

Effect of a Mixed Fermented Loquat Leaf Tea By-Product on the Growth Performance and Meat Quality of Tsushima-Jidori Crossbred Chicken

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In the present study, the effects of dietary supplementation with a mixed fermented loquat leaf tea residue (MFL) were evaluated on muscle α -tocopherol concentration and drip loss of Tsushima-Jidori crossbred chicken. MFL contained significantly less β -carotene, α -tocopherol, and total catechin than that of residues of green tea leaf infusion, although total polyphenol was significantly higher and 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity tended to be higher, indicating increased antioxidant properties. A total of 120 male Tsushima-Jidori crossbred chickens were divided into two groups at 62 days of age. The control group was fed a basal diet (commercial finisher diet containing 16.5% crude protein and 12.77 MJ/kg metabolizable energy) and the test group was fed a basal diet supplemented with MFL at a concentration of 1.0% until 90 days of age. Body mass, body mass gain, feed intake, and tissue mass did not significantly differ between the two groups. Dietary supplementation with MFL significantly increased breast muscle α -tocopherol concentration and reduced muscle drip loss. This was accompanied by a lower muscle K-value, which indicated the freshness of the meat. These results suggested that dietary supplementation with MFL improved the shelf life and water-holding capacity of breast muscles of Tsushima-Jidori crossbred chickens.

Key words: α -tocopherol, drip loss, K-value, meat quality, mixed fermented loquat leaf tea residue, Tsushima-Jidori crossbred chicken

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Introduction

The production and consumption of poultry meat (e.g., chicken, turkey, duck, and goose) have increased worldwide[1]. Broiler chickens are raised specifically for meat production because they grow faster and have higher feed conversion ratios than other chicken types[2]. In Japan, commercial broiler chickens, such as Ross chickens, are popular and yield inexpensive meat[3], but several breeds of chicken yield better meat quality, known as “Jidori” chicken meat. Jidori chicken meat is defined in the Japanese Agricultural Standards (JAS) of the Japanese Min-

istry of Agriculture, Forestry, and Fisheries; JAS 844 defines Jidori chickens in terms of genetic characteristics, feeding period, method of rearing, and stocking density of the birds[4]. Consequently, the retail price of Jidori chicken meat is generally higher than that of broiler chicken meat[5].

The Tsushima-Jidori crossbred chicken is a cross between “Tsushima-Jidori,” which is native to Tsushima Island in the Nagasaki Prefecture of Japan, and “Tatsushamogoro,” which was developed at the National Livestock Breeding Center by crossbreeding the Oh-Shamo and Red Cornish breeds. The Tsushima-Jidori crossbred chicken meets the conditions necessary for classification as a Jidori chicken according to JAS 844; its production is estimated to be 7,000 birds annually[6], and the meat is transported to urban areas, such as Tokyo and Osaka, in cold storage. As Nagasaki is hundreds of kilometers away from these urban areas, preservation of meat freshness and quality is important for the success of Tsushima-Jidori production. However, chicken meat is susceptible to oxidative deterioration because it contains high concentrations of polyunsaturated fatty acids that are more easily oxidized than unsaturated fatty acids[7–9]. The oxidation

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of such lipids in chicken meat has adverse effects on its freshness and quality; for example, generating rancid odors, “off” flavors, drip loss, discoloration, loss of nutritional value, and a reduction in shelf-life[10,11].

Diets supplemented with ingredients with high antioxidant activities reduce lipid peroxidation in chicken skeletal muscles[12–15]. For example, green tea (*Camellia sinensis*) leaves suppress muscle lipid peroxidation and improve the quality of chicken meat due to their substantial radical-scavenging activity[16]. Loquat (*Eriobotrya japonica*) leaves also have potent radical-scavenging activity[17] and enzymes that accelerate the oxidation of catechins, which are present in large amounts in green tea leaves[18]. Recently, a fermented tea extract made from a 1:9 mixture of loquat and green tea leaves reduced serum and liver triacylglycerol concentrations, white adipose tissue mass in rats[19], and visceral fat volume in humans[20]. However, the effects of adding fermented tea to animal feed have not been characterized.

