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Verification of the Effectiveness of an SNP Marker in the Cholecystokinin Type A Receptor Gene for Improving Growth Traits in Okumino-kojidori Chickens

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A significant association was reported between a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) in the cholecystokinin type A receptor gene and growth traits in some Japanese slow-growing chickens. Demonstration tests of the genetic improvement effect by comparing the superior allele-A fixed chickens with conventional ones were carried out considering the effect of different seasons on growth traits in other slow-growing chickens. Meat-type Okumino-kojidori chickens from Gifu Prefecture are a three-way cross of Gifu-jidori improved, White Plymouth Rock, and Rhode Island Red breeds. We used a total of 468 meat-type Okumino-kojidori: 264 individuals from a private hatchery as conventional chickens and 204 A-allele fixed individuals from the Gifu Prefectural Livestock Research Institute as improved chickens. We performed fattening experiments over two seasons: summer and winter. In each season, experimental birds of both sexes were hatched on the same day, raised in the same chicken house, and fed the same diet *ad libitum* for 12 weeks. Body weight was recorded at 3, 6, 9, and 12 weeks of age. SNP genotypes were determined using the mismatch amplification mutation assay. Association between the SNP and growth traits was analyzed using generalized linear models built on sex-based, seasonal, additive, and dominance genetic effects. The observed AA, AC, and CC genotype frequencies in the conventional chickens were 0.158, 0.479, and 0.363, respectively; body weight at 12 weeks and average daily gain from 3 to 12 weeks was superior for the A allele compared to the C allele. The improved chickens were heavier than the conventional ones at 12 weeks. Body weight at 12 weeks in allele-A fixed chickens increased by 3.2% compared to the conventional chickens. We concluded that g.420 C>A is a good selective marker that increases slaughter weight in the meat-type Okumino-kojidori chickens.

Key words: chicken, cholecystokinin type A receptor gene, growth traits, Okumino-kojidori chicken, single nucleotide polymorphism

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Introduction

Since Japan is geologically isolated, chickens in Japan were introduced from overseas at various times. "Jidori" in Japanese originally referred to indigenous chickens thought to be introduced from China more than 2,000 years ago. Gifu-jidori is a Jidori variety native to the Gifu Prefecture, an inland prefecture located in the center of Honshu, Japan. The meat-type Okumino-kojidori is a commercial brand of chicken produced in the Gifu Prefecture. The meat-type Okumino-kojidori chicken is a three-way hybrid chicken produced by crossing Gifu-jidori improved breed cocks, F_1 hybrid hens of a White Plymouth Rock cock, and a Rhode Island Red hen. The Gifu-jidori improved breed is a synthetic breed. Four breeds, i.e. Gifu-jidori, Red Cornish, New Hampshire, and Red Rock, were involved in the establishment of the Gifu-jidori improved breed. The Gifu-jidori improved breed has been maintained to be a hereditary percentage of the Gifu-jidori breed by more than 50%. The number of Okumino-kojidori chicks that initially fed in production farms in FY2017 was about 132,000.

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The meat-type Okumino-kojidori chickens are raised to about 12 weeks (wks) of age in both sexes, while broiler chickens are raised for less than 50 days (d). Shortening the rearing period and/or increasing the slaughter weight of the meat-type Okumino-kojidori is important for the benefit of the producers. Rikimaru *et al*. (2013) first reported a significant association between growth traits and a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) in the 5'-untranslated region of the cholecystokinin type A receptor gene (*CCKAR*) in a Hinai-dori breed native to the Akita Prefecture in northern Honshu, Japan. Recently, associations between the SNP and growth traits in other brands of chickens, Amakusa Daioh cross (Takahashi *et al*., 2019) and Miyazaki-jitokko (Horinouchi *et al*., 2019) chickens have been reported, but reports have not yet validated the effectiveness of genetic improvement by comparing with the conventional chickens. The purpose of this study is to compare genetically improved chickens with conventional ones to test whether the g.420 C>A SNP in *CCKAR* is useful for improving growth traits in Okumino-kojidori chickens.

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.

