Practical Application of Miyazaki Jitokko Chickens Selected for a Superior Allele at a Single Nucleotide Polymorphism Site in the Cholecystokinin Type A Receptor Gene

Shojiroh Horinouchi¹, Hiromi Nakayama¹, Tadahiro Ando² and Hideaki Takahashi³

¹Kawaminami Branch, Miyazaki Prefectural Livestock Research Institute, Kawaminami Town 889-1301, Japan
²Miyazaki Jitokko Cooperative Association, Miyazaki 880-0806, Japan
³Institute of Livestock and Grassland Science, NARO, Tsukuba 305-0901, Japan

This study aimed to examine 1) whether selection for a superior allele at a single nucleotide polymorphism site (SNP; AB604331, g.420 C>A) of the chicken cholecystokinin type A receptor (CCKAR) gene in Miyazaki Jitokko chickens is detectable in commercial poultry farms, and 2) whether the reproductive traits of the Kyushu Rhode hens, as a maternal stock line of the Miyazaki Jitokko chickens, are affected by SNP selection. Conventional and A-allele fixed (improved) Miyazaki Jitokko chicks were hatched on the same day and raised in a battery cage until 7 days of age. The chicks were then deposited at two commercial poultry farms and reared until slaughter at 126 and 163 days for cockerels and pullets, respectively. Body weight on the day of hatching (day 0), at 5 days of age, and at slaughter were measured. The differences in the body weights of the farm and test groups at slaughter were analyzed using the generalized linear model. A-allele fixation increased the body weight at slaughter by approximately +123.5 g and +131.9 g in cockerels and pullets, respectively. No significant differences between the conventional and improved hens were detected in terms of egg-laying rate, fertilization rate, and hatchability in the Kyushu Rhode hens. The data suggest that fattening chicks can be supplied as usual, even if Kyushu Rhode hens are switched from the conventional to improved type. In conclusion, genetic improvements using the CCKAR SNP site as a marker were effectively established in terms of the growth of the Miyazaki Jitokko chickens in commercial farms and the reproductive traits of the Kyushu Rhode hens.

Key words: cholecystokinin type A receptor gene, Miyazaki Jitokko chicken, single nucleotide polymorphism, slaughter body weight

J. Poult. Sci., 58: 12-20, 2021

Introduction

Jitokko is a native Japanese chicken breed in the foothills of Mt. Kirishima—a stunning volcanic mountain range that spans the Miyazaki and Kagoshima Prefectures in the Southern Kyushu subregion of Japan. The distinctive characteristics of the Jitokko breed include primitive plumage, large crests, beards, and short legs, which are inherited by a dominant lethal gene termed Creeper (Cp) (Shibuya et al., 1972). In 1943, the Jitokko breed was declared a natural treasure in Japan. Miyazaki Jitokko chicken, which is a special product of the Miyazaki Prefecture and a popular chicken brand in the Japanese market, has been commercialized since 1990. These chickens were obtained by crossing F₁ cocks (Jitokko sire × White Plymouth Rock dams) with Kyushu Rhode hens (a synthetic breed that results from a cross between Rhode Island Red and White Plymouth Rock). The annual number of Miyazaki Jitokko chicks initially fed in commercial poultry farms is approximately 600,000–700,000 birds (Miyazaki Prefectural Government, 2019). Miyazaki Jitokko chickens are produced by approximately 50 private farms of the Miyazaki Jitokko Cooperative Association (Miyazaki, Japan). The average number of chicks initially fed per farm is 12,000–14,000 chickens. Genetic improvements of the grandparent and parent stock lines of the Miyazaki Jitokko chickens, i.e., the Jitokko, White Plymouth Rock, and Kyushu Rhode breeds, have been
conducted by the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute (Kawaminami Town, Japan).

