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DIFFERENTIATION OF GROWTH, INNER ORGAN DEVELOPMENT, GUT MICROBIOTA, GUT HISTOLOGY AND MEAT QUALITY OF JAPANESE QUAILS FED DIETS CONTAINING VARYING AMOUNTS OF *Satureja spicigera (K. Koch)* Boiss LEAF POWDER

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Abstract: This study investigates the impact of varying amounts of *Satureja spicigera* leaf powder (SLP) (the creeping savory) on the growth, inner organ development, gut microbiota, meat quality, and gut histology of quails. The trial included five treatment groups: SLP0 (control, 0%), SLP1 (0.25%), SLP2 (0.5%), SLP3 (0.75%) and SLP4 (1%). The quails were housed in floor pens with 20 chicks per replicate, and there were 4 replicates for each group, leading to a total of 400 quails. At the end of the study, 1 male and 1 female quails from each replicate (a total of 40 quails) were slaughtered, and samples were collected for analysis. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and visceral organ weights did not change between the groups (P>0.05). Meat quality characteristics (such as sensory criteria) and gut microbiota did not differ between treatment groups. However, a significant reduction in the MDA (Malondialdehyde) level in the breast meat was observed in the SLP1 and SLP2 groups compared to the control group (P<0.05). The breast meat color of the SLP1 and SLP4 groups was significantly lighter than the control group (P<0.05). Villi length (VL) and crypt depth (CD) were greater in the SLP1 group compared to SLP0 and SLP4 groups in the duodenum (P<0.05). The SLP3 group had the highest VL (P<0.01), and the SLP4 group had the lowest CD (P<0.01). Villi length and crypt depth (VL/CD) ratio in SLP3 and SLP4 groups was higher than in SLP0 and SLP1 groups in the jejunum (P<0.05). While no differences in villi length were noted, crypt depth was higher in the SLP3 and SLP4 groups compared to the other groups (P<0.01). The lamina muscularis mucosa (LMM) thickness was lower in the SLP0 group, and VL/CD was lower in the SLP3 group in the ileum (P<0.01). The supplementation of SLP in the diet of Japanese quails enhanced gut health by elevating VL in the duodenum and jejunum, which could enhance digestion. The antioxidant effect of SLP was also evident in the reduction of MDA levels in breast meat. To conclude, *Satureja spicigera* leaf powder can be considered a useful feed additive in quail breeding, particularly for improving intestinal health and for suppressing MDA formation in quail meats.

Keywords: *Satureja spicigera*, MDA, Histomorphology, Quail.

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1. Introduction

After the ban on the use of antibiotics as growth enhancers in animal nutrition, scientists have proposed using medicinal and aromatic plants (MAPs) to support growth without negatively affecting animal, instead of antibiotics. Thanks to their bioactive components, MAPs have effects such as supporting the immune system, reducing disease risks and damages, improving health status, increasing animal welfare, supporting the intestines, and increasing digestion, nutrient absorption, and wound healing (Cedillo-Cortezano et al., 2024). The use of MAPs in animal husbandry can also provide environmental benefits by reducing methane emissions and greenhouse gas levels (Andrić et al., 2023). Among the medicinal aromatic plants, the Lamiaceae family is the largest family containing many subspecies, and most *Thyme, Oregano, Mint,* and *Salvia* species have been used by scientists as yield enhancers, antioxidants and antimicrobial products in animals. However, there are many species belonging to the *Lamiaceae* family whose growth promoting, antioxidant and antimicrobial effects in animals need to be determined. *Satureja* is one of these species. The *Satureja* genus is an important member of this group, which includes thymol and carvacrol components in its structure, along with *Origanum, Thymbra, Coriodymus* and *Thymus* species belonging to the Lamiaceae family, and is represented by a total of 15

