**Indian J Anim Health (2023), 62(2): 305-311**
DOI: https://doi.org/10.36062/ijah.2023.01023

**Research Article**

# Improvement in the gut microstructure and nutrient digestibility by dietary inclusion of nanoencapsulated lavender essential oil in broiler chicken

**S. Adil[1*], M. T. Banday[1], I. U. Sheikh[1], I. A. Baba[1], Z. Kashoo[2], S. Muzamil[3] and S. Shah[4]**

[1]Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST, Srinagar- 191 121, Kashmir, India; [2]Division of Veterinary Microbiology and Immunology, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST, Srinagar- 191 121, Kashmir, India; [3]Division of Veterinary Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST, Srinagar- 191 121, Kashmir, India; [4]Division of Veterinary Pathology, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST, Srinagar- 191 121, Kashmir, India

## Abstract

**This study investigated the effects of different dietary inclusion levels of Lavender essential oil (LEO) in free and nanoencapsulated form on intestinal gross and microstructure changes and subsequent nutrient digestibility in broiler chicken. At 1 week of age, 420 broiler chicks were randomly allocated into 7 treatment groups with 4 replicate pens, each containing 15 birds. The experiment lasted till the birds were 42 days of age. Dietary treatments included a corn-soybean based diet supplemented with 0 (T1), 20 mg/kg Enramycin as antibiotic growth promoter (T2), 300 mg/kg (nanoencapsulating material) chitosan (T3), 200 and 400 mg/kg free LEO (T4 and T5, respectively), 200 and 400 mg/kg nanoencapsulated LEO (T6 and T7, respectively). The results revealed that the dry matter metabolizability (DMM), digestible crude protein (DCP), ether extract (EE), crude fibre (CF), calcium (Ca), and phosphorous (P) digestibility improved in the groups fed LEO in the diet with more significance ($p<0.05$) in the groups wherein nanoencapsulated form of LEO was used. Similarly, the intestinal length and weight, ileal villus height (VH), and villus height to crypt depth (VH:CD) improved significantly ($p<0.05$) in all the LEO groups, particularly in nanoencapsulated groups (T6 and T7) compared to control. In conclusion, nanoencapsulation of LEO is a viable strategy for improving the intestinal gross and microstructure, and subsequent nutrient digestibility in broiler chicken.**

**Keywords**: Broiler chicken, Lavender essential oil, Metabolism trial, Nano-encapsulation

## Highlights

- LEO, free and nanoencapsulated, was used in the diet of broiler chicken.
- Nanoencapsulation of LEO was performed through ionic gelation method.
- DMM, DCP, EE, CF, Ca and P digestibility improved particularly in nanoencapsulated LEO groups.
- Intestinal length and weight improved in groups fed nanoencapsulated LEO based diet.
- Ileal VH and VH:CD ratio increased in the birds belonging to nanoencapsulated LEO groups.

## INTRODUCTION

Administration of sub-therapeutic antibiotics has been used widely to increase weight gain, improve feed efficiency, reduce morbidity and mortality in poultry birds (Zeng *et al.*, 2015), and reduce poultry and human foodborne pathogens (Sims *et al.*, 2004). Although they have been applied in poultry for over 50 years, the use of antibiotic growth promoters (AGPs) has declined (Sneeringer *et al.*, 2015) due to consumer preferences (Brewer and Rojas, 2007) and regulations (Castanon, 2007). Additionally, there are concerns over the development of antibiotic resistance in bacteria (Forgetta *et al.*, 2012) and antibiotic residues in meat and other livestock products which pose a risk to public health and the environment (Jazi *et al.*, 2018). This has led many countries to restrict the use of antibiotics in animal feed. Accordingly, it is necessary to identify cost-effective, safe, and eco-friendly alternatives to antibiotics. Among various safe feed additives, the use of phytogenics and their derivatives like essential oils (EOs) in livestock nutrition has recently received increased attention owing to their important biological activities including antioxidant, antimicrobial, and anti-inflammatory (Windisch *et al.*, 2008; Brenes and Roura, 2010).

