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Research Article

Influences of Lipoic Acid and Acetylated Wood Powder on Muscular Free Amino and Fatty Acids in Broiler Chickens

Yoshio Hamano[1*], Yasuji Kurimoto[2]

[1]Field Education and Science Center, Faculty of Bioresource Sciences, Akita Prefectural University, Ohgata, Akita 010 0451, Japan; [2]Institute of Wood Technology, Akita Prefectural University, Noshiro, Akita 016 0876, Japan.

Abstract | In chickens, α-lipoic acid (LIP) or acetylated wood powder (AWP) as a new feed resource causes changes in profiles of fatty or free amino acids in skeletal muscles or the liver. However, it is unclear whether a combination of these additives functions concomitantly or synergistically to improve meat quality. The present study was conducted to determine the single or combined effects of LIP and AWP on growth performance and free amino and fatty acid profiles in chicken muscle obtained under different feeding states. At 14 days of age, chicks were allocated into four groups and fed diets supplemented with LIP (100 mg/kg) or AWP (20 g/kg) for 26 days as a 2 x 2 factorial design. At 40 days of age, breast muscles as antemortem tissue were harvested from 8 birds under fed conditions. After the remaining birds received feed withdrawal, breast muscle was obtained and stored at 2°C for 3 days. LIP reduced body weight gains when the chickens were concomitantly fed AWP. LIP and AWP increased the feed conversion rates and decreased feed intakes, respectively. However, breast muscle and abdominal fat weights remained constant. No significant interaction between LIP and AWP was detected in the free amino or fatty acid profiles in the ante- and post-mortem breast muscles. AWP reduced total and glucogenic free amino acid concentrations in antemortem breast muscle. LIP decreased total fatty acid concentrations and monounsaturated fatty acid components. In post-mortem breast muscle, the effects observed in antemortem muscle disappeared. In contrast, AWP increased a component of polyunsaturated fatty acids in the breast meat. Therefore, this study suggested that growth and metabolic responses to LIP and AWP occur almost independently, and the results are different depending on feeding conditions or post-mortem metabolism.

Keywords | Acetylated wood, α-Lipoic acid, Chicken meat, Fatty acids, Free Amino acids

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***Correspondence** | Yosho Hamano, Department of Animal Science, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034 8628, Japan;; **Email:** yhamano@vmas.kitasato-u.ac.jp
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INTRODUCTION

α-Lipoic acid (LIP) is a vitamin-like substance that functions as a cofactor in enzyme systems and an antioxidant (Salehi et al., 2019). The administration of LIP promotes the rates of glucose uptake by skeletal muscle in insulin-resistant diabetic rats and alleviates oxidative stress (Salehi et al., 2019; Anthony et al., 2021). In chickens, LIP supplementation has also been reported to improve meat quality (Sohaib et al., 2018). For chicken management, feeding free acetic acid increases feed intake (Hudha et al., 2010) and body weight gain (Ghazalah et al., 2011). However, free acetic acid will be hard to handle in practical use, because this volatile fatty acid causes an undesirable smell. Moreover, administration of calcium acetate as an organic salt form has been reported to adversely impair chicken growth performance (Pinchasov and Jensen, 1989). To solve these issues, acetylated wood powder (AWP), which is produced by acetylation of the wood component, replac-

ing hydroxyl group in the cell wall (Kurimoto and Sasaki, 2013), may be effective as a new feed ingredient.

Intestinal esterase may release free acetic acid from AWP during digestion, similar to α-tocopherol acetate (Jensen et al., 1999). Absorbed acetic acid will be rapidly converted to acetyl-coenzyme A (acetyl-CoA) in the body. On the other hand, because LIP functions as a cofactor for α-ketoacid dehydrogenase complexes such as pyruvate dehydrogenase (Solmonson and Deberardinis, 2018), LIP may affect the increased acetyl-CoA distribution. In normal rats, LIP administration reduces plasma ketone body concentrations and stimulates hepatic ß-oxidation-related gene expression (Valdecantos et al., 2019). However, Khamaisi et al. (1999) reported that LIP treatment reduced hepatic acetyl-CoA concentration suggesting inhibited ß-oxidation in fasting rats. Thus, considering acetyl-CoA metabolism, combined administration of LIP and AWP may modulate amino and fatty acid metabolisms in the liver or skeletal muscle, leading to improved meat quality. However, it is still unclear whether metabolic responses to each substance occur independently or synergistically in antemortem skeletal muscle and subsequent post-mortem chicken meat in broilers.

We aimed to determine the interrelationship between LIP and AWP on growth performance and the free amino and fatty acid profiles in skeletal muscle in chickens.

