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Author	Sarbani Biswas ^{1,3} , Je Min Ahn ^{1,4} , In Ho Kim ^{1,2*}
Affiliation	¹ Department of Animal Biotechnology, Dankook University, 31144, Cheonan, Choongnam, South Korea ² Smart Animal Bio Institute, Department of Animal Biotechnology, Dankook University, 31144, Cheonan, Choongnam, South Korea ³ Department of Animal Science, College of Agriculture and Life Sciences, Chonnam National University, 61186, Gwangju, South Korea ⁴ Daehan Feed Co., Ltd., 22300, Incheon, South Korea
ORCID	Sarbani Biswas: https://orcid.org/0000-0002-6732-286X Je Min Ahn: https://orcid.org/0000-0001-6969-4723 In Ho Kim: http://orcid.org/0000-0001-6652-2504
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First name, middle initial, last name	In Ho Kim
Email address	inhokim@dankook.ac.kr
Address	Department of Animal Biotechnology, Dankook University, No. 29 Anseodong, Cheonan, Choongnam, 330-714, South Korea
Office phone number	+82-41-550-3652
Fax number	+82-41-565-2949

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**Micelle silymarin as a functional feed additive: Enhancing broiler performance and nutrient bioavailability
in laying hens**

Sarbani Biswas^{1,3}, Je Min Ahn^{1, 4}, In Ho Kim^{1, 2 *}

¹ *Department of Animal Biotechnology, Dankook University, Cheonan, Choongnam, South Korea*

² *Smart Animal Bio Institute, Department of Animal Biotechnology, Dankook University, Cheonan, Choongnam, South Korea*

³ *Department of Animal Science, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, South Korea*

⁴ *Daehan Feed Co., Ltd., 22300, Incheon, South Korea*

*** Corresponding author:** In Ho Kim

Email: inhokim@dankook.ac.kr

Phone: 82-41-550-3652 / 82-10-8803-9598; Fax: +82-41-559-7881

Running head: Impact of micelle silymarin on broilers and hens

Abstract

Micelle silymarin (MS), a bioactive compound with antioxidant and anti-inflammatory properties, functions as a functional feed additive, promoting broiler performance and optimizing nutrient bioavailability in laying hens. This study aimed to assess the impact of MS on different aspects of broilers, including growth, digestibility, excreta score, footpad lesion score, tibia ash, meat quality, and blood absorption rate of laying hens. In the first experiment, 576 one-day-old Ross 308 broilers (of mixed sex) were randomly allocated. These birds had an average body weight of 43 ± 0.23 g and were monitored over a 32-day trial period. The broilers were grouped into eight separate cages, each containing 18 birds, assigned to four different treatments: basal diet as the control and basal diet supplemented with 0.02, 0.04, and 0.06% MS. In the second experiment, sixteen Hy-line brown laying hens, aged 61 weeks, were separated into two distinct treatment groups: TRT1, basal diet + 4% powdered silymarin; TRT2, basal diet + 4% MS, with eight repetitions per treatment. Administering graded doses of MS to broilers led to a consistent increase in body weight gain across days 1–9, 9–21, 21–32, and the overall period ($p = 0.021$, 0.011 , 0.019 , and 0.002 , respectively). Additionally, feed intake showed a linear increase ($p = 0.017$) during days 1–9, and dry matter digestibility improved linearly ($p = 0.011$) with higher levels of MS inclusion. As MS concentrations in the broiler diet increased, tibia ash content and breast muscle yellowness linearly increased ($p = 0.038$ and $p = 0.045$, respectively), while pH decreased ($p = 0.004$). However, the excreta score and the footpad lesion score exhibited no significant changes ($p > 0.05$). Furthermore, laying hens that were fed micelle-type silymarin demonstrated a significantly higher silymarin absorption rate in the blood two hours and four hours ($p < 0.001$ for both) after feeding than powdered-type silymarin. Overall, supplementation with MS at 0.06% was identified as the optimal level, enhancing broiler growth performance, nutrient utilization, and meat and bone quality, facilitating the welfare of broiler production, while MS also demonstrated superior bioavailability in laying hens.

Keywords: broiler, blood absorption, hen, micelle silymarin, productivity

INTRODUCTION

Poultry production is the most efficient method to address the shortage of animal protein due to its rapid growth rate and high economic return [1]. As bacteria become more resistant to antimicrobials, using plant-based feed additives instead of antibiotics has gained widespread acceptance in modern livestock management to improve performance [2]. Phytogetic supplements for livestock typically contain natural acids, volatile oils, saponins, flavonoids, other organic components, and inorganic compounds like antibacterial agents and growth stimulants [3]. There is emerging evidence that the phytochemical constituents found in plants and their derivatives can be helpful in several aspects of health and welfare [4].

