**Evaluation of mango saponin in broilers: effects on growth performance, carcass characteristics, meat quality and plasma biochemical indices**

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**Objective:** The objective of the present study was to determine whether mango saponin (MS) could be used as a feed additive in broiler chicks by evaluating growth performance, carcass characteristics, meat quality, and plasma biochemical indices.

**Methods:** A total of 216 1-d-old Arbor Acres male broiler chicks were randomly assigned into three dietary treatments supplemented with 0 (control), 0.14% (MS 0.14%), or 0.28% (MS 0.28%) MS. Each treatment had six replicates (cages) with 12 chicks each. The feeding trial lasted for six weeks.

**Results:** Compared with the control, dietary supplemented with 0.14% or 0.28% MS increased average daily weight gain of chicks in the grower (22 to 42 d) and the whole (1 to 42 d) phases, and the final body weight of chicks on d 42 was higher in MS supplemented groups (p<0.05). Lower L<sub>45 min</sub>* (lightness) and L<sub>24 h</sub>* values, lower b<sub>24 h</sub>* (yellowness) value, and higher a<sub>45 min</sub>* (redness) and a<sub>24 h</sub>* values of the breast muscle were observed in chicks fed with 0.28% MS on d 42 (p<0.05). The total antioxidant capacity in plasma increased in MS 0.14% group on d 21 (p<0.001). Lower contents of plasma total cholesterol and triglyceride were observed in chicks fed with 0.28% MS on d 21 and d 42, whereas the group supplemented with 0.14% MS only decreased plasma triglyceride content on d 21 (p<0.05). The glucose content in plasma decreased in MS 0.28% group on d 42 (p<0.001).

**Conclusion:** Overall, MS could be used as a feed additive in broiler chicks, and the supplemental level of 0.28% MS in diet could improve growth performance, meat quality, and plasma lipid metabolism in broiler chicks.

**Keywords:** Mango Saponin; Growth Performance; Meat Quality; Plasma Biochemical Index; Broiler Chick

**INTRODUCTION**

The phytochemical plants and extracts are nowadays utilized as new feed additives to replace feed antibiotics in animal feeds [1,2]. Mango tree (*Mangifera indica* L.), which is known as an important cosmopolitan fruit species worldwide, grows in almost all ecological zones of the tropical countries of the world [3]. It is a typical multipurpose tree for its industrial and medicinal applications, which has significant economic values. Mango kernels and peels can be used in animal diets as sources of starch or protein [4,5]. Mango leaves have high content of phenolic compounds containing mangiferin, flavonoids, benzophenone, and gallotannins [6]. It has been reported that the mango leave extract is rich in potent antioxidant phenolic compounds [7] and has a high antioxidant activity, which is higher than that of β-carotene [8]. Moreover, the mango leave extract has been demonstrated to have analgesic, anti-diarrheal, anti-inflammatory, antimicrobial, and antifungal activities, as well as hypoglycemic effects in rats [9].
Mango saponin (MS) is a mixture extracted from mango leaves with alcohol as the extraction solvent. It is a phenolic compound containing flavonoids and mangiferin. Mangiferin is a potent antioxidant with strong radical scavenging activity and has high ability to chelate metals [10,11]. It was also reported that mangiferin could inhibit lipid oxidation in rats [11,12]. In addition, flavonoids in plants were reported to act as antioxidants and exhibited health effects in rats [13,14]. Considering the multiple effects of mango leaves extract, mangiferin, and flavonoids on rats, we hypothesized that dietary MS supplementation would be beneficial for broilers.

The MS can be widely obtained by conventional extractions from mango leaves. However, few studies have been carried out on the MS application in poultry. Therefore, the objective of the present study was to determine whether MS could be used as a feed additive in broiler chicks by evaluating growth performance, carcass characteristics, meat quality, plasma antioxidant, and biochemical indices.