MATERIALS AND METHODS

Animal Care and Design

This study was approved by the Animal Care and Use Committee at Akita Prefectural University. In the present study, birds were reared in a windowless and air-conditioned room under continuous lighting and a constant temperature of 25°C. However, relative humidity could not be controlled. The entire room and the instruments of battery brooders and wire cages installed in this room were disinfected three times with Pacoma® containing trimethyl ammonium chloride (Meiji Seika Pharma Co., Tokyo, Japan) before the introduction of chicks. Floor cleaning and excreta removal were performed daily with running water. These wastes were collected and disposed of in a cesspool located in the basement of the chicken house. In addition, ventilation was performed twice a day for 10 min to avoid room temperature changes (10:00 and 16:00). Birds were fed on a commercial starter diet containing 23% crude protein (CP) and 12.5 MJ metabolizable energy (ME) /kg until 21 days and, thereafter, finisher diets containing 16% CP and 13.0 MJ ME/kg *ad libitum*. These concentrations of CP and ME fulfilled the recommended conditions of nutrient requirements in the Japanese Feeding Standard for Poultry (Japan Livestock Industry Association, 2011). In this study, AWP was prepared by the method of (Kurimoto and Sasaki, 2013). The binding rate of the acetyl group introduced into the wood powder was 34.8 ± 2.0 g per 100 g of AWP (Hamano and Kurimoto, 2016). LIP (racemic type) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ninety1-d-old female broiler chicks (Ross strain) were randomly divided into 3 groups of 30 birds each and housed in a heated battery brooder consisting of 3 floors. The temperature in this brooder was gradually lowered from 34°C to 25°C. The starter diet and drinking water were provided using feeders and chick waterers, respectively, until 14 d of age. Then, 14-d-old chicks were transferred to 16 wire cages (60 x 75 x 75 cm) of four birds each. The cages with approximately similar average body weight per cage were allocated into four treatment groups of four cages each (16 birds per treatment) as a 2 x 2 factorial arrangement. The control group was fed basal diets (starter and finisher). The second and third groups were fed a diet supplemented with AWP (20 g/kg) and LIP (100 mg/kg), respectively. In the fourth group, a diet supplemented with both AWP (20 g/kg) and LIP (100 mg/kg) was provided. These experimental diets and drinking water were given *ad libitum* for 26 days. Feed intake for individual cages was measured by the difference between the total amount of feed given and the remaining feed in each feeder at the end of the experiment. At 40 days, the body weights of the chickens were measured in each cage. Eight chickens were randomly selected from each group and quickly killed by arteriovenous exsanguination. Breast muscle (approximately 50 g) was immediately removed and frozen using liquid nitrogen. The other side of the breast muscle and abdominal fat were removed and weighed. The frozen samples were individually vacuum-sealed in a polyethylene bag and stored at -50°C until chemical assays. The remaining chickens (8 birds per group) received 14 h feed withdrawal treatment but had free access to tap water. The fasting and sampling times in each treatment group were shifted and considered to prevent fasting time dependent errors of metabolic state (Hamano and Kurimoto, 2016). After the fasted chickens were killed by arteriovenous exsanguination, the left side of the breast muscle was immediately removed. The individual sample was cooled in a polyethylene bag using running water. Then, the breast meats refrigerated for exactly 72 h (2°C for 3 d) were vacuum-sealed in polyethylene bags and stored at -50°C. Carcass residue after sampling was properly disposed of by an industrial waste disposal company.

Chemical Analysis

A frozen tissue sample was crushed by a stamp mill (model ANS-143PL, Nitto Kagaku Co. Ltd., Aichi, Japan). The freezing temperature of meat samples during pulverization was maintained using liquid nitrogen and dry ice. The tissue powder was stored at -50°C and used for determi-

nations of free amino acids and fatty acid methyl esters (FAMEs).

The free amino acids of breast muscle were assayed following a previous study (Hamano and Kurimoto, 2016) using a commercial kit (EZ-faast for GC-FID, Phenomenex, Torrance, CA, USA). This analytical kit could not measure arginine. Glycine was categorized as an essential amino acid for broiler chickens, similar to previous study (Konashi et al., 2000). In addition, free amino acids were divided into three taste groups perceived as sweetness, bitterness, and umami (Mirzapour-Kouhdasht et al., 2023). FAMEs in the samples were determined by the method previously reported using a gas chromatograph (Hamano, 2016).

Statistical Analysis

All data were statistically analyzed by statistical software using StatView (Abacus Concepts Inc., Berkeley, CA, USA). Data are expressed as means with pooled SEM, and a two-way analysis of variance (ANOVA) model as main factor for LIP and AWP feedings was used to determine differences in data between treatment groups. Differences were considered significant at the level of $P < 0.05$. If a significant interaction between LIP and AWP was detected ($P < 0.05$), significant differences between the experimental groups were analyzed by the Scheffé test ($P < 0.05$).

RESULTS

Growth Performance and Tissue Weights

A significant interaction between LIP and AWP was detected in body weight gains ($P < 0.05$) in Table 1. AWP administration had no effect on body weight gains when chickens were not treated with LIP. In the group fed LIP, body weight gain was decreased by the AWP treatment ($P < 0.05$). Feed intake decreased ($P < 0.05$) in the AWP-fed birds independent of LIP administration. On the other hand, increased feed conversion rates were observed in the group fed LIP ($P < 0.01$) but not in the AWP-fed chickens. Consequently, neither LIP nor AWP affected the weights of breast muscle and abdominal fat.

Tissue Concentrations of Free Amino Acids and Fatty Acid Composition in Early Post-Mortem Breast Muscle of Chickens by Fed Condition

The methionine in early post-mortem muscle was not detected in this study. The total free amino acid contents were reduced ($P < 0.05$, Table 2) in the AWP-fed group but not in the LIP-fed birds. AWP feeding decreased ($P < 0.01$) the tissue concentrations of glycine and phenylalanine, resulting in a reduced total concentration of essential amino acid class ($P < 0.01$). In addition, AWP concomitantly decreased ($P < 0.05$) total concentrations of non-essential amino acids, regardless of LIP. This effect was dependent on the lowered concentrations of asparagine and glutamine ($P < 0.05$). Feeding LIP reduced the concentrations of aspartic acid and glutamine. The class of glucogenic amino acids in the AWP-fed group decreased ($P < 0.05$), regardless of LIP, although branched-chain or ketogenic amino acids remained unchanged.

