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Artigo

The effect of different levels of Lavender essential oil and bacitracin methylene disalicylate supplementation on performance, carcass traits, some blood parameters, small intestinal morphology and microflora in Japanese quails

O efeito de diferentes níveis de óleo essencial de lavanda e suplementação de dissalicilato de metileno de bacitracina sobre o desempenho, características de carcaça, alguns parâmetros sanguíneos, morfologia do intestino delgado e microflora em codornas japonesas

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Abstract: In order to investigate the effect of different levels of lavender essential oil and bacitracin methylene disalicylate antibiotics on performance, carcass traits, some blood parameters, small intestinal tissue morphology and intestinal microbial population in Japanese quails, a study was performed on 400 pieces of Japanese quail per day. The treatments included the group consuming the basic diet without any additives as (control) and the antibiotic bacitracin (0.25g.kg) and 3 levels (150, 200 and 250 ppm) of lavender essential oil per kg of feed in form of completely randomized design with 4 replications and 20 birds (male and female) quail per each replication. In this experiment the daily weight gain, daily feed intake, feed conversion ratio, final live weight, relative carcass weight, breast, thigh, gingival, liver, some blood parameters, intestinal tissue morphology and small intestinal microbial population were examined. The results showed that the use of lavender essential oil and bacitracin antibiotics increased the daily feed intake of quails. The results of different weeks of the experimental period showed an increase in daily weight in quails consuming lavender essential oil and antibiotics compared to the control group. In addition, in general, the feed conversion ratio using essential oils and antibiotics decreased compared to the control group during different breeding periods. The results show a slight decrease in the relative percentage of body weight, liver and an increase in the weight percentage of empty breasts, thighs and carcasses of the studied quails under the influence of lavender essential oils and antibiotics. The Serum albumin levels of quails increased significantly under the influence of experimental diet (P≤0.05). The highest serum globulin level was affected by the use of lavender essential oil with antibiotics at the level of 250 ppm (P≤0.05). The results also showed that the serum level of HDL increased under the influence of essential oil in the diet (P≤0.05). Additionally the serum triglyceride and LDL levels decreased due to lavender essential oil consumption (P≤0.05). Intestinal tissue morphology showed that there was no statistically significant difference in changes in width, length, height and intestinal mucosal muscle in the studied quails. The Ecoli colonies decreased and the number of Lactobacillus bacterial colonies increased (P≤0.05). In general, the results obtained from this study showed the usefulness of using lavender by-products and antibiotics of bacitracin on performance, some carcass traits and blood and morphological parameters of intestinal tissue in experimental quails.

Keywords: lavender essential oil, Bacitracin methylene disalicylate, Carcass, Intestine, Japanese quail.

Resumo: A fim de investigar o efeito de diferentes níveis de óleo essencial de lavanda e antibióticos dissalicilato de metileno bacitracina no desempenho, características de carcaça, alguns

parâmetros sanguíneos, morfologia do tecido do intestino delgado e população microbiana intestinal em codornas japonesas, um estudo foi realizado em 400 pedaços de codornas japonesas por dia. Os tratamentos incluíram o grupo consumindo a dieta básica sem quaisquer aditivos como (controle) e o antibiótico bacitracina (0,25g.kg) e 3 níveis (150, 200 e 250 ppm) de óleo essencial de lavanda por kg de ração na forma de ração inteiramente casualizada delineamento com 4 repetições e 20 aves (machos e fêmeas) codornas por cada repetição. Neste experimento, foram examinados o ganho de peso diário, o consumo diário de ração, a taxa de conversão alimentar, o peso vivo final, o peso relativo da carcaça, peito, coxa, gengiva, fígado, alguns parâmetros sanguíneos, morfologia do tecido intestinal e população microbiana do intestino delgado. Os resultados mostraram que o uso de óleo essencial de lavanda e antibióticos bacitracina aumentaram o consumo diário de ração em codornas. Os resultados das diferentes semanas do período experimental mostraram aumento do peso diário das codornas que consumiram óleo essencial de lavanda e antibióticos em relação ao grupo controle. Além disso, em geral, a taxa de conversão alimentar com óleos essenciais e antibióticos diminuiu em comparação com o grupo controle durante os diferentes períodos de reprodução. Os resultados mostram uma ligeira diminuição na porcentagem relativa do peso corporal, fígado e um aumento na porcentagem do peso dos seios, coxas e carcaças vazias das codornas estudadas sob a influência de óleos essenciais de lavanda e antibióticos. Os níveis de albumina sérica de codornas aumentaram significativamente sob a influência da dieta experimental (P≤0,05). O maior nível de globulina sérica foi afetado pelo uso de óleo essencial de lavanda com antibióticos no nível de 250 ppm (P≤0,05). Os resultados também mostraram que o nível sérico de HDL aumentou sob a influência do óleo essencial da dieta (P≤0,05). Além disso, os níveis séricos de triglicerídeos e LDL diminuíram devido ao consumo de óleo essencial de lavanda (P≤0,05). A morfologia do tecido intestinal mostrou que não houve diferença estatisticamente significativa nas alterações de largura, comprimento, altura e músculo da mucosa intestinal nas codornas estudadas. As colônias de Ecoli diminuíram e o número de colônias de bactérias de Lactobacillus aumentou (P≤0,05). De maneira geral, os resultados obtidos neste estudo mostraram a utilidade da utilização de subprodutos da alfazema e antibióticos da bacitracina no desempenho, algumas características de carcaça e parâmetros sanguíneos e morfológicos do tecido intestinal em codornas experimentais.