**MATERIALS AND METHODS**

**Preparation of mango saponin**

Dry mango leaves were ground to powder and sifted out via a griddle of 40 meshes. The powder was then extracted by ultrasound reflux for 2 hours at 70°C with 50% ethanol as solvent. The ratio of ethanol volume (mL) and sample weight (g) was 40:1. Then, the extract solution was concentrated at a temperature of 50°C using a rotary evaporator (R210, Buchi, Bern, Switzerland) [15]. The mango leave extract (MS) was stored at –70°C until used. Flavonoids and mangiferin contents in MS were analyzed using a spectrophotometer (UV-2700, Shimadzu, Japan) with rutin and mangiferin as the standards, respectively. MS contained 13.72% mangiferin and 18.62% other flavonoids.

**Birds and experimental design**

The experimental protocols and procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences. The experimental design is completely randomized design, and a total of 216 1-d-old Arbor Acres male broiler chicks were housed into 18 wire cages with 12 chicks each. Three experimental diets were randomly assigned to chick cages with 6 replicates per diet. The chicken house was kept at a 24-hour constant-light program. The temperature in the chicken house was set at 33°C for the first three days, and then the temperature dropped by 2°C each successive week until it settled at 24°C. Diets and water were supplied ad libitum in pellet form and by nipple drinkers, respectively. The composition and nutrient levels of the basal diet is shown in Table 1. All chicks were raised in accordance with the regulations of the Arbor Acres Broiler Commercial Management Guide.

**Slaughter and sampling**

On d 21 and d 42, one chick around the average body weight (BW) was selected from each replicate for blood sampling and carcass measurements after the chicks starved for 10 hours. Immediately after 10 s of the chicks were stunned with electricity, the chicks were bled by severing the jugular vein using a handheld knife on one side of the neck to allow bleeding for 180 s. Blood samples (5 mL) were immediately collected in heparinized centrifuge tubes during bleeding from the jugular vein and cooled in ice water. Samples were then instantly centrifuged at 1,800 g for 15 min at 4°C. Then, the plasma samples were kept at –20°C until analysis. After blood sampling, carcasses were defeathered and eviscerated to determine carcass weight (without head, paws, or giblets). The abdominal fat and the breast and leg muscles of each chick were collected and weighed. Abdominal fat referred to the adipose tissue from the proventriculus surrounding the gizzard down to the cloaca. The yield of carcass weight was calculated as a percentage of total BW, whereas the breast and leg muscles yield were calculated as a percentage of carcass weight, and the yield of abdominal fat was calculated as a percentage of carcass and abdominal fat weight. The right side of the breast muscle was then used to determine meat color and muscle pH.

| Table 1. Composition and nutrient level of the basal diets |
| --- |
| **Item** | **Age (d)** | **Starter phase**<sup>1</sup> | **Grower phase**<sup>2</sup> |
| **Ingredient (%)** |  |  |  |
| Corn | 56.37 | 63.48 |
| Soybean meal | 36.55 | 29.24 |
| Soybean oil | 3.00 | 3.50 |
| Salt | 0.35 | 0.35 |
| Dicalcium phosphate | 1.24 | 0.72 |
| Limestone | 1.61 | 1.67 |
| DL-methionine | 0.28 | 0.30 |
| L-lysine HCl | 0.20 | 0.27 |
| Threonine | 0.09 | 0.15 |
| Choline chloride (50% choline) | 0.10 | 0.10 |
| Premix<sup>3</sup> | 0.22 | 0.22 |
| **Nutrient level** |  |  |  |
| AME (MJ/kg) | 12.55 | 12.97 |
| CP (%) | 21.00 | 19.00 |
| Ca (%) | 1.00 | 0.90 |
| Available P (%) | 0.35 | 0.25 |
| Lys (%) | 1.15 | 1.05 |
| Met (%) | 0.55 | 0.48 |
| Met+Cys (%) | 0.81 | 0.78 |
| Thr (%) | 0.74 | 0.70 |
| Trp (%) | 0.21 | 0.18 |

<sup>1</sup> AME, apparent metabolizable energy; CP, crude protein.