A total of 468 meat-type Okumino-kojidori, including 264 conventional chickens introduced from a local private hatchery (Yamamoto hatchery, Minokamo, Japan) and 204 improved chickens that had the fixed A-allele at the g.420 C>A SNP and produced at the Seki Experiment Station, Department of Swine and Poultry Science, Gifu Prefectural Livestock Research Institute (Seki, Japan), were used. The fattening experiments were performed by dividing the chickens at the Seki Experiment Station into two seasons: summer from May 23 to August 15, 2018, and winter from November 27, 2018 to February 19, 2019. Experimental birds from each season were hatched on the same day. The chicks were maintained in the same windowless house with forced ventilation but without forced air-cooling systems during the experimental period. Until 3 wks, the chicks were housed in a circular area (1.2 m in diameter) with 45-cm high chick guards made of zinc coated steel on a concrete floor with a hot water recirculating system for the heat insulation of chicks. After 3 wks, the chicks were raised in concrete-floored pens at 9 birds/m^2. Sawdust was used as a floor covering and the lighting program in the house was set to a photoperiod of 23-hours light and 1-hour dark throughout the experiment. The luminance above the floor was set to about 8 lux until 3 days, about 6 lux from 4 to 7 days, and 2−4 lux starting at the 8th day. The chicks were fed a starter diet (ME, 3,150 kcal/kg; CP, 20.5% (wt/wt)) from 0 to 3 wks and a finisher diet (ME, 3,250 kcal/kg; CP, 18%) from 3 to 12 wks. Food and water were provided *ad libitum* throughout the experiment.

The body weight (BW) of the birds was measured at 3, 6, 9, and 12 wks. Average daily gain (ADG) was calculated by dividing weight gain within an interval by the length of the interval in days. The birds were slaughtered at 12 wks.

Genotyping

Blood was collected from the wing vein and immediately transferred to heparinized tubes. The blood was spotted onto an FTA Card (WB120028; GE Healthcare, Buckinghamshire, UK) and left to dry overnight at room temperature. Extraction of the genomic DNA from the FTA card and genotyping of the g.420 C>A SNP in *CCKAR* were performed as previously described (Rikimaru *et al*., 2013).

Statistical Analysis

Genotype and allele frequencies were calculated using gene counting. SNP-trait association analysis taking into account sex-linked and seasonal effects was conducted in Minitab 18 (Minitab, LLC, State College, PA, USA) using the following generalized linear model (GLM):

$$y=\mu+C_g g+C_s s+C_a a+C_d d+e \quad (1)$$

where y is the response variable for each phenotype; μ is the intercept; the gender effect (g) is a covariate coefficient with C_g having values of 0 and 1 for female and male, respectively; the seasonal effect (s) is a covariate coefficient with C_s having values of 0 and 1 for summer and winter, respectively; the additive effect (a) is a covariate coefficient with C_a having values of 2, 1, and 0; the dominance effect (d) is a covariate coefficient with C_d having values of 0, 1, and 0, for genotypes AA, AC, and CC, respectively; and e is the residual standard error.

The difference between the two groups was estimated using the following GLM:

$$y=\mu+C_g g+C_s s+C_{gr} gr+e \quad (2)$$

where y, g, s, C_g, C_s, and e are as described above and the group effect (gr) is a covariate coefficient with C_{gr} having values of 0 and 1 for the conventional and improved groups, respectively.

The percentage of haplotype variance explained by the model was calculated as described by Rikimaru *et al*. (2012), using the following formula:

$$\text{variance percentage}=100\times(1-F_{var}/R_{var})$$

where R variance (R_{var}) is the residual variance from the reduced model omitting the additive effect but including gender, seasonal, and dominance effects in equation 1; omitting the group effect but including gender and seasonal effects in equation 2. F variance (F_{var}) is the residual variance from the full model in equations 1 and 2.

Results

Of the conventional birds (n=264), 37 AA, 136 AC, and 91 CC birds were detected (Table 1). The genotype frequencies of AA, AC, and CC were 0.140, 0.515, and 0.345, respectively. Allele frequencies of A and C were 0.398 and 0.602, respectively (Table 2). In contrast to the conventional birds, the improved birds (n=204) had the A allele fixed (Table 1 and 2).