EOs are volatile, lipophilic materials extracted from plants, and there are more than 100 kinds of EOs (Wei and Shibamoto, 2007). The activity of EOs is strongly associated with their chemical composition, functional groups and synergistic interactions between components (Nazzaro *et al.*, 2013). In the present study, one such EOs was used,

*Corresponding Author, E-mail: aadilsheikh5@gmail.com

namely lavender essential oil obtained from lavender plant (*Lavandula officinalis*). Lavender oil is one of the most popular and versatile essential oils used in aromatherapy and is believed to have antibacterial, antifungal, anti-inflammatory and antiseptic properties (da Silva *et al.*, 2015). It has many bioactive compounds particularly linalol and linalyl acetate (Fakhari *et al.*, 2005).

However, the direct inclusion of EOs into animal diets has shown limitations, related to the reactive, hydrophobic, and volatile nature of the EOs (Jemaa *et al.*, 2018). The efficacy of phytocompounds has been shown to increase if they are used in a protected such as encapsulated form (Ribeiro-Santos *et al.*, 2017). Encapsulation involves the process wherein small particles are surrounded by a capsule coating, and when encapsulation is done at a nanoscale (1-100 nm), it is known as nanoencapsulation (Quintanilla-Carvajal *et al.*, 2010). Nanoencapsulation is a new technology that can mask the unpleasant taste and aroma of plant based bioactive compounds, besides protecting them against adverse environments such as high temperature, high humidity, and gastric pH, thus releasing their contents in a sustained manner at controlled rates, thereby increasing their bioavailability (Natrajan *et al.*, 2015; Hosseini and Meimandipour, 2018).

Therefore, the present study aimed to investigate the effect of nanoencapsulated LEO as an alternative to AGPs on intestinal gross and histomorphological changes and subsequent nutrient digestibility in broiler chicken.

## MATERIALS AND METHODS

### Experimental site and material

The experiment was conducted at the Instructional Poultry farm of the Division of LPM, FVSc and AH., SKUAST-K, Shuhama, Srinagar. The birds (Cobb strain) and feed ingredients were procured from local dealers. The lavender oil was obtained from aerial parts of lavender plant (*Lavandula officinalis*) grown in the Field station of CSIR, Bonera, Pulwama, Kashmir.

### Nanoencapsulation of lavender essential oil (LEO)

Nanoencapsulation of the LEO in chitosan nanoparticles was done by ionic gelation as per the procedure of Yoksan *et al.* (2010). Chitosan at the concentration of 1 mg/mL was dissolved in 1% acetic acid (w/v), sonicated and centrifuged for 30 min at 10000 rpm. The supernatant was removed and filtered through 1µm pore size filter paper. LEO in a desired ratio was added dropwise to this solution under homogenization to get an emulsion. Thereafter, 1 mg/mL solution of sodium triphosphate pentabasic was added dropwise into the agitated emulsion for about 40 minutes. Centrifugation was done again for 30 min at 10000 rpm at 4°C, and several washings were done with distilled water. The solution was ultrasonicated for 5 min at 40 kHz, frozen immediately and afterwards freeze dried (Lyovapor™ L-200, BUCHI).

### Morphological assessment of LEO loaded CNPs

The morphology of LEO loaded chitosan nanoparticles was studied by Scanning Electron Microscopy (SEM) technique using Hitachi 3600 N Scanning Electron Microscope. The samples were mounted on the grid and coated with gold before taking the SEM images at a magnification of 50KX.

### Experimental diets

The feeding regime comprised of starter diet until 21 days and a finisher diet until 42 days of age. The diets were prepared as iso-nitrogenous and iso-caloric and used throughout the experiment. The ingredient composition of the basal diet is given in Table 1. The nutrient composition percentage of the basal diet is presented in Table 2. All the diets were prepared with the same batch of ingredients, and all diets within a period had the same composition. At 1 week of age, a total of four hundred

**Table 1. Ingredient composition of basal control diet fed to broiler chicken**

| Ingredients (g/kg) | Starter (7-21 days) | Finisher (22-42 days) |
|---|---|---|
| Maize | 550 | 586 |
| Soybean meal | 350 | 320 |
| Fish meal | 40 | 20 |
| Vegetable oil | 30 | 40 |
| Limestone | 7.00 | 10.00 |
| Di-calcium phosphate | 15.00 | 16.00 |
| Salt | 3.00 | 3.00 |
| DL-Methionine | 1.10 | 1.00 |
| Lysine | 1.30 | 1.40 |
| Trace mineral Premix[1] | 1.00 | 1.00 |
| Vitamin Premix[2] | 1.50 | 1.50 |

