

The Effects of dietary level of mulberry leaf powder on growth performance, carcass yields, and some serum lipid levels in Pradu-Hang-Dam chickens during cool and rainy seasons

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Abstract

This study aimed to investigate the effects of using powdered mulberry leaves at different concentrations and during different seasons on the growth performance, carcass characteristics, and blood lipid levels in Pradu-Hang-Dam chickens. One-week-old chickens were divided into three groups: a control group (without mulberry leaf powder (MLP)), and two groups fed diets with 0.5% and 1.5% MLP, respectively. The results revealed that chickens in the control group and those supplemented with 1.5% MLP had the highest feed intake during the cool and rainy seasons, respectively ($P < 0.05$). The daily growth rate was highest in the control groups and 0.5% MLP during the cool and rainy seasons, respectively ($P < 0.05$). Feed intake and average daily gain were higher during the cool season than the rainy season for all mulberry leaf concentrations ($P < 0.05$). The group supplemented with 0.5% MLP had the lowest feed conversion ratio, especially significant in the rainy season ($P < 0.05$). The group supplemented with 1.5% MLP exhibited higher carcass characteristics than the control group during the rainy season ($P < 0.05$). During the cool season, the control group had higher carcass characteristics than the MLP-supplemented group, with overall values higher than the rainy season. The analysis of blood lipid metabolism showed that the triglyceride, cholesterol, and high-density lipoprotein levels in the group supplemented with 0.5% MLP during the cool season were the lowest. In conclusion, the cool season is suitable for raising Pradu-Hang-Dam chickens. Additionally, supplementing the diet with 0.5% MLP was sufficient to induce changes in growth performance, carcass characteristics, and blood parameters.

Keywords: blood lipid, carcass characteristic, growth performance, mulberry leaf power, Pradu-hang-dam chicken, season

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Introduction

Most of the area in Nan Province consists of mountains and highlands, resulting in limited agricultural activities in the region. For this reason, there has been encroachment into forested areas for agriculture, including the cultivation of various crops such as vegetables, field crops, maize, and a variety of fruits like lychee, longan, rambutan, and orange. Consequently, chemical substances have been used in agriculture, leading to contamination and pollution in the environment and affecting overall health. Meanwhile, animal husbandry including cattle, buffaloes, sheep, goats, pigs, and poultry like native chickens, egg-laying chickens, and ducks, also plays a vital role in this province. However, due to the high demand for meat, there is a need to import protein sources from other provinces because most animal farming in Nan Province is done on a small scale or backyard farming. Many farms lack of proper management in terms of housing, nutrition, and health. This results in lower quality and less efficient production. For example, there are approximately 55,384 meat chickens raised by 241 farmers, while there are 99,484 egg-laying chickens and 1,331 farmers engaged in egg production (ICT, 2021). Furthermore, the COVID-19 pandemic has caused economic challenges, with some individuals losing their sources of income and returning to agriculture. In this context, promoting chicken farming can be considered an alternative or supplementary livelihood option. Chicken farming has a short production cycle of about 3-4 months, requires relatively low investment, and doesn't demand extensive land use. Utilizing locally available agricultural materials or crops as poultry feed can significantly reduce production costs since animal feed typically accounts for 60-70% of total production costs (Lawrence *et al.*, 2008). Reducing these costs can lead to more profitable poultry farming ventures.

Chicken farming by farmers in Nan Province involves raising small-sized native chickens using a free-range approach. Since these chickens grow slowly, it is necessary to find supplementary food sources to enhance production efficiency. Mulberry leaves are an alternative that can be used as a supplemental food source for raising chickens. This kind of plant is scientifically known as *Morus alba* Linn, belonging to the Moraceae family. One acre of mulberry cultivation can yield approximately 5,000-7,500 kilograms of mulberry leaves and can be harvested five times a year. Currently, the Mulberry Center under the Patronage of Her Royal Highness Princess Maha Chakri Sirindhorn in Nan Province has been promoting mulberry cultivation for chicken farming in collaboration with various sectors, including the government, farmers, civil society, and private companies. These efforts have resulted in an agreement with Chulmaithai Co., Ltd. on purchasing agricultural products from farmers at fair prices so they can increase their income. This initiative has created a stable market for mulberry leaves, reduced the need for imports, helped mitigate deforestation and environmental degradation, and improved the overall quality of life for farmers. Moreover, it has allowed farmers to produce poultry in an environmentally friendly manner by reducing the

use of chemical substances in agriculture. Previous research studies reveal that mulberry leaves have been used as an alternative protein source in the feeding of various animals, including pigs, small and large ruminants, and poultry as well. These studies have shown that mulberry leaves are nutritionally valuable, with a protein content of approximately 16%, dry matter content of 25%, metabolizable energy content (ME) of 2,240 kcal/kg, fat content of 5.31%, and fiber content of 15.47% (DLD, 2020). It has been found that mulberry leaves can also reduce cholesterol levels in poultry, making them a healthy protein source. In summary, the utilization of mulberry leaves in chicken farming offers an environmentally sustainable and economically viable solution for farmers in Nan Province while also providing an alternative source of high-quality protein for consumers who prioritize health and nutrition. Additionally, this approach can help reduce production costs and enhance the overall livelihood of farmers.