species in Turkiye, 5 of which are endemic (Paşa et al., 2019). Essential oils, plant parts, and plant extracts of *Satureja* species are reported to have antibacterial and antifungal activity due to phenolic compounds such as *thymol* and *carvacrol* (Nasiroleslami et al., 2016). *Thymol* and *carvacrol* have antimicrobial, antibacterial, antioxidant and antifungal effects (Bozdemir, 2019). It has been stated that the antimicrobial and antioxidant activities of the genus *Satureja* are due to the rich essential oil and phenolic content of these species (Kavcı, 2020). *Satureja*, a fragrant species similar to thyme from the *Lamiaceae* family and used as an appetizer, is called the creeping savory. There are very few studies in which the effects of *Satureja* species on the performance of animals were determined. In these studies, *Satureja montana* (Pashtetsky et al., 2020; Vilmosh et al., 2023), *Satureja hortensis* (Seyedtaghiya et al., 2021; Nasiroleslami et al., 2016), and *Satureja khuzestanica* (Rahimi et al., 2021; Arak et al., 2017) were examined for their biochemical content and for use as a feed additive in poultry feeding. No such study on *Satureja spicigera (K. Koch)* Boiss has been found. Therefore, the aim of this study was to determine the biochemical content of the leaf powder of *Satureja spicigera (K. Koch)* Boiss and the possible effects of its addition to quail diets on performance, meat quality, microbiology, and intestinal histology.

2. Materials and Methods

2.1. Animal Material

Coturnix coturnix japonica of mixed sex, at the age of 1 day, were used as biological material in the experiment. At the beginning of the experiment, 5 treatment groups and 4 replicates were formed from 400 chicks, with 80 animals in each group, and all had similar body weights. The chicks were randomly placed in 10 cm sawdust litter pens with 20 chicks in each pen.

2.2. Feed Material

The ration used in the study was obtained from a commercial feed company active in Kırşehir province. The nutrient content of the feed used is given in Table 1.

2.3. *Satureja Spicigera*

Satureja spicigera (K. Koch) Boiss was purchased from a local herbalist in Ordu province. Species identification of *Satureja* plants from herbaria was carried out by Dr. Sibel Ulcay. During the analysis of *Satureja spicigera (K. Koch)* Boiss plant with the NEOS microwave extraction system, it was determined that it contained 2.5% essential oil. The biochemical analysis of the essential oil was carried out in Kırşehir Ahi Evran University Central Research and Application Laboratory, and its detailed content is given in Table 2.

2.4. Conducting the Trial

The experiment was conducted for 42 days. Before the quails were placed in the cages, their live weights were determined by weighing them individually on an electronic scale with a precision of ± 0.01 g, and the average live weight in the cages was calculated. Treatment groups were SLP0: Basal feed (Control), SLP1: Basal diet + 0.25% *Satureja* leaf powder, SLP2: Basal diet + 0.50% *Satureja* leaf powder, SLP3: Basal diet + 0.75% *Satureja* leaf powder, and SLP4: Basal diet + 1% *Satureja* leaf powder. Feed and drinking water were given ad libitum. Live weights and feed intake of quails were determined by weekly weighing. Feed consumption of quails was calculated after weekly weighing by subtracting the excess feed from the total feed amount. Feed conversion ratios were calculated by dividing the feed consumption up to the weighing day by the live weight.

2.5. Slaughtering and Sampling

On the 42nd day of the experiment, feeders and waterers of all treatment groups in the experimental room were removed from the evening to the morning. Animals in all groups were weighed with scales with 1-gram precision, and the average live weights of the treatment groups were determined. After weighing, two quails (one female, one male) with average weights close to the group average were selected from each replicate, and their weights were recorded. These selected animals were cut open and the internal organs were removed. The weights of the heart, liver, and gizzard were determined and the length of the digestive tract was measured and recorded. For histological analysis, 5 cm samples were taken from the duodenum, jejunum, and ileum and kept in 10% formaldehyde until analysis (24 hours).

