

Is a Single Nucleotide Polymorphism Marker in the Cholecystokinin A Receptor Gene Practically Suitable for Improving the Growth Traits of Hinai-jidori Chickens?

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We have previously reported a significant association between the single-nucleotide polymorphism (SNP; g.420 C>A) in the cholecystokinin type A receptor gene (*CCKAR*) and the growth traits of Hinai-dori, a breed of chicken that is indigenous to Japan. Moreover, we have demonstrated that the minor allele of this SNP improved the growth rate in a low-growth line of the Hinai-dori breed. Hence, in the present study, we verified the association between this SNP and the growth traits of the Hinai-jidori chicken: a cross between a Hinai-dori sire and Rhode Island Red dam. In addition, we verified whether the growth rate was improved in Hinai-jidori chickens produced from the parent stocks in which the SNP A/A genotype was fixed by selection (improved Hinai-jidori chickens). The Hinai-jidori female chicks at 4 weeks of age, were subdivided into three genotypic groups (A/A, A/C, and C/C), with 20 chicks in each group, and reared in an open-sided poultry shed until 23 weeks of age. The results showed that the body weight at 23 weeks of age and the average daily gain after 14 weeks of age were significantly higher in group A/A than in group C/C. Subsequently, the improved and the conventional Hinai-jidori chickens were reared until they reached 22 weeks of age to verify the effects on their growth traits. The body weight of the improved Hinai-jidori chickens at 22 weeks was significantly greater than the conventional Hinai-jidori chickens. Moreover, the association between the SNP and body weights of Hinai-jidori chickens at market age (24 weeks) on the production farms showed that the A allele was significantly superior to the C allele. In conclusion, the *CCKAR* g.420 C>A SNP improves the growth rate of commercial Hinai-jidori chickens and could be a candidate marker for improving the growth performance in selective breeding of Hinai-jidori chickens.

Key words: chicken, cholecystokinin type A receptor gene, growth trait, Hinai-jidori, single-nucleotide polymorphism

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Introduction

In Japan, the regional indigenous Japanese breeds are used to produce meat-type chickens. These chickens are termed as “Jidori” in the Japanese Agricultural Standard (Ministry of Agriculture, Forestry and Fisheries of Japan, 1999). The Hinai-jidori chicken is a cross between Hinai-dori sires and Rhode Island Red dams and is a popular Jidori chicken brand in Japan. The characteristic feature of Hinai-jidori chickens

is the higher palatability of their meat in comparison to that of the broiler chickens (Rikimaru *et al.*, 2011a). Nevertheless, they grow slowly, requiring a longer time for shipment as compared to the broilers or other Jidori chickens. Moreover, the business conditions of the meat-type chicken farmers have increasingly worsened due to the high feed prices and rapid fluctuation of the exchange rate. Therefore, improvement of the growth rate is an urgent need at Hinai-jidori production sites.

The change in the growth and body weight (BW) by domestication is related to the selection based on food intake of the chicken (Burkhart *et al.*, 1983; Denbow, 1994; Richards, 2003). Eating behavior is controlled by a neural network comprising complicated mutual relations of hyperphagic substances and the feeding deterrents generated in the central and peripheral nervous systems (Wynee *et al.*, 2005). Hormones, such as ghrelin, cholecystokinin (CCK), and leptin control the start and end points of eating a meal, daily

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food intake, and weight changes through this neural network. CCK is an important gastrointestinal hormone and is one of the gastrointestinal peptides secreted from I cells of the duodenum and jejunum. This process is performed by stimulation of the food flowing into the duodenum after a meal (Buchan *et al.*, 1978). Two G protein-coupled receptors for CCK have also been identified: CCK type A receptor (CCKAR) (Sankaran *et al.*, 1980) and CCK type B receptor (CCKBR) (Innis and Snyder, 1980).

