Supplemental arachidonic acid-enriched oil improves the taste of thigh meat of Hinai-jidori chickens

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ABSTRACT The Hinai-dori is a breed of chicken native to the Akita prefecture in Japan. A cross between the Hinai-dori and Rhode Island Red breeds has been commercialized as the Hinai-jidori chicken, one of the most popular brands in Japan. High arachidonic acid (AA) content is a characteristic feature of Hinai-jidori chicken meat. To elucidate the relationship between AA content and the palatability of the Hinai-jidori chicken, we examined the effects of palm oil (PO), corn oil (CO), and arachidonic acid-enriched oil (AAO) diet supplementation on the fatty acid content and sensory perceptions in thigh meat. Each oil and silicate was mixed at a ratio of 7:3, 5% of fresh matter was added to the finisher diet, and Hinai-jidori chickens were fed these diets for 2 wk before slaughter. In thigh meat, the AA content of the AAO group was significantly more than 2-fold higher than that of the PO and CO groups. Other fatty acid contents were not significantly different among the groups. Sensory evaluation showed that the total taste intensity, umami (L-glutamate taste), kokumi (continuity, mouthfulness, and thickness), and aftertaste of the AAO group were significantly higher than those of PO and CO groups in both chicken soup and steamed minced meat. These data suggest that the palatability of chicken meat can be improved by dietary AA supplementation.

Key words: Hinai-jidori chicken, fatty acid content, arachidonic acid, feed supplement, sensory evaluation

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INTRODUCTION

Globally, chicken meat is obtained from several fast-growing broiler strains provided by commercial breeding companies that use intensive fatting systems to ensure high meat yields. However, some Japanese consumers are willing to pay a high retail price for better quality chicken meat, known as Jidori chicken in Japan. Jidori chickens were raised to increase production of a breed native to Japan while increasing consumer perception of improved animal welfare and a more natural production approach based on rulings of the Japanese Agricultural Standard (Ministry of Agriculture, Forestry and Fisheries of Japan, 1999). Because the chickens require a relatively long growing time and involve considerably high production cost, the retail price of these chickens can be 2 to 5 times higher than that of broilers. In Japan, most high-quality chickens were initially bred by crossing native Japanese breeds with highly selected lines with rapid growth rate or relatively high egg production. The Hinai-jidori chicken, a cross between Hinai-dori (a breed native to the Japanese Akita prefecture) sires and Rhode Island Red dams, is a popular Jidori chicken brand in Japan (Rikimaru and Takahashi, 2007).

A sensory evaluation report demonstrated increased palatability of the Hinai-jidori chicken over broilers (Hatakeyama et al., 1983). Although studies have shown that free amino acids content, including Glu and inosine 5′-monophosphate (**IMP**), may be correlated with chicken meat palatability (Nishimura et al., 1988; Karasawa et al., 1989; Fujimura et al., 1994), it has not yet been elucidated whether these factors actually play a role in flavor. To define candidate substances related to chicken meat palatability, we raised Hinai-jidori and broiler chickens under identical environmental and time conditions and compared the meat quality traits (e.g., free amino acids and IMP content and fatty acid composition) of Hinai-jidori and broiler chicken thigh meat. We found that high arachidonic acid (**AA**, 20:4n-6) content is characteristic of Hinai-jidori chicken meat (Rikimaru and Takahashi, 2010); however, the direct

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Table 1. Content and composition of fatty acids in diets

