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Research article

Effects of *Moringa oleifera* leaf extract on growth performance, blood hematology, immunity, carcass, and meat quality of Mae Hong Son chickens

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Abstract

Moringa oleifera is a nutrient-rich plant with a wide range of beneficial effects on chicken and chicken products. Mae Hong Son chickens, a variety of Thai native poultry, are renowned for their exceptional meat quality, distinct flavor, and remarkable tenderness. Therefore, this present study aimed to investigate the effects of *Moringa oleifera* leaf extract (MOLE) on growth performance, blood hematology and immunity, carcass, and meat quality of Mae Hong Son chickens, respectively. Briefly, 200 birds of Mae Hong Son chickens were subsequently divided into 4 groups, each containing 50 birds. They were fed for 16 weeks with different supplementary as follows: 1. Control group 2. Placebo (maltodextrin + microcrystalline cellulose PH101) 3. 2% of MOLE extract supplementation and 4. 4% of MOLE extract supplementation. The results showed that T4 achieved the best outcomes in terms of body weight and average daily gain, while also having the lowest total white blood cell (WBC) count and the highest ND HI titer at 12 weeks old ($p < 0.05$). On the other hand, T1 had the highest fat percentage and thiobarbituric acid reactive substances (TBARS) values in both the thigh and breast ($p < 0.05$). Additionally, the shear force of T1 was the lowest, both in the thigh and breast ($p < 0.05$). In summary, the potential of MOLE could improve growth performance, hematological parameters, immune response, and meat quality in Thai native chicken production.

Keywords: Blood hematology, Growth performance, Immunity, Meat quality, *Moringa oleifera* leaf extract

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INTRODUCTION

Plants have been cultivated worldwide due to their high medicinal and health properties. Plant extracts called phytobiotics or phytogens, have gained popularity in both traditional and alternative animal medicines (Kuralkar and Kuralkar, 2021). Therefore, *Moringa oleifera* (MO) has widely been produced in Southeast Asian countries, especially in Thailand, India, the Philippines, and Pakistan, respectively. The tree is also known as the "miracle tree" by people in Africa (Gupta et al., 2018). *Moringa oleifera* leaves (MOL) and seed extracts contain a valuable source of bioactive compounds that are known for their high medicinal properties (Ezz El-Din Ibrahim et al., 2022). These compounds have been involved in the treatment of various infectious diseases, modulating the immune system, antioxidant, antidiabetic, and antitumor effects (Dhakad et al., 2019). Their phenolic compounds such as flavonoids, phenolic acids, and glycosides while alkaloids, tannins, saponins, isothiocyanate, and glucosinolates have been extensively studied as natural antioxidants (Rocchetti et al., 2020).

The majority of Thai native chickens are raised in small-scale operations by Thai smallholder farmers in free-range or backyard settings (Choprakarn and Wongpichet, 2007). As a result, they are more exposed to pathogens than chickens raised in closed housing systems. Therefore, it is important to consider ways to boost their immune systems to reduce the incidence of disease and the use of antibiotics in their production. Therefore, safe and healthy production of chicken requires proper microbial control to produce high-quality meat (Ravindran, 2017). Mae Hong Son chicken (MHS) is a Thai native chicken which is found locally throughout Mae Hong Son province in northern Thailand. MHS is characterized by its high meat quality as well as its unique flavor and chewiness. MHS is also considered a symbol of the province's cultural heritage and is often used in local ceremonies and festivals. In Thailand, there is a certain group of consumers who prefer meat from native chickens over broiler chickens, mainly because of their superior taste and chewy texture (Jaturasitha et al., 2017). The utilization of crops to promote animal health, and grain conservation, and to mitigate concerns over drug resistance, high input costs, and the presence of toxic residues in food has witnessed a significant increase. This increase can be attributed to advances in organic livestock production systems (Escosteguy, 2014). This study aims to determine the effects of MOLE on the growth performance, blood hematology, immunity, carcass, and meat quality of Mae Hong Son chickens. The impact of MOLE on these parameters will provide valuable insights into understanding its potential as a natural supplement to improve poultry production, immunity and enhance the nutritional value of chicken meat.