The effects of the SNP on growth traits in the conventional birds are shown (Table 3). Males were significantly heavier than females from 3 to 12 wks. Winter birds were signifi-

Table 1. **Number of individuals of each genotype, and genotype frequencies in the g.420 C>A SNP in the conventional and improved meat-type Okumino-kojidiri chickens**

		Conventional chickens			Improved chickens		
Season	Gender	AA	AC	CC	AA	AC	CC
Summer	Female	10	32	20	52	0	0
Summer	Male	9	36	34	59	0	0
Winter	Female	9	31	15	49	0	0
Winter	Male	9	37	22	44	0	0
Total		37	136	91	204	0	0
Genotype frequency observed		0.140	0.515	0.345	1.0	0	0

Table 2. **Allele frequencies observed in the g.420 C>A SNP observed in the conventional and improved meat-type Okumino-kojidiri chickens**

		Conventional chickens		Improved chickens	
Season	Gender	A	C	A	C
Summer	Female	0.419	0.581	1.0	0
Summer	Male	0.342	0.658	1.0	0
Winter	Female	0.445	0.555	1.0	0
Winter	Male	0.404	0.596	1.0	0
Allele frequency observed		0.398	0.602	1.0	0

cantly heavier than the summer birds. As for the additive effect, the significant differences between birds having the A allele and those having the C allele in BW at 12 wks show that the A allele significantly increases the slaughter live weight compared to the C allele. Birds with the A allele had significantly higher ADG than those with the C allele from 6 to 9 wks and from 3 to 12 wks. As for the dominance effect, significant positive effects on BW at 6 and 9 wks and on ADG in the interval of 3−6 wks were detected.

The effects of the SNP on growth traits between the conventional and improved birds are shown (Table 4). Males were significantly heavier than females from 3 to 12 wks. Winter birds were significantly heavier than summer birds. The improved birds had a significantly heavier BW at 12 wks than the conventional ones, and they also had a higher ADG between 9−12 and 3−12 wks. The percentage increase in BW in the improved birds compared to the conventional ones at 12 wks were estimated to be about 3.2%.

The effects of the SNP on growth traits in all 468 birds are shown (Table 5). As shown previously, males were significantly heavier than females from 3 to 12 wks, and winter birds were significantly heavier than summer birds. As for the additive effect, birds with the A allele are significantly heavier than those with the C allele based on BW at 3, 6, 9, and 12 wks. Birds with the A allele had significantly higher ADG than birds with the C allele between 3−6, 6−9, 9−12, and 3−12 wks. As for the dominance effect, significant positive effects on BW were detected at 6 and 9 wks and on ADG between 3−6 wks.

Discussion

Cholecystokinin (CCK) is well-known as a gut peptide that inhibits food intake in mammals (Gibbs *et al*., 1973). Two G-protein-coupled receptors for CCK, CCKAR (Sankaran *et al*., 1980) and CCK type-B receptor (CCKBR) (Innis and Snyder, 1980), have been reported. CCKAR and CCKBR are predominantly expressed in the gastrointestinal tract and central nervous system, respectively (Wank, 1995). The messenger RNA (mRNA) of CCKAR is mainly distributed in the chicken alimentary tract except for the proventriculus and gizzard, and CCKBR mRNA is predominantly expressed in the brain (Ohkubo *et al*., 2007).

It is known that exogenously administered CCK octapeptide (CCK-8) via intracerebroventricular (Denbow and Myers, 1982), intravenous (Savory and Gentle, 1980), and intraperitoneal (Covasa and Forbes, 1994) routes transiently depresses food intake in chickens. However, these responses were obtained at high CCK-8 doses. The dosage of CCK in these reports was over 1,000 times higher than the physiological plasma concentration described by Mabayo *et al*. (1992). Corwin *et al*. (1991) showed that very low doses of CCKAR antagonist devazepide (DVZ, also called L-364,718 or MK-329), but not large doses of CCKBR antagonist L-365,260, increased food intake in rats. This report is considered to be evidence that endogenous CCK acts on peripheral CCKAR in mammals. CCK-8 and CCK are not believed to cross the blood-brain barrier based on the size of the molecules, but DVZ does cross the blood-brain barrier (Pullen and Hodgson, 1987). It may be that the increased

Table 3. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the conventional meat-type Okumino-kojidori chickens**

Traits	*n*	intercept	gender	season	additive
Body weight (BW, g)					
BW_3wks	264	452.4±5.3	17.4±4.8**	60.1±4.7**	5.3±3.7
BW_6wks	264	1249.3±15.7	32.1±14.0*	330.3±14.0**	8.4±11.1
BW_9wks	264	1914.6±24.5	190.4±21.9**	730.2±21.8**	30.1±17.3
BW_12wks	264	2405.7±33.2	344.6±29.6**	1033.4±29.5**	49.6±23.3*
Average daily gain (ADG, g/day)					
ADG_3−6wks	264	37.9±0.6	0.7±0.5	12.9±0.5**	0.1±0.4
ADG_6−9wks	264	31.7±0.6	7.5±0.6**	19.0±0.6**	1.0±0.4*
ADG_9−12wks	264	23.4±0.9	7.3±0.8**	14.4±0.8**	0.9±0.6
ADG_3−12wks	264	31.0±0.5	5.2±0.4**	15.4±0.4**	0.7±0.3*