Otsuka Long Evans Tokushima Fatty (OLETF) rats, which lack the cholecystokinin type A receptor gene (*CCKAR*) because of a mutation, consume significantly more food than the parental control strain (OLETF) (Moran *et al.*, 1998) and are heavier than the control rats from postnatal day 1 until adulthood (Schroeder *et al.*, 2006). Moreover, the intraperitoneal administration of cholecystokinin octapeptide sulfate (CCK8) diminishes the food intake capacity in wild-type and cholecystokinin type B receptor gene (*CCKBR*)^{-/-} mice as compared to the *CCKAR*^{-/-} mice (Kopin *et al.*, 1999). *CCKAR* has also been identified as a candidate gene for human obesity (Arya *et al.*, 2004), and it has been suggested that *CCKAR* promoter polymorphisms are associated with body fat content (Funakoshi *et al.*, 2000; Miyasaka *et al.*, 2007) or midlife weight gain in humans (Koda *et al.*, 2004). In livestock, associations between a polymorphism in the 5' untranslated region (5' -UTR) of *CCKAR* and food intake, the growth rate, and feeding rate have been reported in pigs (Huston *et al.*, 2006, 2008). Moreover, the association between SNPs of *CCKAR* and food intake or BW in chickens has been recently observed (Dunn *et al.*, 2013; Yi *et al.*, 2018). These observations suggest that *CCKAR* gene regulation indeed plays an important role in feeding regulation and BW.

We have previously identified the quantitative trait loci for BW (10 and 14 weeks of age) and average daily gain (ADG; 4–10 weeks of age and 10–14 weeks of age) in a specific region on the chicken chromosome 4. This experiment was performed on an F₂ resource population produced by crossing low- and high-growth lines of Hinai-dori: a breed of chickens that is indigenous to Japan (Rikimaru *et al.*, 2011b). Moreover, in this geographic region we observed a significant association between growth traits and a single-nucleotide polymorphism (SNP; g.420 C>A) in the YY1-binding site (Shrivastava and Calame, 1994) within the 5' -UTR of *CCKAR*. We showed that the A allele has a better influence on the growth traits than the C allele in the F₂ resource population (Rikimaru *et al.*, 2012, 2013). Furthermore, we demonstrated that this SNP improves the growth rate in the low-growth Hinai-dori breed (Rikimaru *et al.*, 2014). These observations suggest that this particular SNP of *CCKAR* could be a candidate marker for improving the growth performance during selective breeding of Hinai-dori. Recently, significant associations between SNP and growth traits were uncovered in the commercial Jidori chickens Miyazaki Jitokko and Amakusa Daiou (Horinouchi *et al.*, 2018; Takahashi *et al.*, 2018). Nonetheless, it has not been revealed whether there is an improvement in the growth rate of

commercial chickens produced from the parent stocks, using SNP as a selection index.

The purpose of the present study was to reveal a possible association between the above SNP and growth traits of Hinai-jidori chickens. We also sought to verify whether there is an improvement in the growth rate of Hinai-jidori chickens produced from parent stocks in which the SNP A/A genotype was fixed by selection.

Materials and Methods

Experimental Birds and Animal Care

Hinai-jidori female chicks were produced by crossing Hinai-dori cocks with Rhode Island Red hens at the Akita Prefectural Livestock Experiment Station.

All animal care and animal use in this study was in accordance with the Guidelines of the Animal Care and Use Committee of the Akita Prefectural Livestock Experiment Station (2013: No. 8, 2017: No. 9).

Experimental Design and Measurements

The Association between the CCKAR SNP and the Growth Traits of Hinai-jidori Chickens

All the 20 Hinai-jidori female chicks from each group had hatched on the same day (May 22, 2013); they were subdivided into three genotypic groups (A/A, A/C, and C/C) at age 4 weeks. The chicks were reared in an open-sided poultry shed with access to a grass paddock until 23 weeks of age. They were fed a first grower diet (metabolizable energy (ME), 2,850 kcal/kg; crude protein (CP), 18%) from the age of 4 to 10 weeks and a second grower diet (ME, 2800 kcal/kg; CP, 15%) from the age of 10 to 14 weeks, and a finisher diet (ME, 2,900 kcal/kg; CP, 16%) from 14 to 23 weeks. The chickens had *ad libitum* access to water for the duration of the experiment.

BW was measured at 4, 10, 14, 18, and 23 weeks of age. ADG between ages 4 and 10 weeks, 10 and 14 weeks, 14 and 18 weeks, 18 and 23 weeks, and 4 and 23 weeks was calculated from the BW in each week. Feed intake was measured at age 10, 14, 18, and 23 weeks. Feed conversion between 4 and 10 weeks, 10 and 14 weeks, 14 and 18 weeks, 18 and 23 weeks, and 4 and 23 weeks of age, was calculated from the feed intake each week.

Feed intake 30 min after feeding and daily feed intake in each genotypic group (A/A, A/C, and C/C) were measured at the same time point every 3 days at age 13 and 14 weeks. Feed intake 30 min after feeding was measured by means of the feed weights, both before and after feeding. Daily feed intake was measured via the feed weights of the day and the day before.