Silymarin, a medicinal plant extract sourced naturally from milk thistle fruit, is found in countries such as Canada, China, Mexico, West Pakistan, and Kashmir, similar to its presence in northern hemisphere regions. It has reddish-purple blooms and thorny leaves, and 60% silymarin is present in dried seed extracts [5]. It comprises polyphenols and three naturally occurring flavonoid lignan components, including silybin, silydianin, and silychristin [6]. Aside from its antioxidant, anti-carcinogenic, and anti-inflammatory properties, silymarin aids liver tissue regeneration and defends against fatty liver and liver toxicity, which is called hepatoprotective activity [7]. It promotes the release of digestive juices, increases appetite, and enhances broilers' liver and cardiovascular system performance [8]. Additionally, some of the biological mechanisms of micelle silymarin (MS) that protect liver cells involve maintaining membrane stability, scavenging free radicals, stimulating protein synthesis in hepatocytes, and modulating the immune response [4]. Various studies have demonstrated that incorporating silymarin into the diet enhances both productive and reproductive performance while improving animal health [9,10]. Recent research suggests that adding silymarin to the feed can enhance broilers' well-being by boosting growth performance, nutrient absorption, meat quality, gut microbiota, footpad dermatitis, and tibia mineral content [10,11,12]. Assessing the absorption capacity or bioavailability in animals' bodies is crucial for understanding the impact of a feed additive. In the prior experiments, Shanmugam et al. [11] observed an enhanced blood profile in broilers fed a silymarin-included diet. Hossain et al. [13,14] noted an improved blood absorption rate in growing pigs using silymarin as a dietary feed supplement. In healthy birds, silymarin intake led to an increase in serum globulin levels, while reducing albumin concentrations and the activities of serum liver enzymes ALT and AST, compared to birds fed mycotoxin-contaminated diets [15]. However, a comparison of absorption rates in the blood of laying hens among different types of silymarin is scarce.

To date, research on the effects of adding silymarin supplements to broilers and laying hen diets on the performance and welfare of the poultry industry is inadequate. From the above study, enhancing poultry dietary supplements with silymarin could potentially enhance broilers' production performance and the blood absorption rate of laying hens. Therefore, our objective was to investigate the incorporation of silymarin into broilers' diets to evaluate its impact on production efficiency, nutrient utilization, footpad lesion score, tibia ash content, excreta quality, and meat characteristics as a natural growth promoter in Experiment 1, and to examine the rate of silymarin absorption in the blood of laying hens in Experiment 2.

MATERIALS AND METHODS

Ethical statement

This research protocol was reviewed (Ethical Approval numbers: Experiment 1, DK-1-2129; Experiment 2, DK-1-2309) and approved by the Dankook University Animal Care and Use Committee in Cheonan, Republic of Korea.

Preparation of silymarin

The experiment utilized silymarin from Synergen Company (Gyeonggi-do, Republic of Korea) with an active content of 250 grams per kilogram, consisting of silybin (10.8%), silydianin (16.3%), and silychristin (7.0%). Method of processing: The milk thistle seed was pressed to produce milk thistle powder and then infiltrated with alkali water. Following the acetone penetration of alkali water, extract the milk thistle powder, filtering and concentrating the extract to produce a concentrated liquid. After the concentrate extraction with a non-polar solvent, a non-polar diluent layer and an alcohol layer were acquired. Silymarin (80% purity) was obtained by concentrating and drying the acetone layer, then formulated into micelles using various emulsifiers to create variable types of silymarin (20% micelle-type and 2% silymarin).

Experiment 1

Animals, housing, and experimental design

In the present study, a group of 576 male Ross 308 broilers, each at an age of one day and possessing an average body weight of 43 ± 0.23 g, was employed. The experimental design included eight replicates for each treatment, with each cage housing 18 birds. The trial was structured into three distinct feeding phases: Phase 1 (Days 1–9), Phase 2 (Days 9–21), and Phase 3 (Days 21–32). This investigation encompassed four distinct dietary formulations: a control group

receiving the basal diet and three additional groups in which the basal diet was augmented with 0%, 0.02%, 0.04%, and 0.06% MS, respectively. All dietary compositions were meticulously formulated according to the recommendations stipulated by the NRC [16] (refer to Table 1). The experimental diet for broilers was formulated based on NRC [16] guidelines, which remain a widely used standard in broiler nutrition research and provide a reliable reference for maintaining consistency with previous studies involving Ross 308. Temperature conditions were rigorously controlled at 33 ± 1 °C during the first week and subsequently decreased to a stable 24 ± 1 °C with humidity levels maintained at 60%. Each cage was outfitted with a one-sided feeder and a nipple drinker equipped with five nipples, ensuring unrestricted access to feed and water for the birds. The cages, made of stainless steel, were identical in size, measuring 1.75 meters in length and 1.55 meters in width, and were constructed using multi-layer battery cages. Over seven days, fluorescent lighting provided artificial illumination at an intensity of 10 lux for a continuous 24 hours daily, which was subsequently adjusted to 20 hours daily for the duration of the study.

Growth performance and nutrient utilization

The weight of the broilers was documented on the 9th, 21st, and 32nd days. The daily feed consumption and leftover feed (per cage) were evaluated to ascertain the feed intake (FI). At the conclusion of each experimental phase, body weight (BW), FI, and feed conversion ratio (FCR) were analyzed. To calculate mortality, the number of deceased birds and their respective weights were recorded on a daily basis.