<sup>2</sup> Premix supplied per kg of diet: Vitamin A 12,500 IU; Vitamin D<sub>3</sub> 2,500 IU; Vitamin E 15 IU; Vitamin K<sub>2</sub> 2.65 mg; Vitamin B<sub>1</sub> 2 mg; Vitamin B<sub>2</sub> 6 mg; Vitamin B<sub>6</sub> 0.025 mg; biotin 0.0325 mg; folic acid 1.25 mg; Ca-pantothenate 12 mg; niacin 50 mg; Cu 8 mg; Zn 75 mg; Fe 80 mg; Mn 100 mg; Se 0.15 mg; I 0.35 mg.

<sup>3</sup> The nutrient level listed in the table are calculated values.
At the same time, 30.0 g of the left breast muscle was used to measure drip loss, cooking loss, and shear value. All samples were stored at 4°C until analysis.

**Meat quality assay**

Muscle pH values at 45 min and 24 h postmortem (pH<sub>45min</sub> and pH<sub>24h</sub>) were determined by a calibrated waterproof pH meter (pH Spear, Eutech Instruments PTE Ltd., Singapore City, Singapore), which had a spear tip probe for deep penetration into the muscle. Each sample was measured in triplicate at different locations within the muscle, and the average value was calculated as the result.

Meat color was measured in triplicate using a chromometer (Chroma Meter WSC-S, Shanghai Precision and Scientific Instrument Co., Shanghai, China), and the CIE (Commission Internationale de l’Eclairage) Lab system values of lightness (L*), redness (a*), and yellowness (b*) were recorded 45 min and 24 h postmortem. The average value was calculated as the result.

A sample of 30.0 g of the left breast muscle was weighed (W<sub>i</sub>) and trimmed into regular-shaped fillets promptly after sampling. Samples were then dried and reweighed (W<sub>f</sub>) after storing at 4°C for 24 h. Drip loss was calculated as: Drip loss = (W<sub>f</sub> - W<sub>i</sub>) / W<sub>i</sub> × 100%.

The reweighed meat samples of the breast muscle (W<sub>f</sub>) were used to measure cooking loss after determining drip loss 24 h postmortem. Samples were then placed in new zip-sealed polyethylene bags, cooked in a water bath at 85°C for 10 min, which ensured that the internal temperature of the samples reached 85°C, afterwards cooled in running water for a while, and then dried and reweighed as W<sub>f</sub>. Cooking loss was calculated as: Cooking loss = (W<sub>f</sub> - W<sub>i</sub>) / W<sub>i</sub> × 100%.

After testing the cooking loss 24 h postmortem, the cooked meat was used to determine the shear value (24 h postmortem) using a digital meat tenderness meter (Model C-LM3, Northeast Agricultural University, Harbin, China) with the Warner-Bratzler (W-B) shear method. Each sample was divided into three stripes (2×2×4 cm³) with 3 times cut each, and the average value was calculated as the result.

**Plasma index assay**

The total superoxide dismutase (SOD) activity, total antioxidant capacity (TAC), and malondialdehyde (MDA) content in plasma were analyzed using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), and all the parameters were evaluated according to the manufacturer’s instructions. The contents of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), and glucose (GLU) in plasma were determined by an automatic biochemistry analyzer (Zhuyoye-310, Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China) in accordance with each kit (Shanghai Kehua Bio-Engineering Co., Ltd., China).

**Statistical analysis**

The normality of the data and homogeneity of variances were tested at first. All data were analyzed using one way analysis of variance (ANOVA), and means were compared by the Duncan’s [16] multiple range test when ANOVA analysis was significant (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA). A p-value of 0.05 or less was declared significant.

