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Research article

Improving feed efficiency and antioxidant properties in Thai native chickens by supplementing Thai rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) powder

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Abstract

This study aimed to investigate the benefits of adding Thai rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) powder to the diet of Thai native chickens. Using a randomized completely block design (RCBD), 200 mixed-gender chickens were assigned to groups that received varying amounts of rice paddy herb powder (RPHP): 0% (Control), 1% (RPHP1), 2% (RPHP2), and 3% (RPHP3), along with oxytetracycline (OTC) at 0.25% in the diet. The addition of RPHP to the diet did not result in significant changes in body weight, body weight gain, or average daily gain (P>0.05). When compared to the control group, the RPHP-supplemented group had significantly lower feed intake and feed conversion ratio (P<0.05). Both RPHP1 and RPHP2 groups had comparable total feed costs and benefit-cost ratios to the control group (P>0.05). In contrast, the inclusion of OTC increased total feed costs, resulting in a lower benefit-cost ratio (P<0.05). Carcass analysis revealed no significant effects from RPHP supplementation, whereas OTC significantly reduced spleen percentage and spleen index (P<0.05). Except for the lightness of breast meat, which decreased significantly in the RPHP2 group compared to the control group (P<0.05), meat quality did not differ significantly among treatment groups (P>0.05). Additionally, supplementation of RPHP, particularly RPHP3, increased SOD and T-AOC levels in meat while decreasing O2- and MDA levels (P<0.05). Taken together, these findings indicated that Thai native chickens fed Thai rice paddy herb powder increased feed efficiency without changing meat quality or carcass characteristics. The inclusion of RPHP at a concentration of 3% in the diet showed potential in enhancing the antioxidant properties of meat.

Keywords: Antioxidant activity, Growth performance, Meat quality, Thai rice paddy herb powder, Thai native chickens.

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INTRODUCTION

The increasing production of Thai native chicken has issues in both quantity and quality, resulting in inefficiency and insufficient output to fulfill market demand (Choprakan, 1998; Department of Livestock Development, 2017). The Department of Livestock Development, in collaboration with The Thailand Research Fund (TRF), identified four Thai native chicken pure breeds: Pradu Hang Dam, Chee, Dang, and Leung Hang Kao. These breeds are valued for their exceptional carcass quality and durability, and they play a crucial part in local farming and conservation efforts. (Laopaiboon et al., 2012; Hadai et al., 2020) However, the poultry industry confronts significant challenges, including environmental factors that exacerbate natural constraints (Surai et al., 2019; Somkuna et al., 2023). Extreme temperatures, inadequate housing, restricted water access, and disease prevalence all stress Thai native chickens, lowering production efficiency (Wuthijaree et al., 2019; Akinyemi and Adewole, 2021; Loengbudnark et al., 2023). Stressors cause oxidative stress in chickens, resulting in cellular component damage by reactive oxygen species (ROS) such as superoxide radicals (O2-) (Wu et al., 2019). Not only does O2- contribute to oxidative stress, but malondialdehyde (MDA) can increase intracellular damage by increasing the synthesis of secondary oxidation products, such as aldehydes. This mechanism degrades fat molecules within cell membranes, resulting in elevated ROS levels and lipid peroxidation. (Reitznerová et al., 2017; Bertolín et al., 2019; Khang et al. 2024) To mitigate ROS and MDA effects, chickens need to develop an antioxidant defense system. In poultry biology, this defense is divided into third levels: the primary level involves antioxidant enzymes such as superoxide dismutase (SOD), and the secondary level includes non-enzymatic substances such as vitamins C and E, glutathione, phenolic acids, and flavonoids, which act as free radical scavengers and the third level is related to damaged molecules in cellular by regeneration and removal roles in cellular (Surai, 2016; Surai et al., 2019; Munteanu and Apetrei, 2021). These antioxidants inhibit oxidative damage and lipid peroxidation, which are critical for poultry meat quality and cellular redox equilibrium (Zhang et al., 2015; Zhang and Kim, 2020; Munteanu and Apetrei, 2021). Concurrently, environmental stressors weaken chicken immune systems, making them more susceptible to disease and infection. Stress impairs immunity, making chickens more susceptible to health issues. As a result, farmers use antibiotics to prevent disease and manage their flocks (Adams et al., 2023). In the poultry industry, antibiotic resistance is a major concern. In today's world, consumers are becoming more health-conscious, expressing concern about residues found in animal products, particularly antibiotics. These residues have the potential to have a significant impact on health, causing disease and contributing to the emergence of drug resistance issues (Sitthisuang, 2016; Jekmadan et al., 2021). Adopting a natural supplement in place of antibiotics, particularly by employing the potential of locally cultivated medicinal plants, provides an opportunity to expand alternatives and capitalize on the use of medicinal plants in Thai native chicken farming. This will not only benefit farmers' careers but also reduce animal feed costs. Notably, the rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) stands out among the indigenous plants in Ubon Ratchathani Province. (Ekpong et al., 2009; Jekmadan et al., 2021; Minanga and Ampode, 2021)

