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Effects of dietary *Silybum marianum* powder on growth performance, egg and carcass characteristics, immune response, intestinal microbial population, haemato-biochemical parameters and sensory meat quality of laying quails

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ABSTRACT The study aimed to assess the effects of different dietary *Silybum marianum* (**SM**, milk thistle) powder levels on growth performance, productivity, immunity, small intestine, haemato-biochemical parameters, meat quality, and egg and carcass characteristics of laying quails. The experimental subjects consisted of one hundred and eight 43-day-old quails divided into 3 treatments (0, 0.75, and 1.50% SM) with 4 replicates each. The egg characteristics and growth performance of the quails were evaluated. Quails were euthanized for evaluation of carcasses, microbiota, and sensory characteristics of meat. Blood samples were analyzed for haematology and biochemical profile. *SM* at 0.75% and 1.50% significantly ($P < 0.05$) increased feed intake, enhanced egg characteristics (number, weight, width, length, volume, weight of egg yolk, and eggshell thickness), jejunum and ileum length, spleen weight, lactobacillus population, sensory characteristics of meat, red blood cell (**RBC**), hemoglobin, erythrocytic indices, concentration of albumin, globulin and thyroid stimulating hormone (**TSH**). *SM* at 0.75% and 1.50% decreased ($P < 0.05$) carcass weight (abdominal fat, heart, neck, and pancreas), feed conversion ratio (**FCR**) based on eggs produced, percentages of heterophils and lymphocytes, concentration of lactate dehydrogenase, population of coliforms clostridia, and *Escherichia coli*. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatine kinase were not significantly ($P > 0.05$) altered by 0.75% and 1.50% SM. *SM* at both levels (0.75% and 1.50%) may improve growth, egg characteristics, immune response, intestinal morphology and microbiota, meat quality and erythropoiesis, and also lead to decreased cholesterol in laying quails. Economics can be improved, too. The authors recommend adding 1.0% of SM to quail diet.

Key words: quail, *Silybum marianum*, blood and immunity, medicinal plant, egg and meat

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INTRODUCTION

The strong demand for protein brought on by the world's growing population has increased quail production (Nadathur et al., 2017). The quail bird is a significant producer of eggs and meat. Protein supply is increased by its short production cycle, high fertility rate and advantageous feed conversion ratio (**FCR**) as well as low specific farm area requirements (Ali et al., 2020; Hlatshwayo et al., 2023). Farmers use prophylactic antibiotics and growth promoters to boost production and health in the poultry industry. This practice, however, has led to the misuse of antibiotics in poultry production. The injudicious use of antibiotics by farmers, referred to as "antibiotic abuse" has increased the risk of antibiotic-resistant pathogens and antibiotic residues in the meat of poultry (Muaz et al., 2018; Owusu-Doubreh et al., 2023). This has posed a serious health risk of antimicrobial resistance to consumers; hence, triggering treatment failure and other adverse effects on human and animal health (Bacanli & Başaran, 2019; Almansour et al., 2023). While some jurisdictions have enacted stricter rules on antibiotics use in poultry production, still too much is deployed. This fact led the authors to the adoption of the exploration of different alternative additives to increase output and enhance overall health (Okey, 2023). In poultry farming alternative additives such as organic acids, probiotics, prebiotics, oils, enzymes, and medicinal plants are used (Olotu et al., 2023). Medicinal plants have natural active ingredients that have been demonstrated to improve digestion, lower cholesterol, boost immunity, increase appetite, and prevent diseases (Alp, 2023; El-Sabrout et al., 2023).

Silybum marianum (**SM**) (milk thistle) is a plant of the Asteraceae family that can grow annually or biennially. It is a medicinal plant which has been used for centuries in the treatment of disease conditions (Porwal et al., 2019; Valková et al., 2020). It has hepatoprotective, antihypertensive, anti-obesity, antiatherosclerotic, and antidiabetic properties (Marceddu et al., 2022). *SM* contains a large number of active chemical compounds concentrated in its leaves, seeds, and roots (Javeed et al., 2022). The plant contains flavonoids, tannins, oils, vitamins, and minerals (Duke, 2004; Bijak, 2017; Hashem & Kadum, 2023). Silymarin reduces aspartate transaminase (**AST**) and alanine transaminase (**ALT**) which is involved in the treatment of liver diseases (Chahkandi et al., 2023). *SM* enhances immune response and growth performance in Nile tilapia (Chaklader et al., 2024), broiler chickens (Ahmad et al 2020), rabbits (Cullere et al., 2016), catfish (Abdel-Latif et al., 2023), and ducklings (El-Garly et al., 2022). Additionally, it reduces oxidative stress in animals by boosting the body's antioxidant capacity (Guerrini and Tedesco, 2023). Information regarding the ideal dosage of *SM* to increase reproduction in laying quail is scarce.

The aim of this study was to investigate the effect of different levels of dietary *Silybum marianum* powder on growth performance, productivity, immunity, small intestine, haemato-biochemical parameters, meat quality, egg and carcass characteristics of laying quails.

MATERIALS AND METHODS

All relevant ethical guidelines were strictly adhered to by the authors. Rasht Branch, Islamic Azad University, Rasht, Iran, approved all the procedures used during this study.

Experimental Diets and Husbandry

A total of one hundred and eight 43-day-old female quails were obtained from a local hatchery and raised in an environmentally controlled pen. The birds were fed the same commercial diet from 43 to 119 d of age with a mash diet (Table 1). On day 1, 108 quails with an average body weight (**BW**) of 239.519 ± 5.355 g were used as the experimental subjects. The quails were randomly divided into 3 treatment groups (3 levels of *SM* 0, 0.75, and 1.50 %), 4 replicates per treatment, and 9 birds per replicate. The quails were fed and given access to water *ad libitum* from days (d) 43 to 119. Temperature was maintained at 24 °C and relative humidity at 55% to 65% during the study. The lighting program consisted of 19 h of light and 5 h of dark during the study period. Body weights, feed intake, and feed-per-gain ratio FCR were recorded weekly. Feed intake and egg weight were calculated weekly for each replicate during the study.

