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Modulation of Intestinal Histology and Immune Functions Using Different Level of *Salvia Indica* Supplementations with Stocking Density in Local Quails

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Abstract:

Under the direction of Salahaddin University in Erbil's College of Agricultural Engineering Sciences, study was carried out to investigate how local quail performed biochemically, histologically, and immune-wise when varied levels of *Salvia indica* (SI) additive were added at high stocking density. For a 42-day trial, 216 one-week-old local quails were divided into four treatments at random, with three replicates for each treatment, in cages measuring 60 by 30 by 25 centimeters. The following was the design of the feed treatments: Positive control (12 birds/replicate), Negative control 20 birds per replicate at a high stocking density, 20 birds per replicate plus 0.2% of SI additive, and 20 birds per replicate plus 0.4% of SI additive. When compared to a negative control, the results indicated that 0.4% SI supplements with high stocking density produced the best live body weight and weight gain. When compared to positive and negative controls, the cholesterol level of female quails considerably ($P<0.05$) decreased in both SI with high stocking density; however, no significant variations were seen with either of the SI additives with high stoking density. Furthermore, when compared to the positive control, the LDL content in female quails was considerably ($P<0.05$) reduced by both supplements of SI with high stocking density and negative control. When treated with SI, the height of the jejunum villus was significantly ($P<0.05$) higher than when treated with both positive and negative controls. The size of Fabricius follicles was significantly ($P<0.05$) enhanced by the addition of SI treatments, but no significant difference was seen between the two SI additives with high stocking density. In summary, adding *Salvia indica* to a diet with high stocking density enhanced the quail population's biochemical markers, immunological response, and jejunum histology.

1.Introduction

Because of its small size, ease of handling, ability to house a large number of birds in a small space, and high egg production, Japanese quails (*Coturnix coturnix japonica*) have gained importance in the avian kingdom. Levels of production system intensification increased in tandem with the rise in quail populations.

Poultry farmers prefer to increase the number of birds per unit of land, known as stocking density, in order to reduce the expenses associated with housing, equipment, and labour. Estevez (2007) states that excessive stocking density has detrimental effects that include reduced feed consumption, feed conversion ratio, and final weight as well as an increase in foot-pad dermatitis, scratches, worse feathering, bruises, and condemnations. High stocking densities, however, may have a detrimental impact on bird welfare, economics, and productivity, according to study. According to Mtileni *et al*. (2007), hens raised in dense populations mature more slowly, lay fewer eggs, and experience a greater death rate. There are a number of feeding strategies that have been tried to reduce stress's negative effects on hens.

One species of herbaceous perennial plant in the Lamiaceae family is *Salvia indica*. It is indigenous to a large area of Western Asia, which encompasses Iran, Turkey, Palestine, and Iraq. Carl Linnaeus, a taxonomist, wrote the first description of it in 1753. Since the plant is not native to India, it is unclear why he gave it the specific epithet indica. Although *Salvia indica* is categorized as an herbaceous perennial, individual plants frequently die within two years of being in cultivation (Clebsch *et al*., 2003).

Alzawi *et al*. (2021) carried out the research. They found that *S. indica* contained 21 different compounds, including methyl β-D-galactopyranoside (18.3%), 9-Octadecenoic acid (17.4%), and cis-Vaccenic acid (11.0%), in addition to smaller amounts of hexadecanoic acid (5.3%), hexadecanoic acid 5.29%, and β-Eudesmol (4.2%). 0.9% of 2-pentadecanone. Plants are used to help birds feel less stressed. *Salvia indica*, a member of the Labiatae family and one of the world's oldest medicinal herbs, is used in both Eastern and Western civilizations. It is widely used in herbal medicine and is believed to be particularly beneficial in fighting secondary infections and enhancing immunity (Nanekarani *et al*., 2012).

Consequently, the aim of this research was to investigate the influence of various levels of *S. indica* as feed additives with high stocking density on the growth performance and intestinal histology and immune function of local quails.

