

Effect of a Single Nucleotide Polymorphism in the Cholecystokinin Type A Receptor Gene on Growth Traits of the Miyazaki Jitokko Chicken

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The Miyazaki Jitokko chicken, native to the Miyazaki Prefecture in southern Kyushu Island, Japan, is the product of a three-way cross involving the Jitokko, White Plymouth Rock, and Kyushu Rhode breeds. In the present study, associations between a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) of the chicken cholecystokinin type A receptor gene and growth traits in Miyazaki Jitokko chickens were investigated. Unrelated male birds ($n=120$) that had hatched on the same day were raised in the same chicken house and fed the same diet *ad libitum* from day 0 to 17 weeks of age. Body weight was recorded at 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, and 17 weeks and the average daily gain of each interval was calculated from the body weight data. SNP genotyping of each bird was performed using the mismatch amplification mutation assay. The associations between the SNP and growth traits were examined using the Thesias program. The genotype frequencies of AA, AC, and CC were 0.525, 0.383, and 0.092, respectively. AA birds were significantly heavier than CC birds from 4 to 17 weeks of age. In the estimated effect of alleles, body weight from 1 to 17 weeks of age in birds with the A allele was greater than that in birds with the C allele. During the rearing period, the effect of the A allele on average daily gain in the first half was greater than that in the second half. We conclude that the g.420 C>A SNP can be used as a selection marker for the parent stock lines of the Miyazaki Jitokko chickens to increase their growth performance.

Key words: chicken, cholecystokinin type A receptor gene, growth traits, Miyazaki Jitokko chicken, single nucleotide polymorphism

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Introduction

Since Japan is an island nation, there are no truly indigenous chickens. Most of the present Japanese chicken breeds were established from three original breeds—Jidori, Shokoku, and Shamo—introduced at various times from overseas. Jidori, which means native chicken, has retained primitive chicken characteristics and is thought to have been introduced to Japan from China more than 2,000 years ago. Shokoku, which has long hackle and saddle feathers, is thought to have been introduced to Japan from China by Japanese missions to Tang China between the 7th and 9th centuries CE. Shamo is thought to have been derived from a Malay-type chicken introduced to Japan from Thailand between the late-15th and early-17th centuries CE, corresponding to the Nanban trade period for cockfighting. Tokugawa shogunate, the feudal Japanese military government

that ruled between 1603 and 1868, enacted a trade protection policy against foreign countries except for Korea, China, the United Kingdom of the Ryukyu Islands, and the Netherlands from the early-17th to the mid-19th century CE. The policy and social stability in that era had a significant impact on the establishment of Japanese breeds of chicken with special bodily form, plumage, and crowing, as well as for cockfighting. To date, more than 30 distinctive breeds native to Japan have been identified and 17 of the breeds have been designated as natural treasures of Japan (Takahashi *et al.*, 1998).

The Jitokko breed, which was declared a natural treasure in 1943, has been maintained at the foot of Mt. Kirishima, located between Miyazaki and Kagoshima Prefectures in southern Kyushu Island, Japan. The origin of the breed is unclear. The characteristics of the breed are Jidori-type plumage, short legs, large crests, and beard. The short-leg trait in the Jitokko breed is controlled by a dominant lethal gene, Creeper (*Cp*), which is manifested as short legs in heterozygous (*Cp/+*) chickens and embryonic lethality in homozygous (*Cp/Cp*) embryos (Shibuya *et al.*, 1972). Jitokko hobbyists, even in the absence of knowledge on heredity,

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have long selected for and maintained birds with short legs.

In Japan, some native breeds are being used to breed the “Jidori brand” chickens that are defined in the Japanese Agricultural Standard (Ministry of Agriculture, Forestry and Fisheries of Japan, 1999). In the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute (Kawaminami Town, Japan), studies on producing a new Jidori brand of chicken utilizing the Jitokko breed as a founder began in 1985. “Miyazaki Jitokko” is a three-way crossbred chicken produced by crossing F₁ Jitokko sire cocks (*Cp*-free (+/+) with White Plymouth Rock dams) and Kyushu Rhode (a synthetic breed resulting from a cross between Rhode Island Red and White Plymouth Rock) hens. Miyazaki Jitokko chickens have been marketed since 1990, and at present constitute the third largest portion of Jidori brand chickens marketed in Japan.

Miyazaki Jitokko roosters and hens are raised for approximately 120 and 150 days, respectively, while broiler chickens are raised for less than 50 days. Since a practical concern for Miyazaki Jitokko producers is to shorten the rearing period and/or increase the slaughter live weight, an improvement in growth traits is warranted. Recently, a significant association was reported between a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) in the 5'-untranslated region of the cholecystokinin type A receptor gene (*CCKAR*) and growth traits in a native Japanese breed, Hinai-dori (Rikimaru *et al.*, 2013). In the present study, we tested whether the g.420 C>A SNP in *CCKAR* is applicable for improving the growth traits of the Miyazaki Jitokko chicken.

