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Evaluating chia (*Salvia hispanica* L.) seed administration for cholesterol reduction in quail (*Coturnix coturnix*)

Evaluasi pola pemberian biji chia (*Salvia hispanica* L.) dalam menurunkan kadar kolesterol pada burung puyuh (*Coturnix coturnix*)

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ABSTRACT

Chia (*Salvia hispanica* L.) seeds contain phenolic compounds such as flavonols and phenolic acids (myricetin, quercetin, kaempferol, caffeic acid) that act as primary and synergistic antioxidants. These antioxidants reduce cholesterol by inhibiting absorption in the intestine and enhancing bile acid formation, facilitating cholesterol excretion through feces. Additionally, the high protein and fiber content in *S. hispanica* L. seeds reduces appetite and promotes prolonged satiety. This study evaluates the effect of different administration patterns of *S. hispanica* L. seeds on cholesterol reduction in common quail (*Coturnix coturnix*). The research involved five groups: three treatment groups receiving *S. hispanica* L. seeds at varying frequencies (once, twice, three times daily) and two controls (positive, negative), with six *C. coturnix* in each group. The *S. hispanica* L. seed dose was 1.8 mg per 200 g body weight (BW) administered for 30 days. Cholesterol levels were measured at baseline and after treatment and analyzed using one-way ANOVA and Duncan's Post Hoc Test. Results indicated that administering *S. hispanica* L. seeds three times daily significantly reduced cholesterol levels ($p < 0.05$) compared to other frequencies. This research provides a foundation for further exploration into practical applications of *S. hispanica* L. seeds in cholesterol management, both in animals and potentially in humans.

ABSTRAK

Biji chia (*Salvia hispanica* L.) diketahui mengandung senyawa fenolik seperti flavonol dan asam fenolat (mirisetin, kuersetin, kaempferol, dan asam kafeat) yang berperan sebagai antioksidan utama dan sinergis. Antioksidan ini menurunkan kolesterol dengan menghambat penyerapannya di usus dan meningkatkan pembentukan asam empedu, sehingga memfasilitasi ekskresi kolesterol melalui feses. Selain itu, kandungan protein dan serat yang tinggi pada biji *S. hispanica* L. dapat mengurangi nafsu makan dan memberikan efek kenyang yang lebih lama. Penelitian ini mengevaluasi efek pola pemberian biji *S. hispanica* L. yang berbeda terhadap penurunan kolesterol pada burung puyuh (*Coturnix coturnix*). Penelitian melibatkan lima kelompok: tiga kelompok perlakuan yang menerima biji *S. hispanica* L. dengan frekuensi berbeda (satu kali, dua kali, dan tiga kali sehari) dan dua kelompok kontrol (positif, negatif), masing-masing terdiri dari enam *C. coturnix*. Dosis biji *S. hispanica* L. yang diberikan adalah 1.8 mg per 200 g berat badan (BB) selama 30 hari. Kadar kolesterol diukur pada awal dan setelah perlakuan serta dianalisis menggunakan uji ANOVA satu arah dan uji lanjut Duncan. Hasil menunjukkan bahwa pemberian biji *S. hispanica* L. tiga kali sehari secara signifikan menurunkan kadar kolesterol ($p < 0.05$) dibandingkan frekuensi lainnya. Penelitian ini memberikan dasar untuk eksplorasi lebih lanjut mengenai aplikasi praktis biji *S. hispanica* L. dalam pengelolaan kolesterol, baik pada hewan maupun manusia.

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INTRODUCTION

The consumption of chia seeds (*Salvia hispanica* L.) has become increasingly popular due to their association with weight loss and maintenance of ideal body weight. Besides their distinct appearance, *S. hispanica* L. seeds have been reported to offer various health benefits. Numerous studies have highlighted the potential of *S. hispanica* L. seeds in promoting weight reduction, making them a promising candidate for the development of functional food products (Safari et al., 2016).