2. MATERIAL AND METHODS

2.1 Experimental Design

This trail is conducted in Grdarasha Field Research, College of Agricultural Engineering Sciences, Salahaddin University, Erbil. The research was conducted to find the influence of adding different levels of *Salvia indica* (SI) with high stocking density on performance, biochemical, histology and immune function in local quail. A number of 216 one-week old local quails were distributed randomly into four treatments with three replicates per each in cages (60×30×25 cm) for forty-two days of age (35 days trial). The dietary treatments were design as follows; Positive control (12 birds/replicate), Negative control (20 birds/replicate), 0.2% of SI additive with stocking density (20 birds/replicate) and 0.4% of SI additive with stocking density (20 birds/replicate). The chicks had unlimited access to feed and water. The control diet's ingredients are listed in (Table 1).

Table 1: The typical diets composition for the trial (%).

Ingredients	(%)
Corn	19.5
Soybean meal	34.1
Wheat	40.0
Limestone	1.5
Sunflower Oil	1.6
Di calcium phosphate	0.6
Enzyme	0.1
Premix[1]	2.5
Anti-oxidant	0.1
Total	100%
Value calculated [2]	
ME (kcal/kg)	3000 kcal/kg
Crude Protein %	23%
Lysine %	1.19%
Methionine %	0.47%
Calcium %	0.81%
Available phosphate %	0.45%

[1]Premix: each 1 kg contain: Crude protein15%. Crude fat 2%. Crud fiber4%. Calicium4% Phosphorus(Av.) 8.6%. Methionine5.2%. Methionine+cystine5.4%. Lysine2.5%. sodum5%. VitA480.000IU. VitD3120.000IU. VitE1000mg. Vit K80mg. Vit B180mg. Vit B240mg. Vit B3,600mg. Vit B6,120mg. Vit B12 1mg. Niacin1.200mg. Folic acid, 40mg. Biotin,6mg. CholinChlorid12.000mg. Iron,2.800mg. Zinc2.400mg. Copper320mg. Manganese3.000mg. Iodine1100mg. Selenium 8mg.

[2] Feed requirements were estimated according to (NRC, 1994).

2.2 Growth Performance

Body weight and Feed intake was tracked during the whole experiment, and the formula for calculating feed conversion ratio (FCR) was derived by dividing feed intake per unit by weight increase. Weighing the carcass is also done.

2.3 Biochemical Parameters

At day 42 of each treatment, six birds (Three males and three females) were chosen randomly, and blood samples were obtained from the wing vein and preserved in sterile tubes for the measurement of serum biochemical parameters and lipid profiles (triglycerides, LDL HDL, and cholesterol) using an instrument known as the Roche Cobas® 6000 analyzer series (Switzerland).

2.4 Examining the Jejunum and Fabricius Bursa Histologically

At the end of the trial, six birds were randomly chosen from each group and killed via cervical dislocation. For histological analysis, the Fabricius bursa and the jejunum portion of the small intestine were separated and preserved in 10% buffered formalin. The processing involved wax impregnation, sequential dehydration, and clearing. After cutting the microtome into five µm thick pieces, the tissue was mounted onto slides. Hematoxylin and eosin were used to carry out a regular technique that is conventional. The stained slides were examined under a standard light microscope with 10X magnification using a light microscope and digital camera. Image J software was used to assess the area of follicles in Fabricius pictures, villus height in the jejunum, and crypt depth (µm) (Mirza, 2015).

2.5 Statistical Analysis

One-way ANOVA analysis was used to evaluate the data using software that used SPSS version 27 (SPSS, 2020). The summary statistics data comprised means and standard error. The significant differences between the various parameters were determined using the Duncan's test at 0.05 levels (Duncan, 1955).

3.RESULTS AND DISCUSIONS

The results of this investigation showed that adding *Salvia indica* as a supplement to the diet did not improve the growth performance of local quails. The best live body weight and weight gain were recorded for 0.4% SI supplements with high stocking density compared with negative control (High stocking density). Additionally, at the end of the trial, there were no appreciable variations in the carcass weight of the local quails. While, the feed intake of Negative Control decreased significantly (P>0.05) compared to Positive Control and 0.4% SI supplements with high stocking density (Table 2).