Table 1. Nutrient content of feed (%)

Feed Raw Materials	%
Corn	44.00
Soybean meal (%44)	41.15
Meat bone meal	4.00
Soybean oil	6.50
DCP	2.50
L-lysine HCL	0.70
DL-methionine	0.35
Salt	0.30
Vitamin premix*	0.25
Mineral premix‡	0.25
Analysis Results	
ME (kcal/kg)	3080
Crude protein	22.39
Crude cellulose	2.80
Crude ash	6.5
Crude fat	9.00
Calcium	1.60
Usable phosphate	3.80

* 1 kg vitamin premix contains Vitamin A: 12.500 IU; Vitamin D3: 4.000 IU; Vitamin E: 30 mg; Vitamin K3: 4 mg; Vitamin B1: 3 mg; Vitamin B2: 7 mg; Vitamin B6: 5 mg; Vitamin B12: 15 µg; Niacin: 25 mg.

‡ Iron:80 mg; Folic acid:1 mg; Pantothenic acid:10 mg; Biotin:45 mg; Choline:125000 mg; Copper:5 mg; Manganese:80 mg; Zinc:60 mg; Iodine:1 mg; Selenium:0,15 mg.

Table 2. Biochemical content of *Satureja spicigera (K. Koch)* Boiss

Contents	RT	%
Sabinene	5.11	0.32
a-Pinene	6.45	1.45
a-Terpinene	6.98	2.64
1-Limonene	7.76	0.29
1-Phellandrene	8.15	0.30
Ɣ-Terpinene	10.22	11.96
Delta.3-Carene	10.77	0.30
P-Cymene	11.65	19.04
3-Octanol	17.82	0.31
Thujol	18.67	0.15
1-Octen-3-ol	20.03	0.82
Trans Sabinene hydrate	20.44	0.43
Cis Sabinene hydrate	23.38	0.20
Linalool	23.48	1.31
Trans-Caryophyllene	24.53	1.51
Carvacrol methyl ether	25.16	8.76
Dihydrocarvone	25.75	0.13
à-Humulene	26.83	0.05
Verbenol	27.46	0.08
Ledene	28.06	1.75
Bicyclogermacrene	28.78	1.04
D-Carvone	29.04	0.05
Germacrene-D	29.52	0.06
Para-Cymen-8-ol	32.40	0.21
Carvacrol acetate	33.00	0.08
M-Cresol	34.16	0.11
Caryophyllene oxide	35.57	0.40
Spathulenol	39.13	0.39
Thymol	40.71	3.08
Carvacrol	41.35	37.20

2.6. Microbiology of the Cecum

One gram of cecal samples was mixed in 9 ml of peptone water using a vortex to obtain a homogeneous distribution. Serial dilutions (1/10) were prepared from the stock materials. MRS agar was used for lactic acid bacteria (MERCK, 1.10660) at 37°C following 3 days in an incubator, and BEA (Bile Aesculin Azide Agar) Agar (Merck 100072) was used for *Enterococcus spp*. at 37°C following 3 days in an incubator. MEA (Malt extract Agar) Agar (Merck 105398 was used for yeast at 25°C degrees following 3 days in incubator The microbial colonies were counted and expressed as log10 colony forming units (CFU) per gram of caecal content.

2.7. Intestinal Histology

On the 21st day of the study, duodenum, jejunum, and ileum samples taken from slaughtered animals in each treatment group were placed in 10% formaldehyde. In the histology analysis of the study, paraffin blocks were prepared; the samples were cut with a thickness of 5 microns, and the tissues were adhered to the slide. The tissues on the slide were freed from paraffin by passing them through xylene, followed by alcohol to remove the xylene. The cleaned tissue samples were stained with Hematoxylin & Eosin dye and photographed using a digital microscope equipped with an AxioCam ERc 5s 5MP (ZEISS Primo Star, Germany) for proper imaging. The photographs obtained for each treatment group and each sample were measured with the ZEN 2012 SP2 image processing and analysis program.

2.8. Meat Quality

After slaughter, the carcasses were cleaned and stored at +4°C for 24 hours, and pH values were measured in the breast meat of each quail slaughtered. The pH values were measured with a digital pH meter (Testo 205), using a solid electrode at 3 different points of the left breast meat. The electrode was kept immersed in the breast until the values were fixed on the display screen of the pH meter. This fixed value was then read and recorded. In this way, the average of three measurements taken from the breast sample of the quails was calculated and recorded as the pH value of the breast meat.