MATERIALS AND METHODS

Moringa oleifera leaf extract

The fresh leaves of *Moringa oleifera* were collected from Ayutthaya, Phitsanulok, and Phichit provinces. These leaves were identified at the Faculty of Medicine, Thammasat University. Cold succinate extraction was conducted using ethanol, and the resulting extract was carefully weighed and stored at sub-zero temperatures in the refrigerator. For MOL wet granulation, the MOLE was combined with pharmaceutical excipients maltodextrin (PC drug, Bangkok, Thailand), and microcrystalline cellulose PH101 (PC drug, Bangkok, Thailand), utilized the Moisture-Activated Dry Granulation Technique (Shanmugam, 2015). The identification of volatile compounds within the MOLE was executed using gas chromatographic (GC) determination at the Center of Scientific Equipment for Advanced Research, Thammasat University.

Animal raising, diet, and carcass quality

The present study was approved by the protocol of the Animal Care and Use Committee, Chiang Mai Rajabhat University, Thailand (CMRU-IAD 009/2564). This study used Mae Hong Son chicken divided into 4 groups: 1. Control group 2. Placebo (maltodextrin + microcrystalline cellulose PH101) 3. 2% of MOLE supplementation 4. 4% of MOLE extract supplementation, respectively. The birds were randomly divided into four groups of 50 birds each, and each group was further divided into 5 replicates of 10 birds. A total of 200 birds were used in the experiment. All chickens were raised at the Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Chiang Mai, Thailand. In this experiment, day-old MHS chicks were acquired from Mae Hong Son provincial livestock. All chickens were raised under the same environmental conditions (i.e., open-air housing and a vaccination program). A commercial indigenous broiler diet was obtained from the Charoen Pokphand Company that contained 21% crude protein (CP). Accordingly, 3100 kcal of ME/kg was first fed to chicks aged within a range of 1-3 weeks. After that, feed containing 14% CP and 3200 kcal of ME/kg was given to chicks during their growth period until 112 days followed by slaughtering. Weights were measured individually once a week and the average body weight (BW) and average daily gain (ADG) were also recorded, respectively. To evaluate the livability percentage, broilers were checked for mortality once a day according to the method described by Marcu et al. (2013). All chickens were fasted for 12 h and their final BW was reported as described by (Kong et al., 2023). Birds were slaughtered according to the standard commercial practice. All carcasses were dressed after 24 hours of cooling. Subsequently, breast and thigh meat samples were refrigerated at -20°C. After 24 hours of postmortem examination, we weighed the liver, stomach (proventriculus and gizzards combined), heart, spleen, and pancreas. Organ composition was calculated as a proportion of the total body mass. The carcasses were then weighed after 24 hours of storage at 4°C. Additionally, dressing percentage and retail cuts were determined relative to the cold carcass weight.

Blood sampling

The blood samples were randomly collected from 16-weeks-old (80 total chickens) after weighing while repeating the process for each replication for 4 chickens. Blood was drawn from the wing vein and added to anticoagulant (EDTA) tubes to determine hematological indices. The following hematological measurements were estimated as follows; red blood cells (RBCs), hemoglobin concentration (Hb%), hematocrit (%), white blood cells (WBCs), lymphocytes (%), monocytes (%), heterophils (%), basophil (%) eosinophil (%) and heterophil/lymphocyte ratio were determined according to Gross and Siegel (1983) with slight modifications.

Immune response against modified-live Newcastle disease vaccine (NDV)

All week-old birds were vaccinated with a commercial monovalent modified-live Newcastle disease virus (NDV) vaccine, B1 Type, LaSota Strain (Zoetis, USA). The booster dose of NDV was administered to all chickens at 2 weeks and 8 weeks of age. In the 2 and 4 weeks following the 3rd vaccination, 4 chickens of each replication were bled and the hemagglutination inhibition (HI) titer of the individual serum samples was determined using the standard method as described by (Meijer et al., 2006).

Meat quality evaluation

The percentage of moisture, fat and protein was determined according to the method of (AOAC, 1990). The breast and thigh muscles were stored in vacuum-packed containers at -20°C until further analysis. A pH meter (pH meter model 191, Knick, Berlin, Germany) was applied to determine the pH values at 45 min and 24 h postmortem, respectively. The internal temperature was controlled by employing a thermocouple tool. Cooked samples were cut into cubes (1×1×1 cm) to measure shear force using a texture analyzer (model TA. XT plus, stable microsystem, Ltd., London, England). Furthermore, thiobarbituric acid reactive substances (TBARS) values were determined to assess the lipid oxidation at 4°C for 0, 1, 3, 5, and 7 d in ground meat.

Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA) (SAS Institute Inc, 1993) at a significant level of 95%. Tukey's method was carried out to compare treatment mean values. The data are presented as least square mean values for the various treatment groups, the corresponding SEM values, and the probabilities of error (P-values).

RESULTS

Gas chromatography-mass spectrometry analysis

The gas chromatography–mass spectrometry analysis of the extract of MOL identified the presence of several phytochemicals as shown in (Table 1). The most prevalent compounds found in MOLE are hydroxyprogesterone (76.03%), vitamin E (60.92%), octadecatrienoic acid (35.79%), and dianhydromannitol (33.52%), respectively.

Table 1 Gas chromatography-mass spectrometry analysis of MOLE used in the diet of Mae Hong Son chickens.

No	Retension Time	Name of the compound	Molecular Formula	Peak Area (%)
1	71.65	Hydroxyprogesterone	$C_{27}H_{40}O_4$	76.03
2	72.96	Vitamin E (Tocopherol)	$C_{29}H_{50}O_2$	60.92
3	52.17	Octadecatrienoic acid	$C_{20}H_{34}O_2$	35.79
4	18.36	Dianhydromannitol	$C_6H_{10}O_4$	33.52

Growth performance

The effects of MOLE on growth performance as presented in (Table 2). The results indicated that BW and ADG values at 0-16 weeks were the highest in T4 when compared with the other groups, whereas T1 (control group) exhibited the lowest performance ($p < 0.05$). However, there was no significant difference in BW and ADG values at 0-6, 0-10 and 0-14 weeks, respectively.

Table 2 Effects of MOLE on growth performance of Mae Hong Son chickens.

Items	T1	T2	T3	T4	SEM	*p* value
Body weight (g/bird)						
0-6 weeks	330.45	336.98	321.03	331.33	6.13	0.84
0-10 weeks	595.67	599.38	626.41	605.45	10.41	0.96
0-14 weeks	777.97	760.71	788.52	779.4	15.23	0.88
0-16 weeks	899.35^{b}	920.08^{b}	941.13^{ab}	1,020.61^{a}	14.93	0.04
Average daily gain (g/bird/d)						
0-6 weeks	23.6	24.23	22.93	23.67	0.44	0.78
0-10 weeks	14.53	14.8	15.28	14.42	0.23	0.72
0-14 weeks	11.11	10.82	11.27	11.13	0.17	0.84
0-16 weeks	10.71^{b}	10.98^{b}	11.21^{ab}	12.15^{a}	0.18	0.05

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

Blood hematology and immunity

The hematological parameters of 16-week-old MHS chickens, raised with different MOLE supplements were investigated. The study found that the total RBC count, hemoglobin concentration, percentage of hematocrit and all types of WBCs, including heterophil, eosinophil, basophil, lymphocyte, and monocyte showed no significant differences ($p > 0.05$) among the four groups. However, the placebo group with maltodextrin + microcrystalline cellulose PH101 supplement in their diet had the highest total WBC count (4.02 x 10^5 cells/mm^3), while the group with 4% MOLE in their diet had significantly lowest total WBC count (Table 3). The results on the antibody response to the booster dose of inactivated Newcastle disease vaccine at 8 weeks of age, showed that after vaccination for 2 weeks, the HI titer in chickens from all four groups showed no significant differences. However, after the 3rd vaccination for 4 weeks, the group of chickens with a 4% MOLE supplemented in their diet had the highest HI titer, but it was not significantly different from the group with a 2% MOLE supplemented or the control group. The control group had the lowest HI titer, but it was significantly different from the group that received the MOLE supplemented in their diet (Table 4).

Table 3 Effect of MOLE on blood hematological parameters of 16 weeks old of Mae Hong Son chickens.

Items	T1	T2	T3	T4	SEM	*p* value
RBC (x10^{6}cells/mm^3)	2.43	2.58	2.46	2.62	0.05	0.44
Hemoglobin (g/dL)	11.13	11.51	11.35	11.64	0.20	0.83
Hematocrit (%)	33.1	34.6	34.1	33.6	0.63	0.87
WBC (x10^{5}cells/mm^3)	3.89^b	4.02^a	2.84^c	2.04^d	0.27	0.02
Heterphil (%)	24.30	25.30	25.80	23.70	1.28	0.13
Basophil (%)	0	0	0	0	0	0
Eosinophil (%)	0.30	0.10	0.60	0.10	0.09	0.08
Lymphocyte (%)	69.20	72.90	73.10	75.10	1.81	0.76
Monocyte (%)	1.20	1.70	1.20	1.50	0.11	0.22
H/L ratio	0.32	0.38	0.39	0.29	0.02	0.84

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

Table 4 Effects of MOLE on ND HI titer (standard deviation) for Newcastle disease vaccination of 10 weeks and 12 weeks old of Mae Hong Son chickens.