Table 2: Effect of adding *Salvia indica* plants on the growth performance and Dressing Percentage of local quails at six weeks of age (Mean ± standard error).

Growth performance	Treatment				P. value
	Positive CON	Negative CON	0.2% SI	0.4% SI	
Initial weight (g)	65.22±1.15 [a]	65.66±1.30 [a]	64.25±0.66 [a]	65.08±0.60 [a]	0.781
Final weight (g)	223.48±1.65 [a]	213.64±3.09 [a]	219.77±6.12 [a]	224.23±1.38 [a]	0.223
Weight gain (g/bird)	158.26±0.61 [a]	147.97±2.73 [a]	155.52±6.78 [a]	159.15±0.80 [a]	0.210
Feed intake (g/bird)	511.22±8.26 [a]	483.85±9.32 [b]	509.48±9.80 [ab]	527.95±8.14 [a]	0.047
FCR*	3.23±0.49 [a]	3.27±0.89 [a]	3.29±0.21 [a]	3.31±0.06 [a]	0.960
Dressing Percentage %	55.99±2.46 [a]	58.06±2.48 [a]	57.11±0.99 [a]	57.59±1.70 [a]	0.898

* FCR: Feed conversion ratio.

[ab] Significant differences (P<0.05) are seen across data with different letters in the same row direction.

The weight of quails in Negative control decreased but not significantly, while using SI as additive in the diet of quails in high stocking density fixed this problem may be due to content of antioxidant in the plant to decrease the stress on the birds. An alternative to antibiotic growth promoters it can be use phytogenic plants. Animal performance is improved by the action of phytogenic ingredients. According to the current study, quail development and FCR are marginally improved when *Salvia indica* leaves are added to diets in comparison to the Negative control group (high stocking density).

Table 3 showed that there were no significant (P>0.05) differences on the cholesterol content and LDL observed among the treatments in the male quails. While, triglyceride significantly (P<0.05) decreased in SI with high stocking density compared with all other treatments. The HDL content in male quails significantly (P<0.05) was increased in SI with high stocking density and negative control which was high stocked density compared with positive control. While, there the LDL content in male quails compared will all other treatments except positive control. While, the cholesterol content in female quails significantly (P<0.05) decreased in both SI with high stocking density compared with positive and negative controls but not significant differences were observed with both SI additives with high stoking density. While, the triglyceride content in female quails significantly (P<0.05) decreased in adding of 0.2% SI with high stocking density compared will all other treatments. Also, both additives of SI with high stocking density and positive control significantly (P<0.05) increased the HDL content in female quails compared with negative control. While, both additives of SI with high stocking density and negative control significantly (P<0.05) decreased the LDL content in female quails compared with positive control.

Earlier study looked at a variety of feed additives to determine whether it was possible to maximize the amount and quality of the finished goods without compromising the effectiveness of the birds' meal (Nunes *et al*., 2010; Torki *et al*., 2021). The research's findings were supported by the data, which showed that include medicinal herbs in broiler diets had a beneficial impact on FI without affecting BWG because it improves FCR. Bird density had a significant impact on the production index as a growth performance indicator during the last week of the birds' lives, especially during the high density (Tayeb *et al*., 2011). The salvia plant is an essential member of the Lamiaceae family of plants and contains flavonoids and phenols. The maintenance of growth performance in birds with high stocking density following the addition of salvia plants as supplements might be attributed to an increase in enzymatic activity leading to an improvement in the metabolic system.

Moreover, the results are in agreement with (Farhadi *et al*., 2020) who found that cholesterol decreased from 153.75 to 127.50 mg/dL when 0.2% sage was added to the broiler diet. Furthermore, El-Garhy (2018) found that the chicks treated with 12 g salvia powder/kg diet had the lowest plasma triglyceride, cholesterol, HDL, and LDL levels.