The rice paddy herb, a vegetable indigenous to Thailand, is primarily found in the northeastern and northern regions of the country. Normally, that grows usually in flooded paddy fields. (Wanyo et al., 2018). This plant belongs to the category of medicinal plants and is classified into two distinct species: *Limnophila Geoffrey* Bonati and *Limnophila aromatic* (Lam.) Merr. The rice paddy herb is well-known for its potent aroma and sharp flavor. It has medicinal properties that can boost appetite and exhibit germicidal effects. Several studies have reported extraction of rice paddy herb, specifically the *Limnophila aromatic* (Lam.) Merr. species, which comprises flavonoid-class in flavones group such as nevadensin,

nevadensin-7-o-ß glycopyranoside, and other flavone which, contains antioxidant activity (Bui et al., 2004; Kukongviriyapan et al., 2007; Homhual, 2010; Nakchat et al., 2014; Khamkaen et al., 2020). According to studies, the principal chemical components of essential oils in rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) include, limonene and perillaldehyde (Khamkaen et al., 2020). Specifically, limonene and perillaldehyde have been shown to suppress the growth of harmful bacteria in the digestive tract. Perillaldehyde is a powerful antioxidant (Sacchetti et al., 2005; Sandri et al., 2007; Sripontan and Yee, 2020). Furthermore, a study has shown that the essential oils from the rice paddy herbs (*Limnophila aromatic* (Lam.) Merr.) have anti-lipid peroxidation, antioxidant effects and free radical scavenging activity (Sribusarakum et al., 2004; Thaipratum, 2014).

Despite a lack of information regarding the use of rice paddy herb in livestock and medicine, a recent study demonstrated that adding 0.2–0.3% of rice paddy herb powder to the diet of nursery pigs led to growth performance similar to that achieved with antibiotics (Sripontan and Yee, 2020). Even with a supplementation rate of 0.1%, the production cost was much reduced compared to those associated with antibiotics (Sripontan and Yee, 2020)

Similar, to a previous found Wongtha et al. (2019) report that the effects of adding *Limnophila aromatic* extract (LAE) at 1, 3, and 5% in diet can be used as a growth-promoting agent in the raniculture industries. This is consistent with another reported in hybrid catfish with dietary supplementation of nevadensin, a flavonoid isolated from rice paddy herb (*Limnophila aromatica*) at amounts ranging from 10-50 mg/kg in diet improve somatic growth and physiological parameters of hybrid catfish. (Jankham et al., 2020).

Consequently, the purpose of this study was to investigate the effects of supplementing the diet of Thai native chickens with Thai rice paddy herb *(Limnophila aromatic* (Lam.) Merr.) powder, the study specifically examined its effects on productive performance, carcass quality, and antioxidant levels in meat.

MATERIALS AND METHODS

Animals

The animal care and experimental procedures in this study were conducted according to the guidelines and regulations of the Institute of Animals for Scientific Purpose Development (IAD), Thailand, and the Institutional Animal Care and Use Committee (IACUC Permit NO. 06/2565/IACUC) of Ubon Ratchathani University. The Leung Hang Kao Kabinburi native chicks were obtained and raised at Ubon Ratchathani Livestock Research and Breeding Center, Nong Khon, Ubon Ratchathani, Thailand (15.307282, 104.757473) from July to November 2022.

Preparation of the Thai rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) powder

The Thai rice paddy herb was collected from cultivation plots in Wang Yangtok Village, Bung Wai Subdistrict, Warin Chamrap District, Ubon Ratchathani province, Thailand, during the rainy season following the methodology described by Gorai et al. (2014). The roots of the rice paddy herb were removed during processing once it reached the laboratory. Afterward, a hot air oven was used to apply the convection drying method. The temperature was set at 40 °C for one week, and the material was turned over daily to ensure complete drying. Following the modification described in the method outlined by Pimson et al. (2018), the fully dried rice paddy herb was ground into a fine powder with a size of 0.5 mm using a multifunctional grinder. Thai rice paddy herb's nutritional composition is detailed in Table 1.

Table 1 Nutritional composition of Thai rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) powder (% on a dry matter basis).

Nutritional composition	Thai rice paddy herb powder (*Limnophila aromatica* (Lam.) Merr.)
Dry matter	86.02
Crude protein	17.51
Crude fat	1.18
Crude fiber	16.09
Ash	21.45
Nitrogen free extract	43.77
Metabolism energy (Kcal/kg) *	2,275.4

*ME (Kcal/kg) = 37 × % CP + 81 × % EE + 35 × % NFE (Pauzenga, 1985)

Experimental design and treatments

The study employed a randomized complete block design (RCBD) using Leung Hang Kao Thai native chickens at 3 weeks of age. The chickens were of both male and female genders, with a total of 200 chickens. These chickens were evenly distributed into four groups, with each group consisting of 50 chickens. The blocks were partitioned into replications of 10 chickens each, giving a total of 5 treatment groups allocated among the 4 blocks. The dietary treatments included a basal diet made up of commercial feeds with 17% crude protein and a metabolizable energy of 2,800 kcal/kg. These were adjusted based on the dietary requirements for Thai native chickens aged 3-16 weeks, as described by Suebsima et al. (2023). The control group was given the basal diet with no additional substances. Other groups received an additional supplement mixed into the basal diet, with concentrations of 1%, 2%, or 3% RPHP (RPHP1, RPHP2, and RPHP3, respectively), while another group received 0.25% oxytetracycline (OTC).