[1]Trace mineral premix (mg/kg diet): Mg 300, Mn 55, I 0.4, Fe 56, Zn 30 and Cu 4; [2]Vitamin premix (per kg diet): vitamin A 8250 IU, vitamin D3 1200 ICU, vitamin K 1 mg, vitamin E 40 IU, vitamin B1 2 mg, vitamin B2 4 mg, vitamin B1 210 mg, niacin 60 mg, pantothenic acid 10 mg, choline 500 mg

**Table 2. Nutrient composition of basal control diet fed to broiler chicken**

| Attribute | Starter (7-21 days) | Finisher (22-42 days) |
|---|---|---|
| Crude protein* | 223 | 203 |
| Metabolizable energy** (Kcal/kg) | 3062 | 3150 |
| Calcium* | 10.10 | 10.50 |
| Available phosphorous* | 4.70 | 4.40 |
| Lysine** | 13.00 | 11.80 |
| Methionine** | 5.00 | 4.40 |

*Analysed values, **Calculated values

twenty day old broiler chickens were randomly allocated into 7 treatment groups with 4 replicate pens, and each pen contained 15 birds. The experiment lasted till the birds were 42 days of age. Dietary treatments included corn-soybean based diet supplemented with 0 (T1), 20 mg/kg Enramycin as antibiotic growth promoter (T2), 300 mg/kg (nanoencapsulating material) chitosan (T3), 200 and 400 mg/kg free LEO (T4 and T5, respectively), 200 and 400 mg/kg nanaoencapsulated LEO (T6 and T7, respectively).

The birds had unrestricted access to feed and were offered *ad-libitum* drinking water throughout the experiment. The birds were maintained on a recommended lighting schedule. The vaccination was carried out as per area specific prevalent diseases. Chicks were checked twice daily for mortality, if any. All the birds were managed under uniform husbandry conditions.

**Parameters estimated**

**Metabolizable energy bioassay:** In order to study the dry matter metabolizability (DMM), digestible crude protein (DCP), ether extract (EE), crude fibre (CF), calcium (Ca) and phosphorous (P) retention, a metabolism trial lasting for four days was conducted at 5$^{th}$ week of age (35$^{th}$ to 38$^{th}$ day) during which the net feed consumed by each group of birds in the respective dietary treatments was recorded and the droppings voided over same period were collected quantitatively. After completing 34 days of the feeding trial, all the birds were starved for a period of 2 hours (i.e., from 8 to 10 AM) during which the preliminary preparation such as cleaning of feeders and faecal trays, provision of fresh drinking water and weighing of required quantity of feed into respective feeders were carried out. Exactly at 10 AM, the respective feeder and faecal trays were introduced into cage housing, and weighed quantity of feed was offered to all the birds. The droppings of respective dietary group of each bird were collected separately, once daily and transferred into pre-weighed aluminium dishes, weighed again and placed into the forced draft hot air oven at 60±5ºC during all 4 days of collection. On the last day, the feeders were removed at 10 AM to determine the net feed intake and faecal trays were removed to collect the faeces in aluminium dishes. The dropping collected were dried for 4- 5 days in an oven at 60±5ºC till a constant weight was attained, which represented the net dried faecal output. The representative sample of test diets and excreta samples were ground and stored in air-tight containers for further analysis for dry matter, nitrogen, phosphorous (AOAC, 2000) and calcium (Talpatra *et al.*, 1940). The intake, excreted, and retained amount of dry matter, nitrogen, calcium and phosphorous were calculated in grams per bird per day (g/b/d) basis and percent (%) retention was calculated on the basis of total intake by following formulas:

Nutrient retention % = (Nutrient intake-Nutrient in faeces)/Nutrient intake x100

**Intestinal gross and histomorphometry:** On day 42, 4 birds per treatment (1 bird/replicate) were randomly selected and bled by Halal method in the slaughterhouse of the Division of Livestock Products Technology, FVSc & AH, SKUAST-Kashmir. The birds were eviscerated, and samples of intestines were collected. Intestinal length was measured with the help of a measuring scale, and intestines were weighed using a weighing balance. Ileal samples (approximately 2 cm in length) were cut with scissor and washed in physiological saline to remove all the contents and then fixed in 10% buffered formalin solution for subsequent histomorphological investigations. The tissue samples were cut into pieces of thickness 2–3 mm and washed under water for a few hours prior to dehydrating in ascending grades of alcohol and later cleared in benzene and embedded in paraffin. Tissue sections of 4–5 µm thickness were stained using the Harri's Haematoxylin and Eosin method (Lillie and Denton, 1965). Villus height (VH), crypt depth (CD) and VH:CD ratio were estimated.

**Statistical Analysis**

The analysis of data regarding blood biochemical parameters was carried out by using one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) using statistical package SPSS version 21.0. The differences within the means were estimated using Duncan's multiple range test (Duncan, 1955) by considering the differences at a significant level ($p<0.05$).

**RESULTS**

**Morphology:** The morphology of LEO loaded CNPs analysed through scanning electron microscopy is presented in Fig. 1. The ionic gelation resulted in spherical shape of LEO loaded CNPs with smooth surfaced and having aggregations in some locations.

**Metabolic bioassay:** The data pertaining to DMM, DCP, EE, CF, Ca and P retained has been presented in Table 3. The values of DMM (%) in different treatments varied from 73.04 in T1 to 79.61% in T7 groups, DCP values varied from 64.75 in T1 to 74.61% in T7 groups, EE values ranged from 76.06 in T1 to 82.11% in T7 groups, CF values were in between 17.08 in T1 to 21.58% in T7 groups, Ca retention varied from 48.73 in T3 to 55.61% in T7 groups and P retention varied from 53.07 in T1 to 61.15% in T7 groups. Results revealed that supplementation of LEO (free and nanoencapsulated) significantly ($p<0.05$) increased the

**Table 3. Effect of supplementing LEO (free and nanoencapsulated) on nutrient digestibility of broiler chicken**

| Dietary Treatments[2] | Parameters (%)[1] | | | | | |
|---|---|---|---|---|---|---|
| | DMM | DCP | EE | CF | Ca | P |
| T1 | 73.04$^{c}$ | 64.75$^{c}$ | 76.06$^{c}$ | 17.08$^{cd}$ | 49.10$^{c}$ | 53.07$^{d}$ |
| T2 | 76.05$^{b}$ | 69.08$^{b}$ | 79.68$^{b}$ | 15.58$^{d}$ | 52.29$^{b}$ | 57.64$^{bc}$ |
| T3 | 73.47$^{c}$ | 65.14$^{c}$ | 76.30$^{c}$ | 18.29$^{bc}$ | 48.73$^{c}$ | 54.05$^{d}$ |
| T4 | 75.70$^{b}$ | 67.59$^{bc}$ | 78.83$^{b}$ | 19.31$^{b}$ | 50.89$^{b}$ | 56.84$^{c}$ |
| T5 | 75.92$^{b}$ | 68.48$^{b}$ | 78.23$^{b}$ | 19.93$^{ab}$ | 51.50$^{b}$ | 57.15$^{bc}$ |
| T6 | 78.66$^{a}$ | 73.28$^{a}$ | 81.73$^{a}$ | 21.15$^{a}$ | 54.48$^{a}$ | 59.97$^{ab}$ |
| T7 | 79.61$^{a}$ | 74.61$^{a}$ | 82.11$^{a}$ | 21.58$^{a}$ | 55.61$^{a}$ | 61.15$^{a}$ |
| SEM | 0.50 | 0.74 | 0.47 | 0.43 | 0.49 | 0.61 |
| P-value | p<0.05 | p<0.05 | p<0.05 | p<0.05 | p<0.05 | p<0.05 |