To evaluate the digestibility of nutrients, all broilers from randomly selected eight cages per treatment were administered diets containing 0.2% Cr₂O₃ for seven days before excreta collection (days 25–31). The specimens were aggregated by cage using collection tray and combined; a representative sample was preserved in a freezer at –20 °C until further analytical procedures were conducted. The excreta samples were dried at 70 °C in a laboratory-grade electronic oven for seventy-two hours. The collected excreta samples were ground and then passed through a 1-mm sieve. The analytical methods established by the Association of Official Analytical Chemists [17] were employed to assess the dry matter (DM), nitrogen (N), and energy (E) content of both feed and excreta samples. The quantification of chromium was accomplished utilizing ultraviolet spectrophotometry (UV-1201; Shimadzu). The thermal energy produced from the combustion of the samples was evaluated using a bomb calorimeter (Parr 6100; Parr Instrument

Co., Moline, IL) to ascertain energy content. The apparent total tract digestibility (ATTD) for nutrients was determined following the procedure outlined by Biswas et al. [18].

Excreta score

The collection tray was meticulously used to collect excreta specimens from each replicate cage, which were then carefully placed onto blank paper for examination. Subsequently, two independent personnel conducted detailed visual assessments of the excreta, following the scoring criteria outlined by Sampath et al. [19]: Score 1 for dry and firm excreta, Score 2 for mostly dried excreta, Score 3 for moist excreta, Score 4 for wet and loose excreta, and Score 5 for extremely wet excreta.

Footpad lesion score and tibia ash

On day 31, each bird's footpad dermatitis wound score was systematically and precisely calculated. The evaluation of footpad dermatitis was conducted utilizing a four-point scoring scale: 0 denoting the absence of lesions, 1 for minor lesions of the footpad mucosa (less than 1 cm), 2 for more extensive lesions (greater than 1 cm), and 3 for pronounced dorsal swelling. Two independent evaluators assessed the scoring for both feet of the aforementioned broiler specimens, and the mean score derived from their evaluations was employed for subsequent statistical analyses.

Utilizing the diethyl ether extraction method, lipids were extracted from the bones in a Soxhlet apparatus for 48 hours. Following the lipid extraction, the bones were transferred to crucibles and subjected to air-drying in a static furnace at 105 °C for at least two hours. Upon removal, the dehydrated bones were weighed in the crucibles and then subjected to ashing in a furnace maintained at 600 °C for a period ranging from 24 to 48 hours. The residual ash was weighed to calculate the total ash content [20].

Organ weight and meat quality analysis

On day 32, 16 birds (two birds per replicate) were randomly selected from each treatment group (resulting in a total of 64) for meat quality assessment, and trained personnel performed humane slaughter. Random selection was used to avoid sampling bias, and the selected birds' body weights were confirmed to be statistically similar across groups prior to slaughter. The breast muscle, liver, kidney, spleen, bursa of Fabricius, and gizzard were excised and weighed to compute the relative organ weight (g/kg), determined by dividing the organ weight (g) by the live body weight (kg). A portable chroma meter (Konica Minolta, CR-400, Osaka, Japan) was employed to evaluate the parameters of lightness, redness, and yellowness. The values were derived by averaging measurements taken at three distinct

locations on the surface of each sample. The pH of the broiler meat was ascertained using a portable pH meter (Testo 205, Testo, Germany) coupled with two calibration buffers (pH 4.0 and 7.0), and the measurement protocol was executed in duplicate. The water holding capacity (WHC), cooking loss, and drip loss were quantified according to the methodology delineated by Biswas et al. [21].

Statistical analysis

The data was assessed using general linear model techniques within a completely randomized block design (SAS Institute Inc., Cary, NC, USA), with the cage as the experimental unit. A thorough analysis using linear and quadratic polynomial contrasts was employed to study the impacts of raising levels of MS inclusion in the diet. A significance level of $p < 0.05$ indicated significance, while $p < 0.10$ suggested a trend.

Experiment 2

Test animals, husbandry, and test design

The feeding test was carried out for 4 hours on 16 Hy-line brown laying hens that were 61 weeks old. The study design consisted of two treatments: 1) TRT1, a basal diet including powdered silymarin at 4%, and 2) TRT2, a basal diet containing micelle-type silymarin at 4%. Each treatment had eight repetitions. Each hen was housed in an individual pen measuring 38 cm in width, 50 cm in length, and 40 cm in height. The ambient temperature was kept at 26°C, and a lighting regimen of 16 hours of light and 8 hours of darkness was implemented. Each pen was equipped with a nipple drinker and a removable trough feeder, allowing unrestricted access to feed and water. The higher silymarin levels used in laying hens were intended to ensure measurable absorption within the 4-hour sampling period and were not designed to reflect practical dietary inclusion levels, as the aim was to compare short-term absorption rate between silymarin types. This approach was based on a similar design reported by Hossain et al. [13], who observed significantly higher blood absorption of micelle-type silymarin compared to the powdered form in growing pigs over a short 24-hour sampling period.