Table 3. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the conventional meat-type Okumino-kojidori chickens (continued)**

Traits	dominance	F_{var}	R_{var}	Variance (%)
Body weight (BW, g)				
BW_3wks	8.2±5.0	378658	381602	0.8
BW_6wks	35.5±14.7*	3297334	3304751	0.2
BW_9wks	45.7±22.9*	8035992	8130530	1.2
BW_12wks	56.7±31.0	14703859	14960450	1.7
Average daily gain (ADG, g/day)				
ADG_3−6wks	1.3±0.5*	4167.6	4169.9	0.1
ADG_6−9wks	0.5±0.6	5241.1	5352.2	2.1
ADG_9−12wks	0.5±0.8	10025.7	10115.6	0.9
ADG_3−12wks	0.8±0.5	3176.9	3228.4	1.6

**, $P<0.01$; *, $P<0.05$

food intake observed following DVZ administration is due to a central effect rather than the antagonism of endogenous peripheral CCK. Meanwhile, Covasa and Forbes (1994) reported that 1) intraperitoneal DVZ doses ranging from 8 to 32 μg/kg BW had no effect on food intake in free-feeding chickens two hours after injection, 2) high doses of over 90 μg/kg BW of intraperitoneal DVZ increased food intake in free-feeding chickens 90 to 120 minutes after injection, and 3) CCK-8 (14 μg/kg BW) caused a transient reduction in feeding, and this effect was not blocked by pretreatment with a high dose of over 90 μg/kg BW of intraperitoneal DVZ. Covasa and Forbes (1994) therefore questioned the involvement of endogenous CCK as a satiety agent in chickens, as their findings were in contrast to those of the previous findings of DVZ acting as an effective CCKAR antagonist in rats (Corwin *et al*., 1991). After all, there is no evidence that CCK acts as a true satiety signal and decreases food intake under physiologically normal conditions in chickens.

Rikimaru *et al*. (2012) reported significant associations between observed *CCKAR* haplotypes and growth traits using a resource population crossing low- and high-growth lines of Hinai-dori breed native to the Akita Prefecture in northern Honshu Island, Japan. The authors implied that an SNP (g.420 C>A) in the predicted YY1 binding site (Shrivastava and Calame, 1994) in the 5′-untranslated region of the *CCKAR* gene may be associated with growth traits. The authors later issued a follow-up report with evidence that the SNP is associated with growth traits (Rikimaru *et al*., 2013). Shortly after publication of Rikimaru *et al*. (2013, first published in January 25, 2013), Dunn *et al*. (2013, first published in February 26, 2013) reported significant associations between *CCKAR* SNPs and growth traits in a broiler×White Leghorn intercross population. Dunn *et al*. (2013) named the g.420 C>A SNP of Rikimaru *et al*. (2013) as snp.13.786.12116.S.3 and described its segregation with the high- and low-growth haplotype individuals in their population. However, there is a difference between the two research groups in the number of genotypes detected in the *CCKAR* region, including the promoter, exon, and intron regions. Dunn *et al*. (2013) detected 37 distinct genotypes, but Rikimaru *et al*. (2012) detected six distinct genotypes constructed by five haplotypes. The differences in the number of genotypes detected is thought to reflect differences in the resource populations used by the groups. As described above, Dunn *et al*. (2013) analyzed the crossbred population, but Rikimaru *et al*. (2012, 2013) analyzed the linecross population within a local breed. We think that Rikimaru's group would undoubtedly designate g.420 C>A as a candidate SNP responsible for growth traits since they identified a limited number of haplotypes. On the other hand,

Table 4. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the conventional and improved meat-type Okumino-kojidori chickens**