As for the Hinai-jidori production farms, a total of 692 female Hinai-jidori chickens were weighed on the four production farms on the shipping date (165, 166, and 168 days of age).

Verification of the Influence of Selection using the CCKAR SNP on the Growth Traits of Hinai-jidori Chickens

Thirty Hinai-jidori female chicks, produced from parent stocks (Hinai-dori breed and Rhode Island Red (high egg-laying line) in which the SNP A/A genotype was fixed by

selection (improved Hinai-jidori chickens) and 63 conventional Hinai-jidori chicks were hatched on the same day (May 22, 2017). These chicks were raised in a battery cage until age 4 weeks. The chicks were randomly subdivided into four pens at 4 weeks of age and reared in a poultry shed until the age of 22 weeks.

The chicks were fed a starter diet (ME, 3,000 kcal/kg; CP, 24%) from age 0 to 4 weeks, the grower diet (ME, 2,900 kcal/kg; CP, 19%) from 4 to 14 weeks, and the finisher diet (ME, 2,900 kcal/kg; CP, 16%) from the age of 14 to 22 weeks. Water was available without restrictions for the entire duration of the experiment.

BW was measured at 0, 4, 14, and 22 weeks of age. ADG between ages 0 and 4 weeks, 4 and 14 weeks, 14 and 22 weeks, and 0 and 22 weeks, was calculated from the BW each week.

***CCKAR* Genotyping**

Blood was collected from the ulnar vein, spotted onto an FTA Classic Card (WB120205; GE Healthcare, Buckinghamshire, UK), and air-dried at room temperature. DNA was extracted from the FTA Classic Card as described by Rikimaru *et al.* (2013). *CCKAR* genotyping was conducted according to the procedure described by Rikimaru *et al.* (2013). Briefly, a polymerase chain reaction (PCR) solution was prepared from a combination of one forward primer (5' - GAATGTGTGCTGCGTGCTT-3') and two sets of reverse primers (A allele primer: 5' -GGATCCACAGGTTAGCT-GCgAt-3, C allele primer: 5' -GGATCCACAGGTTAGCT-GCgAg-3') to detect the SNP (AB604331: g.420 C>A) in the YY1-binding site within the 5' -UTR of the *CCKAR* gene (Rikimaru *et al.*, 2012). PCR amplification was performed in a reaction volume of 9 μ L, which included 2 pmol of each primer, 4 μ L of 2 \times PCR mix (EmeraldAmp; Takara, Otsu, Japan), and 1 μ L of DNA template. Reactions were carried out in a 96-well plate on a thermal cycler (GeneAmp System 9700; Perkin-Elmer, Foster City, CA, USA) under the following cycling conditions: 30 cycles of 98 $^{\circ}$ C for 10 s and 68 $^{\circ}$ C for 60 s. PCR products (2 μ L each) were then analyzed by electrophoresis on a 2.0% agarose gel with 1 \times Tris-acetate EDTA (TAE) buffer, at 150 V for 30 min in a horizontal gel electrophoresis apparatus (BE-548B; BIO CRAFT, Tokyo, Japan). The amplicons were stained with ethidium bromide for 30 min. The genotype that could be detected only by the reaction containing the allele A and C primer was assumed to be A/A and C/C, respectively. The genotype that could be detected by both the reactions containing alleles A or C primers was assumed to be A/C. *CCKAR* genotyping of each chicken was performed by 4 weeks of age.

As for the Hinai-jidori production farms, blood was collected from the chickens' comb on the shipping date, and *CCKAR* genotyping was performed as described above.

Statistical Analysis

These analyses of the phenotypic values of Hinai-jidori chickens at the Akita Prefectural Livestock Experiment Station were performed in Excel-Statistics 2006 software (Social Survey Research Information, Tokyo). Comparisons among the means were made by Scheffe's multiple-com-

parison test or the *t* test. Differences between the groups were considered significant when the *P* values were less than 0.05. Allele frequencies were calculated by gene counting.

SNP trait association analysis of Hinai-jidori chickens at the Akita Prefectural Livestock Experiment Station was performed by means of the R package (R Core Team, 2016) via the generalized linear model. The hypothesized model for the phenotypic data on each trait of Hinai-jidori chickens was as follows:

$$y = u + C_a a + C_d d + e$$

where *y* is the response variable of each phenotype; *u* is the intercept; the additive effect (*a*) is a covariate coefficient, with *C_a* taking a value of 2, 1, and 0 for genotypes A/A, A/C, and C/C, respectively; the dominance effect (*d*) is a covariate coefficient, with *C_d* taking a value of 0, 1, and 0, for genotypes A/A, A/C, and C/C, respectively; and *e* is the residual standard error.

