence of different genotypes on the meat quality of chicken kept in intensive and extensive farming managements. Acta Alimentario, 27: 63-75.

Le Bihan-Duval E., N. Millet and H. Remignon, 1999. Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. Poult. Sci., 78: 822-826.

Mcllveen, H. and J. Buchanan, 2001. The impact of sensory factors on beef purchase and consumption. Nutr. Food Sci., 31: 286-292.

Mullen, A.M., P.C. Stapleton, D. Corcoran, R.M. Hamill and A. White, 2006. Understanding meat quality through the application of genomic and proteomic approaches. Meat Sci., 74: 3-16.

Nishimura, T., A. Hattori and K. Takahashi, 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. J. Anim. Sci., 77: 93-104.

Romanov, M.N. and S. Weigend, 2001. Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poult. Sci., 80: 1057-1063.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn. Cold Spring Harbor Laboratory, New York, USA, pp: 1112-1125. ISBN: 978-087969577-4.

Sonaiya, E.B., M. Ristic and FW. Klein, 1990. Effect of environmental temperatures, dietary energy, age and sex on broiler carcass portions and palpability. Br. Poult. Sci., 31: 121-128.

Touraille, P.C., J. Kopp, C. Valin and F.H. Richard, 1991. Chicken meat quality 1. Influence of age and growth rate on physico-chemical and sensory characteristics of the meat. Arch. Geflugelkunde, 45: 69-76.

Yamashita, C., Y. Ishimoto, H. Mekada, S. Ebisawa, T. Murai and S. Nonaka, 1976. Studies on the quality of poultry meat. II. Influence of age of chickens on the meat taste. Japanese Poult. Sci., 13: 14-19.

Zhang, X., F.C. Leung, D.K.O. Chan, G. Yang and C. Wu, 2002. Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA and microsatellite polymorphism. Poult. Sci., 8: 1463-1472.