Mixed fermented loquat leaf tea residue (MFL) is a byproduct of the fermented tea manufacturing process in Nagasaki. In the present study, the chemical composition and functional ingredients of MFL were determined and the effects of supplementing the diet of Tsushima-Jidori crossbred chickens with MFL were evaluated using muscle α -tocopherol concentration and drip loss.

Materials and Methods

Preparation of MFL and green tea leaf residue (GTL), determination of chemical composition, and identification of functional ingredients

The MFL was collected as a dried processing residue from a commercial tea manufacturer (Sandai Ltd., Nagasaki, Japan). GTL was used as a comparator during the analysis of the chemical composition and functional ingredients of MFL. GTL was collected as a wet extraction residue of green tea from another commercial tea manufacturer (JA Zen-Noh Nagasaki Omura Fruit Juice Plant, Nagasaki, Japan) and dried using a ventilation dryer at 80 °C for 48 h. Both residues were pulverized and passed through a 1-mm mesh sieve.

The chemical composition of MFL and GTL was analyzed in triplicate in terms of crude protein (CP), crude fiber, ether extract, and crude ash, according to previously described feed analysis methods[21]. The α -tocopherol and β -carotene content of MFL and GTL were measured using the method described in the Standard Tables of Food Composition in Japan[22]. Total catechin content was determined according to the method described by Goto *et al.*[23].

To determine the total polyphenol content and free-radical scavenging activity (FRSA) of the residues, MFL and GTL (250 mg each) were homogenized separately with 5 mL of methanol and incubated at 80 °C for 1 h. The homogenates were then centrifuged at 15,000 \times g for 5 min and the supernatants were collected. The Folin–Ciocalteu method was used to determine the total polyphenol content, according to the method described by Anesini *et al.*[24]. Briefly, 100 μ L of each supernatant was

mixed with 100 μ L 10% sodium carbonate, 50 μ L Folin–Ciocalteu reagent (Nacalai Tesque Inc., Kyoto, Japan), and 750 μ L distilled water. After incubation for 60 min at room temperature, the absorbance of the mixture was measured at 700 nm. Gallic acid (Nacalai Tesque Inc.) was used as the standard and the data obtained were expressed as g/g ingredients.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method was used to measure the FRSA, according to the method described by Nara *et al.*[25]. Briefly, 200 μ L of the supernatant was mixed with 200 μ L 0.2 M 2-(N-morpholino) ethane sulfonic acid buffer, 200 μ L ethanol, and 200 μ L 1.2 M DPPH. After incubation for 20 min at room temperature, the absorbance of the mixture was measured at 517 nm. Trolox (Cayman Chemical Company, Ann Arbor, MI, USA) was used as a standard and the data were expressed as mmol Trolox equivalent/g ingredient.

Animals and experimental design

All experimental procedures were conducted in accordance with the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan[26] and approved by the Livestock Research Division of the Nagasaki Prefectural Agriculture and Forestry Technology Development Center. A total of 120 male Tsushima-Jidori crossbred chickens that hatched on the same day were reared in a windless chicken coop at the Nagasaki Prefectural Agricultural and Forestry Technology Development Center under a continuous lighting program until they were slaughtered at 91 days of age. The chicks were provided with water and a commercial starter diet (22% CP and 12.77 MJ/kg metabolizable energy (ME); Itochu Feed Mills Co., Ltd., Tokyo, Japan) *ad libitum* until 27 days of age, and were then fed a customized diet for Tsushima-Jidori crossbred chickens (16.5% CP and 12.52 MJ/kg ME; Itochu Feed Mills Co.). At 62 days of age, chickens with similar body mass were selected, divided into two groups, and fed one of two experimental diets (60 birds per diet, 20 per pen): a basal diet (a customized diet containing 16.5% CP and 12.52 MJ/kg ME; Itochu Feed Mills Co.) or a basal diet supplemented with 1.0% MFL. Supplementation with 1% MFL was based on preliminary data showing that 1.0% MFL supplementation was more effective among 0.25, 0.5, and 1.0%. Body mass and feed intake of the chickens were measured to calculate the feed conversion ratio.