The percentage of haplotype variance explained by the model was calculated as follows:

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

where variance R (*R_{var}*) is the residual variance from a reduced model, which omits the additive but includes dominance effects; and variance F (*F_{var}*) is the residual variance from the full model, including both additive and dominance effects.

The BW of the Hinai-jidori chickens aged 168 days on the production farms was adjusted for the effect of time in days. ADG was calculated from the shipping BW and the hatching BW, assuming that the hatching weight was 40 g. SNP-trait association analysis was performed by Minitab Statistical Software (Minitab Inc., PA, USA), using the generalized linear model. The assumed model for the phenotypic data of the BW of Hinai-jidori chickens on the production farms was as follows:

$$y = u + C_a a + C_d d + e$$

where *y* is the response variable of each phenotype; *u* is the intercept; the additive effect (*a*) is a covariate coefficient, with *C_a* taking values 2, 1, and 0 for genotypes A/A, A/C, and C/C, respectively; the dominance effect (*d*) is a covariate coefficient, with *C_d* taking values 0, 1, and 0 for genotypes A/A, A/C, and C/C, respectively; and *e* is the residual standard error.

Results

The Association between the CCKAR SNP and the Growth Traits of Hinai-jidori Chickens at the Akita Prefectural Livestock Experiment Station

Table 1 shows the phenotypic values of the genotypes and the effects of the g.420 C>A polymorphism of *CCKAR* on the growth traits of Hinai-jidori chickens. The A/A group had a significantly higher BW (*P*<0.05) as compared to the C/C group at 23 weeks of age, although there were no significant differences in the BW of A/A and A/C groups throughout the experimental period. The ADG from age 14 to 18 weeks in the A/A group was significantly superior (*P*<0.05) to the ADGs of the A/C and C/C groups. The ADGs from age 18 to 23 weeks and 4 to 23 weeks for the A/A group were sig-

Table 1. Phenotypic values of the genotypes and effects of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene on the growth traits of Hinai-jidori chickens

Items	A/A (n=20)		A/C (n=20)		C/C (n=20)		Intercept±SE
	Mean±SE		Mean±SE		Mean±SE		
BW-4 week (g)	335.6±5.6		335.6±5.6		335.6±4.7		335.5±5.3
BW-10 week (g)	1,108.6±28.5		1,141.1±27.9		1,177.4±35.0		1177.4±30.5
BW-14 week (g)	1,730.5±28.6		1,718.6±32.2		1,762.5±39.3		1762.5±33.4
BW-18 week (g)	2,320.1±29.1		2,197.9±41.1		2,265.1±39.4		2265.1±36.5
BW-23 week (g)	2,873.2±48.0	^a	2,743.9±45.7	^{ab}	2,703.4±38.9	^b	2703.4±44.0
ADG 4-10 week (g/day)	18.4±0.7		18.5±0.7		20.0±0.9		20.0±0.7
ADG 10-14 week (g/day)	22.2±0.7		21.6±0.6		20.9±0.6		20.9±20.9
ADG 14-18 week (g/day)	21.1±0.8	^a	17.1±0.8	^b	18.0±0.7	^b	18.0±0.8
ADG 18-23 week (g/day)	15.8±1.0	^a	15.8±1.0	^{ab}	12.8±0.5	^b	12.5±0.9
ADG 4-23 week (g/day)	19.1±0.4	^a	18.1±0.3	^{ab}	17.8±0.3	^b	17.8±0.3

Table 1. Phenotypic values of the genotypes and effects of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene on the growth traits of Hinai-jidori chickens (continued)

Items	additive effects		dominance effects		F _{var}	R _{var}	Variance (%)
	Mean±SE		Mean±SE				
BW-4 week (g)	0.1±3.8	n.s.	0.6±0.6	n.s.	31876	31872	0.01
BW-10 week (g)	-34.4±21.6	n.s.	-28.9±38.0	n.s.	1098279	1040189	5.29
BW-14 week (g)	-16.0±-16.0	n.s.	-27.9±-27.9	n.s.	1271779	1251534	1.59
BW-18 week (g)	27.5±25.8	n.s.	-94.7±45.4	*	1634067	1488283	8.92
BW-23 week (g)	84.9±31.1	**	-44.4±54.8	n.s.	2479608	2165888	12.65
ADG 4-10 week (g/day)	-0.8±0.5	n.s.	-0.7±0.9	n.s.	658.8	625.5	5.05
ADG 10-14 week (g/day)	0.7±0.4	n.s.	0.0±0.8	n.s.	454.5	437.2	3.80
ADG 14-18 week (g/day)	1.6±0.5	**	-2.4±0.9	*	819.9	650.0	20.73
ADG 18-23 week (g/day)	1.6±0.6	**	1.4±1.1	n.s.	945.5	811.3	14.19
ADG 4-23 week (g/day)	0.6±0.2	**	-0.3±0.4	n.s.	139.3	121.6	12.74