Feeding and management

The experimental feed was formulated by incorporating rice paddy herb powder (RPHP) or oxytetracycline (OTC) antibiotics in a specific proportion for each experimental group. The experiment was conducted until the chickens reached a chronological age of 16 weeks. Throughout the experiment, the chickens were given unrestricted access to both water and feed (*Ad libitum*) (Suebsima et al., 2023)

Productive performance

Data on growth performance were collected from chickens between the ages of 3 and 16 weeks. The collected data encompassed various parameters, including initial and final body weights, body weight gain, feed intake, and feed conversion rates (Laeadon et al., 2019; Minanga and Ampode, 2021). The data were meticulously recorded every week, enabling a thorough comprehension of the chicken's growth performance. In addition, the study recorded economic assessment, including overall expenses and benefit-cost ratios (Laeadon et al., 2019; Lloren, 2021). The economic assessment procedure integrated various metrics, including the production efficiency index, economic loss index, and viability percentage (Witabot, 2018; Laeadon et al., 2019; Lloren, 2021), offering a full view of the economic aspects of the study.

Table 2 The effect of Thai rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) powder on the productive performance of Thai native chickens aged 3-16 weeks.

Parameters	Dietary treatment groups					SEM	P-value
	Control	RPHP1	RPHP2	RPHP3	OTC		
IBD (g/bird)	145.65	150.33	154.58	150.40	144.98	5.59	0.161
FBW (g/bird)	1521.72	1451.56	1513.13	1483.32	1549.75	69.55	0.371
BWG (g/bird)	1367.92	1292.96	1358.55	1328.68	1404.78	66.21	0.233
FI (g/bird/day)	73.11^{a}	58.81^{b}	64.64^{b}	61.77^{b}	62.70^{b}	4.47	0.044
ADG (g/bird/day)	15.03	14.21	14.93	14.60	15.44	0.73	0.231
FCR	5.05^{a}	4.27^{b}	4.35^{b}	4.34^{b}	4.15^{b}	0.25	0.014
TFC (THB/kg)	71.43^{c}	75.75^{c}	79.27^{bc}	89.33^{b}	158.74^{a}	8.01	0.001
B/C ratio	1.68^{a}	1.60^{a}	1.52^{ab}	1.35^{b}	0.76^{c}	0.13	0.001
Viability (%)	95.00	95.00	100.00	97.50	100.00	4.28	0.304
ELI	26.64	27.47	30.49	28.38	33.85	3.67	0.100
PI	292.77	301.91	335.09	311.89	372.02	40.36	0.100

$^{a-c}$ Means in the same row with different letters are significantly different.
SEM = Standard error of the mean, IBD; Initial body weight, FBD; Final body weight, BWG; Body weight gain, FI; Feed intake, ADG; Average daily gain, FCR; Feed conversion ratio, THB = The official currency of Thailand, TFC; Total feed cost includes RPHP (the RPHP production process costs 150 THB/kg), B/C; Benefit cost ratio, ELI; Economic loss, PI; Productive index,

Carcass characteristics

The carcass characteristics were examined at 16 weeks of age. Each experimental unit contained two males and two females, from which four chickens were randomly selected. This selection constituted 40% of the total experimental animals. These chickens were made to fast for 8 hours before being transported to the slaughterhouse. Live weights were measured and recorded before the chickens were slaughtered, which included throat vessel cutting, bleeding, plucking, and removal of giblet organs following the modification described in the method outlined by (Suebsima et al., 2023) The weights of giblet organs such as the liver, gizzard, heart (Oloruntola et al., 2018), and spleen were recorded. Additionally, the spleen index was calculated using the formula [spleen weight ÷ final weight × 100], as described in the modified method outlined by Minanga and Ampode (2021). Dressing percentages were calculated by comparing their weights to the live weight. Following that, the carcasses were dissected into retail cuts such as breasts, thighs, drumsticks, and wings, and the weights of each cut were recorded following the described method outlined by (Suebsima et al., 2023). Using the obtained data, the percentage of trimmed parts (% retail cuts) following the method outlined (Suebsima et al., 2023) was calculated.

Meat quality

A male and female chicken were randomly selected from each experimental unit at 16 weeks of age to investigate meat quality (breast and thigh). The pH was measured 45 min post-mortem using an EXTECH ExStik Waterproof pH meter, following the method described by Zhang et al. (2015). Meat samples were collected and vacuum-sealed after being ground with a household blender. Proximate analysis (moisture, CP, and EE) was carried out by the Association of Official Analytical Chemists (AOAC, 1990).

According to Glinubon et al. (2022b), color values (L*, a*, and b*) were assessed using a Hunter Lab Color Flex EZ colorimeter the meat samples were stored at 4 °C for 24 h post-mortem before samples were analyzed. Drip loss was measured by hanging samples in polypropylene bags for 24 h at 4±2 °C. The drip loss percentage was estimated using the equation (weight before - weight after hanging)/weight before × 100%. The thawing loss was determined by weighing meat samples after slaughter, and both cooking loss and thawing loss samples were vacuum sealed with a household machine. The sealed samples for cooking loss were stored at -20 °C, thawed overnight in a refrigerator at 4 °C, and cooked in a water bath at 100 °C for 30 min, or until the internal temperature reached 78 °C, as measured with a thermocouple tester. Following cooling, the bags were carefully cleansed and reweighed, and the cooking loss percentage was calculated.