	Hl titers (log_2)					
	T1	T2	T3	T4	SEM	*p* value
10 weeks old	40.80	43.20	46.40	54.00	4.11	0.52
12 weeks old	6.20^c	8.70b^c	9.70^{ab}	11.60^a	0.60	0.01

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

Carcass and meat quality

The effect of MOLE with different treatments on carcass traits is presented in (Table 5). Live weight, hot carcass, chilled carcass, and carcass percentage values of all groups were not significantly different ($p > 0.05$). The organ composition following the shank, feet and giblet percentages were not significantly different. Similarly, the retail cut percentage in terms of outer breast, inner breast, thighs, drumsticks, and wings were also not significantly different ($p > 0.05$).

Table 5 Effects of MOLE on carcass quality of Mae Hong Son chickens.

	T1	T2	T3	T4	SEM	*p* value
Live weight (g)	817.87	819.47	874.67	909.2	16.11	0.12
Hot carcass (g)	589.65	597.95	661.94	666.31	14.98	0.13
Chilled carcass (g)	571.96	580.01	642.08	646.32	14.53	0.39
Carcass percentage (%)	72.09	72.69	75.69	72.85	0.8	0.13
Organ composition (live weight %)						
Shank and feet	6.7	4.5	3.95	4.59	0.64	0.45
Giblets	13.3	12.79	11.78	12.25	0.28	0.24
Retail cuts (cold carcass weight %)						
Outer breast	14.14	13.01	11	13.76	0.48	0.10
Inner breast	4.51	4.33	3.91	4.42	0.18	0.70
Thighs	17.46	17.36	15.13	17.79	0.57	0.35
Drumsticks	15.23	16.81	13.8	16.6	0.53	0.16
Wings	13.74	14.48	12.35	14.85	0.44	0.21
Skeleton	35.99	35.72	32.33	36.32	1.22	0.64

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

Breast muscle

The results of an assessment of the meat quality traits of breast muscle samples obtained from chicken raised with MOLE at different levels are shown in (Table 6). The protein percentage among different treatments indicated that T2 was the highest, followed by T1, T4 and T3, respectively ($p < 0.05$). Moisture was considered the main constituent of the breast muscle. The moisture content obtained in group T3 was significantly higher than in other treatment groups, respectively. The fat content of T1 (2.44%) was significantly higher than the T2 and T3 treatment groups. While pH values at 45 min and 24 hr postmortem were not significantly different ($p > 0.05$) between the treatment groups. The shear force of T4 had the highest value followed by T3, T2 and T1, respectively, which indicates the tenderness quality of meat. In addition, MOLE treatments significantly decreased the TBARS values on days 5 and 7 while assessed to determine the lipid oxidation of breast muscle.

Table 6 Effects of MOLE on meat quality of breast muscle of Mae Hong Son chickens.

	T1	T2	T3	T4	SEM	*p* value
Chemical composition (%)						
Protein	29.70^{a}	30.17^{a}	28.04^{b}	29.53^{a}	0.457	<.05
Moisture	67.86^{c}	67.50^{c}	70.24^{a}	68.77^{b}	0.388	<.05
Fat	2.44^{a}	2.34^{a}	1.73^{b}	1.70^{b}	0.167	<.05
pH value						
pH 45 min	6.31	6.28	6.34	6.32	0.019	0.75
pH 24 hr.	5.79	5.87	5.84	5.82	0.019	0.52
Shear force value						
Force (N)	15.06^{c}	15.66b^{c}	17.48^{b}	20.97^{a}	0.977	<.05
TBARS (malondialdehyde/kg meat)						
Day 0	0.06	0.06	0.04	0.04	0.004	0.05
Day 1	0.07	0.07	0.06	0.06	0.003	0.25
Day 3	0.13	0.12	0.09	0.09	0.007	0.06
Day 5	0.25^{a}	0.23^{a}	0.18^{b}	0.18^{b}	0.007	<.05
Day 7	0.29^{a}	0.28^{a}	0.25^{b}	0.25^{b}	0.005	<.05