A decrease in total fatty acid concentration occurred as revealed in Table 3 in the LIP-fed chickens ($P < 0.01$) but not in chickens that received AWP. In addition, LIP influenced the individual fatty acid composition in breast muscle, except for palmitoleic (C16:1) and eicosenoic (C20:1) acids. However, AWP did not affect any fatty acid component. In LIP-fed chickens, the saturated fatty acid (SFA) component and SFA/unsaturated fatty acid (USFA) ratio increased ($P < 0.001$) compared with the untreated group. The LIP-enhanced SFA occurred in the individual fatty acids, myristic acid (C14:0, $P < 0.001$), palmitic acid (C16:0, $P < 0.05$), and stearic acid (C18:0, $P < 0.01$). The lowered USFA in LIP-fed chickens depended only on oleic acid (C18:1 ω-9) in monounsaturated fatty acids (MUFAs) rather than in polyunsaturated fatty acids (PUFAs). However, different responses to LIP were shown in individual PUFAs. While LIP reduced linoleic acid (C18:2 ω-6) and linolenic acid (C18:3 ω-3), in contrast, concentrations of five other PUFAs, eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3 ω-6), arachidonic acid (C20:4 ω-6), eicosapentaenoic acid (C20:5 ω-3), and docosahexaenoic acid (C22:6 ω-3), significantly rose in the LIP-fed group. Consequently, the MUFA/PUFA ratio was reduced ($P < 0.05$) by LIP.

Changes in Free Amino Acid Concentration and Fatty Acid Profile in Post-Mortem Breast Meats of Fasted Chickens

In post-mortem breast meat refrigerated for three days, the effects of LIP and AWP on concentrations of free amino acids (Table 4) and the fatty acid profile (Table 5) were different from those on those in early post-mortem breast muscle in chickens under the fed condition. Compared with early post-mortem breast muscle, total concentrations of free amino acids were numerically increased with aging (refrigeration time). Neither LIP nor AWP affected the total concentration of free amino acids. Of essential amino acids, LIP administration increased methionine ($P < 0.05$) and reduced tryptophan ($P < 0.05$). However, AWP did not affect any essential amino acid concentration. While there was a significant interaction between LIP and AWP in the total concentration of non-essential amino acids, a significant difference among the treated groups was not detected by the Scheffé test.

In individual non-essential amino acids, a single administration of LIP increased ($P < 0.05$) the alanine concen

Table 1: Effects of α-lipoic acid (LIP) and acetylated wood powder (AWP) on growth performance and tissue composition of breast muscle and abdominal fat in chickens under fed condition

LIP (mg/kg)	0		100			ANOVA		
AWP (g/kg)	0	2	0	2	SEM	LIP	AWP	Interaction
Body weight gain[1] (kg/bird/ 26 d)	1.77 [ab]	1.77 [ab]	1.86 [a]	1.57 [b]	0.035	NS	*	*
Feed intake[1] (kg/bird/ 26 d)	3.33	3.23	3.69	3.09	0.078	NS	*	NS
Feed conversion rate[1] (feed/gain)	1.87	1.82	1.99	1.96	0.024	**	NS	NS
Tissue wight[2]								
Breast muscle (g)	167	183	176	153	4.9	NS	NS	NS
Abdominal fat (g)	39.0	42.1	48.1	45.6	2.12	NS	NS	NS
Tissue composition[2]								
Breast muscle (g/kg)	74.0	78.1	72.8	73.1	1.16	NS	NS	NS
Abdominal fat (g/kg)	17.2	18.2	20.0	21.7	0.86	NS	NS	NS

[1]Values represent means (n = 4 replicates of 4 birds each) with pooled SEM.
[2] Values represent means (n = 8 birds) with pooled SEM.
* $P < 0.05$; ** $P < 0.01$; NS, not significant.
[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

Table 2: Effects of α-lipoic acid (LIP) and acetylated wood powder (AWP) on free amino acid concentrations in early post-mortem breast muscle of chickens under the fed condition