Cooked meat samples were diced and sheared perpendicular to muscle fibers using a Warner Bratzler device attached to a texture analyzer (Model TA. XT plus), which was operated at room temperature with a 50 kg load transducer and a crosshead speed of 200 mm/min (Glinubon et al., 2022b). Sealed thawing loss samples were defrosted overnight at 4 °C, weighed again, and the percentage of thawing loss was measured.

Thiobarbituric acid reactive compounds (TBARs) in meat samples were determined after 0, 3, 6, and 9 days of storage at 4 °C. Spectrophotometric assays were performed, measuring absorbance at 538 nm (Glinubon et al., 2022a), and TBAR values were calculated and expressed in malondialdehyde (mg/kg of meat) units using the method described by Rossell (1994)

Meat antioxidant levels

Breast meat samples (skin removed) were collected and put into 5 ml cryotubes to be rapidly frozen (snap-frozen) using liquid nitrogen. Before beginning analysis, the meat samples were kept in storage at -80 °C. Initially, phosphate buffer saline (PBS) was used to wash away any blood adhering to the tissue surface (Chen et al., 2011; Cortes et al., 2012). Using ice to keep the environment cold, the tissue was manually chopped and homogenized in a plastic tube containing chilled PBS. Then, 1 g of the tissue was mixed with 9 ml of cooled PBS solution (weight/volume, 1:9), and 10–20 seconds were spent using a homogenizer (PT2100, HiTechTrader, USA) with a 15,000-rpm setting. Centrifugation was performed on the resultant mixture for 15 minutes at 4 °C with 3000 g. For further investigation, the resultant supernatant was carefully collected and stored at -80 °C (Zhang et al., 2015; Wang et al., 2020). The protein concentration in the breast tissue was evaluated individually by a protein analyzer (Optizen: NANO Q, Republic of Korea) (Zhang et al., 2015). The assay kit (Cat #abx298898, Abbexa, Sugar Land, TX, USA) was used to measure the levels of O_2- and MDA (Cat #EEA015, Thermo Fisher Scientific, Waltham, MA, USA) to assess the production of free radicals and the state of lipid peroxidation. In addition, the activity of antioxidant enzymes such as SOD (Cat #EIASODC, Thermo Fisher Scientific, Waltham, MA, USA) and T-AOC (Cat #abx295022, Abbexa, Sugar Land, TX, USA) was measured in the non-enzymatic group of antioxidants.

Statistical analysis

All data were subjected to analysis of variance using the randomized complete block design (RCBD) model procedure in the SAS Studio statistical package (SAS Institute, 2023). Duncan's new multiple-range test was used to determine treatment effects and differences among groups, with P<0.05.

RESULTS

Productive performance

The initial body weight, final body weight, and body weight gain did not show any statistically significant differences (P>0.05) across the different dietary treatments. Nevertheless, it was noted that the control groups displayed significantly higher feed intake in comparison to all other treatment groups. In addition, the feed conversion ratio was significantly lower in the groups supplemented with RPHP compared to the control group (P<0.05).

Regarding economic factors, it was found that the RPHP1 and RPHP2 groups had comparable total feed costs and benefit-cost ratios to the control group (P>0.05). In contrast, the RPHP3 and OTC groups exhibited significantly higher total feed costs and a lower benefit-cost ratio compared to the control group (P<0.05). Nevertheless, there were no significant differences in the viability rate, economic losses index, and productive index among the different treatment groups (P>0.05).

Carcass characteristics

There were no significant differences in live weight, hot carcass weight, dressing percentage, or retail cut percentage, which includes breasts, thighs, drumsticks, and wings (P>0.05). Surprisingly, diets in the OTC group had the lowest spleen percentage and spleen index, indicating a significant difference when compared to other treatment groups (P<0.05). However, there were no significant differences in percentages of liver, heart, and gizzard among the treatment groups (P>0.05) (Table 3).

Table 3 The effects of Thai rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) powder supplementation on carcass characteristics in Thai native chickens at 16 weeks of age.

Parameters	Dietary treatment groups					SEM	P-value
	C	RPHP1	RPHP2	RPHP3	OTC		
LW (g)	1585.75	1554.44	1568.88	1508.88	1664.81	74.68	0.115
HCW (g)	1290.56	1271.50	1250.13	1211.88	1345.13	62.16	0.096
Dressing (HCW%)	81.46	81.76	79.68	80.31	80.78	1.25	0.189
Breasts (HCW%)	12.34	12.93	12.49	12.56	12.84	0.52	0.498
Thighs (HCW%)	14.25	14.16	13.87	14.16	13.75	0.34	0.255
Drumsticks (HCW%)	13.37	13.26	13.01	12.99	13.31	0.38	0.534
Wings (HCW%)	11.88	12.04	12.27	12.07	11.87	0.34	0.487
Liver (LW%)	2.09	1.88	2.06	2.10	1.92	0.22	0.494
Spleen (LW%)	0.37^a	0.33^a	0.36^a	0.29^a	0.16^b	0.07	0.005
Heart (LW%)	0.45	0.44	0.46	0.48	0.47	0.05	0.842
Gizzard (LW%)	3.92	3.48	3.71	3.90	3.75	0.33	0.368
Spleen index	0.39^a	0.36^a	0.32^a	0.30^a	0.17^b	0.07	0.006

$^{a\text{-}b}$ Means in the same row with different letters are significantly different.
External and visceral organs are expressed as a percentage of Hot carcass and Live weight.
SEM = Standard error of the mean, LW; Live weight, HCW; Hot carcass weight.