BW-4 weeks, BW-10 weeks, BW-14 weeks, BW-18 weeks, and BW-23 weeks: body weight measured at 4, 10, 14, 18, and 23 weeks of age. ADG 4-10 weeks, ADG 10-14 weeks, ADG 14-18 weeks, ADG 18-23 weeks, and ADG 4-23 weeks: average daily gain from 4 to 10, from 10 to 14, from 14 to 18, from 18 to 23, and from 4 to 23 weeks of age, respectively. SE: standard error, n.s.: not significant, F_{var}: residual variance from the full mode. R_{var}: residual variance from the reduced model, variance (%) = 100 × (1 - F_{var}/R_{var}).

^{a, b} Within a row, different superscripted letters denote a significant difference ($P < 0.05$). * $P < 0.05$, ** $P < 0.01$.

nificantly higher ($P < 0.05$) than that of the C/C group.

In terms of the additive effect, the A allele was significantly superior ($P < 0.05$) to the C allele on regulating the BW at 23 weeks and ADG from 14 to 18, 18 to 23, and 0 to 23 weeks. Regarding the dominance effect, the A allele had significant effects on the BW at 18 weeks and on ADG from age 14 to 18 weeks as compared to the C allele.

A comparison of the feed intake and feed conversion among genotypes of the g.420 C>A polymorphism of *CCKAR* in Hinai-jidori chickens is shown in Table 2. The A/A and A/C groups manifested significantly greater ($P < 0.05$) feed intake, 30 min after feeding than the C/C group, from 13 to 14 weeks of age, although there was no significant difference in the daily feed intake among the three groups. The A/A group showed the lowest feed conversion among the three groups from age 4 to 23 weeks.

Verification of the Impact of Selection using the *CCKAR* SNP on the Growth Traits of Hinai-jidori Chickens

The phenotypic values and effects of the *CCKAR* g.420 C>A polymorphism on the growth traits of improved and

conventional Hinai-jidori chickens are presented in Table 3. The A allele frequency in the g.420 C>A polymorphism in the improved Hinai-jidori chickens was 1.0, whereas the A and C allele frequencies for the g.420 C>A polymorphism in the conventional Hinai-jidori chickens were 0.484 and 0.516, respectively. The BW of improved Hinai-jidori chickens at 22 weeks of age was significantly higher ($P < 0.05$) than the conventional Hinai-jidori chickens. The ADGs of improved Hinai-jidori chickens from 14 to 22 and 0 to 22 weeks of age, were significantly higher ($P < 0.05$) than the conventional Hinai-jidori chickens. In the improved Hinai-jidori chickens, an additive effect revealed that the A allele was significantly superior ($P < 0.05$) to the C allele, based on the BW at age 14 and 22 weeks and by the ADG from 4 to 14 and 0 to 22 weeks.

The Association between the *CCKAR* SNP and BW of Hinai-jidori Chickens on the Production Farms

The BW of the Hinai-jidori chickens with the *CCKAR* g.420 C>A polymorphism on the shipping date at the production farms is depicted in Fig. 1. Chickens with the A/C

Table 2. Comparison of the feed intake and feed conversion in Hinai-jidori chickens with various genotypes of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene

Items	A/A	A/C	C/C
Daily feed intake (g/birds per day) ¹	101.7±2.3	96.7±2.3	97.0±2.0
Feed intake thirty minutes after feeding (g/birds) ¹	18.1±1.4 ^a	20.1±2.1 ^a	12.8±1.0 ^b
Feed intake 4–23 weeks (g/birds per day) ²	105.0	102.4	100.1
Feed conversion 4–23 weeks ²	5.50	5.65	5.62

¹ Values are mean±SE. Feed intake was measured every day between 13 and 14 weeks of age. ² No statistical tests were performed due to group feeding.

^{a, b} Within a row, different superscripted letters denote a significant difference ($P<0.05$).

