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Effects of lemongrass (*Cymbopogon citratus*) and aloe vera (*Aloe barbadensis*) as feed additives on morphometry of small intestine of Cobb strain chicks



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ABSTRACT

The present study was designed to investigate the effect of lemongrass, aloe vera and combination of lemongrass and aloe vera as dietary supplement to assess histomorphometry of small intestines of Cobb strain broilers under normal and heat stress condition. All the micrometrical parameters showed a significant increase ($P < 0.05$) in duodenum, jejunum and ileum in all the experimental groups (C1, C2, T1, T2, and T3) with the advancement of age. A significant increasing ($P < 0.05$) trend in the height of epithelial cells in all three segments of the small intestine was seen in C1, T1, T2 and T3 groups as compared with the heat stressed (C2) group on day 28 and 42. A significantly higher ($P < 0.05$) villus height of all three segments of intestines was observed in experimental groups (C1, T1, T2 and T3) as compared to C2 (heat stress) group on day 28 and 42. Significantly higher ($P < 0.05$) villus width of intestinal segment was seen in the supplemented (T1, T2 and T3) groups and in C1 (normal temperature) as compared to C2 (heat stress) group. Significantly lower ($P < 0.05$) values for the width of lamina muscularis were recorded in heat-stressed control (C2) when compared to birds reared in normal control temperature (C1).

Keywords: Lemongrass; Aloe vera; Morphometry; Small Intestine; Cobb strain chicks.

Introduction

The poultry industry plays a significant role in the Indian economy. There is a great demand of chicken meat, as many households prefer chicken meat over other meats like mutton, beef, and pork. Cobb strain chicks are regarded as the world's most efficient broiler breed because of the lowest cost of live weight produced, superior performance on lower cost feed rations, most feed efficient and excellent growth rate. Recent ban on the use of antibiotic growth promoters has drawn the concerns of researchers. Commonly known herbs are aloe vera, fenugreek, ashwagandha, moringaoleifera, cinnamon, tulsi, garlic, pepper etc. can be used as natural feed additives and an alternative to antibiotics for poultry (Abd El-Hack *et al* 2022). Lemongrass (*Cymbopogon citratus*) is a feed additive used to enhance the growth and increase the performance in poultry. This grass contains flavonoids, phenolic compounds, terpenoids (Lewis 1986) and essential oils, which may be responsible for its different biological activities such as growth promoter, antibacterial, antidiarrheal, antifungal and antioxidant (Shah *et al* 2011).

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Aloe vera (*Aloe barbadensis*) as an additive to broiler chicken feed, has great potential for improving growth performance, carcass characteristics, haemato-biochemical parameters, intestinal health, immune system response and cost of production.

Chickens, being endothermic animals, can maintain a core body temperature up to 41°C through latent and sensible thermal losses. Therefore, when they are exposed to thermal challenges, they are unable to maintain a balance between the heat generation and heat removal from the body, which results in increased core body temperature, leading to mortality. The present study was aimed to analyze the impact of lemongrass and aloe vera on small intestine morphology of broilers during normal and heat stress conditions.

Materials and Methods

Location of work of the experiment

The study was conducted in the Division of Veterinary Physiology and Biochemistry in collaboration with the Division of Veterinary Anatomy, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu, R.S. Pura, UT of J&K.

Experimental birds under study and housing

A total of 300 day-old Cobb strain chicks were obtained from Venky's Private Limited, Satwari, Jammu. The experimental outset was started in the month of mid-April and ended in the month of May in their growth phase for a period of 42 days.

The birds were reared under standard management practices. The rearing of birds was done in starter phase (day 1 to day 14) and grower phase (day 15 to day 42). The chicks were kept on deep litter system with a floor area @ 0.5 sq ft per chicks with a light intensity of 20 lux and duration of 24 hours of light. The birds were individually weighed before being randomly assigned to 15 pens with 20 birds per pen. The experimental birds were bedded on clean wood shavings and rice hulls with a depth of 8 cm. Chicks were randomly distributed in different groups of control and treatment (C1, C2, T1, T2 and T3) with 3 replicates of each containing 20 chicks.

Dietary regimn

Diets were formulated according to the NRC recommendations (1994). The composition of diets is shown in Table 1.