Fatty acid	Finisher (control)		Palm oil		Corn oil		Arachidonic acid-enriched oil	
	g/100 g of diet	% fatty acid	g/100 g of diet	% fatty acid	g/100 g of diet	% fatty acid	g/100 g of diet	% fatty acid
Crude fat	3.4	—	6.9	—	6.9	—	6.9	—
Myristic acid (C14:0)	0.01	0.3	0.04	0.6	0.01	0.1	0.02	0.3
Palmitic acid (C16:0)	0.45	13.2	1.90	27.5	0.83	12.0	0.84	12.2
Palmitoleic acid (C16:1n-9)	0.02	0.5	0.02	0.3	0.02	0.3	0.02	0.3
Stearic acid (C18:0)	0.11	3.1	0.26	3.7	0.16	2.4	0.34	4.9
Oleic acid (C18:1n-9)	1.07	31.4	2.46	35.6	2.03	29.4	1.29	18.7
Linoleic acid (C18:2n-6)	1.45	42.6	1.80	26.1	3.45	50.0	1.76	25.5
α-Linolenic acid (C18:3n-3)	0.00	0.0	0.02	0.3	0.03	0.4	0.01	0.2
γ-Linolenic acid (C18:3n-6)	0.07	1.9	0.07	1.0	0.07	1.0	0.15	2.2
Eicosatrienoic acid (C20:3n-3+n-6)	0.00	0.0	0.00	0.0	0.00	0.0	0.12	1.7
Arachidonic acid (C20:4n-6)	0.00	0.0	0.00	0.0	0.00	0.0	1.54	22.4
Lignoceric acid (C24:0)	0.02	0.7	0.03	0.4	0.03	0.4	0.32	4.7
Unidentified fatty acids	0.23	6.9	0.34	4.9	0.31	4.4	0.80	11.5

involvement of AA in Hinai-jidori chicken palatability has not yet been established.

Arachidonic acid is a polyunsaturated fatty acid present in animal lipids. The AA is present at the head of the arachidonic acid cascade; more than 20 eicosanoid-mediated signaling pathways control a wide array of cellular functions, particularly those regulating inflammation, immunity, and the central nervous system (Brash, 2001). Recent taste perception studies have shown that even a slight amount of fatty acid is detected by taste bud cells (Mattes, 2009). Recently, we reported that polyunsaturated fatty acid, including AA, enhanced the umami (L-glutamate taste) and kokumi (continuity, mouthfulness, and thickness; Yamamoto et al., 2009) flavor of foods. When foods such as fried potatoes, vegetable soup, and fried rice were cooked with vegetable oil containing AA, palatability indices increased (Yamaguchi et al., 2005, 2007; Kiyohara et al., 2009). These findings suggest that AA may be associated with chicken palatability. To elucidate the relationship between AA content and palatability of Hinai-jidori meat, Hinai-jidori chickens were administered diets containing 3 oils (palm, corn, and AA-enriched) during rearing; meat was subsequently evaluated by biochemical and sensory analyses.

MATERIALS AND METHODS

Bird Housing and Treatment

All animals received humane care as outlined in Science Council of Japan (2006). Hinai-jidori chickens were raised in the Livestock Experiment Station in the Akita Prefectural Agriculture Forestry and Fisheries Research Center (Daisen, Japan). Thirty female chicks hatched on the same day were raised in an open-sided poultry shed and given access to a grass paddock until 20 wk of age. Chicks were fed starter diet [ME, 3,000 kcal/kg; CP, 24% (wt/wt)] from 0 to 4 wk, grower diet (ME, 2,850 kcal/kg; CP, 18%) from 5 to 10 wk, and finisher diet (ME, 2,900 kcal/kg; CP, 16%) from 11 to 20 wk; the diets were specially prepared for Hinai-jidori chickens (Kitanihon Kumiai Feed Co., Sendai, Japan).

Three types of oil, including palm oil (iodine value = 60, melting point = 15°C; J-Oil Mills Inc., Tokyo, Japan), corn oil (J-Oil Mills Inc.), and AA-enriched oil (Suntga40S, Nippon Suisan Co., Tokyo, Japan), were mixed in a volume ratio of 7:3 with silicate (Tixosil 38A, Rhodia Silica Korea Co., Seoul, South Korea). Mixtures were powdery and easy to handle. These mixtures were added to the finisher diet in the amount of 5% of fresh matter, and the 3 experimental diets were prepared. Diet fatty acid profiles are shown in Table 1. Oil enriched with AA is fermented and comprises 95% triglyceride. Of these triglycerides, AA accounts for 40%.

At 20 wk of age, 30 female birds were moved to individual cages and evenly divided into 3 dietary groups: palm oil (**PO**), corn oil (**CO**), and AA-enriched oil (**AAO**). The 3 diets were administered at 100 g/d per bird from 21 to 22 wk of age. Food intake was less than that of ordinary Hinai-jidori chickens to ensure that all of the food was consumed. Water was provided ad libitum.