At 90 days of age, chickens with body masses close to the mean value for each pen were selected for slaughter and meat quality evaluation. After 16 h of fasting, birds were weighed and sacrificed by carotid incision, and the mass of the breast muscles, breast tender muscles, leg muscles, liver, heart, gizzard, and abdominal fat deposits were recorded. The left half of the breast muscle was used to determine drip loss and a portion of the remaining half was minced using a food processor (Magmix 5100, FMI Co., Ltd., Tokyo, Japan) and vacuum-packed in aluminum bags for storage at –20 °C until analysis of the α -tocopherol, nucleic acid, and free amino acid content.

Determination of the drip loss of the meat

Drip loss was measured using the method described by Berri *et al.*[27]. The pectoralis major muscle was weighed immediately

Table 1 Chemical composition and functional ingredients in residues of fermented loquat and green tea infusions

Fraction	Compound	MFL ¹⁾	GTL ¹⁾
Chemical composition	Crude protein (%DM)	24.9 ± 0.02 ^{B2)}	34.5 ± 1.35 ^A
	Crude fiber (%DM)	15.1 ± 2.31 ^{ns}	17.7 ± 1.01 ⁴⁾
	Crude fat (%DM)	2.59 ± 0.08 ^B	5.20 ± 0.13 ^A
	Crude ash (%DM)	5.63 ± 0.12 ^A	3.48 ± 0.08 ^B
Functional ingredients	α-tocopherol (μg/g)	4.33 ± 0.09 ^B	13.1 ± 0.66 ^A
	β-carotene (μg/g)	0.96 ± 0.04 ^B	39.1 ± 1.71 ^A
	Total catechins (mg/g)	10.0 ± 1.58 ^B	40.4 ± 1.85 ^A
	Total polyphenol (mg/g)	209.1 ± 31.9 ^A	113.1 ± 26.8 ^B
	DPPH radical-scavenging activity (μM TE ³⁾ /g)	396.8 ± 22.2 ^α	324.1 ± 41.4 ^β

1) MFL, mixed fermented loquat leaf tea residue; GTL, green tea leaf residue; %DM, % dry mass.

2) Values with different capital letters (A, B) and Greek alphabets (α, β) within the same row are significantly different at the 1% and 10% levels, respectively, using the Student *t*-test (ns: not significant).

3) TE: Trolox Equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

4) Means ± standard deviation (SD).

after dissection, placed in a plastic bag, and stored at 5 °C for 24 h. Subsequently, it was wiped and weighed again, and the difference in mass was regarded as the drip loss, which was expressed as a percentage of the initial muscle mass.

Measurement of the α-tocopherol concentration in breast meat

Briefly, 100 mg of breast muscle was homogenized in 1 mL 10 mM Tris, 150 mM NaCl, and 1 mM EDTA.2Na (pH 7.4). Then, 500 μL of the homogenate was mixed with 1 mL hexane:2-propanol (6:4 v/v) and centrifuged at 20,000 × *g* for 3 min. The supernatant was removed and the pellet was resuspended in 500 μL ethanol containing 0.025% butylated hydroxytoluene. The α-tocopherol concentration of the breast muscle was determined using the LC-2000 Plus High-Performance Liquid Chromatography (HPLC) System (Jasco Co. Ltd., Tokyo, Japan) with an Inertsil ODS-3 Column (4.6 × 250 mm; GL Science Inc., Tokyo, Japan), according to the method of Faustman *et al.*[28]

Measurement of the concentration of ATP-related substances in breast meat

Two grams of breast muscle were homogenized in 10 mL 10% perchloric acid and then centrifuged at 3,000 × *g* for 5 min at 4 °C. The supernatant was poured into a 50 mL flask, which was then filled to the mark with 50 mM K₂HPO₄ after adding 1 mL 10 mM KOH. After mixing, 1 mL of the extract was passed through a 0.2 μm filter and analyzed using HPLC. The concentration of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx) in the breast muscle was determined using the LC-2000 Plus HPLC System (Jasco Co. Ltd.) with an AQ-C18 Column (4.6 × 150 mm; GL Science Inc.), according to the method of Kitada *et al.*[29]. The K value was calculated as the percentage of the total content of ATP, ADP, AMP, IMP, HxR, and Hx that was only HxR and Hx using the following equation:

$$K \text{ value (\%)} = \frac{[HxR] + [Hx]}{[ATP] + [ADP] + [AMP] + [IMP] + [HxR] + [Hx]}$$