Table 1: Composition of the ration

Ingredients	Starter ration (%)	Finisher ration (%)
Maize	60.88	67.5
Soybean meal	30.37	24.5
MBM	5	5
Salt	0.25	0.25
Refined oil	2.7	2.2
Sodium bicarbonate	0.01	0.01
Methionine	0.13	0.09
LSP	0.59	0.29
Vitamin supplement	0.05	0.05
Trace mineral mixture	0.1	0.1

Grouping of the experimental birds

A total of 300 Cobb strain chicks were randomly distributed in five different groups of control (C1 & C2) and treatment (T1, T2 & T3) with 3 replicates of each containing 20 chicks

I. Control group (C1): Birds falling in this group were given the basal diet and were kept under normal temperature

II. Control group (C2): Birds falling in this group were given the basal diet and were kept under heat stress conditions.

III. Treatment 1(T1): Birds in this group were supplemented with lemongrass @ 15mg/kg under heat stress conditions.

IV. Treatment 2(T2): Birds in this group were supplemented with aloe vera @ 1.5mg/kg under heat stress conditions.

V. Treatment 3(T3): Birds in this group were supplemented with the combination of lemongrass and aloe vera @ 15mg/kg and 1.5mg/kg under heat stress condition.

Application of heat stress

The chicks were acclimatized for 3 days after arrival. Heat stress was induced in C2, T1, T2 and T3 groups from day 4th to the end of the experimental period. Temperature was increased by using tarpaulin sheets and room heaters from 28^oC (4th day) to 48^oC on day 17, then maintained at 48^oC up to 42 day of the trial period.

Sample collection

Nine (09) chicks out of each group were sacrificed on day 3, 28 and 42 of experiment and tissue samples from different parts of small intestine (duodenum, jejunum and ileum) from each bird were collected and fixed in 10 per cent neutral buffered formalin (NBF) and processed as per the standard procedure (Luna 1968). Tissue sections of about 5 μ thickness were obtained in clean glass slides and then stained by routine Haematoxylin and Eosin stain and different micrometrical parameters were recorded using an image analyser (Magnus Microscope with camera, MX21i-TrLED).

1. Height of intestinal epithelium (μ m)
2. Villus height (μ m)
3. Villus width (μ m)
4. Width of muscularis laminae (μ m)

Statistical analysis

For all the recorded data in the present experiment, the standard statistical procedures have been followed (Snedecor and Cochran 1994). The data were presented by showing mean and standard error. The significant differences of values for different parameters studied were assessed by two-way analysis of variance. The data were analysed under polynomial contrast and Duncans post hoc multiple comparison at the significance level of 0.05.

Results

Height of epithelial cells

The height of epithelial cells of the duodenum, jejunum and ileum of Cobb strain broiler chicken in different treatment groups at different days of period growth stages are depicted in Table 2. A significantly increase ($P < 0.05$) of epithelial cell was observed in all the groups (supplemented and control) with the advancement of age.

In duodenum, significantly highest ($P < 0.05$) value was recorded in T3 group and significantly lowest ($P > 0.05$) value was observed in the heat stress (C2) group. In jejunum, significantly higher ($P < 0.05$) values were recorded in treatment groups than those of control group during the trial period. Lowest significant value ($P > 0.05$) was observed in heat stress (C2) group. In ileum, the height of epithelial cells showed significant variation among different treatment groups. The significantly highest ($P < 0.05$) value was recorded in treatment (T3) group.

Table 2: Effect of lemongrass and aloe vera supplementation on height of epithelial cells of small intestine (mean \pm SE) in Cobb strain broilers under normal and heat stress conditions