Therefore, we hypothesize that supplementation with powdered mulberry leaves at suitable concentrations can lead to excellent growth rates, good carcass quality, and maintenance of serum chemical substance levels within optimal ranges for Pradu-Hang-Dam chickens, even when raised during non-ideal seasons in tropical areas.

Materials and Methods

Animal ethics statement: The present study was conducted from September 2022 to June 2023 on a farm located at the School of Agricultural Resources, Chulalongkorn University, Nan Province at 14°31'59.99" N, 100°55'0.01" E, Thailand. All procedures in this study were consistent with the ethical standards issued by the National Research Council and were approved by the Ethics Committee for Human and/or Animal Experimentation in meeting No. IACUC004, the School of Agricultural Resources, Chulalongkorn University, Thailand.

Animal and study design: This study utilized a total of 240 chickens and employed a Completely Randomized Design (CRD) experiment to compare the effects of different diets on growth performance, carcass quality, and blood chemical levels in the Pradu-Hang-Dam breed. The chickens were divided into three experimental groups, each further divided into two replicates. In each replicate, there were 20 chickens raised in an open-house system with free range for ten weeks. The experiments were conducted in two periods: from September to December 2022 and from March to June 2023.

Preparation of mulberry leaf powder: Mulberry leaves were collected from the agricultural farm in Mae Charim District, Nan Province, Thailand, at 18°42'41.3" N, 101°06'44.0" E. Harvested parts were measured from the tip of the leaves, at about 30 cm in length. They were washed thoroughly with water until clean and then used only the leaves. After that, all the leaves were placed in a hot-air oven at 60°C for one hour. Next, grind the mulberry leaves into fine powder using a blender and sift through a strainer to achieve a

particle size of approximately 1 mm. Store the powder in a moisture-free container at room temperature to be ready to use.

Diet and housing conditions: For experimental groups, 240 one-week-old Pradu-Hang-Dam chickens of mixed genders were randomly selected and raised in open-type poultry houses. The experiment was conducted twice for each group and was divided into two testing periods: September to December 2022 and March to June 2023. The chickens were raised in pens of the same size, 4 square meters each. They were fed with a diet containing sufficient protein, energy, and nutrients according to the chickens' requirements (Nick, 1994), supplemented with mulberry leaf powder. The grouping and diet details are as follows: Experimental Group 1: Pradu-Hang-Dam chickens raised on a basic diet without mulberry leaf powder (0%), serving as the control group; Experimental Group 2: Pradu-Hang-Dam chickens raised with a basic diet supplemented with 0.5% mulberry leaf powder; and Experimental Group 3: Pradu-Hang-Dam chickens raised with a basic diet supplemented with 1.5% mulberry leaf powder. Chickens were fed twice a day, at 07:00–08:00 a.m. and 04:00–05:00 p.m., with the amount of food provided at each feeding session weighed. The remaining food was weighed daily before the morning feeding, and additional food was given if the remaining amount was less than 10% of the total food provided. Clean water was always sufficiently provided to the chickens throughout the 10-week raising.

Serum biochemical determination: Randomly select three chickens from each experimental group and conduct a fasting period for 2–4 hours on the last day of the experiment to collect blood samples by wing vein puncture, taking 2 milliliters from each chicken, repeated twice. Afterward, the blood samples were centrifuged using a controlled centrifuge at 4°C and a speed of 3,000 rpm for 10 minutes to separate the serum. The serum was then stored at –20°C until further laboratory analysis. The analysis aims to determine the following chemical substances: cholesterol and triglycerides using the enzymatic (OXI/PER) method, and high-density lipoprotein (HDL) using the enzymatic (Detergent direct/Precipitation) method.

Carcass evaluation: At the end of the study, the chickens were fasted for 12 hours, and two chickens per replicate were randomly selected for carcass evaluation. The final live weight of a chicken was taken prior to slaughter by a ventral neck cut with a sharp knife to bleed. Following thorough bleeding, those chickens were manually de-feathered by scalding in hot water. De-feathered chickens were weighed, labeled, and eviscerated. Each carcass was cut into parts and parameters were calculated as described in World's Poultry Science Journal (Hahn and Spindler, 2002), then following data were recorded: 1) the live weight before slaughter, 2) the weight of the body after slaughter and feather removal, 3) the weight of the chicken body after feather removal and removal of internal organs, 4) the weight of internal organs such

as the heart, liver, gizzard, and spleen, 5) the weight of the wings, thighs, and drumsticks, and 6) the weight of the breast meat and remaining parts of the chicken. After that, calculated the percentage of carcass and the percentage of different carcass parts using the recorded values as follows: 1) the weight of the carcass after feather removal compared to the live weight, 2) the weight of internal organs compared to the weight of the carcass after feather removal, 3) the weight of breast meat, thighs, drumsticks, wings, and the skeleton compared to the weight of the carcass after bleeding, and 4) the weight of fat in the abdominal cavity and calculate it as a percentage of the live weight of the chicken.