The cockerels and pullets of the Miyazaki Jitokko chickens are fattened until approximately 120 and 150 days of age, respectively (Miyazaki Jitokko Cooperative Association and Kawaminami Branch and Miyazaki Prefectural Livestock Research Institute, 2013). In contrast to the Miyazaki Jitokko chickens, the rearing period for the broiler chickens is less than 50 days. Miyazaki Jitokko chicken producers aim to increase the body weight at slaughter and shorten the rearing period, since 1) the growth of Miyazaki Jitokko chickens is far slower than that of broilers, and 2) chickens are traded based on the unit price of the body weight at slaughter.

The National Agriculture and Food Research Organization (NARO) and the Akita Prefectural Livestock Experiment Station have reported that a SNP site (AB604331, g.420 C in DDBJ; rs313822901 in NCBI dbSNP) in the 5′-untranslated region of the cholecystokinin type A receptor gene (CCKAR) is associated with growth traits in the Hinai-dori breed of the Akita Prefecture in Honshu, Japan (Rikimaru et al., 2013). The effectiveness of the g.420 C>A SNP as a selection marker for improved growth traits has been investigated in other commercial slow-growing chickens (Amakusa Daioh cross chicken, Takahashi et al., 2019; Miyazaki Jitokko chicken, Horinouchi et al., 2019; Hinaijitori chicken, Rikimaru et al., 2019; Okumino-kojidori chicken, Ishikawa et al., 2020). These studies conclude that the A allele of the g.420 C>A SNP is superior for improving growth traits. However, the effects of marker-assisted selection of the CCKAR SNP in commercial poultry farms are unknown, as the data for the previous studies were obtained in well-maintained poultry houses under uniform environments in prefectural experimental stations and institutes.

Recently, we reported a significant association between the g.420 C>A SNP in CCKAR and growth traits in male Miyazaki Jitokko chickens (Horinouchi et al., 2019). We observed that the body weight from 1 to 17 weeks of age in birds with the A allele was greater than that of birds with the C allele; however, the results of this previous study are not sufficient to confirm that Miyazaki Jitokko chickens that are selected using the SNP marker can be grown in commercial poultry farms, as data on female chickens are absent. To resolve this issue, a validation test is conducted to evaluate the effectiveness of improving the chicken breed by comparing the genetically improved A-allele fixed chickens to conventional ones at commercial poultry farms. In addition, we examined whether improving chickens with the A-allele affects reproductive traits in the maternal stock line, known as Kyushu Rhode, since commercial stocks of the Miyazaki Jitokko chicken were produced using the Kyushu Rhode breed.

**Materials and Methods**

**Experimental Birds**

This research was performed in adherence to the Guide-

**Fattening Experiments on Commercial Poultry Farms**

For conventional Miyazaki Jitokko chicken production, Jitokko, White Plymouth Rock, and Kyushu Rhode lines that were not selected for the g.420 C>A genotype were used. Nine F1 cocks (Jitokko sire×White Plymouth Rock dams) were crossed with 63 Kyushu Rhode hens by natural mating. Meanwhile, improved Miyazaki Jitokko chickens were produced by crossing the A-allele fixed lines of the Jitokko, White Plymouth Rock, and Kyushu Rhode breeds. The A-allele fixed lines were developed using the A-allele of the SNP site as a selection index from the fiscal year of 2015 to the fiscal year of 2017. As shown in Table 1, A-allele frequencies of the three breed lines were gradually raised and the A-allele fixed lines were finally developed.

Six F1 cocks that were obtained by crossing the A-allele fixed Jitokko sires and White Plymouth Rock dams were crossed with 40 A-allele fixed Kyushu Rhode hens by natural mating. Eggs from the conventional and improved Miyazaki Jitokko chickens were collected for three weeks and stored at 15°C and 60% relative humidity until incubation. The eggs were incubated in a setter (MIC-90, Muri Furanki Seisakusho, Nagoya, Japan) at 37.6°C and 55–60% relative humidity. The eggs were turned every hour. On day 18 of incubation, the eggs were moved to a hatcher of the same model set under conditions of 37.4°C and 60–70% relative humidity without egg turning.