Color measurements were performed on the breast meat of quails using a Minolta CR 410 Chroma Meter (Minolta Camera Co., Osaka, Japan), calibrated with a spectrocolorimeter white color plate, (Minolta calibration plate, No. 21733001, Y=92.6, x=0.3136, y=0.3196). After removing the skin from the left breast meat of each quail, color measurements were made at three different points on the breast surface. CIE standards (L* = brightness, a* = redness, and b* = yellowness values) were used in the measurements, and three basic color characteristics (L* 100 = white, 0 = black; a* = red color coordinate ± red-green; b* = yellow color coordinate ± yellow-blue) were considered.

Fat oxidation levels in quail breast meat were determined by the 2-thiobarbituric acid method. 50 mL of distilled water was added to 10 g of quail breast meat at 50°C and homogenized in Ultra-Turrax for 2 minutes. The mixture was taken into distillation tubes, and 47.5 ml of distilled water and 2.5 ml of 4 N HCl solution were added. Paraffin was added to the mixture to prevent foaming, and boiling stones were added to facilitate boiling, and placed in the distillation apparatus. The steam power of the distillation apparatus was set to low mode, and the process was continued until 50 ml of distillate was collected. From the collected amount, 5 ml was separated, transferred to balloon jugs, and 5 ml of TBA reagent was added. For the blind preparation, 5 ml of distilled water and 5 ml of TBA reagent were mixed. The samples were mixed homogeneously with a vortex, kept in a hot water bath for 35 minutes, and cooled in water for 10 minutes. The samples were then read in a spectrophotometer at a wavelength of 538 nm against the blind; the absorbance value was multiplied by 7.8, and the result was expressed as milligrams of MDA per kilogram of sample (Tarladgis et al., 1960).

Sensory quality analysis of meat was carried out by TUBITAK MAM. Quail breast meat samples were kept at -18°C for analysis. One day before the analysis day, they were placed at +4°C and thawed. Quail breast meat was used to ensure that the sample had the same

characteristics in terms of the sensory criteria to be analyzed. Each of the meats numbered between 1-5 was wrapped in baking paper and then in aluminum foil before analysis, and all were cooked at 180℃ for 40 minutes simultaneously. Sensory evaluations of the samples were carried out by 10 expert panelists. In sensory evaluations, color (appearance), smell, flavor, and texture (crispness, juiciness, stickiness, chewiness, and fibrousness) criteria were examined. During the evaluation phase, panelists were given homogeneous samples. The samples were randomly coded with a three-digit number and presented to the panelists on separate plates. Water was given to neutralize their mouths between samples. Changes in the quality of the samples were evaluated using a 1-9 scale. In this scale, a score of 1 corresponds to the worst criterion, and a score of 9 corresponds to the best criterion.

2.9. Statistical Analyses

The trial was conducted in accordance with the randomized plots trial design and the data were analyzed using one-way ANOVA using the IBM SPSS 25 (IBM Corp., Armonk, NY, USA) statistics program. Since the distribution of the data between the group means was homogeneous, the Duncan multiple comparison test was applied.

3. Results and Discussion

Table 3 shows the performance values of the quails after dietary SLP (the creeping savory) to the quail diets. At the end of the study, it was determined that the supplementation of different amounts of SLP to the quail diets had no effect on the performance values; only the BWG value of the SLP3 group was lower than the SLP2 group. However, it was not affected compared to the SLP0 group.

Table 3. Effects of *satureja* leaf powder supplementation on quail performance values

SLP (g/kg diet)	BWG	FI	FCR
SLP0	186.70ab	645.80	3.46
SLP1	189.33a	673.31	3.56
SLP2	185.57ab	654.55	3.53
SLP3	179.90b	626.58	3.48
SLP4	186.62ab	652.56	3.49
SEM	1.31	7.55	0.03
	P Value		
L	0.23	0.44	0.94
Q	0.55	0.85	0.57
C	0.05	0.08	0.52

Values represent means of 4 replicate pens with 2 birds from each pen. a,b,c= Differences between means with different letters in the same row are significant (P<0.05). L= linear. Q= quadratic. C= cubic. BWG= body weight gain, FI= feed intake, FCR= conversion ratio.