Palavras-chave: óleo essencial de lavanda, dissalicilato de metileno de bacitracina, carcaça, intestino, codorna japonesa.

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Introduction

For decades, antibiotic feed additives as growth promoters have been used in poultry diets to improve health and performance (DIBNER AND RICHARDS, 2005). Antibiotics are some feed additives that may use in animal diets to improvise the performance in broiler chicks (BRENES AND ROURA, 2010).

The increasing pressure to reduce or eliminate antibiotics as growth enhancers has initiated new research to find safe and efficient alternatives (SALAJEGHEH et al. 2018).

Among the possible alternatives to phytogenic and herbal products are considered interesting because they have acquired more reliability and acceptability

among consumers as safe and natural additives (HASHEMI AND DAVOODI, 2011).

Herbal components such as phenols, terpenoids and essential volatile oil and extracts have antibacterial and antioxidant properties and they may improve digestion, lower blood lipids and cholesterol and ultimately improve poultry growth (FRANCOIS,2006; YAZDANPARAST AND ALAVI, 2001). Lavender (*Lavandula angustifolia*), belongs to the Lamiaceae family and is a widely distributed aromatic herb which is well known to people as a powerful aromatic and medicinal herb and it is one of such alternatives that could be used as a feed additive. Lavender has antiviral, antibacterial, antifungal, and antioxidation properties (HUI et al. 2010).

Fakhari et al. (2005) declared that lavender oil contained linalool (32.8%), linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%), geranyl acetate (5%), and also lavandulol (4.3%). In another survey, linalool acetate (46.25%) and linalool (35.17%) is considered the major compounds of lavender essential oil (ZARGARI, 2000).

Salarmoini et al (2019) showed that lavender extract at 400 ppm level significantly increased feed intake during the whole rearing period, especially in finisher period and also improved body weight gain and feed conversion ratio during the grower, finisher and entire rearing periods. Additionally they also mentioned that, there were no significant differences in the carcass traits and the relative weight of internal organs of the broilers including breast, thighs, liver, heart, gizzard and abdominal fat. The results of (Yarmohammadi Barbarestani et al, 2020) also showed that the lavender essential oil inclusion had no significant effect on feed intake and body weight gain at any stage of the study. Küçükyilmaz et al (2017) showed that the birds fed diets supplemented with 24 and 48 mg/kg of lavender essential oil had breast meat with higher brightness and higher concentration of superoxide dismutase.

Salajegheh et al (2018) showed that the lavender powder significantly decreased jejunal crypt depth and increased villous height: crypt depth ratio compared to the control group. Malondialdehyde content and cooking loss of meat samples were significantly decreased in birds fed one percentage lavender powder. Overall, the results of this experiment showed that lavender powder might be used to improve growth performance and meat quality in

broiler chickens. Many studies have also been conducted on the effects of dietary herbal essential oil or combinations on the performance of poultry and swine but with varying and conflicting results (NASIRI-MOGHADDAM et al., 2012; RABIEI et al., 2014 and LAUREN AND SOHEIL, 2015).