The conventional and improved Miyazaki Jitokko chicks were hatched on the same day (July 12, 2018) and raised in a battery cage in a conventional poultry research house in the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute until 7 days of age. Among the conventional group, 455 newly hatched chicks, including 245 males and 210 females, were obtained. Among the improved group, 441 newly hatched chicks, including 230 males and 211 females, were obtained. On the day of hatching, wing bands for individual recognition were wrapped around the shins of the chicks; further, all chicks were fed a starter diet (ME, 3,000 kcal/kg; CP, 22% [wt/wt]) supplied by Nichiwa Sangyo (Kagoshima, Japan) until 7 days of age. The chicks were allowed free access to food and water. At 5 days of age, the wing bands were removed and reattached to the upper arm. The body weight of each bird was measured at 0 and 5 days of age. A total of 13 chicks died accidentally by the age of 5 days. At 7 days of age, the chicks were evenly divided into the two commercial Miyazaki Jitokko farms A and B and farmers were unaware of which birds (conventional or improved) they had. Both farms were located in Nichinan (31° N and 131° E), Japan, which ensures that they experienced similar climates. The birds deposited at the two farms were reared in adherence to the Miyazaki Jitokko Management Manual (Miyazaki Jitokko Cooperative Association and Kawaminami Branch, Miyazaki Prefectural...
Conventional and improved chickens were raised in the same herd. Moreover, the birds were fed the same diets supplied by Chubu Shiryo (Nagoya, Japan); they were provided with a starter diet (ME, 3,000 kcal/kg; CP, 22%) from 0 to 3 weeks, a grower diet (ME, 3,000 kcal/kg; CP, 18%) from 4 to 14 weeks, and a finisher diet (ME, 3,000 kcal/kg; CP, 18%) from 15 weeks and thereafter. Feed and water were provided ad libitum throughout the experiment.

Males and females were slaughtered at 126 and 163 days of age, respectively. Prior to slaughtering, the farmers at the two farms randomly captured 430 birds, including 176 males and 254 females, and the body weight at slaughter of each bird was measured. The farmers did not capture birds and collect their blood to determine their CCKAR genotype during fattening, because they did not want to stress the chickens. Therefore, to estimate the A and C allele frequencies in the conventional Miyazaki Jitokko chickens, we analyzed nine F1 cocks (Jitokko sire × White Plymouth Rock dams) and 63 Kyushu Rhode hens that contributed to the production of the conventional chickens. Blood was collected in heparinized tubes from the brachial wing vein, spotted onto an FTA CloneSaver card (WB120028:GE Healthcare, Buckinghamshire, UK), and left to dry overnight at room temperature. The extraction of the genomic DNA from the FTA CloneSaver card and genotyping of the g.420 C>A SNP were performed as previously described (Rikimaru et al., 2013).

### Effects of Selection Using the CCKAR SNP As a Marker on Production Traits in Kyushu Rhode Hens

Thirty chicks each of conventional and improved Kyushu Rhode females were hatched on the same day (April 19, 2018) and raised in the same chicken house in the Kawanami Branch of the Miyazaki Prefectural Livestock Research Institute until 50 weeks of age. The chicken house consisted of solid-floored pens in a conventional poultry research house. To heat-insulate the chicks, a chick-guard and gas-type brooder (Big-G 1200S, Nakajima Seisakusho Co., Nagano, Japan) were used until the birds were 4 weeks of age. The chicks were fed a starter diet (ME, 2,900 kcal/kg; CP, 20%[wt/wt]) from 0 to 4 weeks of age, first grower diet (ME, 2,850 kcal/kg; CP, 17%) from 4 to 10 weeks, second grower diet (ME, 2,750 kcal/kg; CP, 14%) from 10 to 17 weeks, and adult chicken diet (ME, 2,750 kcal/kg; CP, 16%) from 17 to 50 weeks of age. Feed was provided ad libitum from 0 to 7 weeks of age. From 7 to 50 weeks of age, feed was restricted. The rate of ME fill was set to 85%, where ME was calculated based on the formula for the layer hens as previously described in chapter 1.1.2 of the Japanese Feeding Standard for Poultry (Japan Livestock Industry Association, 2011). The birds were placed as a group, and feed and water were provided ad libitum throughout the experiment.