Growth performance: Feed intake was recorded daily, while live body weight and total feed consumption in each replicate were recorded every two weeks to determine feed intake (FI), average daily growth (ADG), and feed conversion ratio (FCR).

Statistical analysis: Data are expressed as sample mean $\pm$ standard error (SEM). Differences in values were considered to exist at a *P*-value of 0.05. Statistical analysis of the index was carried out according to the replication of each group. The differences among the groups were analyzed by One-Way Analysis of Variance (ANOVA) followed by Tukey's test using the IBM SPSS Statistic Version 22.0 (SPSS; IBM, Armonk, NY, USA).

Results

Effects of supplementing mulberry leaf powder on growth performance: According to different experimental groups and different seasons, Pradu-Hang-Dam chickens were raised for a period of 10 weeks with different concentrations of mulberry leaf powder supplementation (Table 1).

Feed intake: During the cool season, the feed intake of the groups raised with a basic diet supplemented with mulberry leaf powder at 0% (control group), 0.5%, and 1.5% were 6,346.83 $\pm$ 162.92, 5,734.05 $\pm$ 62.11, and 5,805.93 $\pm$ 67.26 (g/chicken), respectively. The FI of the group raised with a basic diet was significantly greater than the groups with 0.5% and 1.5% mulberry leaf powder mixed, with statistical significance (*P* < 0.05). The FI of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% in the rainy season were 2,647.39 $\pm$ 31.62, 2,827.17 $\pm$ 54.90, and 2,924.81 $\pm$ 37 (g/chicken), respectively. The FI of the groups raised with a basic diet supplemented with 0.5% and 1.5% mulberry leaf powder was significantly greater than the control group, with statistical significance (*P* < 0.05). The comparison of FI between groups with the same concentration of mulberry leaf powder but in different seasons revealed significantly higher values in the cool season than the rainy season in all concentration groups (*P* < 0.05).

Average daily growth: For the cool season, the average daily growth of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%,

and 1.5% were 24.03 ± 0.12 , 22.69 ± 0.10 , and 21.72 ± 0.07 (g/chicken/day), respectively. The ADG of the control group was significantly greater than the groups with 0.5% and 1.5% mulberry leaf powder mixed, with statistical significance ($P < 0.05$). For the rainy season, the ADG of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 9.77 ± 0.06 , 11.21 ± 0.20 , and 10.83 ± 0.14 (g/chicken/day), respectively. The ADG of the groups raised with a basic diet supplemented with 0.5% and 1.5% mulberry leaf powder was significantly greater than the control group, with statistical significance ($P < 0.05$). The comparison of ADG between groups with the same concentration of mulberry leaf powder but in different seasons revealed significantly higher values in the cool season than the rainy season in all concentration groups ($P < 0.05$).

Feed conversion ratio: For the cool season, the feed conversion ratios of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 3.67 ± 0.11 , 3.51 ± 0.05 , and 3.71 ± 0.04 , respectively. For the rainy season, the FCR of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 3.61 ± 0.03 , 3.36 ± 0.03 , and 3.60 ± 0.04 , respectively. The FCR of the group raised with a basic diet supplemented with 0.5% mulberry leaf powder was significantly lower than the groups with 0% and 1.5% mulberry leaf powder mixed, with statistical significance ($P < 0.05$). The analysis of FCR among groups with identical concentrations of mulberry leaf powder but exposed to different seasons showed no statistically significant differences.

Table 1 Effects of supplementing mulberry leaf powder on the growth performance of Pradu-Hang-Dam chickens.

Parameters	Level of Mulberry Leaf Powder (%)					
	Cool season			Rainy season		
	0	0.5	1.5	0	0.5	1.5
FI (g/chicken)	6346.83 ± 162.92^a	5734.05 ± 62.11	5805.93 ± 67.26	2647.39 ± 31.62	2827.17 ± 54.90^a	2924.81 ± 37.01^a
ADG (g/chicken/day)	24.03 ± 0.12^a	22.69 ± 0.10^b	21.72 ± 0.07	9.77 ± 0.06	11.21 ± 0.20^a	10.83 ± 0.14^a
FCR (feed: gain)	3.67 ± 0.11	3.51 ± 0.05	3.71 ± 0.04	3.61 ± 0.03^a	3.36 ± 0.03	3.60 ± 0.04^a

^{a, b} = Means in the same row with different superscripts differ significantly ($P < 0.05$)

Effects of supplementing mulberry leaf powder on carcass characteristics: The data for the carcass characteristics are presented in Table 2.