Test feed and feeding management

The feeding test was conducted at Dankook University's research farm in Sejong City, under carefully controlled environmental conditions. The test feed, corn-soybean meal, was formulated according to the requirements of NRC [16] (the same as phase 3 broiler feed in Table 1). For the laying hen experiment, the same basal diet used in the broiler trial (formulated according to NRC, [16]) was provided. As this was a short-term (4-hour) study focusing on blood

nutrient absorption, not production performance, the diet was deemed appropriate and nutritionally adequate for the experimental purpose. Birds were allowed to have feed and water freely. The shed temperature was meticulously maintained at 26 °C, and the lighting regimen consisted of 16 hours of light followed by 8 hours of darkness, ensuring the most accurate results.

Sampling and measurement

For blood collection, a meticulous process was followed. A volume of 5 mL of blood was procured utilizing a K₃EDTA vacuum tube via the brachial vein with a sterile needle before the initiation of the test, 2 hours subsequently, and at the end of the test (4 hours). After the blood collection, 6 mL of methanol was incorporated, and the resultant sample underwent centrifugation (10 min, 4 °C, 10,000 × g). Immediately following the centrifugation process, the supernatant was transferred to a new 15-mL tube, concentrated through nitrogen application within a 50 °C water bath, and aliquoted with 6 mL of a 0.2 M phosphate buffer solution (pH 7.2). Silymarin flavonolignans and naringenin were procured from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and distilled water were sourced from Tedia (Fairfield, CT, USA), while HPLC-grade ethyl acetate was acquired from J. T. Baker. Formic acid was also obtained from Sigma-Aldrich.

A stock solution of silymarin was meticulously prepared with a 2 mg/mL concentration in acetonitrile. The gradual incorporation of acetonitrile into this stock solution enabled the formulation of working solutions at concentrations of 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 500 ng/mL, 1000 ng/mL, and 2500 ng/mL. Naringenin was solubilized in acetonitrile to achieve a 20 ng/mL concentration. All stock and working solutions were preserved at -20 °C throughout the analytical procedures. Calibration standards were established by combining silymarin working solutions with total hen plasma, resulting in final concentrations of 1, 2, 5, 10, 20, 50, 100, and 250 ng/mL. A volume of 200 µL of either the calibration standards or hen plasma samples was mixed with 20 µL of the internal standard (IS) solution (naringenin at 20 ng/mL in acetonitrile) and 1 mL of ethyl acetate. The resulting mixture underwent vigorous vortex mixing for a duration of 5 minutes and was subsequently centrifuged at 16,000 × g for 5 minutes. The quantification of silymarin concentrations in hen plasma samples was executed precisely utilizing an Agilent 6470 triple quadrupole LC-MS/MS system (Agilent, Wilmington, DE, USA). The effective separation of silymarin from the baseline in hen plasma was accomplished by utilizing a Synergy polar RP column (2.0 mm × 150 mm, pore size: 4 µm; Phenomenex, Torrance, CA, USA) with a consistent mobile phase comprised of a mixture of water and acetonitrile (15:85, v/v) containing 0.1% formic acid. The temperature of the column was sustained at 30

degrees Celsius, with the mobile phase flowing at a rate of 0.2 millilitres per minute. Employing electrospray ionization in negative ion mode, the LC-MS/MS analysis utilized multiple reaction monitoring transitions at m/z 481.2 to 151.0 for the precisely detected silymarin.

Statistical processing

All data were analyzed utilizing Duncan's multiple range test within the framework of the General Linear Model procedure as implemented in SAS (2013) to ascertain the statistical significance among means, employing the cage as the experimental unit. The threshold for significance was set at $p < 0.05$, with trends considered noteworthy at $p < 0.10$.

RESULTS

Experiment 1

Growth performance

Table 2 demonstrates the influence of elevated dietary MS intake on broiler growth metrics. The broilers' body weight gain (BWG) exhibited a linear increase ($p = 0.021, 0.011, 0.019$, and 0.002 , respectively) corresponding to the incremental MS doses consumed on days 1–9, 9–21, 21–32, and the overall period. During days 1–9, a linear increase ($p = 0.017$) was also observed in FI. Nevertheless, no significant alterations were observed in FCR or mortality rates as the levels of MS in the diet increased.

Nutrient utilization

As indicated in Table 3, the gradual increase in dietary MS levels reveals a linear rise ($p = 0.011$) in DM digestibility. However, no significant alterations ($p > 0.05$) were observed in broilers' nutrient digestibility for N and E.

Excreta score, footpad lesion score and tibia ash

Table 4 elucidates the effects of escalating MS concentrations in the broiler diet on the excreta score, footpad lesion score, and tibia ash. Increasing MS concentrations in broiler diets yielded no statistically significant treatment effects ($p > 0.05$) concerning the excreta or footpad lesion scores. Nevertheless, there was a linear increase in tibia ash content ($p = 0.038$) by MS inclusion.

Organ weight and meat quality

Table 5 presents the ramifications of increasing levels of MS in the flock's dietary regimen on various meat quality parameters. As the dosage of dietary MS increased, a linear increase was observed in breast muscle colour (yellowness)

($p = 0.045$) alongside a reduction in pH level ($p = 0.004$). However, no statistically significant differences were recorded for other meat quality attributes.