Meat quality

The evaluation of meat quality is presented in Tables 4 and 5. There were no differences between the examined parameters for the quality of chicken meat including the pH levels of meats at 45 min post-mortem, the meat color (redness and yellowness) 24 h post-mortem, the water holding capacity (drip loss, cooking loss, and thawing loss), the shear force, the amount of moisture, protein, and fat present, and the TBARs values at 0, 3, 6, and 9 days of storage for both breast and thigh meat (P>0.05). Furthermore, there was no difference in the lightness of thigh meat between dietary treatments (P>0.05). Interestingly, the breast meat of chickens in the RPHP2 group was not as bright as chickens in the control group (P<0.05). Nonetheless, there was no difference in the lightness of the chicken breast meat between the RPHP1, RPHP2, RPHP3, and OTC groups (P>0.05).

Table 4 Effects of Thai rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) powder supplementation on meat quality in Thai native chickens at 16 weeks of age.

Parameters		Dietary treatment groups					SEM	P-value
		Control	RPHP1	RPHP2	RPHP3	OTC		
pH 45 min post-mortem								
Breast		6.27	6.22	6.25	6.31	6.23	0.08	0.544
Thigh		6.71	6.58	6.55	6.54	6.61	0.10	0.191
Color value 24 h post-mortem								
Breast	L*	51.51[a]	48.36[ab]	45.98[b]	49.02[ab]	47.75[ab]	2.11	0.036
	a*	4.26	4.19	4.80	4.40	5.18	0.94	0.554
	b*	15.49	14.25	14.69	14.96	15.23	0.83	0.313
Thigh	L*	43.55	40.81	44.58	42.54	42.21	1.66	0.065
	a*	6.77	7.60	6.63	6.22	6.84	0.62	0.087
	b*	12.27	12.61	11.36	11.69	12.02	1.24	0.655
Drip loss (%)								
Breast		5.83	5.63	5.26	6.67	5.28	1.15	0.439
Thigh		5.20	4.62	4.71	4.48	5.30	0.71	0.424
Thawing loss (%)								
Breast		9.74	10.80	8.69	10.89	10.34	2.11	0.586
Thigh		5.67	5.57	5.44	4.57	6.12	2.04	0.869
Cooking loss (%)								
Breast		27.81	29.20	28.52	28.37	28.74	1.87	0.874
Thigh		34.83	35.91	34.63	35.36	33.95	1.42	0.400
Shear force (N)								
Breast		27.30	30.95	24.07	28.78	36.26	8.22	0.352
Thigh		24.69	23.40	23.26	22.22	24.76	2.98	0.725

[a-b] Means in the same row with different letters are significantly different.
SEM = Standard error of the mean, L*; Lightness, a*; Redness, b* Yellowness.

Meat antioxidant levels

As shown in Figure 1, chickens in the RPHP1, RPHP3, and OTC groups exhibited significantly higher (P<0.05) levels of SOD (11.61, 12.66, and 10.71 U/mg, respectively) compared to the control group (5.56 U/mg). The control group had the highest O_2- level at 0.64 U/mg, significantly higher than the RPHP1, RPHP2, RPHP3, and OTC groups, which had lower levels at 0.22, 0.34, 0.34, and 0.27 U/mg, respectively (P<0.05). Furthermore, the RPHP3 group of chickens displayed the highest T-AOC with a value of 0.32 U/mg, whereas the control group had the lowest level at 0.09 U/mg (P<0.05).

In addition, the control and OTC groups showed higher levels of MDA production at 35.17 and 28.14 μmol/mg, respectively (P<0.05), while the RPHP1, RPHP2, and RPHP3 groups showed decreased MDA production at 14.22, 15.03, and 20.83 μmol/mg, respectively (P<0.05). Nonetheless, no significant changes (P>0.05) were found between the OTC and RPHP3 groups.

Table 5 The effects of Thai rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) powder supplementation on the chemical composition and TBAR value of Thai native chicken meat.

Parameters	Dietary treatment groups					SEM	P-value
	Control	RPHP1	RPHP2	RPHP3	OTC		
Chemical composition							
Breast							
Moisture (%)	70.86	69.94	69.83	70.28	71.18	0.92	0.235
CP (%)	25.81	25.41	24.06	25.05	24.21	1.33	0.325
EE (%)	0.28	0.21	0.31	0.24	0.27	0.15	0.910
Thigh							
Moisture (%)	74.24	74.84	74.19	74.64	73.99	0.69	0.428
CP (%)	22.05	20.93	21.31	22.00	21.94	0.95	0.399
EE (%)	1.39	0.75	1.20	1.20	1.06	0.39	0.270
TBARs value (mg/kg)							
Breast							
Day 0	0.23	0.25	0.26	0.22	0.32	0.07	0.581
Day 3	0.25	0.28	0.26	0.26	0.33	0.04	0.291
Day 6	0.31	0.36	0.40	0.27	0.52	0.13	0.251
Day 9	0.35	0.38	0.47	0.31	0.65	0.27	0.573
Thigh							
Day 0	0.22	0.27	0.26	0.20	0.27	0.04	0.220
Day 3	0.80	0.61	0.86	1.05	1.51	0.47	0.272
Day 6	1.32	0.89	1.39	1.60	1.92	0.67	0.473
Day 9	1.98	1.10	1.50	1.88	2.45	0.88	0.460

SEM = Standard error of the mean, CP; Crude protein, EE; Ether extract, TBARs value; Thiobarbituric acid reactive substance value.