Carcass and Gastrointestinal Tract Traits

The measurements of carcass and gastrointestinal tract (**GIT**) traits were done following the methodology of Sarmad et al. (2020). In summary, 3 birds (119 d of

Table 1. Experimental diet fed to quails during the experimental period (43 to 119 d of age).

Composition	% As fed
Ingredients (%)	
Maize	58.90
Soybean meal 44% protein	30.0
Soybean oil	3.20
Salt	0.20
$CaCO_3$	5.70
$NaHCO_3$	0.10
Dicalcium phosphate	1.20
Vitamin and mineral premix[1]	0.50
DL-methionine	0.20
Calculated analysis	
Metabolizable energy (kcal/kg)	2900
Crude protein (%)	18.0
Calcium (%)	2.50
Available phosphorus (%)	0.15
Methionine (%)	0.47
Methionine + cysteine (%)	0.74
Lysine (%)	1.08

[1]Supplied per kg of feed: Retinol: 9000 IU; Cholecalciferol 2000 IU, Alpha tocopherol 18 IU, Menadione 2 mg, Thiamine 1.8 mg, Riboflavin 6.15 mg, Niacin 30 mg, Pantothenic acid 10 mg, Pyridoxine 3 mg, Biotin 0.1 mg, Folic acid 1 mg, Cobalamin 0.015; Iodine (KI), 0.76 mg; Copper (Copper sulfate), 6.36 mg; Iron (Iron sulfate), 25.12 mg; Selenium (Sodium selenite), 0.1 mg; Manganese (Manganese sulfate) 32.50 mg.

age) per replicate were chosen, weighed, and euthanized. Following their excision, the GIT and carcass were divided into the following sections: breast, neck, wings, thighs, heart, gizzard, liver, pancreas, abdominal fat, duodenum, jejunum, and ileum. Furthermore, the weight of the carcass was determined both with and without the entire GIT. Both absolute (g) and relative (% BW) weights for the organs were determined. Additionally, using a digital caliper (Mitutoyo Digimatic, Japan) with an accuracy of 1 mm, the absolute (cm) lengths of the duodenum, jejunum, and ileum were measured in all of the birds.

Blood Sampling and Analysis

Blood samples were taken via the wing vein from 12 birds per treatment (3 birds/replicate group) at 119 d of age. After being drawn into EDTA tubes, 1 mL of blood per bird was centrifuged at room temperature for 10 minutes at 3000 rpm. Following that, the plasma was kept at −20 °C until analysis. The following parameters were measured in plasma: glucose, uric acid, triglycerides, total cholesterol, high-density lipoproteins (**HDL**), low-density lipoproteins (**LDL**), LDL/HDL, very low-density lipoprotein (**VLDL**), total protein, albumin, globulin, AST, alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**), calcium, phosphorous, creatinine, thyroid stimulating hormone (**TSH**), creatine kinase (**CK**), lactate dehydragenase (**LDH**), red blood cell (**RBC**), haemoglobin, mean corpuscular volume (**MCV**), mean corpuscular haemoglobin (**MCH**) and mean corpuscular haemoglobin concentration (**MCHC**). Standard procedures for commercial laboratory kits were used to conduct biochemical analyses (Pars Azmoon Co., Tehran, Iran; Jafari-Golrokh et al. 2016).

Immune Response Assays

Plasma samples were taken from a total of 12 birds (aged 119 d) from each treatment (3 birds/replicate group). The assessment of immune markers, such as leukocytes (**LE**), heterophils (**HE**), lymphocytes (**LY**), monocytes (**MO**), eosinophils (**EO**), and basophils (**BA**), was conducted using a semi-automated cell counter following the methods of Khazaei et al (2021). Twelve birds (3 birds/replicate group) at 119 d of age were weighed individually, and euthanized. The spleen and bursa of Fabricius (*Bursa Fabricii*) were removed and weighed as immune organs. The organs were weighed and expressed in absolute (g) and relative terms (% BW).

Microbiota Analysis

Following the methods outlined by Dibaji et al. (2014), the microbes studied were Lactobacilli, Escherichia coli, Coliforms, and Clostridium bacteria. In summary, samples were spread out over the proper selective agar and allowed to incubate to obtain viable counts. Three birds from each replicate were chosen on d 14 and 119 d of age and were euthanized via cervical dislocation. The caecal segments were separated, weighed, and then transmitted in sterile Petri plates to the laboratory. To separate the gastrointestinal contents and the bacteria and to prepare the suspension, samples were transferred to sterile tubes containing phosphate-buffered saline (**PBS**) and agitated for around 30 minutes. From the produced suspension, an aliquot (1 mL) was taken out and put into PBS (9 mL) in a different tube. Similar preparations were made for the serial dilutions (1/10), and a drop of each sample was applied to the suitable agar surface. For 48 hours, inoculated plates were incubated aerobically at 37°C. Bacterial colonies were counted using a colony counter. Lactobacilli were cultured on Sharpe agar, Escherichia coli was cultured on eosin methylene blue, and Coliform bacteria were cultured on MacConkey agar. One gram of the original sample was taken into consideration for calculating the average number of bacteria. Logarithmic colony-forming units (**CFU**/g) were used to transform all quantitative data.

Meat Quality Assessment

At 119 d of age, 3 birds per duplicate were chosen and euthanized. The meat from the thighs and breasts was removed from the carcass. The meat from the breast and thighs of 3 quail per replicate was cooked at 180°C for 45 minutes without the addition of oil or spices to evaluate the sensory qualities of the meat. The cooked samples were then given a number, and a 5-person panel of trained individuals scored them on a scale of 0 to 10 for color, scent, taste, crispiness, juiciness, and overall acceptability (Azizi et al., 2022).

Egg Productivity and Characteristics Assessment

The eggs were collected twice a day at the farm. The number, weight, volume, width, length, yolk weight, albumin weight, eggshell weight, and eggshell thickness of eggs were recorded weekly and throughout the study (i.e., 43 d−119 d). The egg shape index was also calculated using the formula below:

Shape index = egg width (mm)/egg length (mm).