Experiment 2

Blood absorption rate

The effect of the blood absorption rate of regular powder-type silymarin and micelle-type silymarin of laying hens is shown in Figure 1. Blood absorption rate was significantly higher in the TRT2 group than in the TRT1 group 2 hours ($p = <.0001$) and 4 hours ($p = <.0001$) after the test.

DISCUSSION

Silymarin is known for its bioactive properties that promote liver health and improve performance and health indicators in livestock. This investigation aimed to assess the effects of varying formulations of silymarin supplementation on performance metrics, health indicators, and blood absorption rates in poultry to ascertain its potential advantages. Among the tested inclusion levels, 0.06% MS resulted in the greatest improvements in BWG, DM digestibility, and tibia ash content, with no adverse effects on excreta quality or footpad condition. Moreover, micelle-type silymarin facilitated superior blood absorption rates in laying hens compared to conventional powder-type silymarin. These findings support the use of 0.06% MS as an effective and welfare-compatible supplementation level for enhancing broiler productivity. Future research should investigate the long-term effects and economic viability of silymarin supplementation. Additionally, challenges such as variability in absorption rates and the need for standardized protocols should be addressed to optimize its use in the poultry industry.

The silymarin extract from milk thistle has received an enormous interest in biological research [22]. The current study corroborates the findings of Tedesco et al. [9] and Shanmugam et al. [11], suggesting that the integration of silybum marianum (SM) seed extract into broiler diets resulted in augmented BWG and FI. Similarly, adding SM to Japanese quail [12] and broiler [23] diets significantly improved FI and BW. In comparison to control birds, the birds fed diets prepared by adding 1000 mg/kg silymarin demonstrated increased daily weight gain (6.35%) and lower FCR (6.6%) during Days 7–35 [22]. In addition, chickens' BWG was enhanced when milk thistle extract SM was added to the drinking water [10]. A linear improvement in FCR and FI was observed in weeks 0–6, 7–12, and 0–12 with increasing MS supplementation levels (0–0.06%) in laying hens [24]. As per Schiavone et al. [25], administering silymarin at doses of 40 ppm and 80 ppm showed no advantageous effects on the growth parameters of broilers. The

253 varying results could be attributed to several factors, including differences diet composition, type of silymarin,
254 variations in dosage, study duration, and genetic variation among the birds [11]. Improved BWG and FI in broilers
255 might be due to the bioactive phytochemical components of silymarin, which cause improvements in the gut
256 environment and alleviate physiological responses in challenge condition [11,26].

257 The plant extract inhibits the proliferation of pathogenic microorganisms within the gastrointestinal tract,
258 thereby allowing advantageous microbes a competitive edge in the nutrient acquisition process, which may enhance
259 nutrient digestion in broiler chickens [27]. In contrast to our findings, the byproduct of SM oil extraction (0–12%) did
260 not significantly impact the nutrient utilization of DM in broiler chicks [23]. Furthermore, broilers administered with
261 silymarin (0–0.06%) seed extract demonstrated a linear enhancement in the digestibility of DM, N, and E [11]. In
262 addition, MS (0–0.2%) inclusion in the fattening pig diet increased the absorption of N [28], and SM (0.05%–0.10%)
263 addition to the weaning pig diet showed no remarkable effect on nutrient digestibility (DM, N, and E) [29]. Using
264 herbal products and plant extracts may enhance digestive efficiency by stimulating the gastric mucosa to produce
265 digestive enzymes and increasing FI, and intestinal health status of the broilers [30], which may elucidate our study's
266 observed modifications in nutrient digestibility.

267 Silymarin, a plant-derived compound known for its potential health benefits, has been studied for its effects on
268 gut health and microbial populations across different animal species, though it's specific impact on broiler excreta
269 scores remains inadequately studied. Given the limited availability of literature on the influence of silymarin
270 administration on broiler excreta scores, we conduct comparisons of its effects in other animal species and with various
271 plant extracts. The current study revealed that incorporating MS into the broiler diet did not result in a significant
272 impact on the excreta score. Similarly, plant extract (gallic acid and quercetin) inclusion into the broiler diet had no
273 impact on excreta score [20,21]. However, Shanmugam et al. [11] reported that incorporating silymarin into a broiler
274 diet might elevate the count of beneficial bacteria, specifically *Lactobacillus*. Additionally, piglet-fed plant extract
275 (0.01%) revealed improved gut health and lower fecal score in growing pigs [31]. Previously, Hossain et al. [13] stated
276 that the inclusion of increasing doses of silymarin in the growing pig diet led to a reduction in *E. coli* counts and an
277 increase in *Lactobacillus* counts. Changes in microbial populations, such as an increase in *Lactobacillus* or a reduction
278 in *E. coli* could potentially influence fecal consistency and excreta scores. The variations in outcomes can be attributed
279 to factors such as the animal species, dosage level, and the plant source of silymarin.

