

9

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Carcass Production and Single Nucleotide Polymorphism Adipocyte Fatty Acid Binding Protein (A-Fabp) Gene on *Cairina moschata*

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14

Abstract. The aim of this study was to determine differences in growth, carcass production and identify polymorphisms of adipocyte fatty acid binding protein (A-FABP) gene in Muscovy ducks from the second generation selection (G2). The research material used 180-day-old Muscovy ducks consisting of male and female ducks with white feathers and male and female ducks with a combination of black and white feathers. Measurement of duck body weight was carried out every week, and ducks are slaughtered at 10 weeks to obtain carcass production data. The data obtained were analyzed by systat-13 program based on variance analysis and Duncan test. The primary design was based on a database of the genebank *Cairina moschata* adipocyte fatty acid binding protein (A-FABP) gene, exons 1, 2 and partial cds (FJ763338.1). The primary base sequence of the A-FABP gene was the primary forward: 5'-TCTGGGGGTGTTATCTGGAG -3' and reverse primer: 5'- ATTTGTCAGTGGCTGTGCTG -3'. The sequencing results of PCR products were analyzed using bioedit version 7.7 to determine the presence of the A-FABP gene polymorphism. The results showed that at the same age male Muscovy ducks produced carcass weight, and thickness of breast meat higher than female ducks. Body weight, carcass weight and parts of the carcass (breast, thigh, back, and wings) of a combination black-white feather male ducks higher than the male white feathers. The abdominal fat on all the ducks relatively the same. The A-FABP gene PCR product was at 176 bp. The results of bioedit analysis showed that at 151 bp, base length there was a mutation from Guanin to Adenin in the observed *Cairina moschata*, both male and female Muscovy ducks with white feathers and black-white combinations. All ducks observed had homozygous AA genotypes. Base changes in SNP c. 151G> A indicate a transition mutation. The study concluded that male Muscovy duck with a combination black- white feathers have highest genetic potential in body weight and carcass production with thick meat breast compared to other ducks. The weight of abdominal fat was relatively the same in male and female manila ducks. The A-FABP gene in manila ducks was monomorphic.

Keyword: carcass weight, monomorphic, muscovy duck, thick meat breast



1. Introduction

The genetic diversity of Muscovy ducks in Indonesia can be seen from the variation color of the wing, head, back, tail and abdomen feathers. Most of the Muscovy ducks are white and only a small portion is black. The color of the feather is controlled by the feather color gene, which is the W (white) gene which is dominant to its partner (Gen w). The w gene is recessive to the W gene and causes feather to become colored [1]. At the same age male Muscovy ducks produce body weight gain and relative growth, higher compared to female ducks. The body weight increase for Muscovy ducks is highest at 3 weeks, the growth rate begins to decline at 4 weeks of age [2]. Muscovy duck has an average carcass percentage of 74% [3].

Today, an increase in poultry meat production is balanced with an increase in the quality of meat, one of which is the fat content of meat. One of the studies on fat metabolism and fatty genetic factors is to study the effect of the Adipocit Fatty Acid Binding Protein (A-FABP) gene on fatty and growth. FABP is included in the superfamily of lipid binding proteins and occurs intracellularly in invertebrates and vertebrates [4]. At high levels of tissue fatty acid (FA) metabolism occurs, such as in the intestine, liver, adipose, and muscle, with high FABP levels parallel to the absorption and utilization of FA [5]. Adipocyte FABP (A-FABP) has traditionally been considered a cytosolic FA companion expressed in adipocytes [6]. A-FABP only binds FA long chains with high affinity. This ligand specificity leads to the emergence of a broad hypothesis that A-FABP plays an important role in the storage of triglycerides and their release in cells [7].

The expression of the A-FABP gene and its protein content in Pekin ducks is higher than that of Muscovy ducks in all nutritional conditions, this indicates a higher intracellular transport in fatty acid adipocytes especially those synthesized by the liver [8]. A-FABP acts as a carrier for the transport of intracellular fatty acids and plays an important role in the development of fat properties. The fatty acid binding protein gene is a group of genes associated with abdominal fat levels, where abdominal fat decreases carcass quality. The FABP gene group, in this case is strongly associated with regulating fat deposits, especially abdominal fat. The study of the A-FABP gene SNP on cucumber is important because it is a waterfowl that has the potential to produce meat, while on the other hand it has a high fat content.

In a previous study it was reported that the combination of black and white feathers had a higher growth rate and body weight compared to white feathers [1]. The purpose of this study was to determine the presence of A-FABP gene polymorphisms in Muscovy duck populations with different feather colors and differences in carcass production.