S. hispanica L. seeds, native to Central America, particularly Mexico and Guatemala, are rich in essential nutrients, including protein (15–25%), fatty acids (30–33%), carbohydrates (26–41%), fiber (18–30%), and minerals (4–5%). Notably, Ganachari et al. (2022) reported differences in moisture content between white and black *S. hispanica* L. seeds, with values of 11.12% and 10.30%, respectively. Moreover, *S. hispanica* L. seeds are an excellent source of omega-3 fatty acids, specifically alpha-linolenic acid, comprising 17.83% of their composition (USDA-AGS-SR21, 2014). Recognized as a novel food by the European Parliament and Council in 2009, *S. hispanica* L. seeds are considered safe for human consumption, posing no significant risks of adverse or allergic reactions (Turck et al., 2019).

The rich composition of *S. hispanica* L. seeds, including dietary fiber, protein, antioxidants, essential fatty acids, and phenolic compounds, enhances their potential as a functional food source. Their bioactive phenolic compounds, such as flavonols and phenolic acids (myricetin, quercetin, kaempferol, and caffeic acid), contribute to their health benefits. These phenolic compounds are key antioxidants that synergistically provide high antioxidant activity (Hrnčič et al., 2020). The antioxidant properties of *S. hispanica* L. seeds not only inhibit cholesterol absorption in the intestine but also promote bile acid formation, facilitating the excretion of cholesterol through feces (Anakonda et al., 2019).

One of the critical public health challenges today is the rising prevalence of obesity in Indonesia. Childhood obesity has also surged, with one in five primary school children and one in seven adolescents being overweight or obese (Kemenkes RI, 2018). Obesity correlates with increased cholesterol levels, which serve as a marker for atherosclerosis (Anakonda et al., 2019). Elevated vascular cholesterol levels result in reduced elasticity and narrowing of blood vessels, leading to arterial blockages. This, in turn, increases the likelihood of atherosclerosis, characterized by the accumulation of fat, thrombocytes, macrophages, and white blood cells in the arterial walls, particularly in the intima and media layers. Common sites for atherosclerosis include coronary arteries, the aorta, and cerebral arteries, all of which can lead to vascular diseases or heart conditions (Laila et al., 2022).

Given that cholesterol is a major contributing factor to vascular and cardiac diseases, and *S. hispanica* L. seeds have gained attention as a potential dietary solution, this study investigates the effect of *S. hispanica* L. seed consumption on cholesterol levels in male *C. coturnix* by varying the frequency of administration. While previous studies on *S. hispanica* L. seeds have focused on their impact on weight loss, high-density lipoprotein (HDL) cholesterol, and dietary patterns (de Souza Ferreira et al., 2015; Dickens et al., 2023; Helal et al., 2023; Munir et al., 2021; Rodrigues et al., 2018), this research emphasizes the administration pattern—once, twice, and three times daily—and evaluates its effectiveness in reducing total cholesterol levels.

MATERIALS & METHODS

Materials

The materials used in this study included male *C. coturnix*, *S. hispanica* L. seeds, and high-fat feed (HFF). Male *C. coturnix*, aged 2–3 months with an average body weight of 100–200 g, were procured from a local poultry farm in Pekanbaru, Indonesia. These birds were chosen as the experimental animals due to their established use as a model for cholesterol studies (Baer et al., 2015). *S. hispanica* L. seeds were sourced from a certified agricultural supplier in Central Java, Indonesia. These seeds were selected as the primary test material for cholesterol reduction because of their high content

of bioactive compounds, including flavonoids and phenolic acids, known for their antioxidant properties and cholesterol-lowering effects.

The HFF was prepared as a cholesterol-inducing diet by mixing standard *C. coturnix* feed with melted beef fat, duck eggs, and propylthiouracil (PTU). The standard *C. coturnix* feed was obtained from a local feed supplier, while the beef fat and duck eggs were purchased from a local market in Pekanbaru. PTU, a pharmacological agent used to inhibit thyroid function and induce hypercholesterolemia, was sourced from a pharmaceutical distributor in Jakarta, Indonesia. All materials were handled and stored under controlled laboratory conditions to maintain their quality and ensure consistency throughout the study.