Traits	Conventional (*n*)	Improved (*n*)	Intercept	Gender	Season
Body weight (BW, g)					
BW_3wks	264	204	451.2±3.7	42.7±3.8**	50.4±3.8**
BW_6wks	264	204	1239.7±13.4	172.5±13.5**	237.0±13.5**
BW_9wks	264	204	1922.5±22.4	421.3±22.6**	539.0±22.6**
BW_12wks	264	204	2430.4±29.9	636.6±30.2**	778.9±30.1**
Average daily gain (ADG, g/day)					
ADG_3−6wks	264	204	37.5±0.5	6.2±0.5**	8.9±0.5**
ADG_6−9wks	264	204	32.5±0.5	11.9±0.5**	14.4±0.5**
ADG_9−12wks	264	204	24.2±0.6	10.2±0.6**	11.4±0.6**
ADG_3−12wks	264	204	31.4±0.4	9.4±0.4**	11.6±0.4**

Table 4. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the conventional and improved meat-type Okumino-kojidori chickens (continued)**

Traits	Group	F_{var}	R_{var}	Variance (%)
Body weight (BW, g)				
BW_3wks	4.7±3.8	767694	770180	0.3
BW_6wks	16.0±13.6	9848212	9877530	0.3
BW_9wks	36.2±22.7	27494092	27644874	0.5
BW_12wks	118.2±30.3**	48944685	50548065	3.2
Average daily gain (ADG, g/day)				
ADG_3−6wks	0.5±0.5	13938.5	13971.9	0.2
ADG_6−9wks	1.0±0.5	15551.8	15658.7	0.7
ADG_9−12wks	3.9±0.6**	19389.0	21136.8	8.3
ADG_3−12wks	1.8±0.4**	10663.5	11036.3	3.4

**, $P<0.01$; *, $P<0.05$

Rikimaru's group cannot deny Dunn's claim that an SNP located at the downstream region of the *CCKAR* gene is responsible for growth traits, since DNA sequences in the region have not been determined in the Hinai-dori resource population.

In "Jidori brand chickens" defined by the Japanese Agricultural Standard (Ministry of Agriculture, Forestry and Fisheries of Japan, 1999), there has been a significant association reported between the SNP and growth traits within the conventional Amakusa Daioh Cross (Takahashi *et al*., 2019) and Miyazaki-jitokko (Horinouchi *et al*., 2019) chickens, respectively. Takahashi *et al*. (2019) and Horinouchi *et al*. (2019) used 144 Amakusa Daioh Cross (72 individuals each of male and female) and 120 male Miyazaki-jitokko chickens, respectively. Previous reports were based on a single fattening experiment of each, but data in this study were obtained from fattening experiments performed in different seasons. So, this report takes into account the seasonal effect on growth traits. Data obtained from previous reports can be compared to the conventional Okumino-kojidori chickens shown in Table 3. There were no significant differences between the A and C alleles in most intervals during the rearing period in the Amakusa Daioh Cross chickens, but the A allele tended to be superior to the C allele for ADG traits. The effect of the A allele on ADG traits in the first half of the rearing period in the Miyazaki-jitokko chickens was significantly higher than that in the second half. The tendency that A allele is superior to the C allele in ADG traits is common in Okumino-kojidori chickens as shown in the Amakusa Daioh Cross and Miyazaki-jitokko chickens, but significant ADG differences between the A and C alleles were observed in the fourth quarter of the rearing period. These data suggest that g.420 C>A SNP is a good selection marker with high versatility that improves growth performance, even if the timing of the ADG improvement effect varies among the three brands of chickens. Moreover, this study offers a great advantage with the comparison between conventional and SNP marker-selected chickens compared to the previous reports (Takahashi *et al*., 2019; Horinouchi *et al*., 2019). We can roughly estimate the number of days saved before slaughtering when the A allele of the g.420 C>A SNP is fixed in the meat-type Okumino-kojidori chickens. As shown in Table 4, the BW difference of means between the conventional and improved chickens at 12 wks was 118.2 g, and the mean of the ADG intercept between 9−12 wks was 24.2 g. This means that the rearing period is shortened by nearly 5 days.

Geographically, Gifu Prefecture is divided into the Mino

Table 5. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the meat-type Okumino-kojidori chickens in total individuals analyzed**

	n	Intercept	Gender	Season	Additive
Body weight (BW, g)					
BW_3wks	468	440.6±5.1	43.4±3.8**	49.6±3.8**	7.6±2.5*
BW_6wks	468	1200.5±18.1	174.9±13.4**	234.1±13.4**	26.7±8.9**
BW_9wks	468	1849.2±30.2	426.2±22.4**	534.0±22.3**	54.5±14.9**
BW_12wks	468	2328.1±40.3	643.8±29.8**	771.4±29.8**	105.4±19.8**
Average daily gain (ADG, g/day)					
ADG_3−6wks	468	36.2±0.7	6.3±0.5**	8.8±0.5**	0.9±0.3**
ADG_6−9wks	468	30.9±0.7	12.0±0.5**	14.3±0.5**	1.3±0.4**
ADG_9−12wks	468	22.8±0.8	10.4±0.6**	11.3±0.6**	2.4±0.4**
ADG_3−12wks	468	30.0±0.6	9.5±0.4**	11.5±0.4**	1.6±0.3**