Table 3. Phenotypic values and effects of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene on the growth traits of improved and conventional Hinai-jidori chickens

Items	Improved Hinai-jidori ($n=30$) ¹		Conventional Hinai-jidori ($n=63$) ²	
	Mean±SE		Mean±SE	Intercept±SE
BW-0 week (g)	38.9±0.6		38.7±0.3	37.7±0.7
BW-4 week (g)	333.6±5.4		327.6±4.2	321.1±8.0
BW-14 week (g)	1,821.4±19.3		1,779.6±16.9	1694.6±29.6
BW-22 week (g)	2,719.8±33.6	^a	2,623.0±27.6	2520.3±50.4
ADG 0–4 week (g/day)	10.5±0.2		10.3±0.1	10.1±0.3
ADG 4–14 week (g/day)	21.3±0.3		20.7±0.2	19.6±0.4
ADG 14–22 week (g/day)	16.0±0.3	^a	15.1±0.3	14.7±0.6
ADG 0–22 week (g/day)	17.4±0.2	^a	16.8±0.2	16.1±0.3

Table 3. Phenotypic values and effects of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene on the growth traits of improved and conventional Hinai-jidori chickens (continued)

Items	additive effects		dominance effects		F_{var}	R_{var}	Variance (%)
	Mean±SE		Mean±SE				
BW-0 week (g)	0.6±0.4	n.s.	0.8±0.6	n.s.	733	709	3.0
BW-4 week (g)	6.2±4.7	n.s.	2.6±6.4	n.s.	94003	92150	2.0
BW-14 week (g)	67.0±17.3	***	31.9±26.9	n.s.	1470420	1259837	14.3
BW-22 week (g)	99.4±29.4	**	13.2±45.8	n.s.	4149494	3662051	11.7
ADG 0–4 week (g/day)	0.2±0.2	n.s.	0.1±0.2	n.s.	114	112	1.8
ADG 4–14 week (g/day)	0.9±0.2	***	0.4±0.3	n.s.	243	207	14.6
ADG 14–22 week (g/day)	0.6±0.4	n.s.	−0.3±−0.6	n.s.	578	555	4.1
ADG 0–22 week (g/day)	0.6±0.2	**	0.1±0.3	n.s.	174	154	11.7

¹ Allele frequency: A, 1.0; C/C, 0.0. ² Allele frequency: A, 0.484; C, 0.516.

BW-0 week, BW-4 weeks, BW-14 weeks, and BW-22 weeks: Body weight measured at 0, 4, 14, and 22 weeks of age. ADG 0–4 week, ADG 4–14 weeks, ADG 14–22 weeks, and ADG 0–22 weeks: average daily gain between 0 and 4, between 4 and 14, between 14 and 22, and between 0 and 22 weeks of age. SE: standard error, n.s.: not significant, F_{var} : residual variance from the full model, R_{var} : residual variance from the reduced model, variance (%) = $100 \times (1 - R_{var}/F_{var})$.

^{a, b} Within a row, different superscripted letters denote a significant difference ($P<0.05$). ** $P<0.01$, *** $P<0.001$.

genotype had a greater BW than those with the C/C genotype on the B farm. BWs of the chickens with the A/A genotype were generally greater than those with the C/C genotype, although there were no significant difference among the farms.

Allele frequency and the effects of the *CCKAR* g.420 C>A polymorphism on the estimated BW of Hinai-jidori chickens at 24 weeks on the production farms are listed in Table 4. The frequencies of alleles A and C for the g.420 C>A polymorphism were 0.563 and 0.437, respectively. The BW (ad-

justed for the effect of time in days) at 168 days of age was 2523.5 g, the additive effect was 31.1 g, and the dominance effect was 1.6 g. Thus, in terms of additive effects, the A allele was significantly superior to the C allele.

Discussion

We have previously demonstrated that the *CCKAR* g.420 C>A polymorphism improves the growth rate of a low-growth line of the Hinai-dori breed, whose growth traits have not been selected (Rikimaru *et al.*, 2013). In the present