The researchers already mentioned that the lavender essential oil can be concluded that lavender essential oil could be used as a growth promoter in broiler nutrition with potential improvements in breast meat quality (ZHENG et al.,2020). The aim of the current study was to investigatethe effect of different levels of lavender essential oil and bacitracin antibiotics on performance, carcass traits, some blood parameters, small intestinal tissue morphology and intestinal microbial population in Japanese quails.

Materials and Methods

Fresh lavender flowers were obtained from Shahre-Kord Agricultural Research Center, Iran. Flowers were dried under shade at room temperature for 72 h and then powdered. Alcoholic extract was obtained by maceration. The lavender powder was soaked in a mixture of ethanol and water (4:1) at the room temperature, in a tightly closed container for three days. The container was shaken two times a day. The mixture was then filtered to separate the solute using rotary evaporator. The extract was dried in an oven at 40 $^{\circ C}$ and stored in airtight container in the refrigerator.

The bird and diets

Total of four hundred seven-day-old, Japanese quails with an average weight of 16 ± 5 g were divided into four treatments and were further subdivided into five replicates with 20 quails each.

The soybean meal and corn grains were analyzed in the lab for determine of dry matter, crude protein, calcium, phosphorus and theirs crude fiber with Association of Official Analytical Chemists (AOAC, 2000) method. The basal diet was balanced based on corn and soybean meal recommended by National Research Council (NRC, 1994). The treatments included the group consuming the basic diet without any additives as (control) and the antibiotic bacitracin methylene disalicylate (0.25g.kg) and 3 levels (150, 200 and 250 ppm) of lavender essential oil per each kg of feed in form of completely randomized experimental design. During the experiment period, diets and fresh water were provided adlibitum.

Performance and Organs percentage

The live body weight gains, feed intake and feed conversion ratio were

calculated weekly. At the end of experimental period (35 days), four chicks form each replicates were slaughtered by cervical dislocation method and then dressing and the internal organs were removed after slaughter and some visceral organ percentage were calculated.

Blood biochemical assay

Blood samples were taken and commercial Pars Azmoon Kits analyzed some bloods chemical. To determine the microbial count, about 5 cm from the length of the ileum was sampled to determine the microbial population.

Intestinal morphology and micro flora assay

In addition, 1 g of ileum content was used to make 10-fold dilution using buffered peptone water and then 0.1 mL of the appropriate ileum dilution was spread on Lactobacillus MRS1 Agar-Hi Media Laboratories to detect lactic acid bacteria and Violet Red Bile Agar to detect Eschechia-Coli, Klebsiella and Lactobacillus bacteria colonies form.

The plates were incubated at 37.5 $^{\circ C}$ for 48 h. After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution and the result was stated as the number of colony forming unit (CFU) in 1 g of the sample.

Statically analyses:

Obtained data were analyzed statistically by one-way ANOVA method using SAS 9.1(2001) software and Duncan's (1995) multiple range tests was used to detect the differences ($P \leq 0.05$) among different treatments means.

Result and Discussion

The effects of Lavender essential oil supplementation on feed intake, weight gain and feed conversion ratio on broilers are presented in Table 2.

As result revealed that use of different levels lavender essential oil had increased feed intake (FI) none significantly ($p \geq 0.05$), and the body weight gain BWG was higher significantly. Additionally, feed conversion ratio (FCR) was at the lowest in 250-ppm lavender essential oil group compared to the control ($p \geq 0.05$). The result showed that the usage of Lavender essence was different influences pre-slaughter weight and carcass yield percentage.

Table 2 – The effect of experimental diets on broilers performance

Treatments	Average FI (g.d)	Average BWG (g.d)	FCR	Pre-slaughter weight (g)	Carcass yeild (%)
Control	18.54	6.99	2.75	244.15	78.36
100ppm lavender essential oil	19.12	7.11	2.60	248.14	79.92
200ppm lavender essential oil	20.36	7.26	2.54	254.12	80.88
250ppm lavender essential oil	20.27	7.34	2.47	259.71	81.63
Bacitracin Antibiotic	20.46	7.45	2.34	260.85	82.40
SEM	0.512	0.416	0.189	11.26	2.44
P-Value	n.s	n.s	n.s	n.s	n.s