Means in a column with different superscripts differ significantly (p<0.05); [1]DMM: Dry matter metabolizability, DCP: Digestible crude protein, EE: Ether extract, CF: Crude fibre, Ca: Calcium, P: Phosphorus; [2]T1: Control, T2: basal diet+10 mg/kg Enramycin, T3: basal diet+300 mg/kg nanochitosan, T4: basal diet+200 mg/kg free LEO, T5: basal diet+400 mg/kg free LE), T6: basal diet+200 mg/kg nanoencapsulated LEO and T7: basal diet+400 mg/kg nanoencapsulated LEO

**Table 4. Effect of supplementing LEO (free and nanoencapsulated) on the gross morphology of small intestine at 42 days post hatch in broiler chicken**

| Dietary Treatments[2] | Parameters | | | |
|---|---|---|---|---|
| | Duodenum (cm) | Jejunum (cm) | Ileum (cm) | Weight of SI[1] (g) |
| **T1** | 29.85$^{c}$ | 64.87$^{d}$ | 61.43$^{d}$ | 98.84$^{b}$ |
| **T2** | 28.37$^{c}$ | 64.04$^{d}$ | 60.55$^{d}$ | 89.23$^{c}$ |
| **T3** | 30.67$^{c}$ | 65.10$^{c}$ | 63.54$^{c}$ | 99.19$^{b}$ |
| **T4** | 31.42$^{b}$ | 67.27$^{b}$ | 65.59$^{b}$ | 101.37$^{ab}$ |
| **T5** | 31.57$^{b}$ | 67.10$^{b}$ | 65.97$^{b}$ | 102.76$^{a}$ |
| **T6** | 32.10$^{a}$ | 69.13$^{a}$ | 68.06$^{a}$ | 104.10$^{a}$ |
| **T7** | 32.40$^{a}$ | 69.63$^{a}$ | 68.61$^{a}$ | 104.45$^{a}$ |
| **SEM** | 0.28 | 0.41 | 0.58 | 0.99 |
| **P-value** | p<0.05 | p<0.05 | p<0.05 | p<0.05 |

Means in a column with different superscripts differ significantly (p<0.05); [1]SI: Small intestine; [2]T1: Control, T2: basal diet+10 mg/kg Enramycin, T3: basal diet+300 mg/kg nanochitosan, T4: basal diet+200 mg/kg free LEO, T5: basal diet+400 mg/kg free LE), T6: basal diet+200 mg/kg nanoencapsulated LEO and T7: basal diet+400 mg/kg nanoencapsulated LEO

**Table 5. Effect of supplementing LEO (free and nanoencapsulated) on the histomorphology of ileum at 42 days post hatch in broiler chicken**

| Parameter[1] | Dietary Treatments[2] | | | | | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | | |
| VH (μm) | 874$^{c}$ | 903$^{bc}$ | 876$^{c}$ | 908$^{bc}$ | 901$^{bc}$ | 927$^{a}$ | 933$^{a}$ | 5.97 | p<0.05 |
| CD | 170$^{a}$ | 159$^{bc}$ | 169$^{a}$ | 157$^{bc}$ | 153$^{bc}$ | 149$^{c}$ | 147$^{c}$ | 2.52 | p<0.05 |
| VH:CD ratio | 5.17$^{c}$ | 5.70$^{bc}$ | 5.21$^{c}$ | 5.83$^{bc}$ | 5.91$^{bc}$ | 6.26$^{a}$ | 6.37$^{a}$ | 0.12 | p<0.05 |

Means in a row with different superscripts differ significantly (p<0.05); [1]VH: Villus height, CD: Crypt depth; [2]T1: Control, T2: basal diet+10 mg/kg Enramycin, T3: basal diet+300 mg/kg nanochitosan, T4: basal diet+200 mg/kg free LEO, T5: basal diet+400 mg/kg free LE), T6: basal diet+200 mg/kg nanoencapsulated LEO and T7: basal diet+400 mg/kg nanoencapsulated LEO

nutrient retention regarding all the above parameters compared to control, with more significance in the groups wherein LEO was supplemented in nanoencapsulated form (T6 and T7). Nutrient retention was found to be highest in T7 (400 mg/kg nanoencapsulated LEO) supplemented group, whereas the lowest percentage was found in the T1 (control) and T2 (Chitosan) groups. The results of the T3 (Antibiotic) group were comparable with that of the free LEO supplemented groups.