Materials and Methods

Experimental Birds

The research was performed according to the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006), and experimental birds received humane care.

We used 120 unrelated male Miyazaki Jitokko chickens that hatched on the same day and had been raised at the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute (Kawaminami Town, Japan). The birds were raised in solid-floored pens in a conventional poultry research house until 17 weeks (wks) of age. For the heat insulation of chicks, a chick-guard and gas-type brooder (Big-G 1200S, Nakajima Seisakusho Co., Nagano, Japan) were used until 2 wks. The birds were fed a starter diet (ME, 3,000 kcal/kg; CP, 22% [wt/wt]) from 0 to 3 wks, a grower diet (ME, 3,230 kcal/kg; CP, 18%) from 3 to 15 wks, and a finisher diet (ME, 3,230 kcal/kg; CP, 18%) from 15 to 17 wks. These diets and water were offered *ad libitum* for the duration of the experiment.

The body weight (BW) of the birds was measured at day of hatch (0 day) and at 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, and 17 wks of age. Average daily gain (ADG) was computed by dividing weight gain between two intervals by the interval in days. At 17 wks, the birds were sacrificed.

CCKAR Genotyping

Blood was collected in heparinized tubes from the wing vein. The blood was spotted onto an FTA CloneSaver Card (WB120028; GE Healthcare, Buckinghamshire, UK) and left to dry overnight at a room temperature of 25–28°C. Genomic DNA extraction from the FTA card and genotyping of the g.420 C>A SNP in *CCKAR* were performed as previously reported (Rikimaru *et al.*, 2013).

Statistical Analysis

Allele frequencies were calculated by gene counting. Comparisons among genotype groups were performed using Fisher's least-significant-difference test. Differences among the groups were considered significant if $P < 0.05$.

Allele frequencies and allele-based association analysis were computed using the Thesias program that is designed to test the effects in unrelated subjects after adjusting for covariates, and is based on the maximum likelihood model described by Tregouet and Garelle (2007). Differences between the SNPs were considered significant at $P < 0.05$.

The variance explained by haplotype was calculated as variance percentage = $100 \times (1 - R/G)$, where R is the residual variance from the residual standard error and G is the global standard error.

Results

The 120 birds comprised 63 AA, 46 AC, and 11 CC genotypes (Table 1). The genotype frequencies of AA, AC, and CC were 0.525, 0.383, and 0.092, respectively. The distribution of the genotypes in the population did not contradict Hardy-Weinberg equilibrium proportions. The allele frequencies of A and C were 0.717 and 0.283, respectively.

We observed an association between three *CCKAR* genotypes (AA, AC, and CC) and growth traits (Table 1). Concerning BW, a significant difference among the groups was observed from 1 to 17 wks and AA birds tended to be heavier than both AC and CC birds. From 4 to 17 wks, AA birds were significantly heavier than CC birds. Concerning ADG, a significant difference among the groups was observed in the intervals 0 day–1 wk, 1–2 wks, 3–4 wks, 4–5 wks, 5–7 wks, and 13–15 wks. In these intervals, the ADG of AA birds tended to be higher than that of AC and CC birds. When the rearing period was divided into approximately four quarters, i.e., 0 day–5 wks, 5–9 wks, 9–13 wks, and 13–17 wks, the ADG of AA birds was significantly higher than that of AC and CC birds at 0 day–5 wks and 5–9 wks.

The effects of cholecystokinin type A receptor alleles on growth traits in the Miyazaki Jitokko chickens are shown in Table 2. Concerning BW, the A allele was significantly superior to the C allele from 1 to 17 wks. In ADG, the A allele was significantly superior to the C allele in the intervals 0 day–1 wk, 1–2 wks, 3–4 wks, 4–5 wks, and 5–7 wks. In the first and second quarters, the ADG of the A allele was significantly superior to the C allele.

Discussion

Cholecystokinin (CCK) is a gut peptide that has been implicated in the control of appetite (Gibbs *et al.*, 1973).