Female body weight was measured at 1, 4, 7, 10, 14, 17, 21, 25, 30, 35, 41, and 46 weeks of age. The weekly egg-laying rate was calculated using the following equation:

\[ y (\%) = \frac{v}{(30 \times 7) \times 100} \]
where \( y \) is the weekly egg-laying rate of each experimental group, \( x \) is the number of eggs collected at one-week intervals, \( 30 \) is the number of hens per experimental group, and \( 7 \) is the number of days in a week.

The mating ratio of F1 cocks (Jitokko sire × White Plymouth Rock dams) to Kyushu Rhode hens within a flock was 1:10 (3 cocks and 30 hens) and was used to obtain fertilized eggs. The conventional and improved F1 cocks mated with the conventional and improved Kyushu Rhode hens, respectively. The F1 cocks were hatched on the same day (April 19, 2018). The number of eggs laid was recorded every day throughout the experiment. The fertilization and hatching rates of the eggs that were collected at 27–28, 29–31, 33–36, and 37–39 weeks of age were surveyed. The collected eggs were stored as described, and randomly chosen eggs were incubated. On day 14 of incubation, fertilized eggs were visually identified using a light-emitting diode, and the fertilization rate, including embryonic deaths, was assessed. The hatching rate was surveyed on day 21 of incubation. After omitting the eggs that failed to hatch and the frail chicks, the number of hatched chicks was determined. The hatching rate was calculated by dividing the number of hatched chicks by the number of fertilized eggs, thereby omitting embryonic deaths.

**Statistical Analysis**

Allele frequencies were calculated by gene counting. The difference between the population means, difference between the two ratios, and equality of the two variances were examined using Student’s-\( t \), \( \chi^2 \), and F-tests, respectively.

The association between improvements in slaughter weight and marker-assisted selection was analyzed using the R package with the generalized linear model, as follows:

\[ y = u + C_{\text{farm}} + C_{\text{group}} + e, \]

where \( y \) is the slaughter weight; \( u \) is the intercept of the model formula; the farm effect (\( \text{farm} \)) is a covariate coefficient of \( C_{\text{farm}} \) with values of 0 and 1 for farms A and B, respectively; the group effect (\( \text{group} \)) is a covariate coefficient of \( C_{\text{group}} \) with values of 0 and 1 for the conventional and improved groups, respectively; and \( e \) is the residual standard error. \( \text{farm} \) and \( \text{group} \) are fixed effects.

**Results**

**Fattening Experiments on Commercial Poultry Farms**

The nine F1 cocks (Jitokko sire × White Plymouth Rock dams) of the non-selected population comprised five AA, two AC, and two CC genotypes. The AA, AC, and CC genotype frequencies were 0.556, 0.222, and 0.222, respectively; whereas, the A and C allele frequencies were 0.666 and 0.333, respectively. Meanwhile, the 63 Kyushu Rhode hens in the non-selected population comprised 27 AA, 32 AC, and 4 CC genotypes. The AA, AC, and CC genotype frequencies were 0.429, 0.508, and 0.063, respectively; whereas, the A and C allele frequencies were 0.683 and 0.317, respectively.

Given that the nine F1 cocks and 63 Kyushu Rhode hens were randomly mated, the frequencies of the AA, AC, and CC genotypes in the conventional Miyazaki Jitokko chickens were estimated to be 0.455 (\( = 0.667 \times 0.682 \)), 0.439 (\( = 0.667 \times 0.317 \), and 0.106 (\( = 0.333 \times 0.317 \), respectively; whereas, the frequencies of the A and C alleles were calculated as 0.675 (\( = (0.455 + 0.455 + 0.439) / 2 \)) and 0.325 (\( = (0.106 + 0.106 + 0.439) / 2 \)), respectively.