Table 5. **Effects of g.420 C>A in cholecystokinin type A receptor gene on body weight and average daily gain in the meat-type Okumino-kojidori chickens in total individuals analyzed (continued)**

	dominance	F_{var}	R_{var}	Variance (%)
Body weight (BW, g)				
BW_3wks	8.9±4.3	752176	757925	0.8
BW_6wks	37.5±15.2*	9624671	9672948	0.5
BW_9wks	58.3±25.4*	26759435	27055204	1.1
BW_12wks	48.9±33.9	47519947	48178629	1.4
Average daily gain (ADG, g/day)				
ADG_3−6wks	1.4±0.6*	13659.6	13706.6	0.3
ADG_6−9wks	1.0±0.6	15178.8	15417.0	1.5
ADG_9−12wks	−0.4±0.7	19123.8	19286.3	0.8
ADG_3−12wks	0.6±0.5	10375.3	10511.6	1.3

**, $P<0.01$; *, $P<0.05$

(southern plain) and the Hida (northern mountainous) regions. The Mino region is a famous hot summer location in Japan. Seki City, where the experiments were performed, is located in the central part of the Mino region. The monthly average temperatures in July and August in Seki City in the last 30 years are 26.5 and 27.8℃, respectively. During the experimental period, the highest recorded temperature in Seki City was 39.6℃. The observation that summer birds were significantly lighter than winter birds could be due to heat stress in summer even if the birds were raised in a windowless house with forced ventilation systems. The data in this study suggest that the SNP-associated genetic improvement effect on growth traits is expressed even under heat stress conditions.

After adding new evidence described here, we think that there is little doubt that the g.420 C>A SNP is a good candidate for marker-assisted selection to improve growth traits with a wide application range in chickens. However, currently there is no explanation as to why g.420 C>A SNP in *CCKAR* affects growth traits. There have been no data showing that CCK at physiologically normal concentration ranges is directly involved in food intake. In addition, it is known that the decreased food intake immediately after intraperitoneal CCK-8 administration is a transient phenomenon and food intake soon returns to normal levels after 2 hours (Covasa and Forbes, 1994). Dunn *et al*. (2013) administered higher than physiological CCK-8 concentrations via intraperitoneal injection into high-, medium-, and low-growth individuals taken from the crossbred population. Even if they observed that high-growth individuals showed minimal reduction of food intake within the 30-minute period after CCK-8 injection, we suspect that the observation reflects physiologically normal eating behavior. The short-term effect of extremely high dose CCK-8 on food intake does not guarantee its long-term effect on food intake. In contrast, Rikimaru *et al*. (2014) observed the following phenomena in Hinai-dori individuals raised in a normal fattening environment: 1) the feed conversion ratio between 4 and 10 wks in AA birds was significantly higher than in CC birds, and 2) there were no significant differences in food intake among the three genotypes (AA, AC, and CC) of g.420 C>A SNP. Recently, Rikimaru *et al*. (2020) reported in the Hinai-Jidori chicken, a cross between a Hinai-dori sire and Rhode Island Red dam, that 1) food intake within the 30-minute period after feeding of AA and AC birds was greater than that of CC birds; however, there was no significant difference in the daily feed intake among the three genotypes of birds, and 2) birds with AA genotype showed the lowest feed conversion among the three genotypes from 4 to 23 weeks. In summary, Rikimaru *et al*. (2014, 2020) suggest that the

metabolic efficiency difference affects the growth traits rather than the food intake. Further comprehensive studies of factors affecting metabolic efficiency are needed to explain why the SNP affects growth traits.

In conclusion, we demonstrated the effectiveness of g.420 C>A SNP in *CCKAR* for improving growth traits in meat-type Okumino-kojidori chickens. We will use the A-allele of the SNP as a marker to select birds in the parent stock lines and to produce the Okumino-kojidori chickens with high growth performance in the near future. Further study is required to explain why this *CCKAR* SNP affects growth traits.

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Conflicts of Interest

The authors declare no conflicts of interest associated with this manuscript.

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