DISCUSSION

In this study, chickens in the rice paddy herb-supplemented and OTC groups had lower feed intake. Nevertheless, the feed conversion ratio for chickens in the rice paddy herb-supplemented and OTC groups decreased compared to the control group. The lower feed conversion ratios observed in the rice paddy herb-supplemented and OTC groups compared to the control group can be attributed to reduced feed intake while maintaining similar body weight and average daily gain. Rice paddy herb contains phenolic compounds like a flavonoid class in the flavone group and the presence of oxytetracycline may have resulted in improved nutrient utilization and metabolic efficiency in the chickens, requiring less feed intake to achieve comparable growth outcomes (Bui et al., 2004; Rajput et al., 2013; Nakchat et al., 2014). This phenomenon highlights the potential of dietary flavonoid interventions to optimize feed utilization and increase overall productivity (Prihambodo et al., 2021). Another possible explanation for the better feed conversion ratio is the substantial presence of flavonoid-class in flavones group chemicals in rice paddy herbs and the various biological actions displayed by perillaldehyde essential oils isolated from these plants. Recent research has investigated the impact of flavonoids and perillaldehyde on many aspects of chicken physiology, such as gut integrity, microbial balance in the digestive tract, and oxidative stress reduction (Bui et al., 2004; Sacchetti et al., 2005; Nakchat et al., 2014; Khamkaen et al., 2020; Zhang and Kim, 2020; Prihambodo et al., 2021) Supplementation with rice paddy herbs may have contributed to these positive outcomes.

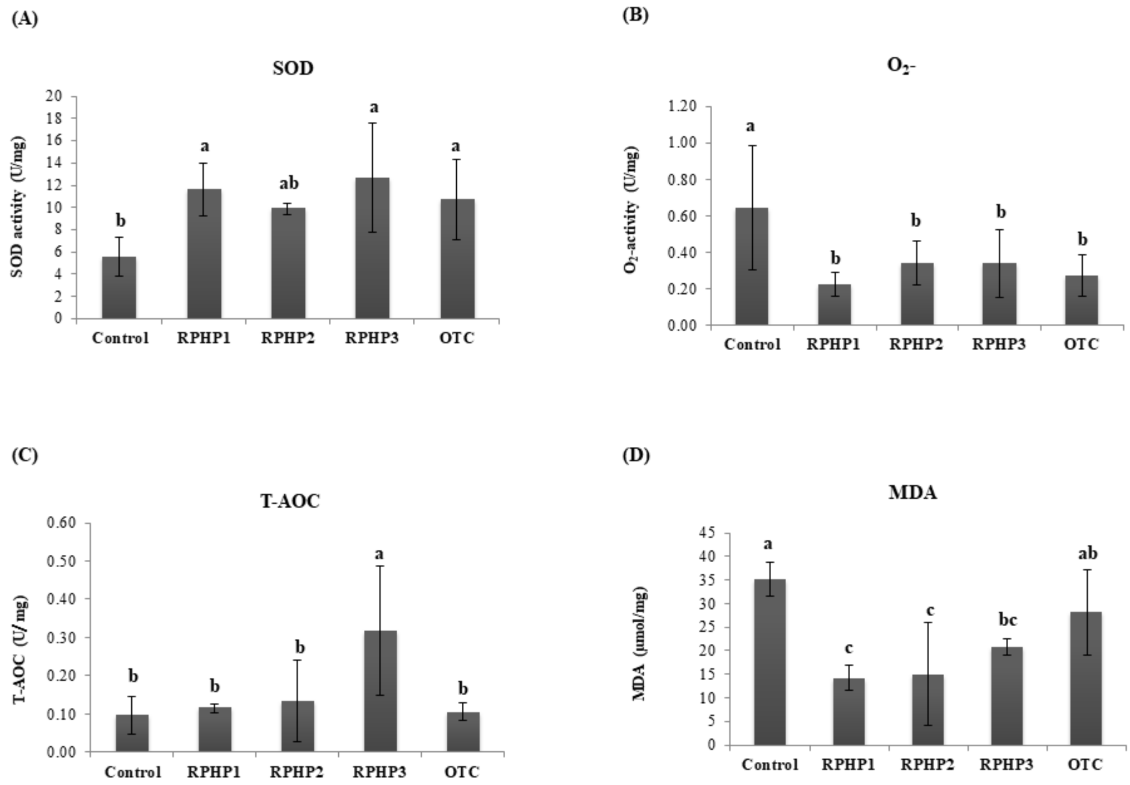

Figure 1 Meat antioxidant levels of Thai native chicken fed with dietary treatment with Thai rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) powder supplementation (T1 control diet no supplement; Control), (T2 1% rice paddy herb powder in diet; RPHP1), (T3 2% rice paddy herb powder in diet; RPHP2), (T4 3% rice paddy herb powder in diet; RPHP3), (T5 0.25% oxytetracycline antibiotic in diet; OTC). SD = Standard deviation, SOD = superoxide dimutase, O_2- = superoxide radical, T-AOC = total antioxidant capacity, MDA = malondialdehyde. (A) as representative SOD, (B) as representative O_2-, (C) as representative T-AOC and (D) as representative MDA. The data are presented as the mean ± SD. Different lower-case letters indicate a significant difference among different precipitation levels (P<0.05).