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

Thigh muscle

The results of thigh muscle samples obtained from chicken raised with MOLE at different levels are shown in (Table 7). The chemical composition in terms of protein, T2 was the highest content followed by T3, T4 and T1, respectively. The moisture content of T4 was the highest, while T2 was the lowest ($p < 0.05$). The amount of crude fat obtained from T1 showed the highest content as compared to T2, T3 and T4, respectively. The pH value at 45 min and 24 hr postmortem was not significantly different ($p > 0.05$) among other groups. The highest shear force was found in T3 while T1 had the lowest value. The thigh muscle treated with the MOLE had significantly lower amounts of TBARS on (3, 5, and 7 days) as compared to the control group, respectively.

Table 7 Effects of MOLE on meat quality of thigh muscle of Mae Hong Son chickens.

	T1	T2	T3	T4	SEM	*p* value
Chemical composition (%)						
Protein	22.78^{c}	25.98^{a}	24.62^{b}	22.96^{bc}	0.793	0.001
Moisture	69.57^{b}	67.34^{b}	71.01^{a}	72.07^{a}	0.602	<.05
Fat	7.66^{a}	6.95^{a}	4.68^{b}	4.37^{b}	0.398	<.05
pH value						
pH 45 min	6.56	6.47	6.48	6.50	0.013	0.06
pH 24 hr.	5.82	5.76	5.79	5.84	0.023	0.64
Shear force value						
Force (N)	12.74^{b}	13.90^{ab}	15.74^{a}	15.53^{a}	0.99	0.01
TBARS (malondialdehyde/kg meat)						
Day 0	0.07	0.07	0.06	0.06	0.002	0.67
Day 1	0.08	0.08	0.07	0.07	0.003	0.11
Day 3	0.19^{a}	0.19^{a}	0.16^{b}	0.16^{b}	0.004	0.001
Day 5	0.28^{a}	0.27^{a}	0.22^{b}	0.21^{b}	0.009	<.05
Day 7	0.32^{a}	0.31^{a}	0.26^{b}	0.24^{b}	0.009	<.05

T1 Control group; T2 Placebo (maltodextrin + microcrystalline cellulose PH101); T3 2% of MOLE supplementation; T4 4% of MOLE supplementation.

$^{a, b, c}$ Means in the same row followed by different letters are significantly different ($p < 0.05$).

DISCUSSION

Growth performance

Our results were in accordance with the previous research studies that have reported that *Moringa oleifera* extract affects the performance of broiler chickens (Alabi et al., 2017; Khan et al., 2017; Rehman et al., 2018; Mahfuz and Piao, 2019). Abdulsalam et al. (2015) investigated the effect of *Moringa oleifera* leaf meal in broiler chickens and found that the supplemented diet could enhance the growth performance in the finishing phase. According to the study, weight gain increased during the final two weeks. Moreover, a growing body of evidence showed that most of the stresses in poultry production, at the cellular level, are associated with oxidative stress (Surai, 2016). The utilization of medicinal plant extracts in poultry farming to alleviate stress caused by overcrowding is imperative and warrants further investigation. In addition, dose-dependent birds with high concentrations of MOLE positively affected their growth performance because of their abundance of phytochemicals, macronutrients, and minerals with high bioavailability (Penalver et al., 2022). This result could be attributed to the high levels of polyphenol and flavonoid contents in a diet that has efficiently metabolized the growth of rabbits (Etchu et al., 2017).

Moreover, broilers fed diets containing 1% and 2% *M. oleifera* extract exhibited higher BWG compared to those on the control diet (Teteh et al., 2013). These findings are consistent with our study. During weeks 0-16, the chickens in the group supplemented with the extract showed higher body weights and average daily weight gain compared to the control groups. Polyphenols transform into absorbable metabolites within the gut microbiota, leading to increased bioavailability. (Iqbal et al., 2020). The composition of gut microbiota plays a critical role in both feed digestion and nutrient absorption (Iqbal et al., 2020). *Clostridium coccoides* were associated with higher body

weight gain (BWG), while Enterobacteria, *E. coli* and *Shigella* were associated with lower BWG in chickens (Rubio et al., 2015). Similarly, the inclusion of MOL at higher levels (15% and 20%) in broiler diets resulted in a higher growth rate and better health status of broilers (Alnidawi et al., 2016). Khan et al. (2017) showed that supplementation with 1.2% MOLE led to significantly higher body weights than in the control group ($p < 0.05$). Moreover, dietary supplementation with MOL at levels ranging from 5% to 20% showed higher growth performance in broiler chickens (Moreki and Gabanakgosi, 2014).