Statistical Analysis

By using the General Linear Model procedure of SPSS (IBM Corp., New York) 3 treatments were set up for the completely randomized design of data analysis (SPSS, 2012). *SM* served as the principal effect in the model. Utilizing the *Tukey post-hoc* test, the variations in treatment means were examined. Differences were considered significant at $P < 0.05$.

RESULTS

Growth Performance

Quails supplemented with 0.75 and 1.50% SM on d 50 to 63 and 78 to 84 showed a significantly ($P < 0.05$) lower FCR based on eggs produced when compared to the control group (Table 2), which is a desired effect that leads to cost savings for the farmer. Quails supplemented with 0.75 and 1.50% SM from d 85 to 98 showed a significantly ($P < 0.05$) higher feed intake than the control group (Table 2). FCR based on eggs produced and feed intake were increased ($P < 0.05$) on d 113 to 119 in SM-supplemented quails compared to the control (Table 2). Overall, the supplementation of quails with SM at 0.75 and 1.50% significantly ($P < 0.05$) increased feed intake and decreased FCR based on eggs produced but did not alter ($P > 0.05$) weight gain (Table 2).

Egg Traits

There was a significant ($P < 0.05$) increase in the number of eggs (at weeks 8, 9, 10, 11, 12, and 16), egg weight (at weeks 8, 9, 12, 16, and 17) weight/number ratio (at week 17), egg length (at week 8), width/length ratio (at week 11), egg volume (at week 8), weight of egg yolk (at weeks 8, 9, 10, 11, 12, 13, 14, 15 and 17) weight of egg albumen (at weeks 8, 9, 12 and 13), eggshell weight (at week 17) and eggshell thickness (at week 8) in quails given 0.75 and 1.50 % SM compared to the control group (Tables 3–4). At week 7 SM did not influence the performance and egg traits parameters (Table 3). Overall, the number of eggs, egg weight, egg width, egg length, egg volume, weight of egg yolk, and eggshell thickness were significantly ($P < 0.05$) increased by supplementation with 0.75 and 1.50 % SM compared to the control group (Table 4).

Weight of Invaluable Body Parts

Supplementing quails with 0.75 and 1.50% SM did not significantly ($P > 0.05$) change the weight of the defeathered body, breast, drumsticks, empty abdomen, wings, or gizzard (Table 5). In comparison to the control group, the weight of the pancreas, heart, neck, and abdominal fat were reduced ($P < 0.05$) in quails supplemented with 0.75 and 1.50% SM (Table 5).

Intestinal Morphology

The length of jejunum and ileum were increased ($P < 0.05$) in quails fed with diet containing 0.75 and 1.50 % SM compared to the control. The length of the duodenum, and weight of the jejunum, ileum, and duodenum were not altered ($P > 0.05$) by the supplementation of SM (Table 6).

Blood Constituents

Supplementation with 0.75 and 1.50 % SM did not significantly ($P > 0.05$) alter fasting blood sugar, HDL, LDL, LDL/HDL, uric acid, total protein, and calcium. The concentration of albumin was increased ($P < 0.05$) with 0.75 and 1.50 % SM supplementation compared to the control group (Table 7). TSH, globulin, hemoglobin, MCH, MCV, and MCHC were increased ($P < 0.05$) in the SM-supplemented quails compared to the control group. The concentrations of VLDL and RBC were not altered ($P > 0.05$) by 0.75 and 1.50 % SM (Table 7).

Liver Enzymes

Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatine kinase were not significantly ($P > 0.05$) altered by 0.75 and 1.50 % SM supplementation. The concentration of lactate dehydrogenase was decreased ($P < 0.05$) in quails fed with diet containing 0.75 and 1.50 % SM (Table 8).

Immune Response

The percentages of heterophils and lymphocytes were reduced ($P < 0.05$) in quails fed with diet containing 0.75 and 1.50 % SM compared to control quails. The percentages of LE, monocytes, eosinophils, and basophils were not altered ($P > 0.05$) by the supplementation of SM (Table 9). Supplementation with SM did not ($P > 0.05$) change the weight of bursa of Fabricius and liver. The quails fed with a diet containing 0.75% SM had an increase in spleen weight ($P < 0.05$) (Table 9).

Intestinal Microbial Population

The population of coliform, clostridium, and *Escherichia coli* were reduced ($P < 0.05$) while Lactobacillus increased ($P < 0.05$) in quails fed with a diet containing SM versus the control quails (Table 10).

Sensory Evaluation of Breast and Thigh Meat

The aroma, taste, color, and juiciness of the breast meat increased ($P < 0.05$) with supplementation of 1.50% SM. Crispy and oral admission of the breast meat were enhanced ($P < 0.05$) with 0.75 and 1.50% SM supplementation. The aroma, taste, and oral admission of thigh meat were increased ($P < 0.05$) with 0.75 and 1.50% SM supplementation. The color, crispiness, and juiciness of the thigh meat were increased ($P < 0.05$) by 1.50% SM (Table 11).

Cost-Effectiveness of Using Silybum Marianum

The profit obtained in quails supplemented with 0.75% and 1.50% SM is expected to be better than at 0% SM, since SM is a low-cost ingredient and the FCR becomes lower, which means that for the same amount of feed, the farmer will obtain more product to sell. The economics have been estimated in Table 12. For a recommendation on optimum dosage, see below.