Statistical analysis

The chemical composition and functional ingredient data for MFL and GTL and the growth performance, tissue mass, and meat quality of the chickens were analyzed using the Student's *t*-test. These analyses were performed using JMP version 13.2.1. Statistical significance was set at *P* < 0.05. The feed component data are expressed as the mean ± standard deviation (SD), and the growth performance, tissue mass, and meat quality are expressed as the mean ± standard error of the mean (SEM).

Results and Discussion

Table 1 lists the chemical compositions of the MFL and GTL functional ingredients. MFL contained smaller amounts of CP and crude fat than that of GTL, but larger amounts of crude ash. Consistent with the lower crude fat content, smaller quantities of fat-soluble functional ingredients (α-tocopherol and β-carotene) were identified in MFL than that in GTL. In addition, the total catechin content in MFL was lower than that in GTL. However, MFL had higher total polyphenol content than that of GTL. Miyata *et al.*[30] have reported that MFL may be prepared by rubbing tea leaves and loquat leaves together and that the chlorogenic acid within the loquat leaves promotes the oxidative polymerization of catechins, resulting in the production of polyphenols. These polyphenols have comparable antioxidant properties to catechins[31] and have a more potent antioxidative activity than α-tocopherol[32]. Consequently, MFL had a significantly higher FRSA than the GTL.

Dietary supplementation with 1.0%–1.5% green tea powder negatively affects feed intake and body mass gain of chickens[33]. In addition, supplementation with either 1.0% or 5.0% tea leaves reduces the size of the abdominal fat deposits in Ise Akadori, a Japanese brand name chicken (a slower growing

Table 2 Effect of feeding manufactured processed residues of mixed fermented loquat leaf tea on growth performance parameters, weight, and meat quality

Measurements	Control	MFL-treated	<i>t</i> -test
Growth performance parameters			
Initial body weight (g)	2187 ± 6 ¹⁾	2190 ± 6	ns
Final body weight (g)	3328 ± 90	3284 ± 16	ns
Body weight gain (g)	1141 ± 93	1095 ± 12	ns
Feed intake (g)	155 ± 6	151 ± 5	ns
Feed conversion ratio	3.67 ± 0.16	3.72 ± 0.10	ns
Survival rate	100 ± 0	100 ± 0	ns
Production score	116 ± 14	109 ± 3	ns
Weight of tissues			
Breast muscle (g)	384 ± 7	389 ± 7	ns
Breast tender muscle (g)	102 ± 2	101 ± 3	ns
Leg muscle (g)	710 ± 6	717 ± 4	ns
Liver (g)	16 ± 0.3	16 ± 0.3	ns
Heart (g)	43 ± 1	44 ± 1	ns
Gizzard (g)	39 ± 2	367 ± 1	ns
Abdominal fat tissue (g)	102 ± 8	93 ± 14	ns

1) Means ± standard error (SE) (ns, not significant). MFL, mixed fermented loquat leaf tea residue.