According to the results of studies showed that cholesterol not significantly (P>0.05) increased in Salvia supplementation with high stocking density compared to the to both groups (Mustafa and Ihsan, 2022). The research using *Salvia indica* with HSD on local quails and broiler's biochemical blood parameters is seldom. To discover more of *Salvia indica* beneficial effects, additional study is needed. These findings also lend support to the use of medicinal herbs as they can reduce bird stress, minimize raring costs, and increase economic returns.

The effects of *Salvia indica* on the quail's histology of the jejunum portion of the small intestine at 42 days of age were displayed in (Table 4**)**. This stress was caused by excessive stocking density. When compared to both positive and negative controls, the height of the jejunum villus improved statistically (P<0.05) in those treated with SI. The largest villi and crypt depth were observed at a rate of 0.4% SI. Also, by increasing the rate of SI in 0.2% to 0.4% significantly increased the villus high compared each other. While, only negative control significantly reduced crypt depth compared all other treatments. At the end of the study, Figure (1) provided a clear illustration of the differences between the groups and the control group. The

present study's results are consistent with earlier research on Japanese quails, which discovered that include medicinal plants in the diet lengthened the villus small intestine, increased the depth of the crypt, and raised the ratio of villi height to crypt depth (Mustafa *et al*., 2022). Therefore, by altering the height and crypt depth of the intestinal villi, medicinal plant leaves may be enhancing the absorption of nutrients. The researchers found that another feature that influences the absorptive level in the intestines is the growth in villi surface area (Saki *et al*., 2017). The current study's findings suggest that adding *Salvia indica* will increase villus height and lessen the stress that birds experience from dense stockings. Hence, it is crucial for villus length since longer intestinal villi are thought to be healthier.

The Bursa Histology results for 42-day-old local quails are displayed in (Figure 2). Furthermore, microscopic images of tissue slices from the Bursa of Fabricius provided a thorough account of the effects of the four therapies (Figure 3). The addition of SI treatments considerably (P<0.05) enhanced Fabricius follicle size when compared to both the positive and negative controls diet; however, no significant difference was seen between the two SI additions with high stocking density. The development of B lymphocytes is controlled by an organ of the immune system known as the Bursa of Fabricius (Alloui *et al*. 2005). No detectable antibodies were produced by small follicle birds against IBDV or the injected antigen. It was demonstrated that the presence of larger follicles was associated with the capacity to elicit Ig responses. However, the small follicles were not able to sustain the complete bursal B-cell development pathway (Mirza, 2015).

Table 3: Effect of adding *Salvia indica* plants on biochemical parameters of local quails at six weeks of age (Mean ± standard error).

Parameters		Treatment				P. value
		Positive CON	Negative CON	0.2% SI	0.4% SI	
Male	**Cholesterol** (mg/dL)	175.63±8.13 [a]	188.20±5.91 [a]	195.00±3.77 [a]	196.86±1.56 [a]	0.087
	Triglyceride (mg/dL)	171.10±3.43 [a]	166.56±9.70 [a]	170.36±6.03 [a]	121.16±3.43 [b]	0.001
	HDL (mg/dL)	84.36±3.58 [b]	93.40±0.81 [a]	96.46±2.25 [a]	94.50±0.28 [a]	0.018
	LDL (mg/dL)	69.00±1.15 [a]	76.33±3.48 [a]	65.50±0.28 [a]	76.33±0.88 [a]	0.63
Female	**Cholesterol** (mg/dL)	289.43±4.19 [a]	247.66±6.42 [b]	212.56±3.03 [c]	196.86±5.81 [c]	<0.001
	Triglyceride (mg/dL)	201.16±1.96 [b]	234.00±4.61 [a]	165.66±5.78 [c]	221.66±6.17 [a]	<0.001
	HDL (mg/dL)	39.40±3.93 [b]	61.53±5.97 [a]	38.16±2.74 [b]	41.33±4.40 [b]	0.038
	LDL (mg/dL)	187.33±8.33 [a]	127.00±2.51 [b]	107.00±4.58 [b]	101.00±4.37 [b]	<0.001

[abc] Significant differences (P<0.05) are seen across data with different letters in the same row direction.