The quality of the footpad is a crucial component of poultry well-being because if the lesion is severe, it will hurt the birds, thus reducing their ability to consume feed and production performance [32]. Wet litter conditions exacerbate footpad dermatitis in broilers, emphasizing the importance of maintaining dry litter to alleviate these issues [33]. Adequate nutrition has also been shown to reduce leg issues in birds by minimizing body mass alterations, thereby lowering the risk of leg weakness [34]. Our research findings indicated no significant effect of silymarin on footpad lesion scores, although there was a linear increase in tibia ash. This aligns with Pisarski and Kwiecien [35], who reported that medicinal plants positively influenced the physical and chemical characteristics of broiler tibia. Similarly, Bendowski et al. [10] observed a reduction in footpad inflammation when milk thistle was added to broilers' drinking water. Conversely, Biswas et al. [20] found no significant impact of gallic acid on footpad lesion scores or tibia ash, but quercetin inclusion (0–0.06%) improved tibia ash linearly without affecting footpad lesion scores [21]. Studies on other species support these findings. In Japanese quail, silymarin supplementation (0–1 mL/kg BW) altered tibia weight, thickness, and width, but not ash, volume, or density [33]. Silymarin has also been shown to increase tibia ash and internal layer thickness in studies involving carbon tetrachloride-treated birds [12]. Furthermore, herbal extracts, in general, have demonstrated the potential to enhance tibial bone calcification [37]. Despite these findings, the precise mechanism through which silymarin influences footpad lesions and tibia ash remains unclear. Further research is needed to understand its role in improving broiler welfare and skeletal health.

The quality of meat, particularly in terms of pH, color, and organ weight, plays a vital role in determining its safety and consumer acceptability. Given the growing consumer concerns about meat safety and quality, it is essential to evaluate factors that influence these attributes. As per Hossain et al. [38], meat pH directly influences shear energy, meat color, and drip loss, due to its correlation with intramuscular acidity. The present findings significantly affected the pH level, breast muscle weight, and meat color. Similar results were observed in studies by Chand et al. [39] and Khaleghipour et al. [22], where the administration of SM in chicken diets led to an increase in spleen weight alongside breast muscle development. Conversely, the SM-added diet decreased the relative organ weights of the spleen in quail [12]. In contrast, Bendowski et al. [10] found no impact of silymarin extract on meat color in broilers, although their study did observe an increase in meat pH with silymarin supplementation. Similarly, broiler-fed diet supplemented with silymarin had improved pH value and decreased spleen weight [11]. Silymarin's natural antioxidant properties and its role in oxidative stability may contribute to improved broiler meat quality and shelf life [25].

The bioavailability and absorption of silymarin can vary significantly depending on its form and administration, influencing its effectiveness in different animal species. Our investigation noted that micelle-type silymarin demonstrated superior blood absorption rates in laying hens when contrasted with powdered-type silymarin. Comparable outcomes were observed in growing pigs, where micelle-type silymarin exhibited enhanced absorption at intervals of 1, 2, 4, 8, 12, and 24 hours post-administration [13]. Yu et al. [40] observed that the administration of MS to a dog's diet resulted in a significantly high absorption rate in the bloodstream. In porcine subjects, the absorption of the flavonoid quercetin was significantly greater in the 300 g-quercetin cohort compared to the 30 g and 150 g cohorts at the 4-hour mark [14]. The seed extract of silymarin is generally absorbed at a rate ranging from 20% to 50% [12]. Silymarin possesses the potential to modulate blood lipid metabolism and concentrations by attenuating cholesterol synthesis in the liver and inhibiting its absorption within the gastrointestinal tract [41]. However, silymarin's oral bioavailability can be limited due to its low water solubility, owing to its non-ionizable structure [40]. Conversely, Micelle-based formulations incorporate silymarin into structures formed by surfactant molecules, creating micelles that enhance the solubility of its hydrophobic components, thereby improving their absorption efficiency [42]. The precise mechanism of silymarin in poultry is not well understood, and additional research is required to elucidate silymarin's mechanism in the bloodstream.

CONCLUSION

In summary, the dietary incorporation of MS extract into broilers and laying hens yielded several noteworthy benefits. In broilers, inclusion of MS particularly at the 0.06% level resulted in enhanced broiler performance as evidenced by improved BWG, voluntary FI, and enhanced nutrient digestibility, while also positively influencing tibia ash levels and certain aspects of meat quality. In laying hens, MS supplementation group led to increased absorption rates in the bloodstream compared to the powdered form, indicating its superior bioavailability. These findings collectively suggest that 0.06% MS can serve as a sustainable and valuable feed supplement within the poultry production industry, contributing to both performance enhancement and animal welfare improvement.