LIP (mg/kg)	0		100			ANOVA		
AWP (g/kg)	0	20	0	20	SEM	LIP	AWP	Interaction
			μmol/g					
Σ Free amino acids	34.7	30.2	32.7	27.0	1.03	NS	*	NS
Σ Essential	9.72	7.79	9.04	7.44	0.318	NS	**	NS
Glycine	5.09	3.27	4.26	3.25	0.262	NS	**	NS
Valine	0.29	0.32	0.35	0.35	0.014	NS	NS	NS
Leucine	0.37	0.40	0.40	0.35	0.011	NS	NS	NS
Isoleucine	0.11	0.13	0.13	0.10	0.006	NS	NS	NS
Threonine	1.85	1.79	1.93	1.54	0.073	NS	NS	NS
Phenylalanine	0.31	0.29	0.33	0.27	0.008	NS	**	NS
Lysine	0.37	0.38	0.43	0.45	0.027	NS	NS	NS
Histidine	1.14	1.04	1.04	0.95	0.032	NS	NS	NS
Tryptophan	0.18	0.17	0.18	0.17	0.002	NS	NS	NS
Σ Non-essential	24.5	22.4	23.6	19.6	0.73	NS	*	NS
Alanine	3.78	4.05	4.30	3.57	0.171	NS	NS	NS
Serine	3.31	2.98	3.48	2.85	0.126	NS	NS	NS
Proline	1.49	1.38	1.50	1.67	0.092	NS	NS	NS
Asparagine	0.91	0.78	0.94	0.75	0.028	NS	**	NS
Aspartic acid	0.62	0.65	0.34	0.44	0.037	***	NS	NS
Hydroxyproline	0.47	0.48	0.44	0.33	0.028	NS	NS	NS
Glutamic acid	4.96	4.16	4.40	4.26	0.180	NS	NS	NS
Glutamine	7.81	6.90	7.18	4.70	0.315	**	**	NS
Tyrosine	0.74	0.71	0.71	0.65	0.017	NS	NS	NS
Cystine	0.41	0.34	0.34	0.37	0.015	NS	NS	NS
Σ Branched-chain[1]	0.76	0.84	0.87	0.80	0.027	NS	NS	NS

Σ Glucogenic[1]	31.3	27.3	29.7	24.3	0.96	NS	*	NS
Σ Ketogenic[1]	0.74	0.78	0.83	0.81	0.028	NS	NS	NS

Values represent means (n = 8) with pooled SEM.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.
[1]Branched chain amino acids: valine, leucine, isoleucine; glucogenic amino acids: alanine, glycine, valine, threonine, serine, proline, asparagine, aspartic acid, glutamic acid, glutamine, histidine; ketogenic amino acids: leucine, lysine.

Table 3: Effects of α-lipoic acid (LIP) and acetylated wood powder (AWP) on total fatty acid concentration and fatty acid composition in early post-mortem breast muscle of chickens under the fed condition

LIP (mg/kg)	**0**		**100**			**ANOVA**		
AWP (g/kg)	**0**	**20**	**0**	**20**	**SEM**	**LIP**	**AWP**	**Interaction**
			mg/g					
Fatty acid concentration	21.9	29.3	17.2	15.3	1.84	**	NS	NS
	mg/100 mg total fatty acids							
C14:0	0.74	0.71	0.88	0.90	0.024	***	NS	NS
C16:0	19.3	19.5	20.4	19.8	0.15	*	NS	NS
C16:1	2.24	2.56	2.58	2.38	0.098	NS	NS	NS
C18:0	7.94	7.42	8.45	8.62	0.155	**	NS	NS
C18:1 ω-9 cis	42.9	44.0	41.0	40.6	0.437	**	NS	NS
C18:2 ω-6 cis	18.5	18.3	17.4	17.8	0.178	*	NS	NS
C18:3 ω-3	1.97	2.01	1.82	1.79	0.030	**	NS	NS
C20:1	0.53	0.52	0.56	0.60	0.015	NS	NS	NS
C20:2	0.30	0.28	0.35	0.37	0.014	*	NS	NS
C20:3 ω-6	0.77	0.72	0.97	1.04	0.040	***	NS	NS
C20:4 ω-6	3.76	3.11	4.35	4.74	0.241	*	NS	NS
C20:5 ω-3	0.25	0.22	0.32	0.35	0.016	**	NS	NS
C22:6 ω-3	0.76	0.65	0.90	1.00	0.058	*	NS	NS
Σ SFA[1]	28.0	27.6	29.7	29.3	0.234	***	NS	NS
Σ USFA[1]	72.0	72.4	70.3	70.7	0.234	***	NS	NS
Σ MUFA[1]	45.7	47.1	44.1	43.6	0.460	**	NS	NS
Σ PUFA[1]	26.3	25.3	26.1	27.1	0.338	NS	NS	NS
Σ ω-6	23.8	22.9	23.7	24.6	0.307	NS	NS	NS
Σ ω-3	2.99	2.87	3.04	3.14	0.055	NS	NS	NS
SFA/USFA	0.39	0.38	0.42	0.41	0.005	***	NS	NS
MUFA/PUFA	1.74	1.88	1.69	1.62	0.039	*	NS	NS
ω-6/ω-3	7.71	7.71	7.47	7.54	0.067	NS	NS	NS

Values represent means (n = 8) with pooled SEM.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.
[1]SFA, Saturated fatty acids; USFA, Unsaturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

Table 4: Effects of α-lipoic acid (LIP) and acetylated wood powder (AWP) on free amino acid concentrations in refrigerated breast meat of fasted chickens

LIP (mg/kg)	**0**		**100**			**ANOVA**		
AWP (g/kg)	**0**	**20**	**0**	**20**	**SEM**	**LIP**	**AWP**	**Inter-action**
			µmol/g					
Σ Free amino acid	39.2	43.4	42.9	39.1	1.03	NS	NS	NS
Σ Essential	13.4	13.9	13.9	14.0	0.38	NS	NS	NS