Table 2. Growth performance of quails fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	D 43−49 Feed intake (g/quail/day)	D 43−49 Weight gain (g/quail/*d*)	D 43−49 Feed conversion ratio	D 43−49 Feed conversion ratio based on eggs produced	D 50−56 Feed intake (g/quail/*d*)	D 50−56 Weight gain (g/quail/*d*)	D 50−56 Feed conversion ratio	D 50−56 Feed conversion ratio based on eggs produced	D 57−63 Feed intake (g/quail/*d*)	D 57−63 Weight gain (g/quail/*d*)	D 57−63 Feed conversion ratio	D 57−63 Feed conversion ratio based on eggs produced
0	33.710[a]	2.265[a]	15.520[a]	1.003[a]	34.153[a]	0.670[a]	52.447[a]	0.693[a]	34.510[a]	1.280[a]	27.480[a]	0.485[a]
0.75	33.765[a]	1.985[a]	18.548[a]	1.030[a]	34.435[a]	0.805[a]	53.010[a]	0.640[b]	34.545[a]	0.742[a]	59.950[a]	0.440[b]
1.50	33.492[a]	1.790[a]	19.530[a]	0.993[a]	34.202[a]	1.070[a]	33.435[a]	0.630[b]	34.528[a]	0.857[a]	46.963[a]	0.448[b]
P-value	0.605	0.537	0.466	0.134	0.243	0.176	0.268	0.004	0.912	0.103	0.256	<0.0001
SEM	0.198	0.293	2.290	0.012	0.117	0.140	9.007	0.010	0.057	0.165	12.963	0.004
	D 64−70				D 71−77				D 78−84			
0	34.593[a]	1.008[a]	34.527[a]	0.363[a]	34.790[a]	0.670[a]	57.063[a]	0.418[a]	35.800[a]	0.613[a]	59.715[a]	0.360[a]
0.75	34.818[a]	0.895[a]	39.098[a]	0.350[a]	35.203[a]	0.795[a]	51.052[a]	0.393[a]	35.660[a]	0.675[a]	56.935[a]	0.325[b]
1.50	34.662[a]	0.882[a]	39.383[a]	0.340[a]	35.398[a]	0.620[a]	58.650[a]	0.398[a]	36.435[a]	0.585[a]	62.585[a]	0.345[b]
P-value	0.365	0.102	0.095	0.050	0.054	0.653	0.818	0.111	0.266	0.662	0.801	0.002
SEM	0.108	0.040	1.547	0.005	0.153	0.135	8.843	0.008	0.333	0.070	5.924	0.005
	D 85−91				D 92−98				D 99−105			
0	35.138[b]	0.590[a]	62.553[a]	0.365[a]	35.527[b]	0.578[a]	61.978[a]	0.320[a]	36.423[a]	0.538[a]	71.020[a]	0.325[a]
0.75	36.338[a]	0.610[a]	62.410[a]	0.372[a]	36.460[a]	0.578[a]	63.828[a]	0.320[a]	36.320[a]	0.602[a]	61.170[a]	0.328[a]
1.50	36.335[a]	0.688[a]	54.792[a]	0.358[a]	36.460[a]	0.530[a]	72.897[a]	0.325[a]	36.025[a]	0.617[a]	59.028[a]	0.320[a]
P-value	0.020	0.640	0.698	0.494	0.011	0.776	0.408	0.664	0.286	0.510	0.386	0.772
SEM	0.278	0.075	7.254	0.009	0.193	0.054	5.872	0.004	0.172	0.050	6.208	0.007
	D 106−112				D 113−119				D 43−119			
0	36.395[a]	0.615[a]	61.560[a]	0.345[a]	36.308[b]	0.550[b]	66.508[ab]	0.360[a]	35.213[b]	0.850[a]	41.328[a]	4.440[a]
0.75	36.403[a]	0.583[a]	62.893[a]	0.305[b]	36.788[a]	0.650[a]	58.288[a]	0.318[b]	35.520[a]	0.810[a]	43.870[a]	4.208[b]
1.50	36.430[a]	0.722[a]	60.065[a]	0.330[ab]	36.732[a]	0.802[a]	47.253[a]	0.315[b]	35.518[a]	0.835[a]	42.930[a]	4.230[b]
P-v1lue	0.967	0.712	0.972	0.017	0.003	0.049	0.059	<0.0001	0.006	0.584	0.422	<0.0001
SEM	0.100	0.123	8.441	0.008	0.075	0.061	4.872	0.004	0.057	0.027	1.318	0.019

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 3. Performance and egg traits of quails at 7th to 12th weeks of age fed diets containing different levels of *Silybum marianum* (SM).