Preparation and characterization of S. hispanica *L. seeds*

In this study, a total of 2 kg of *S. hispanica* L. seeds were utilized. The seeds underwent sorting and characterization at the chemistry laboratory of the Health Polytechnic of the Ministry of Health, Riau. The characterization process included several analyses to determine the chemical and physical properties of the seeds. The moisture loss of the *S. hispanica* L. seeds was measured by placing 1 gram of seeds in a closed porcelain crucible that had been preheated at 105°C for 30 minutes. The seeds were evenly distributed to a thickness of 5–10 mm and dried in an oven at 105°C until a constant weight was achieved. After cooling in a desiccator, the moisture loss was calculated as a percentage of the total weight. This experiment was conducted in triplicate to ensure reliability (Kementerian Kesehatan RI, 2017).

Ash content was determined by incinerating 1.5 grams of *S. hispanica* L. seeds in a preheated silica crucible until all carbon residue was eliminated. The sample was cooled to room temperature and weighed until a constant weight was achieved. The ash content was expressed as a percentage, with three replications performed for consistency (Kementerian Kesehatan RI, 2017). Water content was analyzed using a moisture balance. Two grams of *S. hispanica* L. seeds were weighed, and the water content was determined following the standard procedure (Rani et al., 2015).

Organoleptic properties, including shape, color, taste, and odor, were assessed through sensory evaluation by the research team. This qualitative analysis provided a description of the *S. hispanica* L. seeds' physical attributes, such as their round shape, grayish-brown color, odorless nature, and bland taste (Departemen Kesehatan RI, 2000; Singh & Pandey, 2020). The total flavonoid content of the *S. hispanica* L. seeds was measured using a colorimetric method involving an aluminum chloride complex reagent (Sigma-Aldrich, USA). A standard quercetin solution (1000 ppm) was prepared using quercetin dihydrate (Merck, Germany) and diluted into a series of concentrations (40, 60, 80, 120, and 140 ppm). One milliliter of each dilution was mixed with 1 mL of 10% aluminum chloride ($AlCl_3$) (Sigma-Aldrich, USA) and 8 mL of 5% acetic acid (Merck, Germany). The mixture was incubated for 35 minutes, and the absorbance was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific, USA) to quantify the flavonoid content (Kementerian Kesehatan RI, 2017).

Animal acclimatization

Male *C. coturnix*, aged 2–3 months and weighing 100–200 g, were acclimatized for one week to adapt to laboratory conditions. A total of 25 *C. coturnix* were used, divided into five treatment groups with six *C. coturnix* per group. During acclimatization, their body weight was recorded before and after adaptation, and standard feed and water were provided ad libitum (BPOM, 2021).

Preparation of high-fat feed (HFF) and propylthiouracil (PTU)

HFF was prepared by mixing 4 kg of standard *C. coturnix* feed with 1 kg of melted beef fat and four duck eggs. PTU was prepared as a suspension with a concentration of 0.13%, using a dose of 1.8 mg per 200 g body weight. The PTU suspension was homogenized with 0.5% sodium carboxymethyl cellulose (Na-CMC) (Sigma-Aldrich, USA) and distilled water (Arifin et al., 2015).

Pharmacological activity test

S. hispanica L. seeds were administered at a dose of 1.8 mg per 200 g body weight daily for 30 consecutive days. The *C. coturnix* were divided into five treatment groups to evaluate the effects of different administration patterns on cholesterol levels. The first group served as the normal control and did not receive any treatment. The second group, designated as the negative control, received a high-fat feed (HFF) combined with propylthiouracil (PTU). The third group received HFF and PTU along with *S. hispanica* L. seeds administered once daily, specifically in the morning. The fourth group received HFF and PTU with *S. hispanica* L. seeds administered twice daily, in the morning and evening. Finally, the fifth group received HFF and PTU with *S. hispanica* L. seeds administered three times daily before meals (morning, afternoon, and evening).