A comparison of the body weights for the Miyazaki Jitokko chickens in the two commercial farms is shown in Table 2. Among male birds, significant differences in body weight between the conventional and improved groups at both farms were observed at 0, 5, and 126 days of age. Among female birds, significant differences in body weight between the two groups at both farms were observed at 0 and 5 days of age. At 163 days of age, a significant difference between body weights of the conventional and improved groups was observed at farm A, but not at farm B. The variance in the body weight of the improved males was significantly narrower than that of conventional birds at 126 days of age at both farms.

The effects of the SNP site on growth traits between farms A and B and between the conventional and improved Miyazaki Jitokko chickens are shown in Table 3. Males from farm B were significantly heavier than those from farm A. The improved males demonstrated a significantly higher body weight at 126 days of age compared to their conventional counterparts. The improved females demonstrated a significantly higher body weight at 163 days of age compared to their conventional counterparts. Among males and females, the effects of A-allele fixing on slaughter body weight were estimated to be +123.5 g and +131.9 g, respectively.

**Effects of Selection Using the CCKAR SNP As a Marker on Production Traits in Kyushu Rhode Hens**

The egg-laying curves for the conventional and improved Kyushu Rhode hens indicated no significant differences between improved and conventional hens in terms of weekly egg-laying rates (Fig. 1).

The fertilization rate and hatchability of eggs that were produced from the conventional and improved Kyushu Rhode chickens are presented in Table 4. No differences were found in the number of embryonic deaths, hatchability of fertile eggs, and hatchability of set eggs between the improved and conventional chickens, except for the hatchability of fertile eggs in the interval between 29 and 31 weeks of age.

The effects of selection using the CCKAR SNP on the body weight of the Kyushu Rhode hens are provided in Table 5. The improved Kyushu Rhode chickens were significantly heavier than the conventional hens at 1, 4, and 7 weeks of age. After the onset of restricted feeding, no significant differences were found at 10, 14, 17, 21, 25, 30, 35, 41, and 46 weeks of age. The variance in the body weight of the improved hens was significantly narrower than that of their conventional counterparts at 10, 14, 35, 41, and 46 weeks of age.

**Discussion**

CCK is a well-known gut peptide that inhibits food intake in mammals (Gibbs et al., 1973). Two receptors for CCK,
CCKAR (Sankaran et al., 1980), and CCK type B receptor (CCKBR) (Innis and Snyder, 1980) have been reported. Ohkubo et al. (2007) reported that the mRNA of CCKAR in chickens is mainly distributed in the alimentary tract, except for the proventriculus and gizzard. Moreover, the mRNA of CCKBR is predominantly expressed in the brain (Wank, 1995). The CCK octapeptide (CCK-8), which is exogenously administered via intracerebroventricular (Denbow and Myers, 1982), intraperitoneal (Covasa and Forbes, 1994), and intravenous (Savory and Gentle, 1980) routes transiently, has been found to depress food intake in chickens. However, these responses were obtained at surprisingly high CCK-8 doses. According to Mabayo et al. (1992), the dosage of CCK in these reports was more than 1000 times higher than the physiological plasma concentration. Corwin et al. (1991) were the first to demonstrate that very low doses of the CCKAR antagonist devazepide (DVZ, also termed L-364,718 or MK-329), but not large doses of the CCKBR antagonist L-365,260, increased food intake in rats. This study is considered 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 molecule size, but DVZ crosses the blood–brain barrier (Pullen and Hodgson, 1987). Therefore, the increased food intake that is observed following DVZ administration may be due to a central effect, rather than the antagonism of endogenous peripheral CCK. Covasa and Forbes (1994) reported that 1) intraperitoneal DVZ doses that range from 8 to 32 μg/kg BW exhibited no effect on food intake in free-feeding chickens 2 h after injection, 2) high doses that exceed 90 μg/kg BW of intraperitoneal DVZ increased food intake in free-feeding chickens 90 to 120 min after injection, and 3) CCK-8 (14 μg/kg BW) caused a transient reduction in feeding, and this effect was not blocked by pre-treatment with a high dose of more than 90 μg/kg BW of intraperitoneal DVZ. Covasa and Forbes (1994) thus questioned the involvement of endogenous CCK as a satiety agent in chickens, as their findings were contrary to previous findings that indicate DVZ acts as an effective CCKAR antagonist in rats (Corwin et al., 1991). Overall, no evidence demonstrates that CCK acts as a true satiety signal and decreases food intake under physiologically normal conditions in chickens, regardless of whether CCK and CCK-8 cross the blood–brain barrier.