Blood hematology and immunity

Regarding hematological parameters, the study revealed that chickens supplemented with 2% and 4% MOLE had lower WBC counts compared to the control group. This could be attributed to certain compounds in the flavonoids, particularly quercetin, which have anti-inflammatory and anti-allergic properties. Quercetin has been shown to inhibit the release of histamine and several other inflammatory mediators (Butkhup, 2012; Xu et al., 2019). According to Sreelatha and Padma (2009), MOL and its extracts were found to have wound-healing, anti-inflammatory, and antioxidant effects. Notably, the H/L ratio tended to decrease, and this ratio is accepted as a measure of stress in birds (Gross and Siegel, 1983). A higher H/L ratio indicated an increased stress level in those birds (McFarlane and Curtis, 1989), and the normal value for birds was between (0.30-0.57) (Jain, 1993). The inability to control temperature in open-house chicken farming contributed to chicken stress levels, leading to an increase in glucocorticoid hormones (Jain, 1993), which affected the heterophils. These cells were released from the bone marrow into the bloodstream in response to stress, while the number of lymphocytes decreased due to migration back into the bone marrow and lymphoid tissues.

In conclusion, the study indicated that supplementing MHS chickens with different quantities of MOLE affected their growth performance and hematological values. The findings suggested potential benefits in terms of growth and immune response, as well as the possibility of mitigating stress in chickens through the supplementation of MOLE. It was found that supplementing MOLE in the diet could enhance or stimulate the immune response to Newcastle disease vaccine. When measuring the immune response at 2 weeks after vaccination, there was no significant difference. However, the group supplemented with MOLE maintained a higher level of immune response compared to the control group at 4 weeks after the 3rd vaccination, which was differentially significant. The increase in immune response after vaccination against Newcastle disease aligned with the findings of Al-Garib et al. (2003), respectively. The antibodies were detected at the site of infection and in the blood starting at six days after infection or live virus vaccination and peaking at 21–28 days after infection. In this current study, 2 weeks after the 3rd vaccination, an enhancement of the immune response was observed after vaccination. Regarding the effect of supplementing MOL in broiler's diet conducted by Du et al. (2007), it was found that the supplement could boost the immune response to sheep RBC, which were injected into broiler at 21 and 28 days of age. Previous studies on MOLE supplementation have typically used MOLE dissolved in drinking water. Khan et al. (2021) found that MOLE at concentrations of 60, 90, and 120 mL/L in drinking water stimulated the immune response to NDV. However, Mohamed et al. (2023) found that broiler

chickens supplemented with MOLE at a dose of 200 mg/L in drinking water had no significant difference in NDV HI titer from the control group at 35 days of age. This study is the first to investigate the effects of MOLE-supplementation in the form of granules in the diet of native Thai chickens. Moreover, the results of this current study suggest that MOLE can be a beneficial addition to chicken diets to stimulate the immune response to NDV.

Carcass and meat quality

The results of this present study on carcass measurements are consistent with those of Sugiharto and Toana (2021) and Nkukwana et al. (2014), who found no significant differences in dressing percentage, hot carcass weight and cold carcass weight weights in broilers fed *Moringa oleifera* seed meal (MSM) or *Moringa oleifera* leaf meal (MOLM) compared to the control group. Promket and Ruangwittayanusorn (2021), reported that the carcass yield of Thai native chickens Chee KKU12 and Chee N the live weight, the carcass weight and carcass percentage of both groups were not different ($p > 0.05$). However, *Moringa oleifera* seed extract (MSE) supplementation increased dressing percentage, hot carcass weight, and cold carcass weight in broiler chickens, despite similar slaughter weight values among all treatment groups (Egbu et al., 2022).