Sample Preparation and Storage

At 22 wk of age, the chickens fasted for 18 h and were then slaughtered. The chickens were bled and plucked and their carcasses were manually eviscerated and washed, followed by immediate cooling in ice-cold water until a temperature of 8°C was reached. They were then removed from the water and allowed to drain for 30 min. Carcasses were dissected, the thigh meat was deboned after skin removal, the thigh meat from one leg was minced using a domestic meat chopper (No. 5-A, Veritas, Tokyo, Japan), and minced meat was wrapped in Saran Wrap (Asahi Kasei Co., Tokyo, Japan). Thigh meat from the remaining leg was placed in a plastic bag and vacuum packed. Meat samples were stored at −30°C until further analysis.

Determination of Meat Moisture, Crude Fat, and Fatty Acid Content

Moisture content was determined using a freeze dryer (RL-B07, Kyowa Vacuum Engineering Co., Tokyo, Japan). Crude fat content was determined according to the diethyl ether extraction method (960.39a) of Association of Official Analytical Chemists (1990).

To determine fatty acid profiles, we extracted lipids from 0.1 g of each minced meat sample by using 3 mL of chloroform:methanol (2:1, vol/vol) according to the method described by Iverson et al. (2001). The extract was thoroughly mixed with 1.5 mL of hexane. Following addition of 200 μL of 2 *M* potassium hydroxide in methanol, the contents were vortexed for 30 s. Next, 2 mL of saturated sodium chloride solution was added and mixed thoroughly. The sample was then centrifuged at 1,000 × *g* for 5 min, and the supernatant containing fatty acid methyl esters was recovered. The fatty acid methyl esters were separated using a GC2010 Gas Chromatograph (Shimadzu Co., Kyoto, Japan) and a capillary column (length = 30 m, i.d. = 0.25 mm, film thickness = 0.25 μm; DB-23, Shimadzu). The column was set at an initial temperature of 80°C for 2 min, then increased from 80 to 160°C at 35°C/min, 160 to 185°C at 2°C/min, and then 10°C/min to a maximum temperature of 230°C, which was maintained for 9 min. Other conditions included the following: injection port temperature, 250°C; flame ionization detector temperature, 250°C; helium flow rate, 1.49 mL/min; helium liner flow velocity, 35.4 cm/s. The fatty acids were identified comparing retention times with Supelco 37 Component FAME Mix (Sigma-Aldrich Co., St. Louis, MO). The fatty acid contents were also determined by the official method of the Japan Oil Chemists' Society (2009) using an internal standard (methyl tricosanoate, Sigma-Aldrich Co.).

Sensory Analysis

We conducted sensory evaluation using steamed minced meat and chicken soup to remove the effect of meat appearance and texture.

Steamed Minced Meat Preparation. Thigh meat was thawed overnight at 4°C, the skin was removed, and the meat was minced twice using a meat chopper (no. 5-A, Veritas). Subsequently, 20 g of minced fresh meat was placed in a round plastic container and covered with a lid (8.1 cm diameter × 3.4 cm height; cat. no. 1,741, Inomata Chemical Co., Tokyo, Japan), heated in a microwave oven (500 W; NE-P7, Panasonic Co., Osaka, Japan) for 40 s, and immediately served to subjects for sensory evaluation.

Preparation of Chicken Soup. We prepared chicken soup using the same minced fresh meat as that used for steamed minced meat chicken. In a stainless steel pot, 200 g of minced meat and 600 g of water were mixed and left at room temperature for 20 min. The mixture was heated at a high temperature until boiling and stewed for 30 min. Next, the soup was filtered through 4 sheets of gauze, 6 g of sodium chloride was added, and the soup was diluted with water to 300 g in total. The soup was served to panelists at 60°C in 10-mL portions in a plastic cup (EI-75D, Asahi-Kasei Co., Tokyo, Japan).

The final sodium chloride concentration in the soup was tested using a salinometer (PAL-ES1, Atago Co., Tokyo, Japan), and it was determined that each soup contained 0.8% salt. The Glu content was determined using a kit (L-Glu Assay Kit II, Yamasa Co., Choshi, Japan). The IMP content was determined using HPLC (1100 HPLC series, Agilent Technologies Co., Tokyo, Japan).