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Table 1. Composition of broiler diets (% , as fed-basis)

	Phase 1 (Days 1–9)	Phase 2 (Days 9–21)	Phase 3 (Days 21–32)
Ingredients, %			
Corn (7.5% crude protein)	43.82	47.64	54.01
Soybean meal (48%, crude protein)	35.04	31.28	28.13
Corn gluten meal	13.00	13.00	10.00
Wheat bran	3.00	3.00	3.00
Soy oil	1.12	1.10	0.85
MDCP ¹	1.90	1.92	1.91
Limestone	1.34	1.30	1.32
Salt	0.36	0.36	0.36
Methionine (99%)	0.19	0.18	0.19
Lysine	0.03	0.02	0.03
Mineral mix ²	0.10	0.10	0.10
Vitamin mix ³	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated value			
Metabolizable energy, MJ/kg	13.17	13.17	13.17
Available phosphorus, %	0.54	0.53	0.52
Crude protein, %	23.00	21.50	20.00
Calcium, %	1.09	1.08	1.07
Total Phosphorus, %	0.84	0.82	0.79
Lysine, %	1.25	1.15	1.06
Methionine, %	0.54	0.52	0.50
Crude fat, %	3.82	3.88	3.67
Crude fiber, %	3.55	3.49	3.30
Crude ash, %	7.26	7.05	6.80

¹ MDCP, Mono Dicalcium Phosphate.

² Provided per kg of complete diet: 37.5 mg Zn (as ZnSO₄); 37.5 mg Mn (as MnO₂); 37.5 mg Fe (as FeSO₄•7H₂O); 3.75 mg Cu (as CuSO₄•5H₂O); 0.83 mg I (as KI); and 0.23 mg Se (as Na₂SeO₃•5H₂O).

³ Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D₃, 37.5 IU of vitamin E, 2.55 mg of vitamin K₃, 3 mg of Thiamin, 7.5 mg of Riboflavin, 4.5 mg of vitamin B₆, 24 µg of vitamin B₁₂, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

Table 2. The effect of micelle silymarin inclusion on growth performance in broilers¹

Items	CON	TRT1	TRT2	TRT3	SEM	<i>p</i> value	
						Linear	Quadratic
Day 1–9							
BWG, g	146 ^b	153 ^{ab}	154 ^a	156 ^a	3	0.021	0.333
FI, g	168 ^b	180 ^{ab}	181 ^a	183 ^a	4	0.017	0.240
FCR	1.15	1.17	1.17	1.17	0.04	0.674	0.739
Day 9–21							
BWG, g	628 ^b	650 ^{ab}	654 ^a	656 ^a	7	0.011	0.170
FI, g	866	889	891	895	19	0.318	0.635
FCR	1.37	1.36	1.36	1.36	0.03	0.800	0.789
Day 21–32							
BWG, g	931 ^b	962 ^{ab}	973 ^a	980 ^a	14	0.019	0.387
FI, g	1828	1851	1852	1855	24	0.446	0.681
FCR	1.96	1.93	1.90	1.89	0.04	0.207	0.723
Overall							
BWG, g	1705 ^b	1765 ^a	1782 ^a	1791 ^a	17	0.002	0.165
FI, g	2861	2919	2924	2933	36	0.181	0.502
FCR	1.68	1.65	1.64	1.63	0.02	0.278	0.700
Mortality	4.86	1.39	2.08	1.39	1.31	0.112	0.302

¹ Abbreviation: CON, Basal diet; TRT1, Basal diet + Micelle silymarin 0.02%; TRT2, Basal diet + Micelle silymarin 0.04%; TRT3, Basal diet + Micelle silymarin 0.06%.

BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

SEM, Standard error of means.

^{ab} Means in the equivalent row show the superscripts differ ($p < 0.05$).

Means were calculated using 8 replicates (18 birds/replicate) per treatment.

Table 3. The effect of micelle silymarin inclusion on nutrient digestibility in broilers¹

Items, %	CON	TRT1	TRT2	TRT3	SEM	<i>p</i> value	
						Linear	Quadratic
Dry matter	70.00 ^b	74.43 ^a	74.78 ^a	74.92 ^a	1.24	0.011	0.098
Nitrogen	65.96	68.41	68.69	68.81	1.41	0.174	0.416
Energy	70.94	73.45	73.74	73.92	1.37	0.146	0.405

¹ Abbreviation: CON, Basal diet; TRT1, Basal diet + Micelle silymarin 0.02%; TRT2, Basal diet + Micelle silymarin 0.04%; TRT3, Basal diet + Micelle silymarin 0.06%.

SEM, Standard error of means.

^{ab} Means in the equivalent row show the superscripts differ ($p < 0.05$).

Values of means represent 18 per 8 replicate pens pooled on a pen basis (n = 8) per treatment.

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Table 4. The effect of micelle silymarin inclusion on excreta score, footpad lesion score and tibia ash in broilers¹

Items		CON	TRT1	TRT2	TRT3	SEM	<i>p</i> value	
							Linear	Quadratic
Excreta Score								
Initial		1.63	1.56	1.69	1.63	0.13	0.827	1.000
Day 9		1.63	1.75	1.69	1.75	0.20	0.735	0.879
Day 21		1.63	1.63	1.63	1.56	0.15	0.789	0.842
Day 32		1.50	1.69	1.63	1.63	0.17	0.687	0.589
Footpad lesion score		0.75	0.58	0.34	0.15	0.62	0.817	0.147
Tibia ash, %		51.40 ^b	53.61 ^a	53.71 ^a	53.78 ^a	0.65	0.038	0.139

¹ Abbreviation: CON, Basal diet; TRT1, Basal diet + Micelle silymarin 0.02%; TRT2, Basal diet + Micelle silymarin 0.04%; TRT3, Basal diet + Micelle silymarin 0.06%.