**Means within columns with no common on letter are significantly different ($p \leq 0.05$).

The results of (Salajeghe et al., 2019) showed that supplementation of lavender powder at 1 % level significantly increased feed intake during the finisher and entire rearing periods. In addition, they were noted that the body weight gain and feed conversion ratio improved during the grower, finisher and entire rearing periods Table 3. Laghouati et al (2020) had demonstrated that the addition of lavender oil was accompanied by reductions in liver weights; furthermore, lavender oil had a significant effect on the pH, water content, and fat content of the meat. Manfi et al (2016) showed that the inclusion of bacitracin methylene disalicylate increased and feed consumption, feed conversion ratio ($p \leq 0.05$).

Table 3 – The effect of experimental diets on some visceral organs percentage

Treatments	Gizzard	Liver	Breast	Drumstick	Intestine
Control	2.33	2.95	36.39	24.85	3.51
100ppm lavender essential oil	2.51	2.72	36.76	25.62	3.69
200ppm lavender essential oil	2.74	2.59	37.81	26.31	3.76
250ppm lavender essential oil	2.89	2.38	38.93	26.99	3.92
Bacitracin Antibiotic	2.70	2.32	39.87	26.78	3.96
SEM	0.280	0.130	0.461	0.468	0.438
P-Value	n.s	n.s	n.s	n.s	n.s

**Means within columns with no common on letter are significantly different ($p \leq 0.05$).

Data from this study showed that there were no significant differences between treatments about visceral organs percentage except liver ($p \geq 0.05$).

Nobakht et al (2010) showed that thehighest percent of carcass was observed in experimental group but the lowest percent of it observed in control group. The results of (Kiani et al., 2015) suggested that there was no significant difference at the final days of age between the live weight and other body organs of experimental treatments. They also mentioned that there was no significant difference between the mean of spleen weight in the experimental treatments. They noted that the mean of gizzard weight showed no significant difference between the herbal experimental treatments. Javed et al (2006) suggested that there is a possibility of gathering to antimicrobial herbs made a remarkable decrease in the intestine microbial colony and this prevented from lyses of amino acids and they may use in formation of protein issues and increased the breast percentage. Many herbal plants have stimulatory effects on pancreatic secretions by increasing the secretions of digestive enzymes more amounts of nutrients as if amino acids can be digested and absorbed from the digestive tract and thereby improve carcass traits (MOKHTARI et al., 2018; ZHENG et al., 2020). The increasing the percents of gizzard and liver by positive effects via physically grinding and increasing bile secretion on nutrient digestion and due increased amounts of absorbed amino acids, organs like breast and thigh drawn more growth (MOHITI-ASLI and GHANAATPARAST RASHTI,2017; YARMOHAMMADI BARBARESTANIA, 2020).

Data from Table 4 illustrated that the serum blood triglyceride, cholesterol, glucose and LDL were decreased and HDL increased significantly instead ($p \leq 0.05$) and there were no significantly differences between treatments for blood serum Glucose ($p \geq 0.05$).

Some significant influence of experimental diets on serum blood triglyceride, cholesterol, LDL and HDL was observed ($p \leq 0.05$). Most of spices and herbs enhance the synthesis and excretion of bile acids in the liver. As bile acids had beneficially effects on lipids' digestion and absorption, it could improve the lipids' digestion and absorption, which led to increase in the level of blood triglyceride (SRINIVASAN, 2005).

Table 4 – The effect of experimental diets on some blood biochemical

Treatments	Glucose	Triglyceride	Cholesterol	LDL	HDL
Control	173.34	191.45[a]	211.21[a]	153.22[a]	96.62[c]
100ppm lavender essential oil	172.21	172.22[b]	207.46[b]	150.11[a]	103.76[b]
200ppm lavender essential oil	171.45	168.34[b]	202.24[b]	145.69[ab]	110.23[b]
250ppm lavender essential oil	169.31	156.28[c]	192.37[c]	139.16[b]	114.21[a]
Bacitracin Antibiotic	169.65	149.51[c]	194.45[c]	138.26[b]	127.14[a]
SEM	0.126	18.28	6.28	7.38	7.38
P-Value	n.s	**	**	**	**

**Means within columns with no common on letter are significantly different (p≤ 0.05).

Nobakht et al (2010) mentioned that the experimental groups had significantly difference in blood glucose level (P≤0.05). The Lowest level of blood serum glucose was observed in the chicks were fed by Malva and the highest level of that was related to the control group Table 6.