In addition, a current explanation on the reason that underlies the g.420 C>A SNP in CCKAR affects growth traits. Therefore, we cannot exclude the possibility that the associations that were detected in this study may have resulted from the linkage disequilibrium between the SNP and other linked nuclear DNA polymorphisms that are directly involved in the regulation of growth traits. For example, in the distal region of chromosome 4 where CCKAR is located, the CCKAR SNPs—other than g.420 C>A (Dunn et al., 2013) and the SNPs in other candidate genes (Lyu et al., 2017)—have been suggested to affect growth traits by studies on resource populations of White Leghorn×commercial broilers (Dunn et al., 2013) and New Hampshire×White Leghorn (Lyu et al., 2017), respectively. Regardless, without doubt,
the g.420 C>A SNP is a quality marker for marker-assisted selection to improve growth traits with a wide application range in chickens, since a significant association is reported between the SNP and growth traits in other commercial slow-growing chickens (Amakusa Daioh cross chicken, Takahashi et al., 2019; Miyazaki Jitokko chicken, Horinouchi et al., 2019; Hinai-jidori chicken, Rikimaru et al., 2019; Okumino-kojidori chicken, Ishikawa et al., 2020).

Since a significant difference in the slaughter body weight of males between farms A and B was detected in the present study, environmental factors are suggested to affect growth traits. However, the reason for this difference is currently unknown. The environmental factors of climate and feeding conditions were nearly identical in both farms, and the chicks were maintained in adherence to the same management manual. However, the data from the present study suggest that SNP-associated genetic improvements of growth traits can be detected, even if unexpected environmental factors affect growth traits. Additionally, the variation in body weight was narrower in the selected populations due to A-allele fixation, especially among Miyazaki Jitokko males. A similar standardization of body weight was observed in the Kyusyu Rhode hens during the laying period. These phenomena are likely due to A-allele fixation.

Horinouchi et al. (2019) reported that the difference in body weight at 17 weeks (119 days) of age between the conventional and improved Miyazaki Jitokko male chickens was estimated to be +59.3 g when the A-allele frequency was altered from 0.717 to 1.0. Data from the present study suggest that the increase in slaughter weight was approximately +123.5 g in males at 126 days of age and +131.9 g in females at 163 days of age, when the A-allele frequency was altered from 0.675 to 1.0. Although simple comparisons between these two sets of data are not feasible, the slaughter body weight was shown to be improved by A-allele fixation in the Miyazaki Jitokko chickens both in our experimental station and commercial farms.

For sustainable Miyazaki Jitokko production in commercial farms, securing fattening chicks is one of the most important concerns for local hatcheries. Among the Miyazaki Jitokko chickens, fattening chicks are produced by the Kyushu Rhode hens. In broilers, a negative relationship between genetic selection for growth and reproduction traits has been found. For example, reduced reproductive per-
formance in the guise of erratic ovulation and defective egg syndrome is currently commonplace among broiler breeder hens (Barbato, 1999)—which thus explains concerns that reproductive traits may deteriorate in association with the genetic improvements of growth traits by CCKAR SNP fixation. However, our data demonstrate no significant differences in egg-laying rates, fertilization rates, and hatchability between the conventional and improved Kyushu Rhode hens, which suggests that fattening chicks for practical use can be supplied as usual.