Live weight: For the cool season, the average live weights of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were $1,350.00 \pm 38.73$, $1,275.00 \pm 37.08$, and $1,225.00 \pm 37.08$ g, respectively. For the rainy season, the average live weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was higher than that of the groups with 0.5% and 0%, with values of 982.50 ± 35.83 , 912.50 ± 11.57 , and 852.50 ± 14.62 g, respectively. The live weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was significantly greater than the control group, with statistical significance ($P < 0.05$). According to the comparison of the live weights between groups with the same concentration of mulberry leaf powder but in different seasons, the average live weights in the cool season were significantly higher than those of the rainy season in all concentration groups ($P < 0.05$).

De-feathered weight: For the cool season, the average de-feathered weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were $1,125.00 \pm 37.08$, $1,075.00 \pm 37.08$, and $1,050.00 \pm 38.73$ g, respectively. For the rainy season, the average de-feathered body weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was higher than those of 0.5% and 0%, at 952.50 ± 17.43 , 885.00 ± 15.00 , and 795.00 ± 18.03 g, respectively ($P < 0.05$). It was found that the average de-feathered weights in all concentration groups in the cool season were significantly higher than those of the rainy season ($P < 0.05$).

Carcass weight: For the cool season, the carcass weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 952.20 ± 38.51 , 910.50 ± 33.91 , and 884.00 ± 30.26 g, respectively. For the rainy season, the carcass weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was higher than those of the groups with 0.5% and 0%, with values of 720.00 ± 21.91 , 682.50 ± 18.54 , and 637.50 ± 15.61 g, respectively. The carcass weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was significantly greater than the control group, with statistical significance ($P < 0.05$). According to the comparison of the carcass weights between groups with the same concentration of mulberry leaf powder but in different seasons, it was found that the carcass weights of the cool season were significantly higher than those of the rainy season in all concentration groups ($P < 0.05$).

Dressing percentage (%): For the cool season, the dressing percentages of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 70.49 ± 1.40 , 62.36 ± 7.95 and 72.19 ± 1.62 , respectively. For the rainy season, the dressing percentages of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were $74.83 \pm 1.14\%$, $76.64 \pm 1.62\%$, and $73.53 \pm 2.50\%$, respectively. There was no significant difference in dressing percentage among groups with the same concentration of mulberry leaf powder in different seasons.

Breast weight: For the cool season, the breast weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 171.00 ± 6.51 , 157.75 ± 4.10 , and 156.00 ± 7.28 g,

respectively. For the rainy season, the breast weights of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 85.00 ± 4.97 , 94.25 ± 8.42 , and 103.00 ± 1.67 g, respectively. When comparing the breast weights between groups with the same concentration of mulberry leaf powder but in different seasons, it was found that the breast weights of the cool season were significantly higher than those of the rainy season in all concentration groups ($P < 0.05$).

Thigh and drumstick weight: For the cool season, thigh and drumstick weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 295.00 ± 7.77 , 287.00 ± 11.38 , and 282.25 ± 10.12 g, respectively. For the rainy season, the thigh and drumstick weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was higher than those of the groups with 0.5% and 0%, with values of 202.25 ± 3.87 , 190.50 ± 4.92 , and 176.50 ± 5.45 g, respectively. The thigh and drumstick weight of the group raised with a basic diet supplemented with 1.5% mulberry leaf powder was significantly greater than the control group, with statistical significance ($P < 0.05$). When comparing these weights between groups with the same concentration of mulberry leaf powder but in different seasons, it was found that thigh and drumstick weights of the cool season were significantly higher than those of the rainy season in all concentration groups ($P < 0.05$).

Wing weight: For the cool season, wing weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 117.00 ± 3.21 , 117.75 ± 4.60 , and 118.50 ± 6.20 g, respectively. For the rainy season, the wing weights of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 78.00 ± 2.39 , 87.00 ± 0.84 , and 87.00 ± 1.14 g, respectively. The wing weights of the groups raised with a basic diet supplemented with 1.5% and 0.5% mulberry leaf powder were significantly greater than the control group, with statistical significance ($P < 0.05$). The comparison of these weights between groups with the same concentration of mulberry leaf powder but in different seasons revealed significantly higher values in the cool season than the rainy season in all concentration groups ($P < 0.05$).

Visceral organ weight: For the cool season, visceral organ weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 172.50 ± 1.75 , 164.50 ± 6.31 , and 166.00 ± 10.77 g, respectively. For the rainy season, the visceral organ weights of the groups raised with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 157.50 ± 3.71 , 202.50 ± 10.19 , and 232.50 ± 10.19 g, respectively. The visceral organ weights of the groups raised with a basic diet supplemented with 1.5% and 0.5% mulberry leaf powder were significantly greater than the control group, with statistical significance ($P < 0.05$). The comparison of visceral organ weights between groups with the same concentration of mulberry leaf powder