On the 31st day, after completing the treatment period, the *C. coturnix* were fasted for 24 hours to stabilize their metabolic state. Following this fasting period, the *C. coturnix* were euthanized via cervical dislocation, and blood samples were collected through neck dissection. The collected blood samples were stored in hematocrit tubes for subsequent cholesterol analysis (White, 2021).

Total cholesterol determination

Total cholesterol levels were measured using serum obtained from blood samples centrifuged at 5000 rpm for 15 minutes to separate serum from blood cells. A cholesterol reagent (1000 µL; Greiner, Germany) was mixed with 10 µL of serum in a reaction cuvette and incubated at room temperature for 10 minutes. Absorbance was measured at a wavelength suitable for cholesterol determination 495 nm using a photometer (5010 V5+, Riele, Germany). The instrument was calibrated using a cholesterol standard (200 mg/dL; Greiner, Germany) before analysis (Abdillah et al., 2022).

RESULTS & DISCUSSION

The characteristics of the *S. hispanica* L. seeds used in this study are summarized in Table 1. The results indicate that the seeds exhibit a moisture loss of 8.98 ± 0.26%, water content of 4.4 ± 1.29%, ash content of 4.93 ± 0.17%, and total flavonoid content of 0.0182%. Organoleptic observations revealed that the *S. hispanica* L. seeds have a smooth, glossy surface, are round in shape, and range in color from grayish-brown to dark. These findings align with previous reports that describe *S. hispanica* L. seeds as small, oval, and flat, with average dimensions of 2–2.5 mm in length, 1.2–1.5 mm in width, and 0.8–1 mm in thickness (Ganashri et al., 2022).

Table 1. Characteristics of *S. hispanica* L. seeds (n = 5).

Parameter	Value
Moisture loss (%)	8.98 ± 0.26
Water content (%)	4.4 ± 1.29
Ash content (%)	4.93 ± 0.17
Organoleptic properties	Round shape, grayish-brown color, odorless, bland taste
Total flavonoid (%)	0.0182 ± 0.06

Note: All data are expressed as means ± standard deviation, except organoleptic properties, which are reported qualitatively.

S. hispanica L. seeds are rich in phenolic compounds, including myricetin, quercetin, kaempferol, and caffeic acid, which act as key antioxidants with synergistic effects. These compounds play a significant role in promoting human health by reducing oxidative stress and improving lipid metabolism (Hrnčič et al., 2020). The flavonoid content in *S. hispanica* L. seeds was determined to be 18.20 mg/100 g using the colorimetric method, a simple and sensitive assay suited for low-concentration samples. Flavonoids have been shown to inhibit cholesterol ester transfer protein (CETP), thereby increasing HDL cholesterol levels, and reducing low-density lipoprotein (LDL) cholesterol levels (Trisnandi et al., 2022). This mechanism involves disrupting the transfer of cholesterol esters from HDL to LDL, resulting in improved lipid profiles and cardiovascular protection (Fielding & Havel, 2015).

The experimental *C. coturnix* were successfully acclimatized, with no significant weight changes exceeding 10% during the adaptation period. This ensured the health and suitability of the animals for subsequent testing. *S. hispanica* L. seeds were administered in different frequencies (once, twice, and three times daily) at a dose of 1.8 mg per 200 g body weight over a 30-day period. On the 31st day, the *C. coturnix* were fasted for 24 hours before blood collection and analysis. The results for total cholesterol levels are presented in Table 2.

Table 2. Total cholesterol levels (mg/dL) after *S. hispanica* L. seed administration (n = 6)

Group	Cholesterol level (mg/dL)
I	176.00 ± 3.67
II	341.40 ± 3.10
III	238.80 ± 8.23
IV	209.80 ± 1.30
V	203.20 ± 4.38

Note: All data are expressed as means ± standard deviation.