The number of studies on the performance parameters of the *Satureja* plant in poultry nutrition is scarce. Movahhedkhah et al., (2019) reported that the addition of Summer Savory (*Satureja hortensis L.*) Extract to broiler diets did not affect growth performance. Souri et al., (2015) reported that *Satureja khuzestanica* supplementatiopn to diet did not affect broiler growth. Nasiroleslami et al., (2016) reported that the addition of *Satureja hortensis* (Summer Savory) essential oil (SHEO) to laying hens diet, did not change egg laying performance; similarly, Dehghani et al., (2018) reported that SHEO in quail diets had no effect on performance parameters. The performance values of the present study are consistent with the results of previous studies. According to these results, it was determined that SLP did not affect the performance values of quails. There are few studies on Satureja species regarding performance parameters. To fully demonstrate the effects of *Satureja* species on performance, different studies are needed to determine whether they are effective in different animal species, whether they are more effective in different harvest periods, or whether they are more effective under different stress conditions.

The effects of SLP supplementation to quail diets on internal organ development are given in Table 4. Heart weight, liver weight, gizzard weight, and digestive tract length did not differ among the groups.

Table 4. Effects of slp supplementation on quail ınternal organ development

SLP (g/kg diet)	Heart (g)	Liver (g)	Gizzard (g)	Gut length (cm)
SLP0	1.04	1.77	1.99	29.72
SLP1	1.03	1.85	2.14	28.61
SLP2	0.93	1. 75	2.23	30.20
SLP3	0.99	1.93	2.33	30.61
SLP4	0.92	2.00	2.25	29.94
SEM	0.02	0.09	0.05	0.67
		P Value		
L	0.08	0.46	0.06	0.63
Q	0.83	0.76	0.28	0.96
C	0.77	0.93	0.78	0.46

Values represent means of 4 replicate pens with 2 birds from each pen.

Arak et al., (2013) determined that the addition of 600 ppm *Satureja khuzistanica* essential oil (SKEO) to quail diets decreased Bursa fabricus and spleen weights, but had no significant effect on other internal organ weights. In another study, Dehghani et al., (2018) reported that the addition of different levels of SHEO to quail diet did not effect inner organ developments. Souri et al., (2015) reported that the supplementation of SKEO to broiler diets did not affect the development of internal organs. It was determined that the results of previous studies on different *Satureja* species were compatible with the results obtained from the present study and the addition

of SLP to the ration did not affect the internal organ development of quails. The results of the cecum microbiota are given in Table 5. Cecum microbiota did not differ from other groups.

Table 5. Effects of SLP supplementation on quail cecum microbiota

SLP (g/kg diet)	*Enterococcus spp.*	*Lactobacillus spp.*	*Saccharomyces cerevisiae*
SLP0	6.42	6.42	6.51
SLP1	6.29	6.73	6.68
SLP2	6.43	6.68	6.59
SLP3	6.52	6.65	6.90
SLP4	6.60	6.69	6.94
SEM	0.06	0.06	0.07
		P Value	
L	0.60	0.57	0.21
Q	0.49	0.33	0.80
C	0.55	0.36	0.96

Values represent means of 4 replicate pens with 2 bird from each pen.

Masouri et al., (2017) reported that the addition of 500 mg/kg SKEO to corn-based diets of broiler chickens increased the *Lactobacillus* population in the blind intestine and decreased the total number of bacteria and *Escherichia coli*, whereas the caecal population of *Lactobacillus* decreased in broiler chickens fed wheat-based diets, Mousapour et al., (2020) reported that the addition of 150 mg/kg SHEO to broiler diets significantly increased the number of *Lactobacilli* in the ileal digestive tract and reduced the density of coliforms. Mozafari et al., (2018) reported that the addition of 1.0% or 2.0% *Satureja hortensis* L. powder to broiler diets significantly reduced ileal Escherichia coli population compared to the control group, while Movahhedkhah et al., (2019) reported that the addition of 400 mg/kg Satureja hortensis extract to broiler diets decreased the number of *Escherichia coli* and improved the *Lactobacilli/Escherichia coli* ratio, but had no significant effect on the number of Lactobacilli. Although there was no statistical difference in the number of lactobacilli and yeast in the present research study, it was determined that the addition of SLP increased the lactic acid bacteria and yeast population numerically. Therefore, it can be said that the addition of SLP has positive effects on the beneficial bacterial population in the quail intestines and balances the intestinal microflora; it also positively affects intestinal health and provides better digestion, consistent with previous studies.