2. Methodology

Experimental design, birds, and management

The research material used 180 day old duck produced from second generation selection of Muscovy ducks. The day old ducks (DOD) consisting of male and female were divided into 4 groups: male white feathered DOD, female white feathered DOD, male black-white feathered DOD, and female black-white combination feathers. Each group was consisted 45 DOD and kept in a 5x4 m² size colony cage. The feed given in adlibitum was measured, as follows: in the initial period (age 1-21 days) ducks were given complete feed with nutrient content, metabolic energy 3300 kcal / kg, crude protein 20.5%, crude fat 5% , crude fiber 5.7%, Ca 0.95% Ca and P 0.8%. Feed for duck on growth period (age 3-12 weeks) was fed with nutrient content: ME 2905.45 (kcal / kg), PK 16.59, crude fat 3.91%, crude fiber 4.36%, Ca 1.817% and P 1.327%. At the age of 10 weeks the duck weighed and sampled 5 ducks in each group, slaughtered to obtain carcass and abdominal fat data.

Blood sample and DNA isolation

Blood sample, 3 ml was taken from venous auxiliaries, put in a tube filled with anticoagulant (ETDA) and stored in the fridge. Deoxyribo Nucleic Acid (DNA) total genome was extracted from blood samples and isolated with DNA Isolation Kit (Geneaid). DNA isolation results were examined using 1% agarose gel electrophoresis.

Primary design and amplifying DNA fragment with PCR

The primary design was based on a database of the genebank *Cairina moschata* adipocyte fatty acid binding protein (A-FABP) gene, exons 1, 2 and partial cds (FJ763338.1). The primary base sequence of the A-FABP gene is primary forward: 5' - TCTGGGGGTGTTATCTGGAG -3' and primary reverse: 5' - ATTTGTCA GTGGCTGTGCTG -3'. Polymerase Chain Reaction (PCR) consists of several steps, namely DNA pre-denaturation at 94°C for 5 minutes, DNA denaturation at 94°C for 30 seconds, annealing at 62°C for 45 second and elongation at 72°C for 1 minute. The final extension was performed at 72°C for 18 minutes. PCR conducted 35 cycles. PCR products are subject to electrophoresis test with 1.5% agarose gel. The PCR products were visualized by using UV light.

DNA sequencing

PCR's product was carried out by Genetics Science-Indonesia-Ltd. Sequencing and nucleotide sequences and electropherograms Nucleotide A (Adenine), black for nucleotide G (Guanine), blue for nucleotide C (Cytosine) and red for nucleotide T (Thymine). Sequence product was read using software Sequence Scanner v1.0, in the form of electrophoresis consisting of nucleotide sequences from A-FABP gene sample of Muscovy ducks.

SNP program genotyping was determined through BioEdit v7.2.0, by aligning sequence products according to the sequence in the GeneBank FJ763338.1 database from the ClustalW menu (an accessory application). Alignment results were seen in electropherogram, to obtain SNPs in a particular position to use for genotyping. The base sequence of the A-FABP gene in Muscovy ducks (*Chairina moschata*) was in 176-bp. The SNP was confirmed based on the electroferogram results and used for genotyping.

Statistical analysis

Differences in carcass production and abdominal fat measurements among groups were evaluated by ANOVA and Duncan multiple range test (DMRT) with Systat version 13 program.

3. Result and Discussion

Genetic diversity based on the adipocit genes fatty acid binding protein

The PCR results show that A-FABP gene from the ducks with white feather or a combination black-white feather was successfully replicated as shown in **Figure 1** with a fraction of the A-FABP gene on 176 bp. PCR products are then sequenced and aligned. The results of *Cairina moschata*'s A-FABP gene alignment obtained Single Nucleotide Polymorphism (SNP) located at 151 bp base length, which showed a mutation from Guanine to Adinin (**Figure 2**). Base changes in SNP c.151G> A indicate a transition mutation. Transition mutations occur because of substitution between one purine base (adenine and guanine) and other purine base or one pyrimidine base (thymine and cytosine) with another pyrimidine base [9].



Figure 1: PCR product Adipose fatty acid binding protein gene on 176 bp

Previous reports showed that mutations in intron as well as silent mutations in coding regions and other polymorphisms without obvious functional relevance were useful for the evaluation of associations with production traits. Hence, the above described SNPs of A-FABP gene allows the incoming association analysis [10]. Adipocyte fatty acid binding protein (A-FABP) is mostly expressed in adipose tissue and has been considered as the most important candidate gene for IMF deposition [11].

The fatty acid binding protein gene is a group of genes associated with abdominal fat levels, where abdominal fat decreases carcass quality. The FABP gene group, in this case is strongly associated with regulating fat deposits, especially abdominal fat. The fatty acid binding protein gene produces a protein that functions to bind fatty acids produced from the process of anabolism/biosynthesis in liver cells, precisely in the cytoplasm. These fatty acids will be secreted into the blood vessels to be deposited in various cells /tissues of the body in need [12].

The results of the sequencing showed that the A-FABP gene was monomorphic by producing one type of genotype, AA for white and a combination black-white feather ducks. The gene is monomorphic, so it does not show heterozygosity in white and a combination black-white feather ducks. Previous studies have shown that with diversity in the FABP genes, with genotypes that have mutations in the FABP genes, it has an impact on increasing abdominal fat deposits and can affect carcass quality as well as livestock growth [13].