	SM (%)	Number of egg (Egg/Quail/*d*)	Egg weight (Gr/*d*)	Weight / number ratio (Gr/*d*)	Egg Width (Cm/Egg)	Egg length (Cm/Egg)	Width/ length ratio	Egg volume (Cc/egg)	Weight of egg yolk (Gr/yolk)	Weight of egg albumen (Gr/albumen)	Egg shell weight (Gr/Shell)	Egg shell thickness (Mm/shell)
	0	0.325[a]	3.735[a]	11.483[a]	7.100[a]	8.175[a]	0.872[a]	9.525[b]	4.040[a]	5.950[a]	1.492[a]	0.213[a]
Seventh week of age	0.75	0.325[a]	3.638[a]	11.183[a]	7.075[a]	8.050[a]	0.880[a]	10.375[a]	4.055[a]	5.930[a]	1.198[a]	0.213[a]
	1.50	0.337[a]	3.760[a]	11.145[a]	7.075[a]	8.050[a]	0.880[a]	9.975[ab]	4.045[a]	6.030[a]	1.070[a]	0.215[a]
	P-value	0.173	0.296	0.057	0.811	0.059	0.680	0.014	0.075	0.750	0.118	0.849
	SEM	0.005	0.055	0.092	0.031	0.036	0.007	0.158	0.004	0.097	0.131	0.004
	0	0.445[b]	5.478[b]	12.218[a]	7.270[b]	8.148[b]	0.893[a]	10.775[a]	4.110[b]	6.127[b]	1.980[a]	0.260[b]
Eighth week of age	0.75	0.500[a]	5.980[a]	11.973[a]	7.607[a]	8.445[a]	0.897[a]	10.925[a]	4.148[a]	6.200[a]	1.625[a]	0.298[a]
	1.50	0.505[a]	6.060[a]	12.040[a]	7.315[b]	8.418[a]	0.867[b]	10.525[a]	4.155[a]	6.175[a]	1.710[a]	0.288[a]
	P-value	0.013	0.003	0.637	0.002	<0.0001	0.025	0.056	0.002	0.018	0.394	0.002
	SEM	0.012	0.094	0.184	0.051	0.027	0.007	0.100	0.007	0.015	0.182	0.005
	0	0.663[c]	7.890[b]	11.910[a]	7.450[a]	8.500[a]	0.875[a]	12.075[a]	4.245[b]	6.242[b]	1.423[a]	0.238[a]
Ninth week of age	0.75	0.730[a]	8.670[a]	11.878[a]	7.525[a]	8.575[a]	0.875[a]	12.125[a]	4.278[a]	6.280[a]	1.320[a]	0.253[a]
	1.50	0.695[b]	8.640[a]	12.447[a]	7.625[a]	8.500[a]	0.898[a]	11.800[a]	4.278[a]	6.267[a]	1.902[a]	0.242[a]
	P-value	0.008	<0.0001	0.071	0.050	0.291	0.087	0.358	0.003	0.009	0.087	0.596
	SEM	0.001	0.066	0.168	0.042	0.036	0.007	0.163	0.005	0.007	0.173	0.010
	0	0.883[b]	10.608[a]	11.985[a]	7.525[a]	8.550[a]	0.878[a]	12.750[a]	4.270[b]	6.275[a]	1.440[a]	0.260[a]
10th week of age	0.75	0.930[a]	11.098[a]	11.955[a]	7.600[a]	8.650[a]	0.878[a]	12.500[a]	4.298[a]	6.297[a]	1.360[a]	0.273[a]
	1.50	0.945[a]	11.242[a]	11.903[a]	7.600[a]	8.600[a]	0.883[a]	12.725[a]	4.305[a]	6.290[a]	1.307[a]	0.270[a]
	P-value	0.007	0.050	0.882	0.291	0.161	0.814	0.141	0.005	0.100	0.719	0.083
	SEM	0.011	0.161	0.117	0.036	0.033	0.006	0.088	0.006	0.007	0.114	0.004
11th week of age	0	0.765[b]	9.315[b]	12.160[a]	7.650[a]	8.550[b]	0.895[a]	13.525[a]	4.293[b]	6.305[a]	1.563[a]	0.268[b]
	0.75	0.830[a]	10.035[a]	12.095[a]	7.600[a]	8.725[a]	0.870[b]	13.275[a]	4.320[a]	6.302[a]	1.473[a]	0.295[a]
	1.50	0.815[a]	9.945[a]	12.228[a]	7.775[a]	8.675[ab]	0.898[a]	13.350[a]	4.322[a]	6.325[a]	1.580[a]	0.283[ab]
	P-value	<0.0001	0.046	0.898	0.065	0.044	0.030	0.401	0.002	0.185	0.918	0.040
	SEM	0.006	0.186	0.201	0.046	0.042	0.007	0.127	0.005	0.009	0.196	0.006
12th week of age	0	0.900[b]	11.058[c]	12.280[a]	7.800[a]	8.675[b]	0.903[a]	13.725[a]	4.323[b]	6.320[b]	1.638[a]	0.310[a]
	0.75	0.965[a]	12.153[a]	12.603[a]	7.875[a]	8.850[a]	0.893[a]	13.575[a]	4.358[a]	6.345[a]	1.900[a]	0.318[a]
	1.50	0.952[a]	11.730[b]	12.317[a]	7.800[a]	8.750[ab]	0.895[a]	13.575[a]	4.358[a]	6.353[a]	1.608[a]	0.315[a]
	P-value	<0.0001	<0.0001	0.294	0.291	0.024	0.360	0.519	0.000	0.012	0.363	0.100
	SEM	0.006	0.100	0.148	0.036	0.036	0.005	0.103	0.004	0.006	0.151	0.002

[a,b,c]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 4. Performance and egg traits of quails at 13rd to 17th and overall weeks of age fed diets containing different levels of Silybum marianum (SM).

Levels of SM (%)	NE (eggs/quail/d)	EWe (g/d)	EWe/NE ratio (g/d)	EWi (cm)	EL (cm)	EWi/EL ratio	EV (cc)	WEY (g)	WEA (g)	ESW (g)	EST (mm)
13th week of age											
0	0.863	10.722	12.413	8.000	9.025	0.888	13.925	4.338[b]	6.352[b]	1.723	0.310
0.75	0.875	10.822	12.350	8.075	9.075	0.890	13.725	4.393[a]	6.390[a]	1.568	0.315
1.50	0.872	11.320	12.982	8.075	8.975	0.900	13.850	4.358[b]	6.372[a]	2.253	0.315
SEM	0.869	0.319	0.433	0.440	0.377	0.426	0.384	0.001	0.004	0.410	0.274
P-Value	0.088	0.280	0.363	0.246	0.148	0.107	0.098	0.007	0.006	0.362	0.112
14th week of age											
0	0.933	12.395	13.300	8.100	9.125[b]	0.888	14.125	4.378[b]	6.373[b]	2.550	0.338
0.75	0.955	12.635	13.215	8.100	9.050[c]	0.895	13.950	4.448[a]	6.408[a]	2.360	0.338
1.50	0.937	12.445	13.290	8.175	9.200[a]	0.888	14.075	4.432[a]	6.385[b]	2.472	0.333[a]
SEM	0.334	0.195	0.527	0.291	0.003	0.274	0.591	0.000	0.002	0.127	0.634
P-Value	0.061	0.090	0.056	0.136	0.022	0.074	0.121	0.007	0.005	0.059	0.074
15th week of age											
0	0.948	12.478	13.157	8.250	9.150	0.900	14.250	4.418[b]	6.393[b]	2.348	0.355
0.75	0.948	12.325	12.990	8.200	9.175	0.893	14.100	4.465[a]	6.418[a]	2.108	0.353
1.50	0.953	12.548	13.175	8.225	9.200	0.893	14.400	4.462[a]	6.405[a]	2.307	0.358
SEM	0.952	0.784	0.560	0.687	0.687	0.660	0.413	0.010	0.029	0.453	0.622
P-Value	0.073	0.228	0.130	0.060	0.140	0.147	0.152	0.010	0.005	0.138	0.064
16th week of age											
0	0.928[b]	11.755[b]	12.652	8.325	9.175	0.905	14.425	4.453[c]	6.412[b]	1.788	0.368
0.75	0.980[a]	13.223[a]	13.490	8.250	9.200	0.895	14.325	4.523[a]	6.430[a]	2.538	0.370
1.50	0.937[b]	12.270[b]	13.102	8.225	9.175	0.895	14.550	4.477[b]	6.417[a]	2.208	0.373
SEM	0.012	0.290	0.129	0.430	0.811	0.432	0.527	0.000	0.030	0.168	0.676
P-Value	0.010	0.017	0.260	0.054	0.131	0.056	0.136	0.008	0.004	0.254	0.054
17th week of age											
0	0.940	11.223[b]	11.933[b]	8.325	9.325	0.890	14.525	4.498[b]	6.420[b]	1.015[b]	0.377
0.75	0.948	12.928[a]	13.633[a]	8.325	9.375	0.885	14.500	4.610[a]	6.458[a]	2.565[a]	0.385
1.50	0.953	12.982[a]	13.635[a]	8.375	9.325	0.895	14.700	4.592[a]	6.440[b]	2.602[a]	0.385
SEM	0.814	0.166	0.026	0.634	0.634	0.548	0.498	0.008	0.012	0.033	0.141
P-Value	0.064	<0.0001	<0.0001	0.242	0.242	0.056	0.126	<0.0001	0.007	<0.0001	0.053
Overall week of age											
0	0.780[b]	9.695[b]	12.315[b]	7.798[b]	8.763[b]	0.890	13.058	4.305[c]	6.288	1.723	0.300[b]
0.75	0.818[a]	10.320[a]	12.488[a]	7.838[a]	8.833[a]	0.888	13.035	4.355[a]	6.315	1.820	0.310[a]
1.50	0.810[a]	10.268[a]	12.573[a]	7.840[a]	8.805[a]	0.892	13.047	4.343[b]	6.312	1.908	0.308[a]
SEM	0.004	0.040	0.053	0.012	0.005	0.274	0.985	0.003	0.067	0.111	0.002
P-Value	<0.0001	<0.0001	0.021	0.009	0.011	0.052	0.091	<0.0001	0.068	0.055	0.001