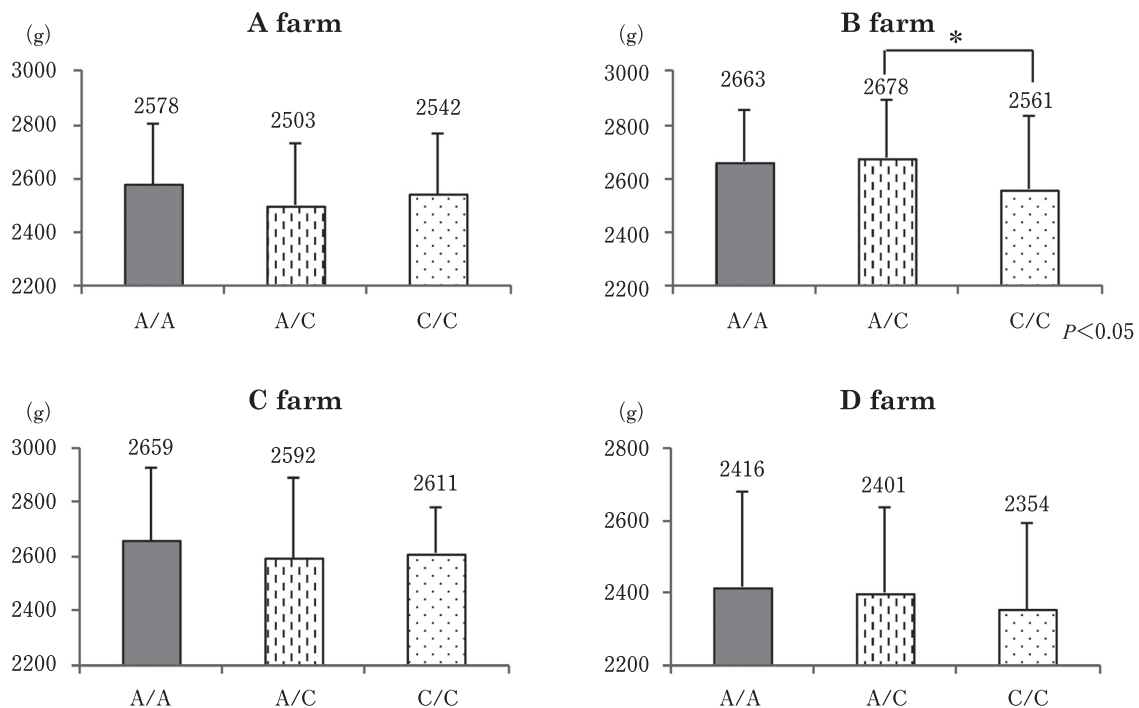


Fig. 1. The BW of Hinai-jidori chickens with various genotypes of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene at the production farms on the shipping date

Table 4. Genotype frequencies and effects of the g.420 C>A polymorphism of the cholecystokinin type A receptor gene on Hinai-jidori chickens' BW (adjusted for the effect of time in days) at 168 days of age on the production farms

Item	n	Allele frequency		Body weight±SE	additive effects	dominance effects		n.s.
		A	C		Mean±SE	Mean±SE		
Average body weight (g)	692	0.563	0.437	2513.5±25.5	31.1±15.6	*	1.6±19.6	

SE: standard error, n.s.: not significant, * $P<0.05$.

study, we investigated the association of this SNP with BW, daily weight gain, feed intake, and feed conversion of Hinai-jidori chickens. Next, we compared the growth of Hinai-jidori chickens (produced from the parent stocks in which the SNP A/A genotype was fixed), and conventional Hinai-jidori chickens. This experiment was conducted to test whether the SNP improves the growth rate of Hinai-jidori chickens by selection. Moreover, we investigated the association of this SNP with the BW of Hinai-jidori chickens at market age on the production farms.

The A allele had significant effects on the growth traits after 14 weeks of age in comparison to the C allele, and the A/A group had significantly greater BW than the C/C group at 23 weeks of age. The feed conversion in the A/A group was the lowest, though the feed intake of this group was the highest among the three groups from 4 to 23 weeks of age. This result is in agreement with the findings by Rikimaru *et*

al. (2013, 2014), who reported that BW, ADG, and feed conversion in the A/A group are superior to the C/C group in the Hinai-dori breed. Recently, Takahashi *et al.* (2018) observed that the A allele has a significant influence on the growth traits as compared to the C allele in Amakusa Daioh cross chickens. Horinouchi *et al.* (2018) observed that the BW of the A/A group is greater than that of the C/C group in the Miyazaki Jitokko chickens, suggesting that the A allele of the *CCKAR* g.420 C>A polymorphism improves the growth rate, and that this phenomenon is not limited to purebred and crossbred chickens. It should be noted that the differences in ADG among the genotypes were observed from 4 to 14 weeks of age in the Hinai-dori breed, whereas the differences were observed after age 14 weeks in the Hinai-jidori chickens. As for Amakusa Daioh cross chickens and Miyazaki Jitokko chickens, the association between the SNP and ADG was observed starting from an earlier age (Takahashi *et al.*,

2018; Horinouchi *et al.*, 2018). Therefore, the time when the SNP affects growth performance may vary depending on breeds or crossbreeding.