Glycine	5.43	5.04	4.61	4.78	0.229	NS	NS	NS
Valine	0.82	1.01	1.06	0.99	0.043	NS	NS	NS
Leucine	1.29	1.46	1.47	1.42	0.054	NS	NS	NS
Isoleucine	0.54	0.65	0.68	0.69	0.028	NS	NS	NS
Threonine	2.21	2.39	2.52	2.43	0.090	NS	NS	NS
Methionine	0.15	0.11	0.19	0.17	0.012	*	NS	NS
Phenylalanine	0.65	0.70	0.74	0.72	0.022	NS	NS	NS
Lysine	0.83	1.10	1.20	1.15	0.058	NS	NS	NS
Histidine	1.23	1.20	1.24	1.44	0.044	NS	NS	NS
Tryptophan	0.16	0.16	0.14	0.13	0.005	*	NS	NS
Σ Non-essential	25.9	29.6	29.0	25.2	0.76	NS	NS	*
Alanine	6.27^{b}	7.54^{ab}	7.93^{a}	6.84^{ab}	0.215	NS	NS	**
Serine	4.00	4.36	4.47	4.52	0.158	NS	NS	NS
Proline	1.80	1.71	2.26	2.11	0.120	NS	NS	NS
Asparagine	0.85	0.91	1.00	0.89	0.026	NS	NS	NS
Aspartic acid	2.03	2.22	2.14	1.74	0.064	NS	NS	*
Hydroxyproline	0.42	0.46	0.46	0.38	0.020	NS	NS	NS
Glutamic acid	2.95^{ab}	3.93^{a}	3.31^{ab}	2.41^{b}	0.183	NS	NS	**
Glutamine	5.93	6.81	5.37	4.41	0.268	**	NS	NS
Tyrosine	1.09	1.03	1.21	1.31	0.044	*	NS	NS
Cystine	0.53^{b}	0.58^{b}	0.82^{a}	0.56^{b}	0.032	*	NS	**
Human taste classification[1]								
Σ Sweetness	20.9	22.6	23.5	22.2	0.55	NS	NS	NS
Σ Bitterness	7.65	3.38	8.97	8.95	0.275	NS	NS	NS
Σ Umami	4.98^{ab}	6.15^{a}	5.45^{ab}	4.16^{b}	0.232	NS	NS	**

Values represent means (n = 8) with pooled SEM.
* $P < 0.05$; ** $P < 0.01$; NS, not significant.
^{a,b}Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.
[1]Sweet: glycine, alanine, hydroxyproline, proline, serine, threonine; Bitter: asparagine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine; Umami: glutamic acid, aspartic acid.

Table 5: Effects of α-lipoic acid (LIP) and acetylated wood powder (AWP) on fatty acid composition in refrigerated breast meat of fasted chickens

LIP (mg/kg)	**0**		**100**			**ANOVA**		
AWP (g/kg)	**0**	**20**	**0**	**20**	**SEM**	**LIP**	**AWP**	**Inter-action**
	mg/g							
Fatty acid concentration	33.6	29.1	32.1	22.6	1.93	NS	NS	NS
	mg/100mg total fatty acids							
C14:0	0.66	0.70	0.69	0.75	0.015	NS	NS	NS
C16:0	20.6	20.1	20.7	20.5	0.131	NS	NS	NS
C16:1	3.12	2.76	3.01	2.56	0.085	NS	*	NS
C18:0	6.95	7.22	7.19	7.92	0.122	*	*	NS
C18:1 ω-9 cis	44.4	43.1	44.4	42.8	0.32	NS	*	NS
C18:2 ω-6 cis	17.9	18.9	17.6	17.8	0.18	NS	NS	NS
C18:3 ω-3	1.91	1.99	1.93	1.82	0.032	NS	NS	NS
C20:1	0.44	0.46	0.45	0.49	0.012	NS	NS	NS
C20:2	0.22	0.26	0.24	0.26	0.009	NS	NS	NS

C20:3 ω-6	0.58	0.68	0.60	0.75	0.036	NS	NS	NS
C20:4 ω-6	2.62	3.14	2.44	3.52	0.192	NS	*	NS
C20:5 ω-3	0.16	0.18	0.18	0.25	0.014	NS	NS	NS
C22:6 ω-3	0.42	0.52	0.49	0.57	0.032	NS	NS	NS
Σ SFA[1]	28.2	28.0	28.6	29.2	0.18	*	NS	NS
Σ USFA[1]	71.8	72.0	71.4	70.8	0.18	*	NS	NS
Σ MUFA[1]	48.0	46.4	47.9	45.8	0.35	NS	**	NS
Σ PUFA[1]	23.8	25.7	23.5	25.0	0.32	NS	**	NS
Σ ω-6	21.1	22.7	20.7	22.1	0.29	NS	**	NS
Σ ω-3	2.49	2.69	2.61	2.65	0.032	NS	NS	NS
SFA/USFA[2]	0.39	0.38	0.40	0.41	0.004	*	NS	NS
MUFA/PUFA	2.02	1.82	2.05	1.84	0.038	NS	**	NS
ω-6/ω-3	8.45	8.40	7.88	8.33	0.089	NS	NS	NS

Values represent means (n = 8) with pooled SEM.
* $P < 0.05$; ** $P < 0.01$; NS, not significant.
[1]SFA, Saturated fatty acids; USFA, Unsaturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

tration, but this effect was not found with the combined AWP supplementation. Although a significant interaction between LIP and AWP was shown in the aspartic acid concentration ($P < 0.05$), a significant difference among the treated groups was not detected by the Scheffé test. The glutamic acid concentration decreased due to LIP feeding occurring only when chickens received AWP supplementation ($P < 0.05$). In addition, LIP reduced the glutamine concentration and adversely increased the tyrosine concentration ($P < 0.05$), regardless of AWP feeding. The cystine concentration increased ($P < 0.05$) with LIP administration, but this effect was inhibited by the combination with AWP. On the other hand, this study classified free amino acids concerning amino acid-inducing human taste perception. Concentrations of the umami taste-related amino acids of aspartic acid and glutamic acid were reduced ($P < 0.05$) by LIP only when chickens were fed AWP. However, concentrations of other amino acids related to bitter and sweet tastes remained unchanged.