Table 1. Association of the g.420 C>A genotypes of the cholecystokinin type A receptor gene with growth traits in Miyazaki Jitokko chickens

Traits	AA (n=63)	AC (n=46)	CC (n=11)
Body weight (BW, g)			
BW 0 day	42.0±3.5	40.8±3.0	40.9±2.6
BW 1 wk	126.7±11.0 ^a	120.3±8.6 ^b	123.5±8.9 ^{ab}
BW 2 wks	258.9±22.4 ^a	246.7±18.1 ^b	248.2±15.7 ^{ab}
BW 3 wks	453.3±40.7 ^a	433.7±40.8 ^b	433.3±30.9 ^{ab}
BW 4 wks	704.5±71.4 ^a	672.2±62.5 ^b	651.5±30.1 ^b
BW 5 wks	964.2±97.9 ^a	915.6±87.9 ^b	895.5±44.6 ^b
BW 7 wks	1581.1±159.9 ^a	1508.4±135.1 ^b	1434.5±62.2 ^b
BW 9 wks	2166.0±201.0 ^a	2062.7±179.9 ^b	1998.7±117.5 ^b
BW 11 wks	2652.4±230.0 ^a	2550.1±226.0 ^b	2491.3±177.7 ^b
BW 13 wks	3135.7±251.9 ^a	3042.3±260.2 ^{ab}	2931.0±198.7 ^b
BW 15 wks	3600.2±252.3 ^a	3538.0±280.9 ^{ab}	3334.8±240.2 ^c
BW 17 wks	3960.1±242.3 ^a	3872.9±316.3 ^{ab}	3729.1±247.7 ^b
Average daily gain (ADG, g/day)			
ADG 0-1 wk	12.1±1.4 ^a	11.4±1.1 ^b	11.8±1.2 ^{ab}
ADG 1-2 wks	18.9±2.0 ^a	18.1±1.7 ^b	17.8±1.2 ^{ab}
ADG 2-3 wks	27.8±3.2	26.7±3.7	26.4±2.6
ADG 3-4 wks	35.9±5.8 ^a	34.1±4.7 ^{ab}	31.2±4.0 ^b
ADG 4-5 wks	37.1±5.6 ^a	34.8±4.8 ^b	34.9±3.1 ^{ab}
ADG 5-7 wks	44.1±5.2 ^a	42.3±4.9 ^{ab}	38.5±3.2 ^c
ADG 7-9 wks	41.8±5.8	39.6±8.0	40.3±6.5
ADG 9-11 wks	34.7±7.4	34.8±8.3	35.2±5.7
ADG 11-13 wks	34.5±6.3	35.2±5.5	31.4±3.9
ADG 13-15 wks	33.2±8.6 ^{ab}	35.4±6.8 ^a	28.8±9.9 ^b
ADG 15-17 wks	25.7±10.2	23.9±11.6	28.2±14.0
ADG 0-5 wks	26.3±2.8 ^a	25.0±2.5 ^b	24.4±1.3 ^b
ADG 5-9 wks	42.9±4.6 ^a	41.0±5.1 ^b	39.4±4.1 ^b
ADG 9-13 wks	34.6±4.4	35.0±5.5	33.3±3.4
ADG 13-17 wks	29.4±5.6	29.7±5.6	28.5±6.3

Values represent mean±standard deviation.

^{a-c} Within a row, means without a common superscript are significantly different ($P<0.05$).

Two receptors for CCK—CCKAR (Sankaran *et al.*, 1980) and the CCK type B receptor (CCKBR) (Innis and Snyder, 1980)—have been described. CCKAR and CCKBR are predominant in the alimentary tract and brain, respectively (Wank, 1995). It has been suggested that CCKAR polymorphisms might affect appetite based on already-known CCK functions (Dunn *et al.*, 2013). However, why CCKAR polymorphisms affect growth traits is unknown. We cannot exclude the possibility that the associations found in this study might be produced by linkage disequilibrium between the SNP and other linked DNA polymorphisms directly involved in the regulation of growth traits. In fact, the distal region on chromosome 4, where CCKAR is located, is one of the hot spots where quantitative trait loci affecting growth traits have been reported (Hu *et al.*, 2016).

The significant association between CCKAR haplotypes and growth traits was first reported by Rikimaru *et al.* (2012) using an F₂ resource population created by crossing low- and high-growth lines of the Hinai-dori breed. Sequential reports from the author's group have shown that a significant difference in allele frequency between low- and high-growth lines was caused by long-term selection for growth perform-

ance, and that the A allele of the g.420 C>A SNP in CCKAR improved growth traits within the Hinai-dori breed (Rikimaru *et al.*, 2013, 2014). A significant association was observed between the g.420 C>A SNP and growth traits using the Amakusa Daioh cross chicken, an F₁ hybrid between Amakusa Daioh (native to the Kumamoto Prefecture) sires and the Kyushu Rhode dams (Takahashi *et al.*, 2019). This report provides an additional line of evidence that the g.420 C>A SNP is a useful marker and its application is practicable for improving the growth traits of Jidori brand chickens.