[a,b,c]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 5. Means weight of invaluable body parts of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	Defeather Body weight (gr)	Empty abdomen weight (gr)	Breast Weight (gr)	Drumsticks Weight (thighs) (gr)	Wings Weight (gr)	Neck Weight (gr)	Heart Weight (gr)	Gizzard (ventriculus) weight (gr)	Pancreas weight (gr)	Abdominal Fat Weight (gr)
0	244.325[a]	204.875[a]	63.450[b]	26.450[a]	7.350[a]	5.200[b]	1.253[b]	4.325[a]	0.258[b]	1.305[a]
0.75	244.575[a]	204.150[a]	67.650[a]	28.400[a]	7.625[a]	5.500[a]	1.465[a]	4.600[a]	0.310[a]	1.100[b]
1.50	245.575[a]	202.475[a]	65.275[ab]	27.575[a]	7.750[a]	5.475[a]	1.395[a]	4.175[a]	0.300[a]	1.157[b]
P-value	0.854	0.172	0.016	0.119	0.396	0.011	0.001	0.062	0.000	0.000
SEM	1.653	0.839	0.805	0.594	0.202	0.060	0.024	0.110	0.006	0.022

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 6. Weight and length means of small intestine of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

	Weight (g)			Length (mm)		
SM (%)	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum
0	3.813[a]	2.255[a]	1.587[b]	35.750[b]	13.175[b]	8.500[a]
0.75	3.818[a]	2.268[a]	1.785[a]	41.075[a]	14.875[a]	9.125[a]
1.50	4.092[a]	2.512[a]	1.697[ab]	41.775[a]	14.825[a]	9.150[a]
P-value	0.267	0.109	0.028	0.002	0.016	0.113
SEM	0.129	0.086	0.042	0.869	0.374	0.220

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

DISCUSSION

The increased feed intake in quails supplemented with 0.75 and 1.50 % SM in the present study agrees with the findings of Al-Kafagy and Hammod (2021) who reported increased feed intake in quails supplemented with seeds and leaves powder of SM. Supplementation of SM increased feed intake in Japanese quails fed a diet contaminated with aflatoxin (Khaleghipour et al., 2019). The appetizing effects of SM may cause an increase in feed intake (Khazaei et al., 2022). The findings of El-Garhy et al. (2022) and Hassaan et al. (2019) that dietary supplementation with SM increased the body

Table 7. Means of blood constitutes of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	Fasting Blood Sugar (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDL /HDL ratio	Uric acid (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Calcium (mg/dl)
0	346.750[a]	241.000[a]	271.000[a]	173.250[a]	90.750[a]	0.525[a]	8.820[a]	5.500[a]	4.308[b]	15.925[a]
0.75	345.500[a]	210.250[b]	255.500[b]	182.750[a]	86.000[a]	0.468[a]	8.185[a]	5.750[a]	4.660[a]	16.700[a]
1.50	347.250[a]	225.000[ab]	263.750[ab]	183.000[a]	90.500[a]	0.495[a]	8.220[a]	6.225[a]	4.738[a]	17.225[a]
P-value	0.956	0.008	0.020	0.067	0.550	0.125	0.147	0.063	0.001	0.078
SEM	4.257	5.177	3.117	2.884	3.341	0.018	0.231	0.188	0.053	0.353

SM (%)	Phosphorus (mg/dl)	Creatinine (mg/dl)	TSH (μIU/ml)	VLDL (mg/dl)	Globulin (g/dl)	Hemoglobin (g/dl)	RBC (10*6/μL)	MCH (pg)	MCV (fL)	MCHC (g/dl)
0	9.150[b]	2.895[a]	2.238[b]	73.250[a]	2.037[b]	21.748[b]	6.623[a]	74.718[b]	176.620[b]	52.233[b]
0.75	9.925[a]	2.565[ab]	2.590[a]	71.750[a]	2.268[a]	23.320[a]	6.998[a]	77.613[a]	188.380[a]	55.963[a]
1.50	9.525[ab]	2.340[b]	2.527[a]	71.500[a]	2.365[a]	23.680[a]	7.227[a]	77.628[a]	185.347[a]	55.120[a]
P-value	0.028	0.039	0.003	0.622	0.001	0.010	0.124	0.003	0.002	0.009
SEM	0.165	0.128	0.055	1.339	0.041	0.364	0.187	0.491	1.622	0.676