The total fatty acid concentrations in Table 5 were not affected by dietary supplementation with LIP or AWP. In fatty acid composition, feeding of LIP increased the total SFA component resulting in reduced USFA ($P < 0.05$). This effect depended only on enhanced stearic acid (C18:0, $P < 0.05$). In USFA, LIP did not affect total MUFA or PUFA components. On the other hand, AWP feeding increased stearic acid (C18:0, $p<0.05$), similar to LIP, but did not affect total SFA. Among USFAs, AWP decreased ($P < 0.01$) MUFAs resulting mainly from lowered palmitoleic acid (C16:1, $P < 0.05$) and oleic acid (C18:1 ω-9). Conversely, PUFAs increased ($P < 0.01$) in the AWP-treated group compared with controls. This main effect resulted from enhanced arachidonic acid (C20:4 ω-6). Consequently, the MUFA/PUFA ratio was reduced ($P < 0.01$) by AWP treatment.

DISCUSSION

Growth Performance

In broiler chickens, the effects of dietary LIP administration on body weight gain and feed intake are not necessarily dose-dependent and inconsistent (Sohaib et al., 2018). The present results of body weight gains and feed intakes remained unchanged in LIP-fed chickens at a level of 100 mg/kg. However, the feed conversion rates increased with the LIP treatment. The present study could not explain the reason for this negative observation. In addition, the decreased feed intakes induced by AWP feeding were not in agreement with our previous report (Hamano and Kurimoto, 2016). Acetic acid administration has been reported to lower feed intake, and the anorectic effect was associated with dietary energy concentration in broiler chicks (Pinchasov and Elmaliah, 1995). Moreover, it was interesting that reduced body weight gain in the AWP-fed chickens occurred when LIP was concomitantly supplemented. This might result from reduced feed intakes. The authors have hypothesized that intestinal esterase desorbs acetic acid (acetyl groups) from AWP (Hamano and Kurimoto, 2016), since a previous report confirmed carboxylesterase-induced hydrolysis of α-tocopherol acetate (Jensen et al., 1999). However, the evidence for our hypothesis remained uncertain in the present study. Further examinations are needed to demonstrate this hypothesis. Consequently, these results suggested that LIP administration was unfavorable for the growth rate when chickens were fed AWP concomitantly.

Free Amino Acid Profile in Breast Muscle

In the present study, the free amino acid profile of ear-

ly post-mortem breast muscle would be considered as the profile in the antemortem tissue. Most of the free amino acids in breast muscle were not affected by LIP. In addition, a class of glucogenic amino acids remained unchanged, while concentrations of several amino acids (aspartic acid and glutamine) decreased with the LIP treatment. A previous study found that LIP stimulates *in vivo* glucose uptake with enhanced insulin sensitivity in the whole body in chickens (Hamano, 2006). Otherwise, LIP administration stimulates hepatic and muscular gluconeogenesis-related genes in Zebrafish (Huang et al., 2020). Khamaisi et al. (1999) adversely indicated the inhibitory influence of LIP on gluconeogenesis derived from alanine. However, LIP feeding, at least, did not affect the muscle-free amino acid profile in the present study.

AWP feeding reduced the free amino acid concentrations of both essential and non-essential classes in breast muscles. Most of the free amino acids reduced by AWP were glucogenic amino acids. A similar response has been found in the liver, but not in skeletal muscle (Hamano and Kurimoto, 2016). The response of free amino acids to AWP observed in the breast muscle would be dependent on hepatic amino acid metabolism. AWP might affect the flux and utilization of the amino acid carbon given to acetyl-CoA in the liver. In addition, the lowered feed intakes (protein intake) induced by AWP as described above probably facilitated the reduced free amino acid contents in the muscle. However, whether acetic acid supplementation elicits the reduced free amino acid in either liver or skeletal muscle was not examined. Thus, this study indicated that LIP had no interrelationship with AWP-induced free amino acid metabolism, especially in glucogenic amino acids in the early post-mortem (antemortem) condition.