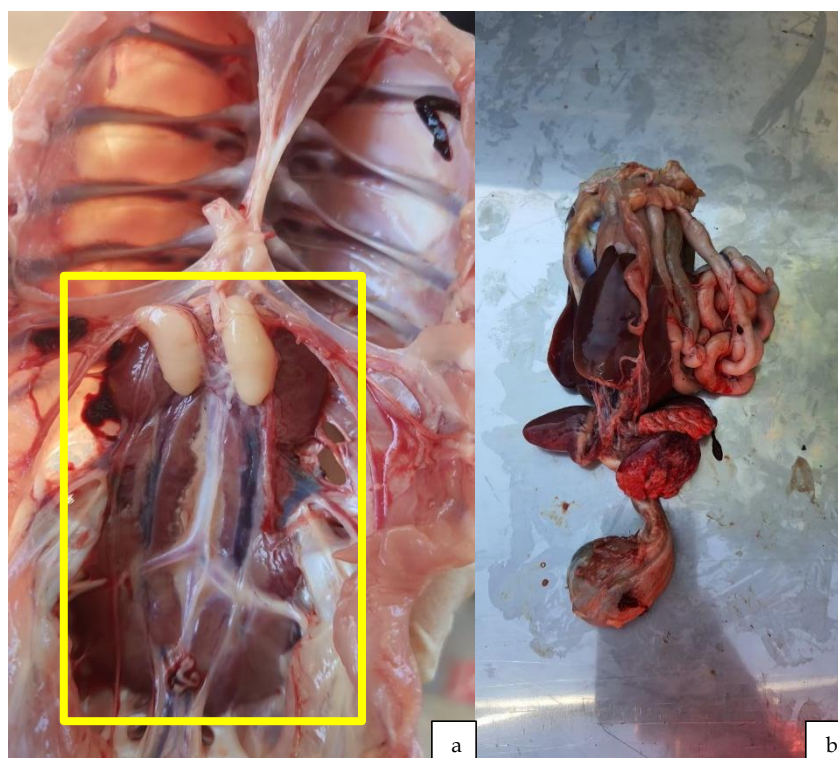
but in different seasons revealed significantly higher values in the cool season than the rainy season in all concentration groups ($P < 0.05$).

Abdominal fat pad weight: For the cool season, the abdominal fat pad weights of the groups with a basic diet supplemented with mulberry leaf powder at 0%, 0.5%, and 1.5% were 2.13 ± 0.83 , 2.88 ± 0.95 , and 4.00 ± 0.84 g, respectively. For the rainy season, the abdominal fat pad weights in all experiment groups were equal to zero (Fig. 1). When comparing the abdominal fat pad weights between groups with the same concentration of mulberry leaf powder. Still, in different seasons, it was found that the values of the cool season were significantly higher than those of the rainy season in all concentration groups ($P < 0.05$).

Table 1 Mean weight of different carcass characteristics of Pradu-Hang-Dam chickens in different concentrations of mulberry leaf powder supplement.

Parameters	Cool season			Rainy season		
	Level of Mulberry Leaf Powder (%)					
	0	0.5	1.5	0	0.5	1.5
Live weight (g)	1350.00±38.73	1275.00±37.08	1225.00±37.08	852.50±14.62 ^b	912.50±11.57	982.50±35.83 ^a
De-feathered weight (g)	1125.00±37.08	1075.00±37.08	1050.00±38.73	795.00±18.03 ^b	885.00±15.00	952.50±17.43 ^a
Carcass weight (g)	952.50±38.51	910.50±33.91	884.00±30.26	637.50±15.61 ^b	682.50±18.54	720.00±21.91 ^a
Dressing percentage (%)	70.49±1.40	62.36±7.95	72.19±1.62	74.83±1.14	76.64±4.46	73.53±2.50
Breast weight (g)	171.00±6.51	157.75±4.10	156.00±7.28	85.00±4.97	94.25±8.42	103.00±1.67
Thigh & drumstick weight (g)	295.00±7.77	287.00±11.38	282.25±10.12	176.50±5.45 ^b	190.50±4.92	202.25±3.84 ^a
Wing weight (g)	117.00±3.21	117.75±4.60	118.50±6.20	78.00±2.39 ^b	87.00±0.84	87.00±1.14 ^a
Visceral organ weight (g)	172.50±1.75	164.50±6.31	166.00±10.77	157.50±3.71 ^b	202.50±10.19	232.50±10.19 ^a
Abdominal fat pad (g)	2.13±0.83	2.88±0.95	4.00±0.84	0	0	0

^{a, b} = Means in the same row with different superscripts differ significantly ($P < 0.05$)

**Figure 1** No fat deposited in the abdominal area (a) including no fat surrounding the visceral organs such as the gizzard, spleen, liver, and heart (b).**Analyzing the levels of chemical substances in serum:**

The effects of supplementing mulberry leaf powder in the diet on lipid metabolism are shown in Table 3.