**Intestinal gross and histomorphometry:** The results pertaining to gross morphology of small intestines (Table 4) and ileal histomorpology are presented in Table 5 and Fig. 2. The length of different parts of small

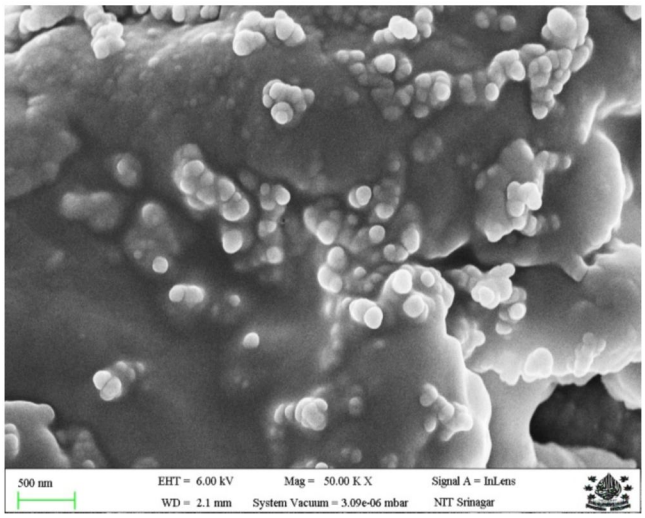


**Fig. 1. Scanning electron microscopy (SEM) of LEO loaded Chitosan nanoparticles**

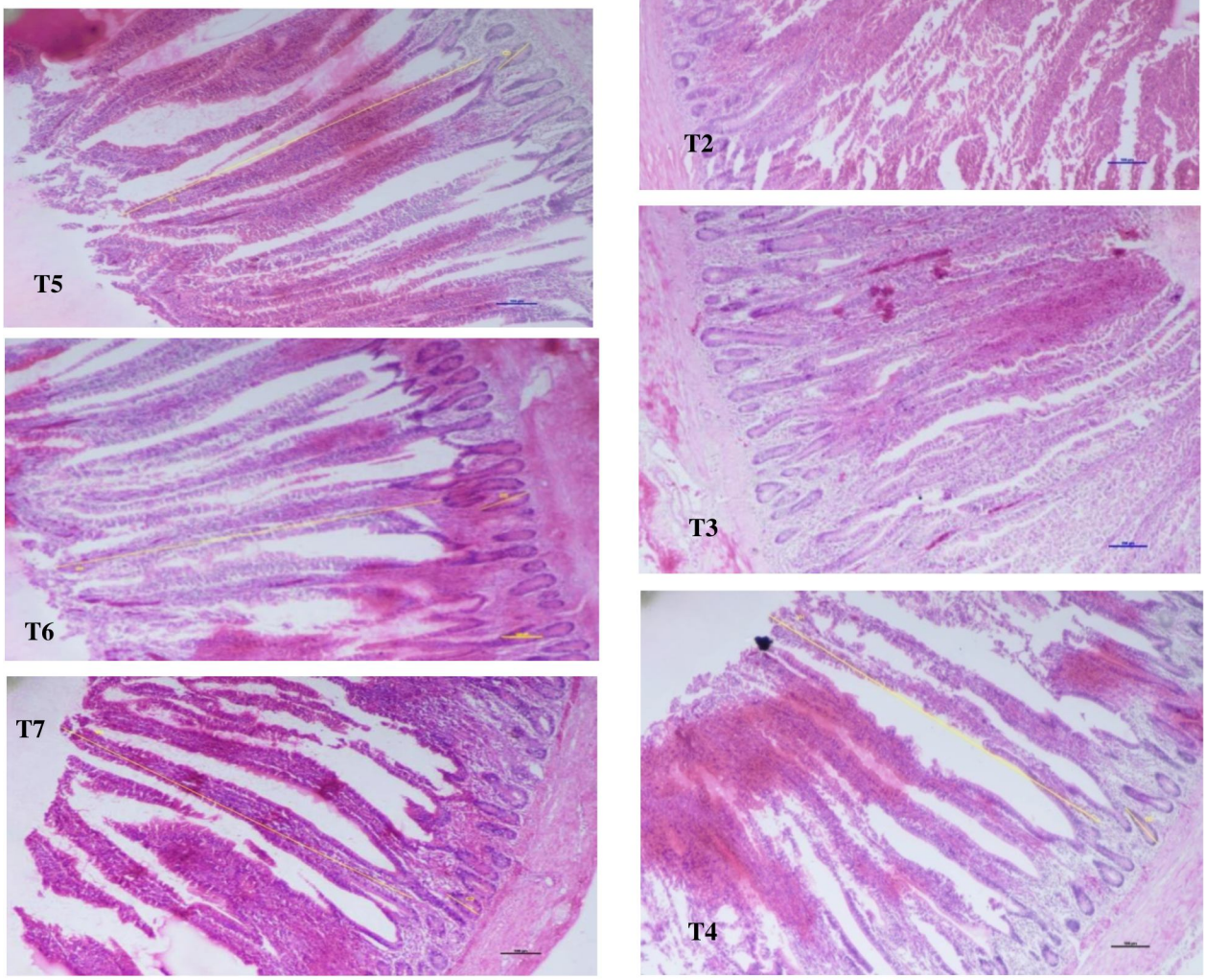


**Fig. 2. Histological micrographs of ileum of broiler chicken in various treatments (T1 to T7) (Scale bar = 100 µm)**

intestine, i.e., duodenum, jejunum and ileum varied significantly between dietary treatments. The length (cm) of duodenum ranged from 28.37 in T2 to 32.40 in T7, jejunum between 64.04 in T2 to 69.63 in T7, and ileum between 60.55 in T2 to 68.61 in T7. The weight (g) of the small intestine varied from 89.23 in T2 to 104.45 in T7. Histomorphology of ileum revealed that VH (μm) varied from 874 in T1 to 933 in T7, CD ranged from 149 in T7 to 170 in T1 and VH:CD ratio varied from 5.71 in T1 to 6.37 in T7 groups. Nanoencapsulation of LEO was found to significantly ($p<0.05$) improve the gross morphology, i.e., length and weight of small intestines than the control group and improved the VH and VH:CD ratio compared to control and other treatment groups. The maximum increase in all the parameters was observed in T7 (400 mg/kg nanoencapsulated LEO) group.

## DISCUSSION

**Metabolic bioassay:** The digestibility study makes it possible to understand the percentage of nutrient utilization absorbed in the digestive tract and constitutes the fraction of the feed that can be used for metabolism (INRA, 1989). Determination of nutrient digestibility is a quantitative assessment of the nutritional and physiological phenomena associated with digestion capacity and gut functionality (Soumeh *et al.