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 8. Means of liver enzymes of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	Aspartat amino transferase (AST) (U/L)	Alanin amino transferase (ALT) (U/L)	Alkaline Phosphatase (U/L)	Creatine Kinase (U/L)	Lactate Dehydragenase (U/L)
0	178.500[a]	92.500[a]	362.500[a]	151.250[a]	328.000[a]
0.75	177.500[a]	87.750[a]	310.000[a]	145.500[a]	296.500[b]
1.50	172.000[a]	85.750[a]	302.750[a]	154.250[a]	291.000[b]
P-value	0.361	0.466	0.055	0.288	0.006
SEM	3.274	3.802	16.172	3.714	6.513

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 9. Means of immune response and organs related with immune system of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	Leukocytes (%)	Heterophile (%)	Lymphocyte (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Bursa of Fabricius Weight (g)	Liver Weight (g)	Spleen Weight (g)
0	9.618[a]	47.203[a]	84.063[a]	11.400[a]	12.655[a]	12.965[a]	0.283[a]	3.675[a]	0.965[b]
0.75	9.533[a]	41.993[b]	81.823[b]	11.220[a]	11.970[a]	12.555[a]	0.303[a]	3.750[a]	1.265[a]
1.50	9.595[a]	42.370[b]	82.682[b]	11.257[a]	11.880[a]	12.698[a]	0.295[a]	3.850[a]	0.973[b]
P-value	0.920	0.000	0.009	0.829	0.132	0.628	0.075	0.169	<0.0001
SEM	0.152	0.538	0.392	0.217	0.265	0.297	0.005	0.060	0.017

[a,b]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 10. Intestinal microbial population of quails at 119th d of age fed diets containing different levels of *Silybum marianum (SM)*.

SM (%)	Coliform (CFU/g) (log10)	Clostridium (CFU/g) (log10)	Lactobacillus (CFU/g) (log10)	*Escherichia coli* (CFU/g) (log10)
0	6.357[a]	5.898[a]	7.303[b]	7.905[a]
0.75	6.320[b]	5.767[b]	7.668[a]	7.865[b]
1.50	6.333[b]	5.787[b]	7.690[a]	7.808[c]
P-value	0.021	0.007	<0.0001	0.001
SEM	0.008	0.023	0.029	0.012

[a,b,c]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 11. Mean sensory evaluation of breast and thigh meat of quails at 119th d of age fed diets containing different levels of *Silybum marianum* (SM).

SM (%)	Breast meat						Thigh meat					
	Aroma	Taste	Color	Crispy	Juiciness	Oral admission	Aroma	Taste	Color	Crispy	Juiciness	Oral admission
0	4.938[b]	5.000[b]	6.000[b]	4.688[c]	6.063[b]	4.500[c]	3.000[c]	3.000[c]	5.063[b]	5.000[b]	6.000[b]	3.125[c]
0.75	5.625[b]	5.625[b]	6.625[b]	6.125[b]	6.188[b]	5.813[b]	4.000[b]	3.625[b]	5.313[b]	5.062[b]	6.125[b]	4.187[b]
1.50	6.750[a]	7.750[a]	8.062[a]	7.062[a]	6.937[a]	6.875[a]	5.125[a]	4.375[a]	6.125[a]	6.125[a]	7.250[a]	5.125[a]
P-value	0.007	<0.0001	0.001	<0.0001	0.001	0.000	<0.0001	0.001	0.001	<0.0001	<0.0001	<0.0001
SEM	0.305	0.208	0.249	0.159	0.120	0.256	0.093	0.167	0.135	0.081	0.110	0.123

[a,b,c]Values in the same column with different superscript letters are significantly different ($P < 0.05$); SEM: Standard error of mean.

Table 12. Cost-effectiveness of using *Silybum marianum* as a dietary supplement.

Item	0%	0.75%	1.5%
1. Cost per kg of feed (IRR)	236,000	236,500	237,000
2. Feed intake per bird (kg)	2.67	2.699	2.699
3. Cost of feed consumed per bird (IRR)	631580.4	638436.5	639750.2
4. Cost of *Silybum marianum* per bird (IRR)	0	10.12	20.25
5. Cost of production per bird (row 4/0.75) (IRR)	842107.2	851248.6	853000.3
6. Egg selling price per bird (IRR)	1,185,600	1,243,360	1,231,200
7. Meat selling price per bird (IRR)	213,180	203,148	209,418
8. Egg + meat selling price per bird (IRR)	1,398,780	1,446,508	1,440,618
9. Profit per bird (item 8 minus item 5; IRR)	556672.8	595259.4	587617.7

Note: All other costs were assumed constant.
IRR: Iranian rial.

weight of Muscovy ducklings and fish, respectively, disagree with the outcome of the present study. The disparity may have resulted from the different species and concentrations of SM used in the research. Silymarin in SM stimulates RNA polymerase I to boost ribosomal protein synthesis resulting in weight gain (Saller et al., 2007). Based on the eggs produced in this investigation, the FCR was found to be lower in laying hens, which is consistent with the findings of Faryadi et al. (2021). The feed consumption per unit production (eggs, meat, and milk) is measured by FCR. The overall expense of feed is improved with a lower FCR, and it is efficient for animals with low FCR to convert feed into output.

The increased egg quality (egg number, weight, width, length, volume, eggshell thickness, and weight of egg yolk) obtained by supplementation with 0.75 and 1.50% SM in the present study aligns with the findings of both Hosseini and Shalaei (2015) and Nobakht (2015). Both reported increased egg production, egg weight, egg mass, yolk color and eggshell percentage in laying hens supplemented with SM. The increase in egg quality may be due to the strong antioxidant effect of SM in enhancing the antioxidant capacity of the body, scavenging reactive oxygen species, and improving the immune responses of the birds (Serçe et al., 2016; Bendowski et al., 2022; Elnesr et al., 2023). The increase in mineral nutrients (phosphorus) may have contributed to the enhanced eggshell thickness in the present study. Phosphorus and calcium are important minerals for skeletal integrity and eggshell thickness (Skřivan et al., 2016; Dijkslag et al., 2023).