Sensory Evaluation. Experienced panelists were selected by the Oils and Fats Fundamental Technology Laboratory, J-Oil Mills Inc. We performed 5 grades of scoring tests using a reference sample (Kiyohara et al., 2009). The panelists tasted 2 pairs of chicken samples (PO–AAO and CO–AAO), which were classified by oil in the diets. In these cases, PO and CO were used as reference standards against AAO. In the evaluation of steamed minced meat, 7 parameters were evaluated: total taste intensity, sweetness, sourness, umami, kokumi, aftertaste, and oiliness. In the evaluation of chicken soup, saltiness was evaluated instead of oiliness. Panelists recorded whether the test sample was comparatively stronger or better (score = +2, +1) or weaker or worse (score = −1, −2) than the reference sample (score = 0). The steamed minced meat and chicken soup were evaluated by 22 and 18 panelists, respectively.

Statistical Analyses. All statistical analyses were performed using SPSS 15.0J for Windows software (SPSS Inc., Chicago, IL). Except for sensory evaluation data, comparisons among the treatment means were assessed by performing Tukey HSD and Dunnett T3 tests at a significance level of $P < 0.05$. Sensory evaluation data were analyzed by Wilcoxon's signed rank test at a significance level of $P < 0.05$ or $P < 0.01$.

RESULTS

The moisture and crude fat levels in thigh meat from the 3 test groups are shown in Table 2. No significant differences were found in the moisture and crude fat contents among the groups. The fatty acid content of thigh meat is shown in Table 3. The AA of AAO content was significantly (greater than 2-fold) higher than

Table 2. Moisture and crude fat in thigh meat of Hinai-jidori chickens fed experimental diets[1]

Item	Palm oil	Corn oil	Arachidonic acid-enriched oil
n	10	10	10
Moisture (%)	74.1 ± 2.9	71 ± 0.7	71.7 ± 0.4
Crude fat (%)	7.3 ± 0.6	7.5 ± 0.8	6.5 ± 0.5

[1]Values are mean ± SD.

Table 3. Fatty acid content (mg/g) of thigh meat of Hinai-jidori chickens fed experimental diets[1]

Item	Palm oil	Corm oil	Arachidonic acid-enriched oil
n	10	10	10
Myristic acid (C14:0)	0.36 ± 0.03	0.3 ± 0.04	0.32 ± 0.04
Palmitic acid (C16:0)	13.1 ± 0.99	11.86 ± 1.57	11.68 ± 1.36
Palmitoleic acid (C16:1n-9)	2.18 ± 0.26	2.18 ± 0.37	1.81 ± 0.26
Heptadecanoic acid (C17:0)	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Stearic acid (C18:0)	3.77 ± 0.22	3.6 ± 0.38	3.96 ± 0.36
Oleic acid (C18:1n-9)	20.34 ± 1.65	20.06 ± 2.75	18.17 ± 2.03
Linoleic acid (C18:2n-6)	9.57 ± 0.83	10.36 ± 1.08	9.88 ± 1.66
γ-Linolenic acid (C18:3n-6)	0.09 ± 0.01[a]	0.09 ± 0.02[a]	0.17 ± 0.02[b]
Linolenic acid (C18:3n-3)	0.34 ± 0.03	0.33 ± 0.04	0.34 ± 0.04
Eicosenoic acid (C20:1n-9)	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.01
Eicosadienoic acid (C20:2n-6)	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.01
Eicosatrienoic acid (C20:3n-3+n-6)	0.1 ± 0.01[a]	0.08 ± 0.01[a]	0.19 ± 0.02[b]
Arachidonic acid (C20:4n-6)	0.92 ± 0.03[a]	0.8 ± 0.05[a]	2.16 ± 0.19[b]
Docosahexaenoic acid (C22:6n-3)	0.25 ± 0.01[a]	0.21 ± 0.02[b]	0.21 ± 0.01[b]
Unidentified fatty acids	1.89 ± 0.19	1.95 ± 0.24	2.08 ± 0.18

[a,b]Means within a row with no common superscript differ ($P < 0.05$).

[1]Values are mean ± SD.

that of PO and CO. The γ-linolenic acid and eicosatrienoic acid contents of AAO were significantly higher than that of PO and CO. The docosahexaenoic acid content of PO was significantly higher than that of CO and AAO.