In post-mortem muscle, the effects of LIP and AWP on the free amino acid profile in refrigerated chicken meat were different from those in antemortem breast muscle. Neither LIP nor AWP affected the total concentration of essential or non-essential free amino acids. This different response would be dependent on feeding conditions and aging time-related catabolism in muscle. However, it was unclear whether this response to LIP was attributable to stimulated proteolysis of post-mortem muscle protein during refrigeration. Fasting is generally performed to lower the contamination risk of carcassses due to microorganisms derived from digested feed and feces in the digestive tract (Xue et al., 2021). The effects of LIP and AWP on meat quality might be affected by the fasting condition, which alters the catabolic state by stimulating fatty acid mobilization with hepatic ketogenesis (Hamano, 2007). From the viewpoint of human taste, free amino acids were categorized into three classes sensed as sweet, bitter, and umami (Mirzapour-Kouhdasht et al. , 2023). When chickens received AWP, concentrations of the free amino acids sensed as umami (glutamic acid and aspartic acid) were lowered by the concomitant administration of LIP. Overall, in antemortem muscle, LIP did not affect the reduced concentration of free amino acids induced by AWP, but the interaction between LIP and AWP observed in several free amino acids was varied to lower their concentrations in post-mortem chicken meats.

Fatty Acid Profile of Breast Muscle

The total fatty acid concentration in early post-mortem muscle was decreased by LIP. The first author has reported that an increase in total fatty acid concentration occurred in early post-mortem muscle when broilers were fed LIP at a level of 200 mg/kg, but not in the muscle treated with LIP at the lower level of 100 mg/kg (Hamano, 2016). Moreover, in that previous report, the supplemented level of LIP (200 mg/kg) enhanced both the concentration and components of MUFAs, especially oleic acid (C18:1 ω-9), in the breast muscle. However, in the present study, LIP increased the proportion of SFA and conversely decreased MUFA. This effect on total SFA and USFA was also confirmed in breast meat refrigerated for three days, while the LIP effect on individual fatty acid was not shown in early post-mortem muscle, except for increased stearic acid (18:0). In addition, Arshad et al. (2013) observed that a single supplementation with LIP reduced SFA and MUFA in leg meat but did not in breast meat of broilers. These different findings are of great interest to investigate the possible role of LIP action in the regulation of fatty acid metabolism. In fasted rat hepatocytes, R-LIP, a natural enantiomer, lowers the free palmitic acid (C16:0) oxidation rate (Walgren et al., 2004). The liver is the primary site of most lipogenesis for avian species (Emami et al., 2020). The alterations in the fatty acid profile induced by LIP might depend not only on its dosage but also be related to hepatic fatty acid metabolism.

AWP treatment did not affect the fatty acid composition in early post-mortem muscle concerning the LIP treatment. Rather, the effect on the fatty acid profile appeared in post-mortem breast meats refrigerated for three days. AWP decreased the MUFA components (palmitoleic acid, C16:1; oleic acid, C18:1 ω-9) and increased the PUFAs dependent on ω-6 PUFA. A previous report indicated that AWP feeding of fasted chickens increased only the component of arachidonic acid (C20:4 ω-6) in breast meat refrigerated for 24 hours (Hamano, 2016). These differences would also be associated with the feeding condition and time course of post-mortem metabolism of muscle. Thus, AWP treatment had no interrelationship with LIP administration in the regulation of fatty acid metabolism, while these compounds affected this metabolism independently.

CONCLUSIONS

The present study found that metabolic responses of skeletal muscle to LIP and AWP varied in proportion to different feeding conditions and the progress of post-mortem metabolism. There was no synergetic or positive interrelationship between LIP and AWP to improve meat quality. Taken together with previous studies, a suitable dose of LIP and AWP for the improvement of meat flavor should be considered in association with feeding and dietary conditions that are thought to affect their metabolic actions. In addition, feeding AWP as an unprecedented feed additive affected growth performance and muscle metabolism. However, it remains unclear whether the desorption of acetic acid from AWP is caused by digestive enzymes. Further experiments are needed to determine the suitable and beneficial usage of LIP and AWP for chicken meat production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

NOVELTY STATEMENT

There are no studies on combined administration of LIP and AWP to affect fatty and free amino acid metabolisms in chickens. This study is the first to demonstrate that these metabolic effects of LIP and AWP occur independently and that aspects of these effects on chicken skeletal muscle vary with feeding states. The findings will provide useful information when considering how these additives can be used to control metabolic state or meat quality.

AUTHORS CONTRIBUTION

Hamano Y. designed the study and performed practical work in animal experiments, data analysis, and writing the manuscript. Kurimoto Y. prepared acetylated wood powder and evaluated the quality of the wood powder.

REFERENCES

Anthony RM, Macleay JM, Gross KL (2021). Alpha-lipoic acid as a nutritive supplement for humans and animals: An overview of its use in dog food. Animals (Basel). 11: 10.3390/ani11051454. https://doi.org/10.3390/ani11051454

Arshad MS, Anjum FM, Khan MI, Shahid M (2013). Wheat germ oil and α-lipoic acid predominantly improve the lipid profile of broiler meat. J. Agr. Food Chem. 61: 11158-11165 https://doi.org/10.1021/jf4041029.