Cool season: The triglycerides levels in the group supplemented with 1.5% mulberry leaf powder were the highest, followed by the groups supplemented with 0% and 0.5% mulberry leaf powder, with values of 38.83 ± 4.42 , 38.00 ± 3.27 , and 32.50 ± 4.05 (mg/dl), respectively. The cholesterol levels in the group supplemented with 1.5% mulberry leaf powder were the highest, followed by the groups supplemented with 0% and 0.5% mulberry leaf powder, with values of 119.33 ± 6.82 , 116.00 ± 3.55 and 97.33 ± 6.71 (mg/dl), respectively ($P < 0.05$). The HDL levels in the group supplemented with 1.5% mulberry leaf powder were the highest, followed by the groups supplemented

with 0% and 0.5% mulberry leaf powder, with values of 45.17 ± 3.02 , 42.33 ± 2.22 and 39.00 ± 1.86 (mg/dl), respectively ($P < 0.05$).

Rainy season: The triglycerides levels in the group supplemented with 1.5% mulberry leaf powder were the highest, followed by the groups supplemented with 0.5% and 0% mulberry leaf powder, with values of 64.17 ± 10.75 , 42.83 ± 2.86 , and 33.50 ± 4.96 (mg/dl), respectively ($P < 0.05$). The cholesterol levels of the group supplemented with 0.5% mulberry leaf powder were the highest, followed by the groups supplemented with 1.5% and 0% mulberry leaf powder, with values of 109.50 ± 6.65 , 109.00 ± 13.95 and 90.00 ± 7.59 (mg/dl), respectively. The FHDl levels in the group supplemented with 0.5% mulberry leaf powder were the highest, followed by the groups

supplemented with 1.5% and 0% mulberry leaf powder, with values of 39.00 ± 2.25 , 38.83 ± 3.31 , and 34.00 ± 1.63 (mg/dl), respectively.

When comparing the lipid metabolism values between seasons, it was found that the triglyceride levels of the groups supplemented with 0.5% and 1.5% mulberry leaf powder in the rainy season were

significantly higher than those in the cool season. On the other hand, the cholesterol and HDL levels of the group supplemented with 0% mulberry leaf powder in the rainy season were significantly lower than those in the cool season, according to statistical significance ($P < 0.05$).

Table 2 The effects of supplementing mulberry leaf powder in the diet on blood parameters.

Parameters	Cool season			Rainy season		
	Level of Mulberry Leaf Powder (%)					
	0	0.5	1.5	0	0.5	1.5
Triglycerides (mg/dl)	38.00±3.27	32.50±4.05	38.83±4.42	33.50±4.96 ^a	42.83±2.86	64.17±10.75 ^b
Cholesterol (mg/dl)	116.00±3.55 ^b	97.33±6.71 ^a	119.33±6.82 ^b	90.00±7.59	109.50±9.65	109.00±13.95
HDL (mg/dl)	42.33±2.22	39.00±1.86	45.17±3.02	34.00±1.63	39.00±2.25	38.83±3.31

^{a, b} = Means in the same row with different superscripts differ significantly ($P < 0.05$)

HDL = High-Density Lipoprotein

Discussion

This study was conducted during the rainy season and the cool season in Nan Province, focusing on investigating the effects of supplementing mulberry leaf powder in the diet for Pradu-Hang-Dam chickens at different concentrations in different seasons. Therefore, the independent variables in this study are the concentrations of mulberry leaf powder and the seasons of feeding, while the dependent variables include growth performance, carcass quality, and blood lipid metabolism.

From the experiment, it was found that in the cool season, Pradu-Hang-Dam chickens of the group with 0% mulberry leaf powder had the highest feed intake, followed by the 1.5% and 0.5% groups, respectively. Conversely, in the rainy season, Pradu-Hang-Dam chickens with 1.5% mulberry leaf powder had the highest feed intake, followed by the 0.5% and 0% groups, respectively. Regarding the daily growth rate, in the cool season, chickens of the group with 0% mulberry leaf powder had the highest rate, followed by the 0.5% and 1.5% groups, respectively. In contrast, in the rainy season, chickens of the group with 0.5% mulberry leaf powder had the highest rate, followed by the 1.5% and 0% groups, respectively. When comparing between seasons, it was found that the feed intake and average daily gain values were higher in the cool season than the rainy season for all concentrations of mulberry leaf powder, with statistical significance. The feed conversion ratio of the group with 0.5% mulberry leaf powder was the lowest in both the rainy season and the cool season, especially significant in the rainy season. However, when comparing between seasons, there was no significant difference between groups with the same concentration of mulberry leaf powder.

For the rainy season, the live weight, de-feathered weight, carcass weight, visceral organ weight, thigh and drumstick weight, and breast weight of the 1.5% mulberry leaf powder group were the highest, followed by the 0.5% and 0% groups, respectively. The abdominal fat pad weight remained consistent across all experimental groups. The wing weights of the 1.5% and 0.5% mulberry leaf powder groups were equal but higher than that of the 0% group, with the 1.5% mulberry leaf powder group showing significant differences from the 0% mulberry leaf powder group.