Moreover, according to the study, phytochemicals derived from plants and containing bioactive secondary metabolites are important for animal health. Rice paddy herb contains phytochemicals such as limonene which have been recognized for their antimicrobial properties (Sandri et al., 2007; Sripontan and Yee, 2020; Jachimowicz et al., 2022), contributing to improved gut health and effective microbial control. Likewise, the OTC group in the diet can potentially protect animal health by inhibiting pathogens in the digestive tract. As a result, despite a decrease in feed intake, the observed better feed conversion ratio indicates a more effective conversion of feed into body mass.

A previous study found that supplementing anti-oxidation herbs, such as *Andrographis paniculata*, at 0.6% in the diet of Leung Hang Kao chickens resulted in the lowest feed consumption while had no negative impact on feed conversion ratio (Hadai et al., 2020). Similarly, Klin-ubon and Siriboon (2021) demonstrated that adding 1-3% of the antioxidant herb *Centella Asiatica* (CA) to the diet of Pradu Hang Dam native chickens resulted in the lowest feed conversion ratios, with a significant decrease in consumption found with only 3% of CA provided. Likewise, Thuy et al. (2023) found that supplementing Tam Hoang chickens' diets with herbal powder containing antioxidants and antimicrobials, such as garlic powder at 1 g/kg, resulted in an improved feed conversion ratio.

Including OTC into the diet resulted in a considerable increase in total feed cost, resulting in a noticeable decrease in the benefit-cost ratio. In contrast, chickens fed a diet supplemented with rice paddy herb, particularly at 1% and 2%, had comparable total feed costs and benefit-cost ratios to those in the control group, while displaying improved efficiency in chicken productivity. The antibiotic-supplemented group had higher total feed expenditures and a poorer benefit-cost ratio due to antibiotic-related expenses. When comparing the total feed cost and benefit-cost ratios between the rice paddy herb-supplemented group and the control group, it is prudent to consider previous research. For example, a study by Laeadon et al. (2019) found that supplementing broiler diets with tamarind seed coat powder, which contains phenolic compounds and other antioxidants, at 300 mg/kg led to improved production efficiency, greater outputs, and economic performance. Similarly, Punyatong et al. (2024) found that supplementing Thai native crossbred chickens with fresh *Azolla pinnata* replacement, which is recognized for its natural antioxidant capabilities, at amounts ranging from 5-25% as an ingredient in feed formulation resulted in lower feed costs per gain.

There were no noticeable variations in carcass features across the treatment groups in this investigation, as indicated by comparable body weight gain, average daily gain, and final body weight across all groups. This is consistent with previous studies on the antioxidant capacity of herbal spices. For example, Yanin et al. (2016) found that increasing the diet with cayenne pepper did not result in significant differences in carcass quality. Similarly, in a study conducted by Kongrith et al. (2019) to investigate the impacts of Plai (*Zingiber cassumunar* roxb) and Ginger (*Zingiber officinale* roscoe) as antioxidant sources in broiler diets, no differences in carcass quality measures were found. Our findings highlight the enormous potential for OTC-containing diets to improve immune system protection. This result is supported by the association between spleen weight and immune system function in chickens, where a lower spleen weight and index indicate a strong immune system and overall health (Minanga and Ampode, 2021).

In terms of meat quality, the lightness of breast meat was reduced in chickens fed a 2% rice paddy herbs diet. Meat color is widely acknowledged as an important indicator of meat quality, with deeper hues often being deemed less appealing than lighter ones. The antioxidant capabilities of rice paddy herbs have been recognized as effective in suppressing lipid peroxidation and may be related to potentially limiting the oxidation process in autoxidative mechanisms (Sribusarakum et al., 2004; Bertolín et al., 2019). This inhibition works by either blocking the initiation or propagation of oxidative chain reactions. As a result, the oxidation of red oxymyoglobin to metmyoglobin can cause an unattractive brown color in meat (Sribusarakum et al., 2004; Kanani et al., 2017). Similar to this current study in broiler, found that adding sweet basil leaf powder to the diet, which contains antioxidants at 1% and 2%, did not affect pH, drip loss percentage, cooking loss percentage, or thawing loss percentage (Pastsart et al., 2021). Our findings contrast with another study in Mae Hong Son chickens found that the effect of *Moringa oleifera* leaf extract on the diet, as a source of phenolic compound and other antioxidants at 2% and 4%, decreased TBAR values at 3, 5, and 7 days of storage for both breast and thigh meat except TBARs values at 3 days for breast meat and adding *Moringa oleifera* leaf extract at 2% and 4% decrease fat for both breast and thigh meat (Marupanthorn et al., 2024). As a result, diets including rice paddy herbs powder had only minor effects on most meat quality indicators, which could be attributed to insufficient supplementation doses and the comparatively low antioxidant content of post-mortem chicken carcasses. It is worth noting that, while these antioxidants are present in live chickens, they may be lost during post-mortem care and processing during storage reducing their potency (Jekmadan et al., 2021; Khang et al., 2024).