*, 2019). Nutrient digestibility refers to the extent to which dietary nutrients are absorbed and assimilated as they pass through the bird's GIT (Nhlane *et al.*, 2020). Elbaz *et al*. (2022) reported a significant improvement in CP and DM digestibility in birds fed EOs compared to control fed birds. Other workers also reported improved total tract apparent retention in EOs groups compared to the control. Similar reports at high levels of EOs have been reported by Dalkiliç and Guler (2009). Kumar *et al*. (2017) also reported improvement in the digestibility of nutrients in broilers by supplementation of phytogenics in the diet. Hernandez *et al.* (2004) studied the effect of 200 ppm essential oil extract from oregano, cinnamon, and pepper; and 5,000 ppm labiatae extract from sage, thyme, and rosemary fed with the standard diet and found that plant extract supplementation improved apparent whole-tract and digestibility of the nutrients and dry matter digestibility. Park and Kim (2018) and Mohebodini *et al.* (2021) also reported improved nutrient digestibility and retention in broilers by using EOs in the diet. Prasad *et al.* (2009), however, reported that EOs didn't improve the digestibility of nutrients in the gut of birds. The difference in the results has been attributed to the fact that it may be due to the type of EOs used, inclusion level, etc., as some oils irritate the wall of the intestinal lining, leading to some inflammation (Su *et al.*, 2020).