**RESULTS**

**Growth performance**

There was no significant difference of dietary MS supplementation on the BW of chicks at 21 d of age (p>0.05, Table 2). However, the final BW of chicks at 42 d of age was increased in MS supplemented groups compared with the control (p<0.05), and no difference was observed between the MS 0.14% and MS 0.28% groups (p>0.05). Dietary MS supplementation did not affect the average daily weight gain (ADG) of chicks in the starter phase (1 to 21 d, p>0.05). However, the ADG in MS supplemented groups was higher than that in the control in the grower (22 to 42 d) and the whole (1 to 42 d) phases (p<0.05), and there was no significant difference between the MS 0.14% and MS 0.28% groups (p>0.05). No significant differences in feed intake and feed efficiency were observed among treatments in the starter, grower, or whole phases during the experimental period (p>0.05).

**Carcass characteristics**

Carcass parameters were not affected by dietary MS supplementa-
tation or the day of age (p>0.05, Table 3). No significant differences were observed in dressing percentage, breast or leg muscle yield, or abdominal fat of chicks at the age of 21 d or 42 d (p>0.05).

### Meat quality

The breast muscle quality of chicks at 42 d of age is listed in Table 4. No significant differences were observed in cooking loss (p = 0.084) and shear value (p>0.05) among three dietary treatments, but the drip loss was decreased in MS 0.28% treatment compared with the control (p = 0.005). There were no changes in pH values of the breast muscle neither in 45 min nor in 24 h postmortem in the experiment (p>0.05). Compared with the control, dietary 0.28% MS supplementation increased a* values in both 45 min (p = 0.005) and 24 h (p<0.001) postmortem and decreased L* values in both 45 min and 24 h (p<0.05) postmortem and b* value in 24 h (p = 0.001) postmortem. Dietary 0.14% MS supplementation only decreased b* values in both 45 min (p<0.05) and 24 h (p = 0.001) postmortem compared with the control.

### Plasma antioxidant and biochemical indices

Dietary supplementation of 0.14% MS increased the plasma TAC content in chicks at 21 d of age compared with the control (p<0.001, Table 5), but there was no significant difference between the MS 0.28% treatment and the control (p>0.05). Furthermore, no difference in plasma TAC content was observed among the treatments at 42 d of age (p>0.05). On d 21 and d 42, lower plasma TC content was observed in the MS 0.28% compared with the control (p<0.05). However, no significant difference in plasma TC content was observed between the MS 0.14% and the control (p>0.05). At 21 d of age, compared with the control, MS 0.14% decreased the TG content in plasma, and this effect was more pronounced.

### Table 3. Effect of dietary mango saponin supplementation on carcass characteristics of broiler chicks

| Item | Control | Mango saponin levels (%) | SEM | p-value |
|------|---------|--------------------------|-----|---------|
|      |         | 0.14                     | 0.28|         |
| At 21 d of age (%) |         |                          |     |         |
| Dressing percentage | 71.8 | 70.0 | 71.1 | 0.44 | 0.271 |
| Breast muscle yield | 21.2 | 19.8 | 20.5 | 0.39 | 0.363 |
| Leg muscle yield | 18.7 | 19.1 | 18.9 | 0.22 | 0.774 |
| Abdominal fat | 1.53 | 1.47 | 1.49 | 0.08 | 0.952 |
| At 42 d of age (%) |         |                          |     |         |
| Dressing percentage | 74.1 | 74.1 | 75.0 | 0.48 | 0.642 |
| Breast muscle yield | 25.1 | 26.2 | 25.0 | 0.52 | 0.688 |
| Leg muscle yield | 19.9 | 20.0 | 20.8 | 0.39 | 0.633 |
| Abdominal fat | 1.98 | 1.97 | 1.96 | 0.07 | 0.991 |

SEM, standard error of the mean.
1) n = 6.
2) Percentage of body weight at slaughter.
3) Percentage of carcass weight after eviscerated and without head, paws or giblets.
4) Percentage of carcass and abdominal fat weight.