Rikimaru *et al.* (2014) reported that there are no significant differences in feed intake among the genotypes from 4 to 10, 10 to 14, and 4 to 14 weeks of age in the Hinai-dori breed. Nevertheless, there was no significant difference in daily feed intake between 13 and 14 weeks of age, whereas the A/A and A/C groups had significantly higher feed intake than the C/C group, 30 min after feeding. There are no significant differences in the feed intake at 24 hours or long-term after intraperitoneal administration of CCK8 to *CCKAR*^{-/-} mice (Kopin *et al.*, 1999; Bi *et al.*, 2004), although *CCKAR*^{-/-} mice manifested significantly greater feed intake than the wild-type mice immediately after administration of CCK8 (Kopin *et al.*, 1999). Houston *et al.* (2008) demonstrated that the *CCKAR* g.179 A>G polymorphism affects the feeding rate (g/min) in commercial pig lines. Dunn *et al.* (2013) confirmed that the high-growth haplotype of *CCKAR* yields a minimal response to CCK after intraperitoneal administration of CCK8, whereas the low-growth haplotype reduce the feed intake 30 min after feeding in chickens. These observations suggest that *CCKAR* polymorphisms participate in the short-term inhibition of feed intake but have no long-term impact.

As expected, improved Hinai-jidori chickens had significantly higher BW than the conventional Hinai-jidori chickens. In conventional Hinai-jidori chickens, the A allele exerted significant effects on the growth traits as compared to the C allele after 14 weeks of age. This phenomenon was also observed among the genotypic groups in Hinai-jidori chickens. It was revealed that the growth rate of Hinai-jidori chickens was improved by selecting the parent stock for the *CCKAR* g.420 C>A polymorphism as a DNA marker. This is the first report showing that the A allele of the *CCKAR* g.420 C>A polymorphism improves the growth rate of commercial chickens by selection of the parent stocks. Calculating based on body weight and standard deviation at 22 weeks of age, the coefficient of variation in the body weight of the improved Hinai-jidori chickens (0.068) was smaller than that of the conventional Hinai-jidori chickens (0.084) (data not shown). It is believed that body weight variation could be reduced by fixing the A/A genotype through selection of the parent stocks. Nonetheless, it is unknown why this SNP affects the growth traits in chickens. It is likely that other linked DNA polymorphisms may be directly involved in the regulation of feed intake and the chicken growth rate by linkage disequilibrium. Dunn *et al.* (2013) found that the high-growth haplotype of *CCKAR* yields lower the expression of *CCKAR* mRNA in the brain, intestine, and exocrine organs. However, they reported that the level of orexigenic Agouti-Related Protein is high in the hypothalamus in the population produced by crossing white leghorn and broiler chickens. Therefore, the alteration of meal patterns due to the decreased level of CCK-mediated satiety signaling may contribute to the growth rate of chickens. Further research is needed to elucidate the association

between *CCKAR* polymorphisms and the growth rate.

The BW of chickens on the shipping date at the production farms was higher in the A/A group than in the C/C group. The estimated shipping BW at 24 weeks of age indicated that the A allele had a significant influence on the growth rate relative to the C allele, which is in line with the results from the Akita Prefectural Livestock Experiment Station in this study. The frequency of the A allele in the Hinai-jidori production farm population was 0.563 in this study. Additionally, the additive effect of the A allele was 31.1 g. It was calculated that the additive effect in the Hinai-jidori population was 35.0 g (62.2×0.563). We estimated that the BW may be increased by 27.2 g (62.2-35.0 g) per bird if the A allele is fixed in the Hinai-jidori population. If the purchase unit price of Hinai-jidori chickens is 827 yen per kg, then there is an expected increased income of 22.5 yen per bird. The number of produced Hinai-jidori chickens per farm and the total production number in 2017 were approximately 5,200 and 518,000 birds, respectively (Akita Prefectural Government, 2018). According to these data, an increased income of 112.5 thousand yen per farm and approximately 11.66 million yen a year can be expected. This result suggests that application of the *CCKAR* g.420 C>A polymorphism for breeding is practical at Hinai-jidori production sites and may increase the income of farmers.

Our findings suggest that this SNP of *CCKAR* is a promising candidate marker for improving growth performance during the selective breeding of Hinai-jidori chickens.

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Conflicts of Interest

The authors declare no conflict of interest.

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