EOs favourably affect gut functions by stimulating digestive secretions, e.g., bile, mucus etc. and increase enzyme activity (Brenes and Roura, 2010). EOs enhance secretion of trypsin, amylase and jejunal chyme (Jang *et al.*, 2007) and reduce adherence of pathogens to the intestinal wall and also improve morphology of intestines (Windisch *et al.*, 2008; Brenes and Roura, 2010). Lee *et al*., 2003 also demonstrated that ileal digestibility coefficients for starch and protein in broiler chicken fed diets containing EOs were higher owing to the greater activity of amylase. Additionally, other researchers (Chowdhury *et al.*, 2018; Mohedodini *et al.*, 2021) attributed the enhanced nutrient digestibility to the effect of EOs on intestinal morphology, gut microbial balance, antioxidative and immunity status of broiler chicken.

**Intestinal gross and histomorphometry**

The intestinal gross and mucosa architecture reveals useful information about gut health of chicken. Improvement in the length and weight of intestines was recorded in the groups fed lavender essential oil in the diet compared to control. The antimicrobial, anti-inflammatory and antioxidant properties of EOs have been attributed to play an important role in intestinal morphometric development (Du *et al.*, 2016). Improvement in the length of intestines means an improvement in the surface area for digestion and absorption of nutrients, thus supporting the results of increased nutrient digestibility in the groups fed LEO in the diet as recorded in our study. Further, the small intestinal villi are considered to be the important components involved in nutrient absorption. Poor nutrient absorption, lower disease resistance and performance have been attributed to shorter villi and deeper crypts (Xu *et al.*, 2003). The increased VH has been associated with increased activity of digestive enzymes, which in turn improves digestibility (Baurhoo *et al.*, 2007), thus supporting the results of improved nutrient digestibility found in the present study. In our study, dietary inclusion of LEO (free or nanoencapsulated) resulted in improved VH in the ileum of birds compared to control, with the highest improvement in nanoencapsulated LEO groups (T6 and T7). Chowdhury *et al.* (2018) also reported an increase in the small intestine VH of broilers fed EOs comprised diets than control. Other researchers have recorded similar findings (Elbaz *et al.*, 2022). El-Baroty *et al.* (2010) reported that EOs prevents villi damage by increasing antioxidant enzyme activity, which helps in better VH. Likewise, other workers (Park and Kim, 2018) associated EOs effect of improvement in the microstructure of intestines with reduction in the production of toxins by EOs through microbial modification in the intestines. In the present study, the CD was reduced in all the LEO

supplemented groups. As per Samanya and Yamauchi (2002), the size of CD can reflect the intensity of renewal of intestinal epithelial cells and intestinal health. Deeper crypts have been associated with faster tissue turnover and high demand for new tissues because of tissue damage (Xu *et al.*, 2003). Furthermore, an increased VH:CD ratio was observed in LEO supplemented groups, particularly nanoencapsulated groups. Thus, the overall improvement in the intestinal gross and microstructure in our study might have improved the digestive and absorptive functions, thereby supporting the fact of improved nutrient digestibility in LEO groups, particularly nanoencapsulated LEO groups than the control group.

On the basis of our study, it can be concluded that LEO supplementation, especially in nanoencapsulated form, proved to be a viable strategy as against antibiotic feed additives in improving the gross and histological architecture of intestines and subsequent nutrient digestibility in broiler chicken.

**Conflict of interest:** Authors have no conflict of interest in this study.

**Author's contribution**: SA, MTB, IUS: Conceptualized the idea; SA: Carried out the experiment; IAB, MAW: Helped during the trial and collection of samples; ZK, SM, SS: Assisted in processing of the samples.

**Ethical Statement:** The study has been approved by the Institutional Animal Ethics Committee of the Faculty of Veterinary Sciences, Shuhama, SKUAST-K.

**ACKNOWLEDGEMENT**

We are highly thankful to Dr. Shahid Rasool, Scientist Incharge, Field Station Bonera Pulwama, CSIR for his help in obtaining LEO. We appreciate the help and support of the Department of Food Science and Technology, University of Kashmir, Srinagar, India for facilitation during nanoencapsulation of LEO.

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*Received- 16.02.2023, Accepted- 19.07.2023, Published- 12.09.2023 (Online), 01.12.2023 (Print)*
**Section Editor:** Dr. A. K. Patra, Associate Editor