The sensory evaluation results of steamed minced meat are shown in Table 4. In a pairwise comparison between PO and AAO, the total taste intensity, sourness, umami, kokumi, and aftertaste of AAO were significantly higher than that of PO. In a pairwise comparison between CO and AAO, the total taste intensity, sweetness, umami, kokumi, and aftertaste of AAO were significantly higher than that of CO.

The sensory evaluation results of chicken soup are shown in Table 5. In a pairwise comparison between PO and AAO, the total taste intensity, sweetness, sourness, umami, kokumi, aftertaste, and saltiness of AAO were significantly higher than that of PO. In a pairwise comparison between CO and AAO, the total taste intensity, sweetness, umami, kokumi, aftertaste, and saltiness of AAO were significantly higher than that of CO.

The Glu and IMP contents of PO, CO, and AAO chicken soup are shown in Table 6. No significant differences were found in the Glu content among PO, CO, and AAO. The IMP content of AAO was significantly higher than that of PO and CO. The umami intensity of each soup was calculated from the observed Glu and IMP content in consideration of their synergistic effects reported by Yamaguchi (1967). The differences in the umami intensity among the 3 groups were less than 1%. The data showed that differences between the groups of the umami intensity could not be attributed to the Glu and IMP contents because Yamaguchi (1967) reported that the differential threshold of umami between samples is 21%.

DISCUSSION

It is widely agreed that sweet, sour, salty, bitter, and umami taste qualities exist in humans. Recent studies have suggested that fats and oils can be implicated in gustatory sense (Chaudhari and Roper, 2010). Al-

Table 4. Sensory evaluation of steamed minced meat of Hinai-jidori chickens fed experimental diets

	Pair[2]	
Item[1]	PO–AAO[3]	CO–AAO[4]
Total taste intensity	0.72**	0.56**
Sweetness	−0.03	0.34*
Sourness	0.34*	0.03
Umami	0.59**	0.50*
Kokumi	0.66**	0.75**
Aftertaste	0.69**	0.50*
Oily	0.03	0.06

[1]Umami = L-glutamate taste; kokumi = continuity, mouthfulness, and thickness.

[2]PO = palm oil; CO = corn oil; AAO = arachidonic acid-enriched oil.

[3]Average AAO score of each subject when PO score is 0.

[4]Average AAO score of each subject when CO score is 0.

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

Table 5. Sensory evaluation of chicken soup made with meat of Hinai-jidori chickens fed experimental diets

	Pair[2]	
Item[1]	PO–AAO[3]	CO–AAO[4]
Total taste intensity	0.86**	0.75**
Sweetness	0.27*	0.27*
Sourness	0.55**	0.11
Umami	0.68**	0.64**
Kokumi	0.73**	0.77**
Aftertaste	0.86**	0.61**
Saltiness	0.61**	0.45*

[1]Umami = L-glutamate taste; kokumi = continuity, mouthfulness, and thickness.

[2]PO = palm oil; CO = corn oil; AAO = arachidonic acid-enriched oil.

[3]Average AAO score of each subject when PO score is 0.

[4]Average AAO score of each subject when CO score is 0.

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

Table 6. Glutamic acid and inosine 5′-monophosphate content in chicken soup made with meat of Hinai-jidori chickens fed experimental diets[1]

Item	Palm oil	Corn oil	Arachidonic acid-enriched oil
Glutamic acid (mg/100 mL)	119 ± 1.1	123 ± 0.8	116 ± 2.7
Inosine 5′-monophospate (μg/100 mL)	547 ± 3.9[a]	546 ± 2.0[a]	560 ± 2.1[b]
Intensity of umami[2]	785	788	798

[a,b]Means within a row with no common superscript differ ($P < 0.05$).

[1]Values are mean ± SD; n = 3.

[2]The value is expressed as the content of monosodium glutamate (MSG; mg/100 mL) in consideration of synergistic effect between glutamic acid and inosine 5′-monophospate (IMP) according to Yamaguchi (1967). The value (y) was calculated as y = u + Yv, where u = MSG content (mg/100 mL), Y = 1,218, and v = IMP content (mg/100 mL).

though dietary fat is predominantly in the form of triglycerides that are not effective taste stimuli, lingual lipase may yield sufficient free fatty acids (**FFA**) to act as a chemical cue. Kawai and Fushiki (2003) reported that lingual lipase secretion is constant and yields FFA quickly enough to enable detection by a fat sensor on the tongue surface.