### Table 4. Effect of dietary mango saponin supplementation on meat quality of broiler chicks on d 42

| Item | Control | Mango saponin levels (%) | SEM | p-value |
|------|---------|--------------------------|-----|---------|
|      |         | 0.14                     | 0.28|         |
| Drip loss (%) | 3.93 | 3.45 | 2.65 | 0.18 | 0.005 |
| Cooking loss (%) | 23.2 | 20.8 | 21.0 | 0.50 | 0.084 |
| Shear value (N) | 14.7 | 11.8 | 11.4 | 0.77 | 0.179 |
| 45 min postmortem |         |                          |     |         |
| pH | 6.45 | 6.48 | 6.53 | 0.02 | 0.401 |
| L* | 51.7a | 48.9b | 47.7b | 0.70 | 0.048 |
| a* | 6.33a | 6.59a | 7.82a | 0.22 | 0.005 |
| b* | 18.6a | 16.2a | 17.4a | 0.39 | 0.027 |
| 24 h postmortem |         |                          |     |         |
| pH | 5.84 | 5.91 | 5.92 | 0.02 | 0.434 |
| L* | 58.9a | 58.8a | 55.0b | 0.71 | 0.018 |
| a* | 5.46a | 5.98b | 7.25b | 0.25 | <0.001 |
| b* | 21.3a | 18.1a | 19.7a | 0.40 | 0.001 |

SEM, standard error of the mean; L*, lightness; a*, redness; b*, yellowness.
1) n = 6.
2) Means within a row with no common superscripts differ significantly (p<0.05).
obvious in MS 0.28% treatment (p<0.001), whereas, at 42 d of age, plasma TG content was only decreased in MS 0.28% treatment compared with the control (p<0.05). There was no significant difference in plasma GLU content among the treatments on d 21 (p>0.05), but it was decreased in MS 0.28% treatment on d 42 compared with the control (p<0.001). No significant differences in MDA, HDL-C, LDL-C, SOD, ALT, AST, TP, and ALB contents in plasma were observed among three treatments neither on d 21 nor on d 42 (p>0.05).

**DISCUSSION**

**Growth performance**

In the present study, dietary MS supplementation increased the growth rate of broilers in the growing period. This finding was completely a new result, because, to the best of our knowledge, no study has investigated the MS effect on growth performance in poultry. However, a similar result was observed in fish, which reported that the tilapia, carp, and tortoise could grow more quickly when were fed with MS, and the enhanced immunity status may account for the improved growth [15]. In the current study, the positive responses of ADG and BW to dietary MS supplementation indicated its beneficial role in broiler growth. Moreover, the feed efficiency was marginally improved in MS supplemented groups. These results may be attributed to the health-promoting properties of the mango leave extract, such as analgesic, antioxidant, antimicrobial, anti-inflammatory, and antifungal activities [17,18]. In addition, an enhanced health status in MS supplemented groups may also account for the improved performance observed in the present study, as evidenced by the improved plasma antioxidant and biochemical parameters in broilers fed with MS (Table 5). However, it is implied that there was no dose-response effect on broiler growth with increasing levels of MS in diet, as no difference was observed in growth performance between the 0.14% and 0.28% MS supplemented groups. In this respect, the supplemental level of 0.14% MS in diet is more economical than the 0.28% supplemented group. The ADG and BW were not affected in the starter phase and greatly increased in the grower phase by dietary MS supplementation, which indicated that a durative time was needed for MS to exert the growth promotion effects in broiler chicks.