For the cool season, the live weight, de-feathered weight, carcass weight, thigh and drumstick weight, and breast weight of the 0% mulberry leaf powder group were the highest, followed by those of the 0.5% and 1.5% groups, respectively. The visceral organ weight of the 0% mulberry leaf powder group was the highest, followed by those of the 1.5% and 0.5% groups, respectively. The abdominal fat pad weight and wing weight of the 1.5% mulberry leaf powder group were the highest, followed by those of the 0.5% and 0% groups, respectively, but without statistical significance. Overall, in the cool season, values were higher compared to those in the rainy season. However, dressing percentages (%) did not show significant differences among concentrations within the same season. Nonetheless, values were higher in the rainy season for all concentrations.

Blood lipid metabolism, including triglycerides, cholesterol, and HDL of the 0.5% mulberry leaf powder group in the cool season, had the lowest values and increased in the 0% and 1.5% groups, respectively. The cholesterol levels of the 0.5% mulberry leaf powder group were significantly lower than those of the 0% and 1.5% groups. In the rainy season, triglyceride levels of the 0% mulberry leaf powder group had the lowest value and increased in the 0.5% and 1.5% groups, respectively. The triglyceride levels of the 0% mulberry leaf powder group were significantly lower than the 1.5% group. Cholesterol and HDL levels of the 0% mulberry leaf powder group in the rainy season had the lowest values and increased in the 1.5% and 0.5% groups, respectively. When comparing between different seasons, triglyceride levels of the 0% mulberry leaf powder group were higher in the cool season compared to the rainy season. For the 0.5% and 1.5% mulberry leaf powder groups, triglyceride levels were higher in the rainy season compared to the cool season, with significant differences observed only in the 0.5% and 1.5% groups. Cholesterol and HDL levels of the 0% and 1.5% mulberry leaf powder groups were higher in the cool season compared to the rainy season. For the 0.5% mulberry leaf powder group, cholesterol and HDL levels were higher in the rainy season compared to the cool season, with significant differences observed only in the 0% mulberry leaf powder group.

The study conducted within two different seasons yielded the research findings as mentioned above. These findings align with previous studies indicating that high ambient temperatures result in decreased feed intake, metabolic disturbances, and increased oxidative stress (Kpomasse *et al.*, 2021). Chickens are sensitive to high temperatures and can experience heat stress, especially in hot climates or during heatwaves. Heat stress can lead to decreased appetite as chickens try to conserve energy and reduce heat production by eating less. When temperatures rise, chickens need to expend more energy to regulate their body temperature through panting and other cooling mechanisms. This increased metabolic rate can result in chickens feeling less hungry because their energy is being diverted to thermoregulation rather than digestion. High temperatures can negatively impact the efficiency of the digestive system in chickens. Digestion requires energy, and in hot weather, chickens may prefer to conserve energy for cooling rather than digesting food. Additionally, high temperatures can alter the microbial balance in the chicken's digestive tract, affecting the breakdown and absorption of nutrients from feed. Chickens need to drink more water to stay hydrated in hot weather. As a result, they may fill up on water, leaving less room in their stomachs for feed. High temperatures can cause feed to spoil more quickly, leading to decreased palatability and reduced consumption by chickens. To mitigate the effects of high temperatures on feed intake, poultry farmers often employ strategies such as providing adequate ventilation, shade, and access to cool, fresh water. Additionally, formulating feeds with ingredients that are more resistant to spoilage and ensuring proper storage practices can help maintain feed quality and encourage consumption, even in hot weather conditions. Hence, during the cool season characterized by lower temperatures compared to the rainy season, chickens exhibit increased food consumption, aligning with the findings of this study.

In addition, high ambient temperatures also impact the metabolism system of chickens, including the metabolism of carbohydrates, lipids, and proteins, as shown in the following details. 1) The reduced growth and increased fat deposition observed in broiler chickens (Park and Kim, 2017) under warm tropical conditions suggest alterations in glucose utilization and its regulation by insulin (Tesseraud and Temim, 1999). Additionally, reports indicate that chickens exposed to hot conditions may develop insulin resistance, leading to difficulties in glucose uptake by muscle cells (Bray and York, 1979; Quinteiro-Filho *et al.*, 2012). This insulin resistance may reduce tissue sensitivity to insulin and impair insulin secretion. In the case of insulin resistance, where cells become less responsive to insulin's actions, the normal regulatory effects on lipid metabolism can be disrupted. When tissues, especially muscle cells, develop insulin resistance, they may struggle to uptake glucose. Consequently, the body may increase insulin production to compensate, potentially contributing to lipid metabolism imbalance. 2) Prolonged exposure to heat stress also alters the composition of fatty acids in fatty tissues, such as abdominal fat, by elevating the levels of saturated fatty acids. Elevated fat content,