Emami NK, Jung U, Voy B, Dridi S (2020). Radical response: Effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. Antioxidants (Basel). 10:35. https://doi.org/10.3390/antiox10010035

Ghazalah AA, Atta AM, Elkloub K, Moustafa ME, Shata RFH (2011). Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. Int. J. Poult. Sci. 10: 176-184. https://doi.org/10.3923/ijps.2011.176.184

Hamano Y (2006). Effects of dietary lipoic acid on plasma lipid, *in vivo* insulin sensitivity, metabolic response to corticosterone and *in vitro* lipolysis in broiler chickens. Br. J. Nutr. 95: 1094-1101. https://doi.org/10.1079/BJN20061766

Hamano Y (2007). Continuous infusion of lipoic acid rapidly reduces plasma ß-hydroxybutyrate with elevation of non-esterified fatty acids in broiler chickens. Br. J. Nutr. 97: 495-501. https://doi.org/10.1017/S0007114507381312

Hamano Y (2016). Alteration of fatty acid profile and nucleotide-related substances in *post-mortem* breast meat of α-lipoic acid-fed broiler chickens. Br. Poult. Sci. 57: 501-514. https://doi.org/10.1080/00071668.2016.1184227

Hamano Y, Kurimoto Y (2016). Effects of acetylated wood powder on growth performance, hepatic and muscular free amino acid profiles, and inosine 5'-monophosphate concentration of breast meat in broiler chickens. Br. Poult. Sci. 57: 643-654. https://doi.org/10.1080/00071668.2016.1190809

Huang CC, Sun J, Ji H, Kaneko G, Xie XD, Chang ZG, Deng W (2020). Systemic effect of dietary lipid levels and α-lipoic acid supplementation on nutritional metabolism in zebrafish (*danio rerio*): Focusing on the transcriptional level. Fish Physiol. Biochem. 46: 1631-1644. https://doi.org/10.1007/s10695-020-00795-8

Hudha N, Ali MS, Azad MaA, Hossian MM, Tanjim M, Bormon SC, Rahman MS, Rahman MM, Paul AK (2010). Effect of acetic acid on growth and meat yield in broilers. Int. J. BioRes. 1: 31-35.

Japan Livestock Industry Association (2011). Japanese Feeding Standard for Poultry. National Agriculture and Food Research Organization, NARO (ed), Tokyo.

Jensen SK, Engberg RM, Hedemann MS (1999). All-*rac*-αlpha-tocopherol acetate is a better vitamin e source than all-*rac*-αlpha-tocopherol succinate for broilers. J. Nutr. 129: 1355-1360. https://doi.org/10.1093/jn/129.7.1355

Khamaisi M, Rudich A, Potashnik R, Tritschler HJ, Gutman A, Bashan N (1999). Lipoic acid acutely induces hypoglycemia in fasting nondiabetic and diabetic rats. Metabolism. 48: 504-510. https://doi.org/10.1016/S0026-0495(99)90112-9

Konashi S, Takahashi K, Akiba Y (2000). Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. Br. J. Nutr. 83: 449-456.

Kurimoto Y, Sasaki S (2013). Preparation of acetylated wood meal and polypropylene composites I: Acetylation of wood meal by mechanochemical processing and its characteristics. J. Wood Sci. 59: 209-215. https://doi.org/10.1007/s10086-012-1319-x

Mirzapour-Kouhdasht A, Mcclements DJ, Taghizadeh MS, Niazi A, Garcia-Vaquero M (2023). Strategies for oral delivery of bioactive peptides with focus on debittering and masking. NPJ Sci. Food. 7: 22. https://doi.org/10.1038/s41538-023-00198-y

Pinchasov Y, Jensen LS (1989). Effect of short-chain fatty acids on voluntary feed of broiler chicks. Poult. Sci. 68: 1612-1618 https://doi.org/10.3382/ps.0681612.

Pinchasov Y, Elmaliah S (1995). Broiler chick responses to anorectic agents: Dietary acetic and propionic acids and the blood metabolites. Ann. Nutr. Metab. 39: 107-116. https://doi.org/10.1159/000177850

Salehi B, Berkay Yilmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, Akram M, Riaz M, Capanoglu E, Sharopov F, Martins N, Cho WC, Sharifi-Rad J (2019). Insights on the use of α-lipoic acid for therapeutic purposes. Biomolecules. 9: 356. https://doi.org/10.3390/biom9080356

Sohaib M, Anjum FM, Nasir M, Saeed F, Arshad MS, Hussain S (2018). Alpha-lipoic acid: An inimitable feed supplement for poultry nutrition. J. Anim. Physiol. Anim. Nutr. (Berl.) 102: 33-40. https://doi.org/10.1111/jpn.12693

Solmonson A, Deberardinis RJ (2018). Lipoic acid metabolism and mitochondrial redox regulation. J. Biol. Chem. 293: 7522-7530. https://doi.org/10.1074/jbc.TM117.000259

StatView ver. 4.0 (1992) Abacus Concepts, Ink., Berkeley, CA, USA.

Valdecantos MP, Pérez-Matute P, Prieto-Hontoria P, Moreno-Aliaga MJ, Martínez JA (2019). Impact of dietary lipoic acid supplementation on liver mitochondrial bioenergetics and oxidative status on normally fed wistar rats. Int. J. Food Sci. Nutr. 70: 834-844. https://doi.org/10.1080/09637486.2019.1572716

Walgren JL, Amani Z, Mcmillan JM, Locher M, Buse MG (2004). Effect of R(+)α-lipoic acid on pyruvate metabolism and fatty acid oxidation in rat hepatocytes. Metabolism. 53: 165-173. https://doi.org/10.1016/j.metabol.2003.09.008

Xue G, Cheng S, Yin J, Zhang R, Su Y, Li X, Li J, Bao J (2021). Influence of pre-slaughter fasting time on weight loss, meat quality and carcass contamination in broilers. Anim. Biosci. 34: 1070-1077. https://doi.org/10.5713/ajas.20.0560