In the Amakusa Daioh cross chickens, no significant differences were observed between the A and C alleles in most intervals of the rearing period (Takahashi *et al.*, 2019). In the Miyazaki Jitokko chickens, the effect of the A allele on ADG in the first half of the rearing period was significantly greater than in the second half. Since the Amakusa Daioh cross and Miyazaki Jitokko chickens are derived from a common maternal stock line, i.e., the Kyushu Rhode, the discrepancies observed in the ADG may reflect the difference in paternal stock lines. Meanwhile, a common trend of the superiority of the A allele over the C allele in ADG throughout the experimental period was observed in both the

Table 2. Phenotypic values of growth traits and effects of cholecystokinin type A receptor alleles on the growth traits in Miyazaki Jitokko chickens

Traits	Phenotypic values		A	C	Variance (%)
	Mean±SD	LRT	Mean±SE	Mean±SE	
Body weight (BW, g)					
BW 0 day	41.4±3.3	3.0	—	—	
BW 1 wk	123.9±10.3	5.9*	62.9±0.6	59.5±1.4	2.3
BW 2 wks	253.2±21.0	7.7**	128.9±1.1	120.9±3.1	3.1
BW 3 wks	444.0±40.9	6.0*	225.9±2.4	212.1±6.1	2.4
BW 4 wks	687.3±67.6	9.8**	351.8±3.7	323.0±11.4	3.9
BW 5 wks	939.3±93.8	9.8**	480.9±5.2	441.0±15.5	3.9
BW 7 wks	1539.8±151.2	12.8**	790.6±8.1	717.6±25.6	5.1
BW 9 wks	2111.1±195.2	11.9**	1081.4±10.7	990.1±30.5	4.7
BW 11 wks	2598.4±230.3	8.0**	1324.4±13.3	1235.4±34.2	3.2
BW 13 wks	3081.2±257.3	7.9**	1568.6±15.2	1469.7±38.3	3.1
BW 15 wks	3552.0±271.1	8.1**	1805.9±16.6	1700.5±38.5	3.2
BW 17 wks	3905.5±280.1	7.5**	1982.4±18.4	1877.8±41.2	3.0
Average daily gain (ADG, g/day)					
ADG 0-1 wk	11.8±1.3	4.6*	6.0±0.1	5.6±0.2	6.3
ADG 1-2 wks	18.5±1.8	6.6*	9.4±0.1	8.8±0.3	2.5
ADG 2-3 wks	27.2±3.4	3.1	—	—	
ADG 3-4 wks	34.8±5.4	8.6**	18.0±0.3	15.8±0.8	3.4
ADG 4-5 wks	36.0±5.2	4.9	—	—	
ADG 5-7 wks	42.9±5.2	11.4**	22.1±0.3	19.8±0.8	4.5
ADG 7-9 wks	40.8±6.8	1.9	—	—	
ADG 9-11 wks	34.8±7.5	0.0	—	—	
ADG 11-13 wks	34.5±5.9	0.7	—	—	
ADG 13-15 wks	33.6±8.3	0.2	—	—	
ADG 15-17 wks	25.2±11.1	0.2	—	—	
ADG 0-5 wks	25.7±2.7	9.5**	13.1±0.1	12.0±0.4	3.7
ADG 5-9 wks	41.9±4.8	7.7**	21.4±0.3	19.6±0.7	3.1
ADG 9-13 wks	34.6±4.7	0.2	—	—	
ADG 13-17 wks	29.4±5.6	0.1	—	—	

SD, standard deviation; SE, standard error; LRT, loglikelihood ratio test statistics.

** $P < 0.05$; * $P < 0.01$.

Miyazaki Jitokko and Amakusa Daioh cross chickens. Since the difference in ADG between A and C alleles accumulated during the rearing period, it can be inferred that the difference in BW between the A and C alleles continued to widen gradually until slaughter age.

From the data in the present study, we can estimate that the days until slaughter can be reduced if the A allele of the g.420 C>A SNP is fixed in the Miyazaki Jitokko population. As shown in Table 2, the mean BW of the present population at 17 wks was 3905.5 g. The allele BW value of the A allele at 17 wks was 1982.4 g. The mean BW of AA homozygotes was almost double the A allele value, at approximately 3964.8 g. The difference in BW between the present population and A allele-fixed populations at 17 wks was estimated to be approximately 59.3 g (= 3964.8 - 3905.5 g). Since the mean ADG of the present population at the 15-17 wks interval was 25.2 g/d, the data suggest that there is a practical merit in shortening the rearing period by at least 2 days. Further study is needed to verify whether the estimated weight gain is reproduced in practice just prior to shipping time.

In conclusion, we have demonstrated a significant association between the g.420 C>A SNP in *CCKAR* and growth traits in Miyazaki Jitokko male chickens. We will utilize the data obtained for marker-assisted selection of the parental stock lines of the Miyazaki Jitokko chicken, i.e., the Jitokko, White Plymouth Rock, and Kyushu Rhode breeds maintained at the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute. When these three lines are fixed in terms of the A allele at the SNP site, we will carry out a demonstration test of the genetic improvement effect, by comparing the A allele-fixed animals with conventional Miyazaki Jitokko chickens in production farms.

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