The results reveal significant differences in cholesterol levels among the groups. The negative control group exhibited the highest cholesterol levels (341.40 ± 3.10 mg/dL), reflecting the hypercholesterolemic effects of the high-fat feed (HFF) and PTU. In contrast, the administration of *S. hispanica* L. seeds three times daily (203.20 ± 4.38 mg/dL) resulted in cholesterol levels closest to those observed in the normal control group (176.00 ± 3.67 mg/dL). Statistical analysis using one-way ANOVA and Duncan's post hoc test confirmed that the three-times-daily administration was significantly more effective in reducing cholesterol levels than once- or twice-daily administration ($p = 0.000$).

The efficacy of *S. hispanica* L. seeds in reducing cholesterol can be attributed to their high antioxidant activity and bioactive compounds. Phenolic acids, such as myricetin and quercetin, act as primary antioxidants, enhancing bile acid formation and facilitating cholesterol excretion via feces (Ullah et al., 2016). Additionally, the protein and fiber content in *S. hispanica* L. seeds contributes to reduced appetite and prolonged satiety, indirectly supporting cholesterol reduction through improved dietary regulation. Previous studies have also highlighted the benefits of *S. hispanica* L. seeds in managing cardiovascular risks. For instance, *S. hispanica* L. seed extracts have been shown to improve blood lipid profiles and control hyperlipidemia caused by high-fat diets (Munir et al., 2021). Similarly, *S. hispanica* L. seeds have been reported to increase HDL cholesterol levels naturally, further supporting their role in lipid metabolism (Dickens et al., 2023). The results of this study corroborate these findings, emphasizing the importance of optimizing *S. hispanica* L. seed administration patterns to achieve maximum efficacy.

The results of this study indicate that administering *S. hispanica* L. seeds three times daily is the most effective pattern for cholesterol reduction. This finding provides a strong foundation for exploring the broader applications of *S. hispanica* L. seeds as a functional food ingredient in cholesterol management. However, further research is needed to investigate the long-term effects of *S. hispanica* L. seed consumption and its potential translation to human dietary interventions.

CONCLUSIONS

The study demonstrates that *S. hispanica* L. seeds effectively reduce cholesterol levels in male *C. coturnix* induced with hypercholesterolemia using a high-fat diet and propylthiouracil (PTU). Group I (normal control) exhibited the lowest cholesterol levels (176.00 ± 3.67 mg/dL), serving as the baseline for normal cholesterol levels in the test animals. In contrast, Group II (high-fat diet with PTU, negative control) showed the highest cholesterol levels (341.40 ± 3.10 mg/dL), confirming that a high-fat diet with PTU significantly increases cholesterol levels. The addition of *S. hispanica* L. seeds demonstrated a cholesterol-lowering effect compared to the negative control group. Group III (*S. hispanica* L. seeds administered once daily) reduced cholesterol levels to 238.80 ± 8.23 mg/dL, while Group IV (*S. hispanica* L. seeds

administered twice daily) further reduced cholesterol levels to 209.80 ± 1.30 mg/dL. Group V (*S. hispanica* L. seeds administered three times daily) showed the most significant reduction in cholesterol levels, reaching 203.20 ± 4.38 mg/dL, which approached the levels of the normal control group. A dose-dependent effect was observed, with increasing the frequency of *S. hispanica* L. seed administration from once to three times daily resulting in progressively lower cholesterol levels. These findings suggest that *S. hispanica* L. seeds are an effective intervention for lowering cholesterol levels induced by a high-fat diet with PTU, with the optimal results achieved through three-times-daily administration. This study highlights the potential of *S. hispanica* L. seeds as a functional food for managing hypercholesterolemia.

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ETHICS STATEMENT

This study was conducted in accordance with ethical guidelines for animal research and was approved by the Ethics Committee of Poltekkes Kemenkes Riau, Pekanbaru, Indonesia, under approval number LB.02.03/6/06/2023. All experimental procedures involving animals adhered to the principles outlined in the Declaration of Helsinki and the internationally recognized guidelines for the care and use of laboratory animals. Efforts were made to minimize animal suffering, and all *C. coturnix* were acclimatized prior to the study to ensure their well-being. Blood collection and euthanasia were performed humanely following standard protocols.

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