Table (4): Effect of adding *Salvia indica* plants on jejunum histology of local quails at six weeks of age (Mean ± standard error).

Treatments	Histology of Jejunum	
	Villi height (um)	Crypt Depth (um)
Positive CON	688.10±8.73 [c]	95.33±0.57 [a]
Negative CON	623.23±8.34 [d]	92.93±0.81 [b]
0.2% SI	722.76±6.42 [b]	97.00±0.81 [a]
0.4% SI	802.30±11.96 [a]	97.46±0.63 [a]
***P.* value**	<0.001	0.008

[a,b,c,d] Letter differences between groups in the same column indicate a statistically significant difference (P<0.05).

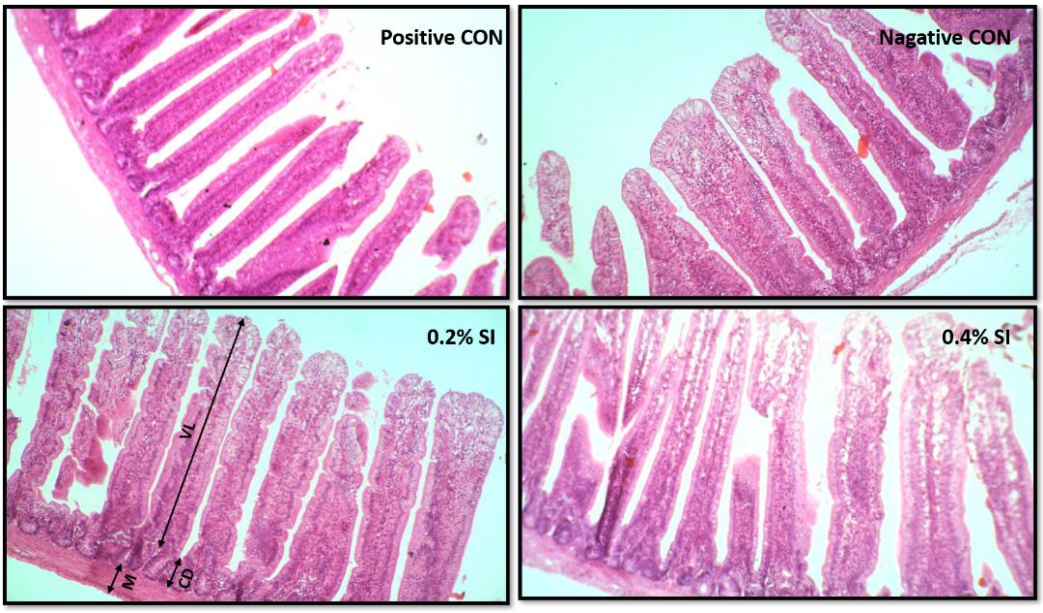

Figure (1): A jejunum slice stained with hematoxylin and eosin from birds given diets at 42 days of age. The groups consist of *Salvia indica* 0.2% with HSD, *Salvia indica* 0.4% with HSD, Positive Control Group, and Negative Control Group. The letters VL, CD, and M stand for Villus length, Crypt depth, and Muscular, respectively (10X magnification).