While there was no significant difference between the pH levels and color (L* and b*) values (Table 6) of the breast meat compared to the control group, the a* value of the SLP4 group decreased compared to those of the SLP1 and SLP2 groups. MDA levels were significantly lower in SLP1 and SLP2groups compared to the control group (P<0.05).

Table 6. Effects of SLP supplementation on quail meat properties and mda levels

SLP (g/kg diet)	pH	L*	a	b	MDA
SLP0	6.00	36.64	12.12_{ab}	5.91	0.71_{a}
SLP1	6.15	38.19	12.79_{a}	6.62	0.51_{b}
SLP2	6.07	37.70	12.31_{a}	6.13	0.51_{b}
SLP3	6.17	37.99	11.73_{ab}	6.04	0.62_{ab}
SLP4	5.96	37.08	10.90_{b}	5.59	0.59_{ab}
SEM	0.05	0.34	0.21	0.14	0.02
		P Value			
L	0.58	0.43	0.05	0.21	0.33
Q	0.19	0.10	0.07	0.10	0.02
C	0.80	0.69	0.52	0.39	0.03

Values represent means of 4 replicate pens with 2 bird from each pen. a,b,c: Differences between means with different letters in the same row are significant (P<0.05). L*= brightness; 0 = black and 100 = white, a*: redness; – 60 = green and 60 = red, b*: yellowness; – 60 = blue and 60 = yellow.

The number of studies on the effects of *Satureja* on poultry meat quality is limited. Among these limited studies, Souri et al., (2015) found that the addition of 2% and 1% *Satureja khuzestanica* extract to drinking water decreased the 24-hour pH of broiler thigh meat. While the pH of breast meat was not affected and the redness, yellowness, and brightness of breast meat were not affected by plant extract applications, 2% and 1% *Satureja* khuzestanica extract decreased the redness and yellowness values in thigh meat. They reported that the difference between the results may be due to the different forms of the plants and the difference in the color pigment ratios contained in the plants. Azarbad et al., (2019), stated that the addition of SKEO in different forms to broiler diets significantly affected the MDA levels in breast meat, and this study supports previous findings that SLP suppressed MDA formation in SLP1 and SLP2 groups compared to the SLP0 group (P<0.05). Therefore, *Satureja spicigera* showed antioxidant properties by suppressing the formation of reactive oxygen species (ROS) in the breast tissue of quails. However, the effects of SLP, essential fatty acids, or their extracts obtained by different methods on MDA and different antioxidant enzymes in various animal species need to be determined.

The change in the sensory criteria of quail breast meat is given in Table 7. It was determined that the meat color of the SLP1 and SLP4 groups was lighter than the control group, and this difference was found to be significant (P<0.05), while no difference was detected in terms of other sensory criteria (odor, crispness, stickiness, chewiness, fibrousness, juiciness, and general flavor). No study was found to determine the effects of the Satureja plant on the sensory properties of camel meat. Therefore, this is the first study to determine the effects of SLP on the organoleptic properties of poultry, especially quail breast meat. When previous studies on the effects of

different aromatic plants on the organoleptic properties of breast meat were examined, Arjun et al., (2022) reported that the addition of *Mint* leaf powder (0.75%, 1.5%, 2.25%, 3%) to the ration resulted in sensory evaluations for all parameters of meat in Japanese quails being significantly higher in the group with 3% mint leaf powder addition. Anar, (2022) reported that *Thyme* and *Rosemary* essential oils added to the ration, alone or in mixtures, did not cause any difference in terms of sensory characteristics in the breast and thigh meat of broiler chickens and shared similar results. The results obtained from this study were similar to those of previous studies. Only in Arjun et al., (2022) all traits were affected in the group in which mint was added to the ration at the 3% level. Since the highest dose was 1% in this study, its effects may not have been significant. Therefore, according to these results, it can be concluded that the doses of aromatic herbs are effective in influencing organoleptic properties in poultry meat.