SEM, Standard error of means.

^{ab} Means in the equivalent row show the superscripts differ ($p < 0.05$).

Table 5. The effect of micelle silymarin inclusion on meat quality and organ weight in broilers¹

Items	CON	TRT1	TRT2	TRT3	SEM	<i>p</i> value	
						Linear	Quadratic
Relative organ weight, %							
Breast muscle	9.73	10.37	10.41	10.59	0.04	0.194	0.591
Liver	3.06	3.29	3.29	3.32	0.14	0.110	0.370
Spleen	0.09	0.13	0.14	0.14	0.12	0.090	0.241
Kidney	0.84	0.91	0.74	0.71	0.07	0.194	0.427
Bursa of Fabricius	0.16	0.23	0.23	0.24	0.03	0.112	0.385
Gizzard	1.42	1.15	1.57	1.16	0.18	0.461	0.909
Breast muscle color							
Lightness (L*)	57.54	55.92	54.45	53.15	1.80	0.118	0.933
Redness (a*)	10.78	12.28	11.88	12.54	1.54	0.505	0.797
Yellowness (b*)	14.69 ^b	15.72 ^{ab}	15.83 ^{ab}	16.35 ^a	0.48	0.045	0.611
pH value	7.69 ^a	7.41 ^b	7.39 ^b	7.30 ^b	0.07	0.004	0.208
Cooking loss, %	17.61	20.00	19.85	20.27	4.38	0.704	0.829
WHC, %	43.88	57.25	58.64	52.94	5.45	0.285	0.130
Drip loss, %							
Day 1	1.98	1.85	1.47	2.07	0.55	0.965	0.534
Day 3	2.81	2.78	1.93	3.54	0.54	0.602	0.179
Day 5	5.46	4.81	3.63	5.48	0.61	0.693	0.086
Day 7	6.53	6.06	4.69	6.64	0.81	0.784	0.187

¹ Abbreviation: CON, Basal diet; TRT1, Basal diet + Micelle silymarin 0.02%; TRT2, Basal diet + Micelle silymarin 0.04%; TRT3, Basal diet + Micelle silymarin 0.06%.

SEM, Standard error of means.

^{ab} Means in the equivalent row show the superscripts differ ($p < 0.05$).

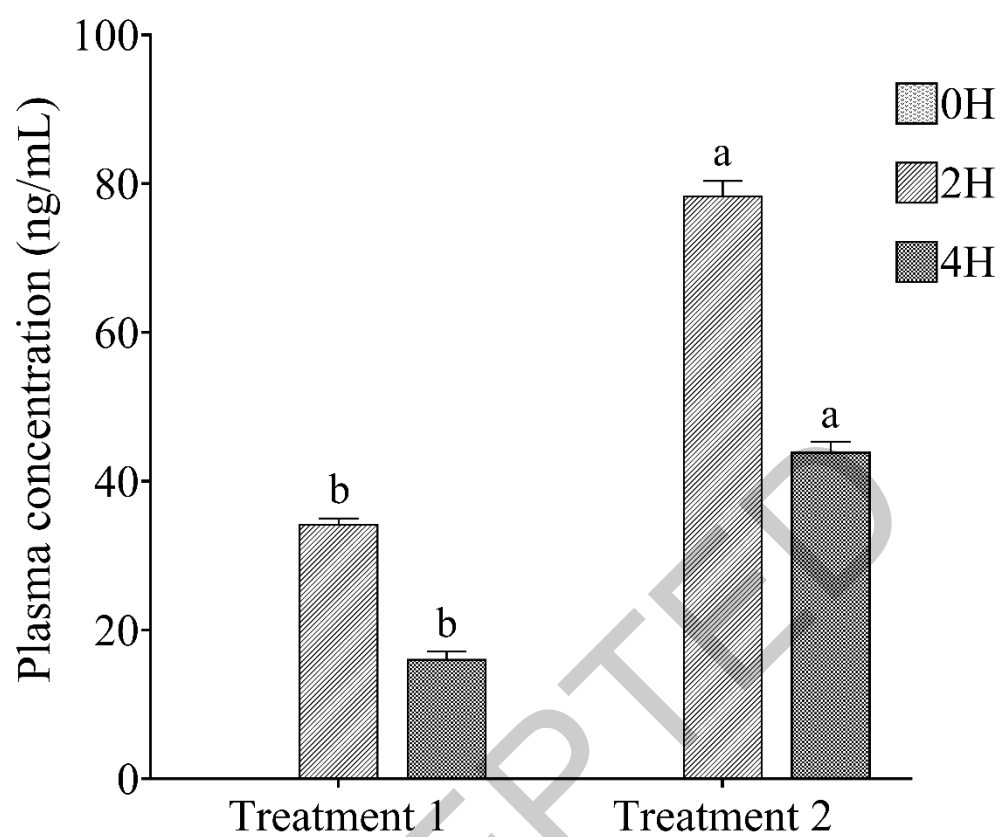


Figure 1: The effect of the blood absorption rate of regular 4% powder-type silymarin (treatment 1) and 4% micelle-type silymarin (treatment 2) of laying hens.