In the current study, adding rice paddy herbs into the diet at 3% resulted in significant increases in SOD and T-AOC levels, which led to a decrease in O_2- and MDA content. This beneficial effect was more noticeable when compared to other treatment groups, emphasizing the importance of rice paddy herb The antioxidant capabilities of rice paddy herbs have been recognized as effective in suppressing lipid peroxidation and may be related to potentially limiting the oxidation process in autoxidative mechanisms containing flavones group and rice paddy herb inclusion levels in alleviating oxidative stress within cells (Bui et al., 2004; Nakchat et al., 2014; Munteanu and Apetrei, 2021). Elevated T-AOC activity implies a lower production of oxidative stress, whereas chickens fed the control diet showed higher O_2- activity and decreased SOD activity, implying probable O_2- damage in the control group. The superoxide radical is a major free radical that causes significant cellular damage (Ahmad et al., 2012; Zhang et al., 2015). Furthermore, the control group showed raised MDA levels, indicating lipid peroxidation, and increased oxidative stress (Wang et al., 2020; Wu et al., 2019). This is consistent with previous studies on the antioxidant capabilities of herbal products in animals. For example, Dityana et al. (2024) found that supplementing a broiler diet with encapsulated *Medinilla javanensis* fruit extract, which is high in flavonoids, at 600 and 800 mg/kg increased SOD levels and decreased MDA levels in serum. Similarly, Norkeaw et al. (2022) studied the effects of dietary supplementation with 500 mg/kg of green tea extract, which is recognized for its powerful antioxidant capabilities, on lipid oxidation in pigs. Similarly, Pastsart (2018) found that adding *Piper samentosum* (Wild betel) leaves to the diet at 2% and 3% concentrations, which are recognized for their antibacterial and antioxidant capabilities, increased SOD activity in broiler breast meat.

As a result, adding 3% rice paddy herbs to the diet appears to increase antioxidant levels in chicken breast meat, improving cellular defense against oxidative stress. Rice paddy herb is well-known for its high content antioxidant for flavones group (Bui et al., 2004; Kukongviriyapan et al., 2007; Nakchat et al., 2014) which function as powerful antioxidants, protecting cells and interrupting harmful oxidative chains. Flavonoids improve overall antioxidant capacity by effectively scavenging O_2- and boosting the production of copious simple phenolic acids (Zhang and Kim, 2020). This increased T-AOC helps SOD neutralize free radical activity, resulting in a balanced and healthy cellular redox state (Surai et al., 2019; Munteanu and Apetrei, 2021). The rice paddy herb plant shows anti-lipid peroxidation activities (Sribusarakum et al., 2004). Furthermore, lower MDA levels indicate that antioxidant enzymes and non-enzymatic antioxidants, particularly in the RPHP supplementation groups, have the potential to improve animal health by scavenging lipid peroxidation, mitigating the negative effects of damaged macromolecules, promoting oxidative injury regeneration, and suppressing free radical production within cells (Hosseindoust et al., 2020; Zhang et al., 2015; Munteanu and Apetrei, 2021).

CONCLUSIONS

The present study demonstrates the effects of supplementing the diet of Thai native chickens with Thai rice paddy herb (*Limnophila aromatic* (Lam.) Merr.) powder. It was found that RPHP supplementation did not affect body weight, weight gain, or average daily gain. The RPHP-supplemented group had a lower feed intake and feed conversion ratio than the control group. RPHP1 and RPHP2 had similar feed costs and benefit-cost ratios to the control. The benefit-cost ratio decreased as OTC raised feed costs. Carcass characteristics showed no RPHP treatment effects. Except for breast meat lightness, which decreased in the RPHP2 group compared to the control group, meat quality did not differ between treatment groups. Furthermore, supplementing with RPHP, particularly RPHP3, increased SOD, T-AOC, and decreased O_2-, and MDA levels in meat. Our findings suggested that RPHP, especially RPHP3, may reduce oxidative stress in meat by increasing antioxidant levels in Thai native chicken meat.

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AUTHOR CONTRIBUTIONS

C. Jekmadan: Investigation; Methodology; Data curation; Formal analysis; Visualization; Software; Writing - original draft.
C. Yuajit: Investigation; Conceptualization; Supervision; Writing - review & editing.
S. Suwalee: Investigation; Methodology; Software; Formal analysis; Writing - review & editing.
B. Suebsima: Conducting Resources; Validation, Supervision; Conceptualization; Writing - review & editing.
J. Glinubon: Investigation; Conceptualization; Supervision; Writing - review & editing.
N. Tantisirin: Validation; Writing - review & editing.
W. Kaewluan: Investigation; Supervision; Conducting Resources.
C. Siriboon: Conceptualization; Validation; Supervision; Writing - review & editing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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