particularly at subcutaneous levels, diminishes the quality of broiler chicken carcasses (Tesseraud and Temim, 1999). 3) Under hot conditions, protein utilization, and efficiency decrease in broiler chickens, indicating alterations in bird metabolism (Tesseraud *et al.*, 1999). Reduced protein deposition may be associated with decreased capacities of protein synthesis (Temim *et al.*, 2000), as well as altered insulin signaling in muscles (Boussaid-Om Ezzine *et al.*, 2010). Nutritional strategies aimed at alleviating the effects of heat stress, such as reducing crude protein levels (Attia *et al.*, 2020^a; Attia *et al.*, 2020^b; Awad *et al.*, 2014; Cheng *et al.*, 1999), have resulted in poor performance of broiler chickens in tropical environments (Awad *et al.*, 2020). Furthermore, heat stress also increases oxidative stress levels, wherein the imbalance of body redox because of heat stress is one of the most significant disturbances leading to decreased broiler performance (Quinteiro-Filho *et al.*, 2012; Al-Zghoul *et al.*, 2019; Hu *et al.*, 2019). The unexpected rise in reactive oxygen species levels causes protein denaturation, lipid peroxidation, and deoxyribonucleic acid (DNA) damage (Ighodaro and Akinloye, 2017).

Mulberry leaves, containing flavonoids classified as nutraceuticals, possess antioxidant properties. They are considered a high-quality forage plant resource due to their high crude protein content (22%–29.8%), balanced amino acid composition, vitamins, and other bioactive substances such as flavonoids, polysaccharides, superoxide dismutase (SOD), sitosterol, isoquercitrin, α -amino acids, and 1-deoxynojirimycin (Moeser *et al.*, 2017; Xiang *et al.*, 2020). According to the use of mulberry leaf powder as a supplement in a diet with varying concentrations in the cool season, it was found that chickens in the 0% mulberry leaf powder group consumed more than those in the 0.5% and 1.5% groups, with significant effects on ADG higher than the 0.5% and 1.5% groups, respectively, in significance between all pairs. In the rainy season, chickens in the 1.5% and 0.5% mulberry leaf powder groups consumed more than those in the 0% group, affecting ADG significantly. This indicates that mulberry leaves, with antioxidant properties, enable chickens to consume more under unfavorable conditions (So-In and Sunthamala, 2022). Additionally, when analyzing the FCR, the 0.5% group had the lowest values in both seasons.

In the cool season, carcass characteristics such as live weight, de-feathered weight, carcass weight, thigh and drumstick weight, wing weight, and visceral organ weight showed no significant differences. However, in the rainy season, the 1.5% mulberry leaf powder group exhibited higher values than the 0% group, with statistical significance, while the 0.5% group did not show significance. This relationship corresponds with FI and ADG. However, the breast weight and abdominal fat pad weight in each season with the same concentrations of mulberry leaf powder mixed in the diet had no significant effects. On the other hand, when comparing between different seasons, the cool season had higher values than the rainy season. As for dressing percentages (%), there were no significant differences within the same season's concentrations, but values were higher in the rainy season compared to those in the cool season for

all concentrations. Thus, mulberry leaf powder affects carcass quality, consistent with previous studies suggesting that mulberry leaf extract reduces oxidative stress, activates antioxidant enzyme activities, and enhances broilers' growth performance under stress conditions (So-In and Sunthamala, 2022). This implies that a 0.5% concentration of mulberry leaves in the diet is beneficial for broilers' growth performance and carcass quality in both seasons. Incorporating mulberry leaves into the diet can be effective during unfavorable environmental conditions.

From the triglycerides levels of the 0.5% and 1.5% mulberry leaf powder groups of chickens, it was found that these levels were significantly lower in the cool season compared to the rainy season. This fluctuation correlates with the amount of food consumed, unlike the 0% mulberry leaf powder group, which had higher triglyceride levels in the cool season compared to the rainy season, based on food intake. In the cool season, the cholesterol levels of the 0.5% mulberry leaf powder group were the lowest compared to other groups and were also lower than those of the rainy season. The HDL levels of the 0.5% and 1.5% mulberry leaf powder groups were the highest in the cool season and the rainy season, respectively. As for the 0% mulberry leaf powder group, cholesterol and HDL levels were higher in the cool season compared to the rainy season, significantly influenced by food intake. These findings resulted from supplementation with mulberry leaves, which contain anthocyanins, a component known for reducing blood fat levels. This aligns with previous studies that found flavonoids, also present in mulberry leaf extracts, act as antioxidants, delaying or acting as oxidation inhibitors. This helps inhibit the chain reactions of free radicals, thereby combating oxidative stress, reducing cell damage, lowering blood sugar, mitigating symptoms of diabetes, and reducing blood cholesterol levels. The study found that the levels of triacylglycerol, total cholesterol, and low-density lipoprotein cholesterol decreased while the ratio of high-density lipoprotein cholesterol to triacylglycerol and high-density lipoprotein cholesterol to low-density lipoprotein cholesterol increased.

In conclusion, higher temperature and humidity in the rainy season reduce chicken food intake, impacting metabolism and increasing oxidative stress. This results in slower growth and lower carcass quality than the cool season. Supplementing with 0.5% mulberry leaf powder also lowers triglycerides and cholesterol but increases HDL levels. Thus, feeding Pradu-Hang-Dam chicken 0.5% mulberry leaf powder improves their growth, carcass quality, and blood biochemistry.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

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