Many studies have described the FFA gustatory transduction mechanism in humans, mice, and rats. Delayed rectifying potassium channels sensitive to select FFA have been identified in isolated taste receptor cells from rat fungiform papillae (Gilbertson et al., 1997). The CD36 and G-protein coupled receptors (i.e., GPR120 and GPR40) have recently been identified as putative FFA taste receptors (Laugerette et al., 2005; Cartoni et al., 2010). Laugerette et al. (2005) reported that CD36 is expressed in some type II (sweet, bitter, and umami) receptor cells in mouse taste buds. Cartoni et al. (2010) reported that GPR120 and GPR40 are mainly expressed in type II and type I cells, respectively. These data suggest that FFA may affect taste perception of salty, sweet, bitter, and umami based on taste receptor distribution; however, Reckmeyer et al. (2010) reported that linoleic acid (**C18:2n-6**) and oleic acid (**C18:1n-9**) did not increase or decrease the intensity of sweet, salty, sour, and umami tastants in humans. Oike et al. (2006) reported that AA activates the TRPM5 cation channel, which is located on taste pathways of sweet, bitter, and umami in type II receptor cells. Taken together, these data suggest that AA may directly affect taste perception in type II receptor cells modulating the TRPM5 channel; however, signal transduction via putative taste receptors (CD36, GPR120, and GPR40) by C18:2n-6 and C18:1n-9 may not affect the taste perceptions of sweet, salty, sour, and umami.

Our data show that dietary AAO may lead to significant increment of AA in the fatty acid content and composition of Hinai-jidori chicken thigh meat. The meats from the PO and CO groups generally contained increased palmitic acid (**C16:0**) and C18:2n-6 contents; however, significant increments of C16:0 and C18:2n-6 were not observed. Because most meat and soup characteristics (except AA content) were not significantly different among PO, CO, and AAO groups, our sensory data suggests that AA in meat may improve taste perception of total taste intensity, umami, kokumi, aftertaste, and saltiness. This is the first report to show that chicken meat palatability can be improved by dietary AA supplement. This finding is in good agreement with the above-mentioned hypothesis that AA may directly affect the taste perception of sweet, bitter, and umami via the TRPM5 channel. However, even if the content of the other fatty acids (e.g., C18:2n-6, C18:1n-9, and C16:0) were not significantly different among the 3 groups, we do not deny the possibility that they may affect taste perception. Therefore, further studies are needed to elucidate the effect of each fatty acid on the palatability of chicken soup and meat.

The reason that saltiness of AAO was stronger than that of PO and CO remains unclear. One possibility is that an interaction may exist between the transient receptor potential channel vanilloid subtype 1 (**TRPV1**) and AA in type III taste receptor cells. The amiloride-insensitive salt taste receptor is the predominant salt taste transducer in mammalian species, including humans (Lyall et al., 2004). Epithelial Na^+ channel has been proposed as an amiloride-sensitive salty receptor (Kretz et al., 1999). Additionally, Lyall et al. (2004) proposed a TRPV1 variant (TRPV1t) as an amiloride-insensitive salty taste receptor candidate. Treesukosol et al. (2007) reported that TRPV1 knockout mice showed diminished gustatory response to salty taste. Hwang et al. (2000) reported that 12-(S)-HPETE, an AA metaborate, and fatty acids (e.g., AA, C18:2n-6, and C18:3n-3) activate the TRPV1 channel. Because the AA content of AAO was significantly higher than that of PO and CO and no significant differences existed in the contents of C18:2n-6 and C18:3n-3 among PO, CO, and AAO groups, AA may activate the TRPV1 channel in type III receptor cells, thereby enhancing the saltiness.

In conclusion, we have shown that AA content in chicken meat can be manipulated by AA diet supplement and that chicken meat containing higher levels of AA tastes better than that containing low levels of AA. Our data strongly supports our previous hypothesis that AA is related to the palatability of Hinai-jidori chickens because this experiment was conducted using

Hinai-jidori chickens. In future studies, we plan to examine whether AA supplementation can improve the taste of broilers.

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