The finding of an insignificant effect of SM on the weight of some body parts of the laying quails agrees with those of Stastink et al. (2016) and Shahsavan et al. (2021) who reported the insignificant effect of SM on carcass characteristics in broiler chickens. The decreased weight of abdominal fat obtained in the present study is in line with the findings of Schiavone et al. (2007) who reported decreased abdominal fat with dried extract of SM in broiler chickens' diet. Silybin may have contributed to the reduction in fat by increasing adiponectin gene and protein expressions, which can improve β-oxidation of free fatty acids and decrease the production of new free fatty acids by hepatocytes, thereby preventing lipid accumulation (Yao et al., 2011).

The increased length of jejunum and ileum obtained in the present study with SM supplementation agrees with the findings of Kalantar et al. (2014) who showed that SM supplementation in broiler chickens significantly increased intestinal length and weight. The weight of the small intestinal segment was insignificantly affected by SM, which is in contrast to the results of Kalantar et al. (2014), who found increased small intestine weight. The amount of SM and animal species utilized in the study could be the reason for the discrepancy. Higher fiber contents in SM diets promote intestinal motility and activity, increasing length and weight of that organ (Brownlee, 2011; Laifa et al., 2022). According to Surai (2015) and Wang et al. (2020), SM possesses anti-inflammatory and antioxidant properties that enhance the health of microbiota and may contribute to improved intestinal function and features.

Hassaan et al. (2019) and Zaker-Esteghamati et al. (2021) reported increased albumin and globulin with SM supplementation in fish and broiler chickens, respectively, which is in line with the present study's findings. The increase in the proteins may be due to the stimulation of RNA polymerase I known for synthesizing ribosomal RNA (rRNA) which is translated into proteins (Blumenthal et al., 2000; Yu et al., 2018). The present findings of increased haemoglobin, and erythrocytic indices (within normal range) in the laying quails supplemented with SM. These results are in line with the findings of El-Damrawy et al. (2023) who demonstrated increased erythrocyte concentration in broiler chickens supplemented with SM to prevent the effects of aflatoxin B1. The result indicates that SM improved erythropoiesis, the process of generating red blood cells. In addition, the findings from the present study show that SM increased TSH release, resulting in enhanced thyroid hormone synthesis and release. The increase in thyroid hormone may have influenced the increased erythropoiesis. The thyroid hormone enhances erythropoiesis through hyperproliferation of immature erythroid progenitors and secretion of erythropoietin by inducing erythropoietin gene expression (Shalet et al., 1966; Bauer et al., 1998). In line with the present finding of elevated TSH in quails supplemented with SM, Ataei et al. (2024) reported increased thyroid functions with the administration of SM. Thyroid hormone also plays an important role in regulating growth and metabolism. The increase in certain growth performance indicators in quail supplemented with SM in the present study could be attributed to increased TSH (Mullur et al. 2014).

The results of Khatami et al. (2023), who showed a considerably lowered VLDL concentration in broiler chicks treated with SM, are incongruent with the insignificant effect of SM on the concentration of VLDL found in the present study. Very low-density lipoprotein is regarded as bad cholesterol and considered harmful.

El-Garhy et al. (2022) reported an insignificant effect of SM on AST, which agrees with the present study's finding. The decreased lactate dehydrogenase obtained in the present study is consistent with the report of Lukanov et al. (2018) in male Japanese quails supplemented with silymarin. Silymarin has hepatoprotective properties that are used in treating various liver diseases (Tighe et al., 2020). Silymarin has been shown in numerous studies to possess potent antioxidant properties and impede lipid peroxidation in liver toxicity caused by a broad range of agents (Surai et al., 2015; Abd Eldaim et al., 2021).

The insignificant effect of SM on some LE agrees with the finding of Lukanov et al. (2018) who reported the same effect on LE of quails. Reduced heterophils and lymphocytes could be due to the antioxidant effect of SM, which shields the body from stressors that reduce these cell types (Bendowski et al., 2022). Higher weight of the spleen was obtained with SM supplementation, indicating improved immunity in the quails. The finding is consistent with that of Morovat et al. (2016), who observed elevated weight of immune organs in heat-stressed broiler chicks given SM supplementation.

The results of Jafari et al. (2016), who reported a decrease in the population of *Escherichia coli* and an increase in the population of Lactobacillus in broiler chickens supplemented with SM, are consistent with the decrease in Escherichia coli and the increase in lactobacillus population caused by SM in the present study. It shows the antioxidant effect of SM in suppressing the population of pathogenic microbes in the intestinal tract. The increase in the population of lactobacillus increased lactic acid production making the environment more acidic. The acidic condition may be responsible for the decrease in the population of the pathogenic microbes (Zaker-Esteghamati et al., 2020).

Štastnik et al. (2016) reported increased taste and color of breast meat from broilers administered with SM,which agrees with the findings of the present study. Janocha et al. (2021) showed that SM in chicken diets had a positive effect on the increased meat flavor of the muscles. *SM* having antioxidant effects might have inhibited the action of free radicals to preserve the taste, color, juiciness, and crispiness of the meat. Bendowski et al. (2022) reported that SM increased antioxidant capacity in the pectoral muscle of broiler chickens.

Therefore, *SM* has positive effects on quails, and the results show that it can bring increased profit in the poultry industry. The profit obtained with *SM* supplemented groups can be higher than in all other groups. It shows that quails given *SM* performed significantly better than the other group, see Table 12. Detailed economics will depend on the conditions of the individual farm, and it is expected that they increase with scale. A dosage of 1% of SM is recommended from this work, which gives a sufficient safety margin for overdosing and is easy to add to feed. Future work can study the effect of SM on additional avian species.

CONCLUSION

Supplementing laying quails with 0.75 or 1.50% *SM* improved growth performance, health, productivity performance and meat quality. *SM* may be used as an additive to boost production in laying quails. The SM can contain mycotoxins as found in dietary supplements for humans, where care must be taken for avoidance. Since SM grows basically all over the world and is a nondemanding plant, it can become an interesting additive in the future. The authors recommend adding 1% of SM to the feed of quails.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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