**Meat quality**

In the present study, lower drip loss and better meat color (higher a* value, lower L* value, and b* value) were observed in broiler chicks fed with 0.28% MS supplemented diet, which indicated that the meat quality was improved with dietary MS supplementation at a level of 0.28%. The meat quality was reported to be affected by the lipid metabolism in broilers [19], and the muscle energy metabolism was also an important factor for determining the final meat quality of animals through glycogen metabolism or TG accumulation [20]. A previous study showed that the mango leave extract played a dominant role in the integrated regulation of sugar and lipid homeostasis in vitro and in vivo, and the ethanolic extract of mango leaves decreased plasma GLU and TG contents in a dose-dependent manner in mice [21]. Similarly, lower plasma TC, TG, and GLU contents were observed in the 0.28% MS treatment. Decreased TC and TG contents indicated the lipid metabolism was ameliorated in broilers. In this respect, the changes of lipid metabolism in broilers possibly contributed to the improved meat quality of broilers in 0.28% supplemented group in our study. Moreover, the meat quality of broilers could be improved by dietary supplementation of antioxidant compounds [22], and several studies have reported that mangiferin or flavonoids could be used as natural antioxidants [1,23,24]. In this respect, mangiferin and flavonoids contained in MS may also account for the increased meat quality in the 0.28% MS treatment. In the present study, dietary MS supplementation could improve the meat quality of the breast muscle in broiler chicks, and a dietary supplemental level of 0.28% had more significant influence than 0.14%.

**Plasma antioxidant and biochemical indices**

In the present study, apart from the supplementation of 0.14% MS which increased plasma TAC content on d 21, no significant differences in plasma SOD and MDA contents were observed among different treatments. However, it was reported that the mango leave extract had significant antioxidant activity on serum and liver in rat [11]. The difference between the previous study and our result was probably due to the different status of animals, as the rats were in stressed status in the previous study, whereas the broilers were in normal status in the present study. The transient phenomenon of higher plasma TAC content in 0.14% supplemented group on d 21 possibly resulted from the presence of mangiferin and flavonoids in MS, since several studies have shown their antioxidant capacity in rats [11,23]. However, no improvement of plasma antioxidant status was observed in broilers with 0.28% MS supplementation. The antioxidant system of an animal is aimed at finding a balance between pro-oxidative and anti-oxidative stressors in the body [25]. In this case, the oxidative balance was probably already attained in chicks by the supplementation of 0.14% MS in our study, and no better effect could be achieved with higher MS supplementation (0.28%).

Plasma TG and TC contents reflect the lipid metabolism status in the body, and the excess accumulation of TG and TC leads to metabolic disorders in broilers [26]. Lipid oxidation, especially in the plasma lipids, produces free radicals and, therefore, impairs the health of animals [27]. In the present study, dietary 0.28% MS supplementation decreased plasma TC and TG contents in chicks. This result implied that the lipid metabolism status was improved in broilers supplemented with 0.28% MS. It could be explained by the presence of mangiferin in MS, which was reported to decrease plasma TC and TG contents in rats [28,29]. In addition, flavonoids in MS also contributed to
the improved lipid metabolism of broilers fed with 0.28% MS, as reported in an earlier study [30]. Decreased TC and TG contents in plasma were observed in 0.28% MS supplemented group rather than in 0.14% group, which indicated that more MS was needed to show the lipid inhibition effect in broilers in the present study. The amelioration of plasma lipid metabolism in broilers fed a diet containing 0.28% MS may contribute to the increased meat quality and growth performance of broilers in our present study.

It has been reported that plasma ALB, TP, and GLU contents are reliable indicators of the function of the liver and, together with plasma AST and ALT activities, are closely correlated with the degree of hepatic lipidosis in animals [31,32]. Dietary 0.28% MS supplementation decreased the GLU content in plasma at the age of 42 d, which is in accordance with a previous study [21]. Furthermore, no differences in ALT and AST activities and TP and ALB contents were observed with dietary MS supplementation in the present study. Collectively, the results of plasma antioxidant and biochemical indices indicated that dietary supplementation of MS had no adverse effect to broilers in our study.

IMPLICATIONS

In conclusion, MS could be used as a feed additive in broiler chicks, and the supplemental level of 0.28% MS in diet could improve growth performance, meat quality, and plasma lipid metabolism in broilers in the present study. This finding encourages further research in the application of MS as a feed additive in farm animals.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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