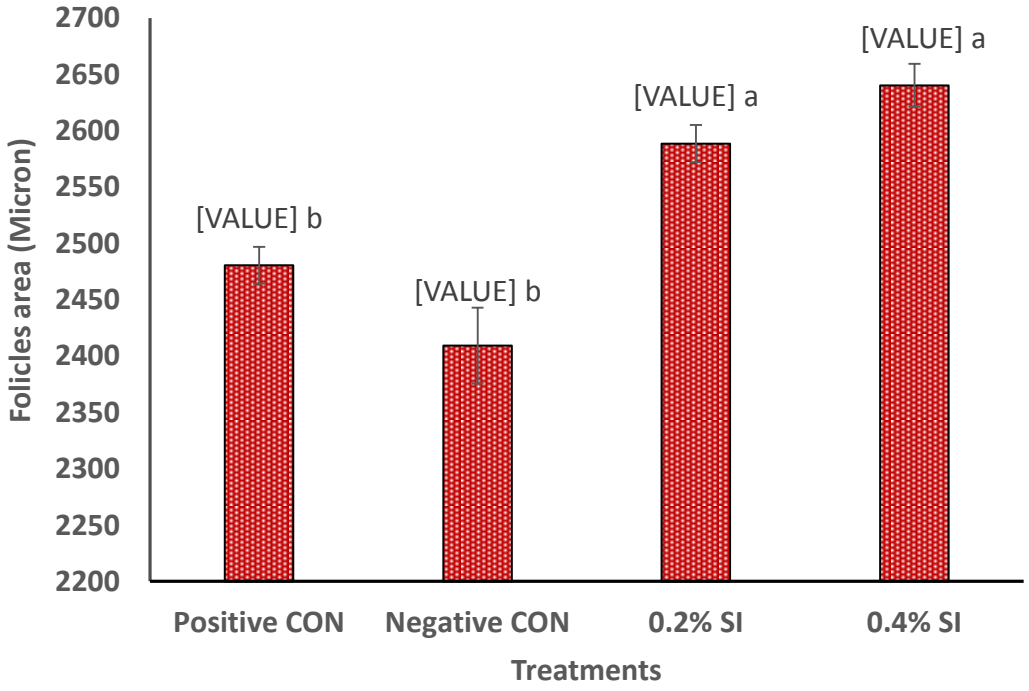

Figure (2): illustrates the impact of *Salvia indica* additive combined with stocking density on the Bursa of Fabricius's follicles area (Micron) at the end of the trail.

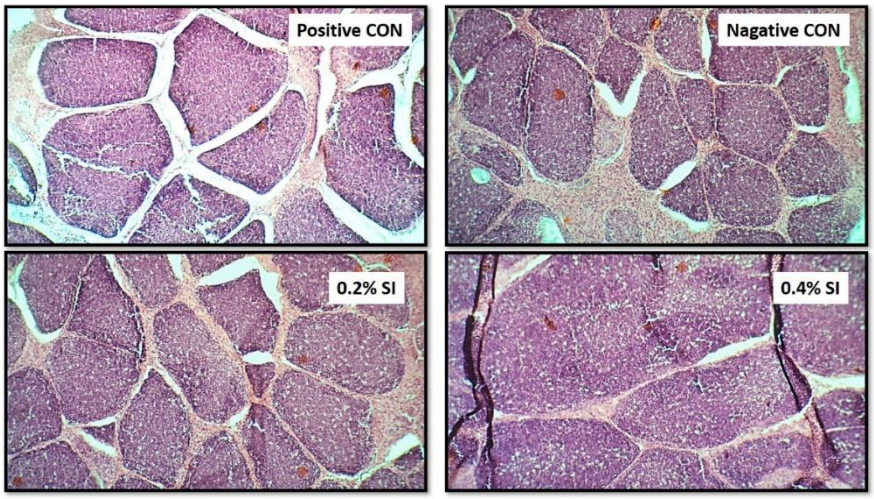

Figure (3): At the end of trail, the Follicles Bursa of Fabricius slice stained with eosin and haematoxylin. The groups including; Positive Control Group, Negative Control Group (HSD), *Salvia indica* 0.2% with HSD and *Salvia indica* 0.4% with HSD (Magnification 10X).

4.CONCLUSIONS

In conclusion, adding *Salvia indica* plant as feed additive with high stocking density to local quails feed improved productivity by increasing the number of birds per area without any effect on the performance, by improving the feed conversion ratio, *Salvia indica* plant can be used as an efficient feed additive to enhance the growth performance and decreased the stress on the birds. Thus, these effects may lead to increased economic efficiency in the quail meat manufacturing process. Additionally, the size of Fabricius follicles increased, and the Jejunum portion of the small intestine had more villi and crypt depth.

Conflict of interest

No conflicts of interest are disclosed by the authors.

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