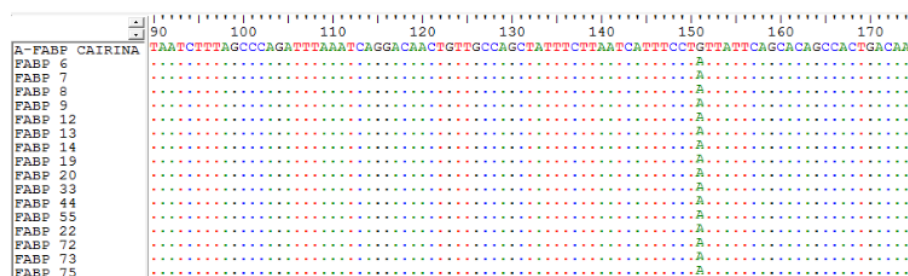


Figure 2. Results of gene A-FABP sequencing on Muscovy duck

Carcass production and abdominal fat in Muscovy duck

Table 1. showed differences in body weight and carcass production in male and female Muscovy ducks based on their feather color, but the weight of abdominal fat is relatively the same. Analysis of

variance shows that male Muscovy ducks have a higher body weight than female ducks ($P < 0.01$). The male Muscovy duck with a combination black and white feather produced the highest carcass production. The parts of the carcass (breast, thigh, back and wings) and breast meat thickness differed significantly between male and female Muscovy ducks based on feather color ($P < 0.01$).

Table 1. Body weight, carcass weight, breast meat thickness and abdominal fat of Muscovy duck (g)

Muscovy ducks	Body weight (g)	Carcass weight (g)	Breast (g)	Thigh (g)	Back (g)	Wings (g)	Meat thick (g)	Abdominal fat (g)
Male white	2404.60 ^b	1480.60 ^b	325.50 ^b	371.00 ^b	485.80 ^b	280.28 ^b	13.06 ^b	19.50
Male black-white	2669.73 ^b	1777.92 ^c	479.98 ^c	401.82 ^c	584.57 ^c	303.84 ^b	14.31 ^c	25.40
Female white	2096.61 ^a	1133.02 ^a	281.58 ^a	301.69 ^b	340.58 ^a	160.90 ^a	12.19 ^b	19.07
Female black-white	1922.14 ^a	1130.44 ^a	324.80 ^b	285.47 ^a	315.92 ^a	165.68 ^a	10.55 ^a	19.80

Note: superkrip different letters in the same column indicate significant difference based DMRT ($P > 0.05$).

The results showed that male and female Muscovy ducks had very different growth rate and body weights, according to previous studies [2]. Muscovy ducks yielded superior values for body weight, weight gain, average feed intake and feed conversion ratio (3903.75, 3659.65, 10744.90 and 2.94, respectively) [14]. Numerous studies have shown that the carcass composition and the meat yield of ducks vary by breed. In this study, male Muscovy ducks with combination black-white feather yielded a higher dressing percentage of 66.59 compared to others. The breast and thigh were a part of the carcass with a higher proportion of meat than the back and wings. This research is in accordance with Wawro et al. [3] who reported the highest values for breast and leg muscle weight were observed in the carcasses of Muscovy males.

Dimorphism appeared to be highly and positively correlated with body weight of males, and positively, but moderately correlated with body weight of females in both bred [2]. Different GH and IGH-I level in male and female ducks caused a higher growth in males than females, or commonly known as sexual dimorphism. Higher body weight gain in male Muscovy is due to dimorphism in Muscovy duck, which that is the different growth rate because of different growth hormone concentration in male and female duck. Growth hormone (GH) level in blood plasma of female Muscovy was highly varied (14-24 ng x ml⁻¹) during the first 4 weeks, while the highest GH plasma in male Muscovy was during the first 7 weeks [15].

An interesting result to note is that abdominal fat in male and female Muscovy ducks with variations in feathers does not show significant differences. Fatness is one of the carcass quality indicators in poultry, this result indicates that Muscovy ducks with high body growth and weight are also followed by accumulation of abdominal fat, so that the weight and percentage of abdominal fat are relatively the same. Different FABP regulate different cellular processes to modulate intracellular fatty acid concentrations. Adipocyte fatty acid binding protein (A-FABP) is expressed in many tissues, especially in adipose tissue, and is associated with lipid metabolic enzymes [11]. The results of the identification of the A-FABP gene SNP showed monomorphic, so there was no difference in fat synthesis, especially abdominal fat.

4. Conclusion

Male Muscovy duck with a combination black- white feathers have highest genetic potential in body weight and carcass production with thick meat breast compared to other ducks. The proportional weight gain is followed by an increase in abdominal fat, so that the weight of abdominal fat is relatively the same in male and female manila ducks. The A-FABP gene in manila ducks is monomorphic.

Acknowledgement

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