Given the data in the present study, a revenue increase for commercial farms due to A-allele fixation of Miyazaki Jitokko chickens can be estimated by the following equation:

\[ \text{Annual revenue increase} = \frac{(123.5 + 131.9)}{2} \times a \times b, \]

where \( a \) is number of Miyazaki Jitokko chickens annually shipped; \( b \) is the price per unit of the body weight at slaughter (kg). In actuality, the number of Miyazaki Jitokko chickens annually shipped was 504,000, and the price per unit of the body weight at slaughter was 620 yen per kg in the fiscal year of 2017 (Miyazaki Prefectural Government, unpublished data). The income of commercial farms is thus estimated to increase annually by 39,903,696 yen. Given the fact that 50 commercial farms had produced Miyazaki Jitokko chickens during the fiscal year of 2017, the increase in income per farm is estimated to be approximately 798,000 yen.

In conclusion, we demonstrated that the marker-assisted selection of the CCKAR SNP site in commercial poultry farms can improve the growth traits of the Miyazaki Jitokko chickens. Therefore, we plan to launch the supply of chicks of parent stocks with A-allele fixation to private hatcheries. Within the next few years, A-allele fixed Miyazaki Jitokko chickens will be supplied to all commercial farms.

Acknowledgments

This work was financially supported by the Project of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on developing regional strategies). The authors thank the technical staff of the Kawaminami Branch of the Miyazaki Prefectural Livestock Research Institute and the farmers who cooperated with this experiment for their kind assistance.

Conflict of Interest

The authors declare no conflicts of interest.
Table 5. Comparison of the body weight of conventional and improved Kyushu Rhode hens throughout the experiment

| Body weight (g) | Improved   | Conventional | a   | b   |
|----------------|------------|--------------|-----|-----|
| 1 week         | 98.0±8.0   | 82.2±9.5     | **  |     |
| 4 weeks        | 513.9±26.5 | 448.1±66.0   | **  |     |
| 7 weeks        | 1183.7±58.9| 1111.9±156.1|     | *   |
| 10 weeks       | 1345.7±110.0| 1335.7±189.9|     | **  |
| 14 weeks       | 1674.3±149.3| 1644.9±218.6|     | **  |
| 17 weeks       | 1831.1±167.3| 1778.4±170.9|     |     |
| 21 weeks       | 2052.5±190.0| 2052.1±223.0|     |     |
| 25 weeks       | 2458.4±234.8| 2386.5±224.8|     |     |
| 30 weeks       | 2881.9±206.8| 2890.8±275.5|     |     |
| 35 weeks       | 3033.1±184.5| 2964.2±324.0|     | **  |
| 41 weeks       | 3111.8±188.1| 3016.2±364.6|     | **  |
| 46 weeks       | 3162.1±185.7| 3064.4±365.0|     | **  |

Data are expressed as means±SD.

a, difference between the two population means; b: quality of the two population variances

*P<0.05, **P<0.01

References

Barbato GF. Genetic relationships between selection for growth and reproductive effectiveness. Poultry Science, 78: 444–452. 1999.

Corwin RL, Gibbs J and Smith GP. Increased food intake after type A but not type B cholecystokinin receptor blockade. Physiology & Behavior, 50: 255–258, 1991.

Covasa M and Forbes JM. Effects of the CCK receptor antagonist MK-329 on food intake in broiler chickens. Pharmacology Biochemistry and Behavior, 48: 479–486, 1994.

Denbow DM and Myers RD. Eating, drinking and temperature responses to intracerebroventricular cholecystokinin in the chick. Peptides, 3: 739–743. 1982.

Dunn IC, Hocking PM, Meddle SL, Wilson PW, Wardle C, Law AS, Bishop A, Hindar C, Robertson GW, Burt DW, Ellison S JL and Morrice DM. Decreased expression of the satiety signal receptor CCKAR is responsible for increased growth and body weight during the domestication of chickens. American Journal of Physiology-Endocrinology and Metabolism, 304: E909–E921. 2013.

Gibbs J, Young RC and Smith GP. Cholecystokinin decreases food intake in rats. Journal of Comparative and Physiological Psychology, 84: 488–495. 1973.