Hosseini (2011) showed that the serum total cholesterol, Triglycerides and LDL concentration were significantly reduced in-group of herbal extract compared to the control group (P≤0.05). Also the concentration of serum HDL and were not significantly reduced in groups compared to the control groups. According to (Lee, 2003), high level of fibers can increase the excretion of bile and this can decrease the blood cholesterol level (P≤0.05).

Table 6– The effect of experimental diets on intestinal morphology

Treatments	Width (nm)	Length (nm)	Height (nm)	Mucus (nm)
Control	7.55	38.26	5.11	1.61
100ppm lavender essential oil	7.11	39.11	5.26	1.44
200ppm lavender essential oil	7.26	39.38	5.41	1.29
250ppm lavender essential oil	7.16	39.45	5.55	1.21
Bacitracin Antibiotic	6.98	40.26	5.61	1.12
SEM	0.036	1.26	0.021	0.016
P-Value	n.s	n.s	n.s	n.s

**Means within columns with no common on letter are significantly different (p≤ 0.05).

The result of experimental diets on small intestine (ileal) microflora has shown in table 7. Data form morphological study of small intestine showed that there were significant differences between treatments (P≤0.05). The Lavender essential oil supplementation can decrease Escherichia Coli and increase Lactobacillus colonies instead (P≤0.05).

Table 7– The effect of experimental diets on ileal microflora

Treatments	Escherichia Coli (CFU)	Lactobacillus (CFU)
Control	5.79^{b}	6.14^{a}
100ppm lavender essential oil	6.08^{ab}	5.66^{a}
200ppm lavender essential oil	6.19^{ab}	5.42^{a}
250ppm lavender essential oil	6.68^{a}	4.72^{ab}
Bacitracin Antibiotic	7.26^{a}	4.46^{b}
SEM	1.72	1.16
P-Value	**	**

**Means within columns with no common on letter are significantly different (p≤ 0.05).

The beneficial effects of herbal plants or their active substances may include the stimulation of appetite and increase feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, and antioxidant activities (KHEIRI et al., 2015). According to the results obtained by (Jafari et al., 2011) carried out on various levels of Malva extract and great burdock at 200 ppm in the fourth group, which was a mixture of the two extracts, the highest separated weight belonged to the great burdock, while the weight of herbal group was greater than that of the control. Considering the presence of natural anti-inflammatory and anti-septic materials such as tannins, anthocyanins, flavonoids and loco-anthocyanins found in Lavender, the increased level of absorption was significant (RABIEI et al., 2014). Kiani et al (2015) demonstrated that in morphological terms, however, the villi colon length and crypt depth showed a significant difference at day 42of aviculture experimental period. Laghouati et al (2020) showed that administration of lavender oil had a measurable effect on the endogenous intestinal population of Lactobacillus, and the bacterial load (including those of Escherichia coli and Staphylococcus aureus) was significantly reduced (P≤0.05). It can be inferred from those experiment that Malva at 400 mg leaves an effect, thus leading to improved intestinal villi length and crypt depth, and ultimately greater absorption brought about by Lavender's anti-inflammatory and anti-microbial properties (Brenes, 2010). The presence of harmful bacteria populations in the gastrointestinal tract may cause breakdown of amino acids and thereby reduce their absorption, some chemical compounds are present in

Lavender as antimicrobial substances may reduce the harmful bacteria populations' colonies in the gastrointestinal tract and improve the levels of absorbed amino acids (Manafi et al., 2014). Also (Lee et al ,2003) found that the existence of harmful microbes in digestive system causes an increase protein and amino acids lysis of nutrients, di-amination activity of proteins and amino acids and rapid decomposition of these molecules due to secretary substances from bacteria like urease(Jangs et al.,2007).

Conclusions

In conclusion, we could explain that lavender essential oil had beneficial acts on performance, carcass traits, some blood biochemical and intestinal flora in Japanese quails. Lavender has anti-inflammatory, astringent, laxative properties. This improvement may be due to the biological function Softhis herbal to improve growth or that may be due to its role as stimulant, carminative, enhanced digestibility antimicrobial properties. However further studies are needed for more explanations.

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