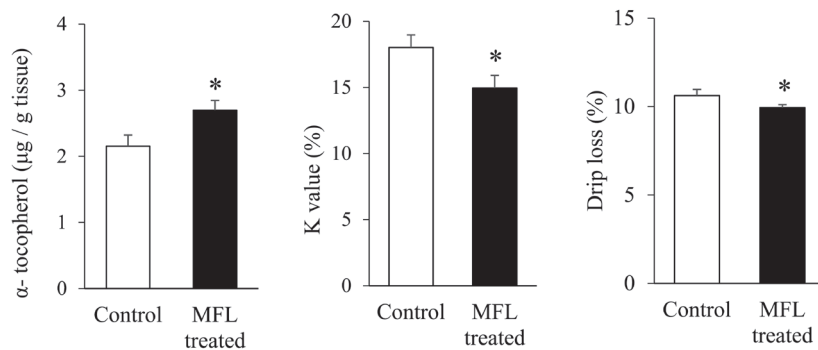


Fig. 1. Effect of feeding manufactured processed residues of mixed fermented loquat leaf tea on α -tocopherol, K-value and drip loss in the breast muscle of Tsushima-Jidori crossbred chickens. Results are expressed as means ± standard error (SE). * $P < 0.05$, unpaired *t*-test. MFL, mixed fermented loquat leaf tea residue.

breed than broilers, but not Jidori), and broilers[34]. These effects of green tea powder may be the result of its high catechin content because catechin inhibits lipid absorption in the rat intestine[35]. However, in the present study, dietary supplementation with 1.0% MFL did not affect the growth performance (final body mass, body mass gain, feed intake, feed conversion ratio, survival rate, and production score), meat yield (breast muscle, breast tender muscle, and leg muscles), or organ mass (liver, heart, gizzard, and abdominal fat deposits) of the Tsushima-Jidori crossbred chickens (Table 2). Because MFL contained less catechin than that of GTL, supplementation of $\leq 1.0\%$ with this

residue did not have negative effects on the growth performance or meat yield of the Tsushima-Jidori crossbred chickens.

In the present study, dietary supplementation with MFL significantly increased the α -tocopherol concentration of the breast muscle of Tsushima-Jidori crossbred chickens (Figure 1). α -Tocopherol and its derivatives have roles as antioxidants, inhibiting lipid peroxidation in biological membranes by scavenging peroxy radicals[36,37]. Kim et al.[38] have reported that dietary supplementation with α -tocopherol improves the meat quality of broiler chickens, i.e., supplementation with 200 IU of α -tocopherol increases lipid stability and delays microbial

growth in the skeletal muscle of broiler chickens. Additionally, feeding polyphenol-rich grape seeds to broilers results in higher α -tocopherol concentrations in plasma and stored meat[39]. These results suggested that the reason for higher α -tocopherol concentration in the breast meat might be due to the rich polyphenol content in the MFL. In the present study, dietary supplementation with MFL reduces muscle K-value, which is an index of meat freshness. ATP in the muscle of slaughtered chickens is enzymatically degraded to ADP, AMP, IMP, HxR, and Hx in sequence[40]. Numata *et al.*[41] have used the K value to determine the freshness of fish; however, it may also be used as an index of chicken meat freshness. Feeding tea leaves to broilers slows the increase in the K-value of chicken meat[42]. In addition, polyphenols derived from tea leaves inhibit metalloproteinase activity[43] and ATP degradation in fish meat[44], which has an ATP degradation pathway similar to that of chicken. Thus, the polyphenols present in MFL may have contributed to maintaining chicken meat freshness in the present study. Because vitamin E reduces the leakage of sarcoplasmic components from myocytes by maintaining cell membrane stability[45], MFL supplementation decreased muscle drip loss of breast muscle, accompanied by increasing muscle α -tocopherol concentration. Taken together, these results suggested that dietary supplementation with MFL improved the shelf life and water-holding capacity of the breast muscle of Tsushima-Jidori crossbred chickens.

In conclusion, MFL contained large amounts of polyphenols and had high FRSA. Dietary supplementation with 1.0% MFL for 4 weeks increased the α -tocopherol content and reduced the K-value and drip loss of Tsushima-Jidori crossbred chickens. These data implied that meat quality might be improved during finishing without affecting the growth or meat yield of chickens.

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Author Contributions

Shogo Matsunaga, Satoru Fukagawa, and Daichi Ijiri conducted the experiments; Shogo Matsunaga, Satoru Fukagawa, Akira Ohtsuka, and Daichi Ijiri designed the experiments; Shogo Matsunaga, Satoru Fukagawa, Kiriko Nakamura, Akira Ohtsuka, and Daichi Ijiri analyzed the data; and Shogo Matsunaga, Satoru Fukagawa, and Daichi Ijiri wrote the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

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