In this present study, chemical composition showed reduced fat content in both breast and thigh muscles. According to Mickdam et al. (2022), investigated that the broiler receiving 3% and 5% of MOLM showed a decreased amount of fat in the carcass. The reduction in fat percentage could be attributed to the difference in fat metabolism caused by supplementation of MOLM. Xie et al. (2018) reported that supplementation of MOLM decreased fat synthesis and inhibited fat accumulation by inhibiting adipogenesis and promoting lipolysis. Moreover, the regulation of lipid homeostasis is related to the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoAR), the rate-controlling enzyme of cholesterol synthesis inside the cells. A membrane receptor, a low-density lipoprotein receptor (LDLR), is controlled by cholesterol levels inside the cell and acts to remove LDL cholesterol from the blood circulation, which contributes to decreased plasma cholesterol levels (Powell and Kroon, 1994). Sangkitikomol et al. (2014) found that MOLE could decrease plasma cholesterol and lipid synthesis by suppressing HMG-CoAR, PPARα1, and PPARγ gene expression, thereby maintaining lipid homeostasis. This suggests that MOLE can help to make thigh muscle healthier by reducing its fat content.

The results of this study on pH values are consistent with those reported by Łukasiewicz et al. (2013), who found no significant differences in the ultimate pH (5.70-5.76) of broiler meat caused by plant additives. Nkukwana et al. (2015) also found no significant difference ($p > 0.05$) in breast meat pH measurements of broilers fed MOLM at 1%, 3%, or 5% of DM intake. The shear force values were significantly higher in chicken meat-fed MOLE than in the other group. This suggests that the MOLE may have made the meat tougher. However, it is important to note that the fat content of the meat was also significantly lower in the MOLE fed chickens. There is a relationship between fat content and tenderness in meat. Meat with a higher fat content is generally more tender than meat with a lower fat content. This is because fat

helps to lubricate the muscle fibers, making them easier to break down. The shear force values contrast with those reported by Egbu et al. (2022). They found that shear force values declined when MSE was administered through drinking water.

TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. MDA is a secondary product of lipid oxidation and is used as an indicator of oxidative stability (Jiang and Xiong, 2016). Divya et al. (2014) found that the TBA values were also lowest at the highest level of *Moringa oleifera* and the highest TBA level in the control group. This suggests that *Moringa oleifera* supplementation can inhibit lipid oxidation in chicken meat, which is a defence mechanism against the formation of excessive free radicals. Lipid peroxidation is a complex process that occurs in aerobic cells when molecular oxygen interacts with polyunsaturated fatty acids (Verma et al., 2009). This process can lead to meat spoilage, especially when the meat is exposed to oxygen, heat, and light. The presence of multiple double bonds in polyunsaturated fatty acids allows them to react with free radicals, which can damage cells and tissues. Cui et al. (2018) reported that the antioxidant properties of *Moringa oleifera* supplementation can help to scavenge free radicals and prevent lipid peroxidation. MOL is rich in various metals such as selenium (Se), manganese (Mn), copper (Cu), and zinc (Zn), which can play a vital role in the action of antioxidant enzymes. In addition, MOL also has a high concentration of natural antioxidant substances, including α-tocopherol, ascorbic acid, carotenoids, polysaccharides, flavonoids, saponins, phenolics, tannins, and proanthocyanidins. The results of this present study suggested that MOLE can be a beneficial addition to broiler chicken diets to improve meat quality with low fat and lipid oxidation.

CONCLUSIONS

Utilizing Moringa oleifera leaf extract in chicken diets can lead to positive outcomes, including improved chicken growth performance, enhanced immune response to the Newcastle disease vaccine, and certain meat quality attributes. Notably, the group receiving the highest level of MOLE supplementation (T4) demonstrated the most promising growth performance and exhibited the highest antibody titer, which could signify a more robust immune response. Hematological values and carcass traits showed relatively minor variations. However, our study highlights the promising potential of *Moringa oleifera* leaf extract as a dietary supplement, particularly in improving specific aspects of chicken production. Therefore, our findings have the potential to enhance sustainable poultry farming practices and foster the creation of healthier, more nutritious poultry products.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.M.; methodology, K.M., N.K., W.W., and N.C.; validation, K.M., W.K., P.S., and N.C.; data curation, K.M., N.K., and N.C.; formal analysis K.M. and N.C.; investigation, K.M. and N.C. project administration, K.M.; resources, K.M. and P.S.; writing original draft, K.M. and N.C.; writing-review and editing, K.M. and N.C.; All authors read and agreed with the final manuscript.

CONFLICT OF INTEREST

The authors declared no competing interests.

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