Table 7. Effects of SLP supplementation on sensory quality characteristics of quail meat

SLP (g/kg diet)	Color	Odor	Brittleness	Stickiness	Chewability	Fiber	Wateriness	Overall flavor
SLP0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
SLP1	8.0	8.8	8.5	8.5	8.6	8.5	8.3	8.6
SLP2	8.5	9.0	8.0	8.4	8.6	8.4	8.4	8.5
SLP3	8.3	8.8	8.8	8.3	8.5	8.3	8.3	8.5
SLP4	7.6	8.8	8.5	8.5	8.4	8.5	8.8	8.6
SEM	0.15	0.17	0.12	0.11	0.09	0.10	0.12	0.12
				P Value				
L	0.01	0.10	0.09	0.42	0.32	0.25	0.18	0.72
Q	0.91	0.65	0.06	0.18	0.51	0.12	0.03	0.33
C	0.23	0.54	0.09	0.75	0.52	0.96	0.79	0.83

Values represent means of 4 replicate pens with 2 birds from each pen. a,b,c: Differences between means with different letters in the same row are significant (P<0.05).

Table 8. Effects of SLP supplementation on duodenum histology

SLP (g/kg diet)	Duodenum VL	Duodenum CD	Duodenum LMM	Duodenum VL/CD
SLP0	977.28_{bc}	47.50_{b}	76.33	20.99
SLP1	1124.27_{a}	56.17_{a}	77.51	20.12
SLP2	1041.99_{ab}	50.82_{ab}	74.31	21.05
SLP3	966.31_{bc}	51.20_{ab}	77.71	19.09
SLP4	864.54_{c}	44.16_{b}	76.29	20.39
SEM	20.73	1.17	2.56	0.49
		P Value		
L	0.00	0.14	0.99	0.54
Q	0.00	0.00	0.95	0.73
C	0.12	0.39	0.95	0.68

Values represent means of 4 replicate pens with 2 birds from each pen. a,b,c: Differences between means with different letters in the same row are significant (P<0.05). VL= villi length, CD= crypt depth, LMM= the lamina muscularis mucosa thickness, VL/CD= villi length and crypt depth ratio.

Table 9. Effects of SLP supplementation on jejunum histology

SLP (g/kg diet)	Jejunum VL	Jejunum CD	Jejunum LMM	Jejunum VL/CD
SLP0	557.09_{b}	38.98_{ab}	61.30	14.77_{cd}
SLP1	542.98_{b}	41.13_{a}	68.39	13.44_{d}
SLP2	559.47_{b}	34.14_{b}	60.23	16.65_{bc}
SLP3	675.18_{a}	36.77_{ab}	68.61	19.00_{ab}
SLP4	584.58_{b}	29.09_{c}	62.10	20.21_{a}
SEM	8.98	0.92	1.39	0.50
		P Value		
L	0.00	0.00	0.85	0.00
Q	0.34	0.12	0.35	0.20
C	0.00	0.81	0.97	0.04

Values represent means of 4 replicate pens with 2 birds from each pen. a,b,c: Differences between means with different letters in the same row are significant (P<0.05). VL= villi length, CD= crypt depth, LMM= the lamina muscularis mucosa thickness, VL/CD= villi length and crypt depth ratio.