Horinouchi S, Nakayama H and Takahashi H. Effect of a single nucleotide polymorphism in the cholecystokinin type A receptor gene on growth traits of the Miyazaki Jitokko chicken. Journal of Poultry Science, 56: 96–100. 2019.

Innis RB and Snyder SH. Distinct cholecystokinin receptors in brain and pancreas. Proceedings of the National Academy of Sciences of the United States of America, 77: 6917–6921. 1980.

Ishikawa S, Asano M, Sakai K and Takahashi H. Verification of the effectiveness of an SNP marker in the cholecystokinin type A receptor gene for improving growth traits in Okuminoko-jidori chickens. Journal of Poultry Science, 57: 107–113. 2020.

Japan Livestock Industry Association. Japanese feeding standard for poultry. National Agriculture and Food Research Organization ed. 2011.

Lyu S, Arends D, Nassar MK and Brockmann GA. Fine mapping of a distal chromosome 4 QTL affecting growth and muscle mass in a chicken advanced intercross line. Animal Genetics, 48: 295–302. 2017.

Mabayo RT, Furuse M, Yang S and Okumura J. Medium-chain triacylglycerols enhance release of cholecystokinin in chicks. Journal of Nutrition, 122: 1702–1705. 1992.

Miyazaki Jitokko Cooperative Association and Kawaminami Branch and Miyazaki Prefectural Livestock Research Institute. Miyazaki Jitokko management manual. a revised edition in 2013. https://www.pref.miyazaki.lg.jp/contents/org/nosei/chikusan/chikusangyo/20180522150217.html. Accessed on October 20, 2019. (in Japanese)

Miyazaki Prefectural Government. Livestock in Miyazaki Prefecture in 2019. http://www.pref.miyazaki.lg.jp/shinsei-chikusan/shigoto/chikusangyo/20180522150217.html. Accessed on October 20, 2019. (in Japanese)

Ohkubo T, Shamoto K and Ogino T. Structure and tissue distribution of cholecystokinin-1 receptor in chicken. The Journal of Poultry Science, 44: 98–104. 2007.

Pullen RGL and Hodgson OJ. Penetration of diazepam and the non-peptide CCK antagonist, L364,718 into rat brain. Journal of Pharmacy and Pharmacology, 39: 863–864. 1987.

Rikimaru K, Komatsu M, Suzuki K, Uemoto Y, Takeda H and Takahashi H. Association between cholecystokinin type A receptor haplotypes and growth traits in Japanese Hinai-dori crossbred chickens. Molecular Biology Reports, 39: 4479–4484. 2012.

Rikimaru K, Sato Y, Ito Y, Fukuda S, Sasaki S and Takahashi H. Is a single nucleotide Polymorphism marker in the cholecystokinin A receptor gene practically suitable for improving the growth traits of Hinai-jidori chickens? Journal of Poultry Science (in press). https://doi.org/10.2141/jpsa.0190041. Accessed on October 11, 2019.

Sankaran H, Goldfine ID, Deveney CW, Wong KY and Williams JA. Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. The Journal of Biological Chemistry, 255: 1849–1853. 1980.

Savory CJ and Gentle MJ. Intravenous injections of cholecystokinin and caerulein suppress food intake in domestic fowls. Experientia, 36: 1191–1192. 1980.
Science Council of Japan Guidelines for proper conduct of animal experiments. http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2.pdf. Accessed on October 20, 2019.
Shibuya, T, Fujio Y, and Kondo K. Studies on the action of Creeper gene in Japanese chicken. Japanese Journal of Genetics, 47: 23–32. 1972.
Takahashi H, Katayama M, Michishita K and Yamashita H. The A allele of the cholecystokinin type A receptor g.420 C>A polymorphism improves the growth traits of the Amakusa Daioh cross chicken. Journal of Poultry Science, 56: 91–95. 2019.
Wank SA. Cholecystokinin receptors. American Journal of Physiology–Gastrointestinal and Liver Physiology, 269: G628–G646. 1995.