Table 10. Effects of SLP supplementation on ıleum histology

SLP (g/kg diet)	Ileum VL	Ileum CD	Ileum LMM	Ileum VL/CD
SLP0	462.31	30.19_{c}	45.15_{cd}	15.52_{ab}
SLP1	454.43	32.71_{bc}	54.83_{a}	14.34_{ab}
SLP2	501.82	31.03_{c}	53.22_{d}	16.61_{a}
SLP3	454.12	40.41_{a}	54.15_{bc}	11.42_{c}
SLP4	483.45	35.98_{b}	57.81_{b}	13.65_{b}
SEM	7.13	0.82	1.39	0.40
		P Value		
L	0.39	0.00	0.01	0.00
Q	0.72	0.00	0.39	0.00
C	0.66	0.05	0.14	0.11

Values represent means of 4 replicate pens with 2 birds from each pen. a,b,c: Differences between means with different letters in the same row are significant (P<0.05). VL= villi length, CD= crypt depth, LMM= the lamina muscularis mucosa thickness, VL/CD= villi length and crypt depth ratio.

In the duodenum, *Lamina muscularis* mucosa (LMM) thickness and VL/CD did not change (P>0.05). However, the duodenum villi length of the SLP1 group was higher than in the SLP0 group (P<0.05), and the duodenum crypt depth of the SLP1 group was also higher (P<0.05). However, jejunum villi length of SLP3 group was significantly higher than the control group, while jejunum crypt depth of SLP4 group was significantly lower than in the SLP0 group (P<0.05); jejunum LMM thickness did not differ from groups. In addition, the VL/CD ratio of the jejunum in the SLP3 and SLP4 groups was statistically higher than in the SLP0 group (P<0.05). Villi length did not change among the groups in the ileum. However, the ileal CD of SLP3 and SLP4 groups was found to be higher than the SLP0 group, while the ileal lamina muscularis mucosa thickness of SLP1, SLP3 and SLP4 groups was found to be higher than the SLP0 group (P<0.05). In addition, the ratio of ileum VL/CD of the SLP3 group was lower than that of the control group (P<0.05). Masouri et al. (2017) found that the addition of SKEO decreased the VL of the duodenum and jejunum in broiler chicks fed wheat-based diets, increased the VL and the VL/CD, and decreased the CD of the duodenum in the group with 500 mg/kg SKEO added to the diets compared to the control group. Mousapour et al., (2020) found that the VL of the group supplemented with 150 mg/kg SHEO in broiler diets was significantly lower than those of the control group and other groups. Rahimi et al., (2021) found that the addition of SKEO (400 and 500 mg/kg) and garlic powder (2% and 4%) to the diet significantly increased the VL, VL/CD and villus surface area in the intestinal morphology of male broiler chickens. Dehghani et al., (2018) reported that SHEO, when added to the diet, enhanced the VL of duodenum, jejunum, and ileum in quails, while the CD decreased significantly. It is thought that these differences seen in the results of the research may be due to the changes in the microflora of the digestive system of the animals and that the chemical compounds contained in the plant species used that affect the morphology of different parts of the intestine.

4. Conclusion

In this study, the biochemical composition of the leaf powder of *Satureja spicigera (K. Koch)* Boiss was determined based on the findings obtained. The inclusion of *Satureja spicigera (K. Koch)* Boiss leaf powder in quail diets was observed to increase duodenal villus length and crypt depth in the SLP1 group, while in the SLP3 and SLP4 groups, it enhanced villus length and crypt depth in the jejunum, thereby improving intestinal health and digestion. Additionally, a significant reduction in malondialdehyde (MDA) levels was observed in the breast meat of quails in the SLP1 and SLP2 groups compared to the control group, indicating an antioxidant effect. All these findings were discussed in detail and contribute to the existing literature. However, as this is a preliminary study aimed at identifying the various beneficial effects of *Satureja spicigera*, a plant commonly cultivated in the Black Sea region, on poultry, further research is warranted. Future studies should aim to elucidate the mechanisms of action, investigate the potential for commercial cultivation of *Satureja spicigera*, and explore its application as a natural feed additive.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.D.	İ.C.
C	60	40
D	60	40
S	60	40
DCP	60	40
DAI	60	40
L	60	40
W	60	40
CR	60	40
SR	60	40
PM	60	40
FA	60	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures were approved by the Local Animal Care and Ethics Committee of Kırşehir Ahi Evran University, 684429034-03 (approval date: 28 February, 2024, Protocol code: 68429034/03).

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