Groups	Days of Experiment		
	03	28	42
Duodenum (μm)			
C1	23.29 ^{aA} \pm 0.93	37.59 ^{bB} \pm 1.34	40.67 ^{cB} \pm 0.77
C2	23.19 ^{aA} \pm 1.25	31.04 ^{bA} \pm 0.85	30.33 ^{bA} \pm 0.89
T1	31.17 ^{bB} \pm 0.86	41.39 ^{bC} \pm 0.92	42.04 ^{bB} \pm 0.91
T2	34.3 ^{cC} \pm 2.95	39.36 ^{bC} \pm 0.95	41.47 ^{bB} \pm 1.02
T3	35.47 ^{cC} \pm 0.95	44.51 ^{bD} \pm 0.99	48.10 ^{cC} \pm 0.85
Jejunum (μm)			
C1	34.28 ^{aB} \pm 0.95	64.47 ^{bB} \pm 6.99	66.59 ^{bB} \pm 0.96
C2	31.60 ^{aA} \pm 0.69	60.50 ^{bA} \pm 0.97	62.58 ^{bA} \pm 0.98
T1	37.71 ^{aC} \pm 1.30	68.61 ^{bC} \pm 1.42	71.76 ^{cC} \pm 1.14
T2	36.461 ^{aBC} \pm 0.98	71.79 ^{bD} \pm 1.05	71.43 ^{bC} \pm 0.96
T3	40.81 ^{aD} \pm 1.25	71.54 ^{bD} \pm 0.98	72.35 ^{bC} \pm 0.93
Ileum (μm)			
C1	24.50 ^{aA} \pm 0.96	37.42 ^{bB} \pm 0.89	41.41 ^{cB} \pm 0.95
C2	23.50 ^{aA} \pm 0.94	29.49 ^{bA} \pm 0.89	36.55 ^{cA} \pm 0.95
T1	26.59 ^{aB} \pm 1.01	35.48 ^{bB} \pm 0.88	42.35 ^{cB} \pm 0.89
T2	28.46 ^{aBC} \pm 0.91	38.38 ^{bCD} \pm 0.89	45.55 ^{cC} \pm 0.93
T3	30.67 ^{aC} \pm 1.05	40.57 ^{bD} \pm 0.99	49.53 ^{cD} \pm 0.94

Mean \pm SE with different superscripts in row wise (a, b, c) and column wise (A, B, C, D, E) differ significantly ($P < 0.05$).

Villus height

The villus height of the duodenum, jejunum and ileum of Cobb strain broiler chicken in treatment and control groups at different growing stages are illustrated in Table 3. With the advancement of age, a significant increase ($P < 0.05$) of villus height of all the three segments of small intestine was observed in all the experimental groups.

Villus height of the duodenum showed significant variation among different experimental groups. Significantly higher ($P < 0.05$) values were observed in T3 group followed by T1 group, during the whole trial period.

Significantly lower ($P>0.05$) values were recorded in C2 (heat stress) group when compared with normal temperature group (C1) and supplemented (T1, T2 and T3) groups.

Villus height of the jejunum showed significantly higher ($P<0.05$) values in treatment groups as compared to control groups. Significantly lower ($P>0.05$) values were obtained in C2 (heat stress) group as compared to C1 (normal temperature) and other treatment groups during different days of experiment.

When compared the villus height of ileum portion of small intestine, significantly higher ($P<0.05$) values were recorded in T3 group as compared to the other treatment groups (T1 and T2) and control groups. Between T1 and T2 groups, significantly higher ($P<0.05$) villus height was found in T2 group on day 28th and 42nd of experiment. Significantly lower ($P<0.05$) villus height was recorded in C2 (heat stress) group as compared to C1 (normal temperature) group and other treatment groups (T1, T2 and T3) during the trial period.

Table 3: Effect of lemongrass and aloe vera supplementation on villus height of small intestine (Mean±SE) in Cobb strain broilers under normal and heat stress conditions

Groups	Days of Experiment		
	03	28	42
Duodenum(µm)			
C1	525.48 ^{ab} ±0.96	987.39 ^{bb} ±0.89	991.43 ^{cb} ±0.95
C2	459.47 ^{aA} ±0.89	929.43 ^{bA} ±0.87	934.51 ^{cA} ±0.92
T1	622.40 ^{ab} ±0.94	1230.39 ^{bd} ±0.90	1262.41 ^{cd} ±0.95
T2	618.47 ^{ac} ±0.91	1051.32 ^{bc} ±0.94	1129.57 ^{cc} ±1.03
T3	656.37 ^{ae} ±0.90	1286.37 ^{be} ±0.88	1298.38 ^{ce} ±0.93
Jejunum(µm)			
C1	101.40 ^{ab} ±0.92	596.18 ^{bb} ±0.82	627.49 ^{cb} ±0.90
C2	98.43 ^{aA} ±0.89	424.40 ^{bA} ±0.86	521.48 ^{cA} ±0.89
T1	421.54 ^{ac} ±3.96	737.37 ^{bc} ±0.93	756.09 ^{cc} ±0.46
T2	437.38 ^{ad} ±0.88	738.54 ^{bc} ±0.90	759.57 ^{cd} ±0.77
T3	439.38 ^{ad} ±0.89	743.58 ^{bd} ±0.88	779.39 ^{ce} ±0.93
Ileum(µm)			
C1	76.43 ^{ab} ±0.88	387.49 ^{bb} ±0.96	421.39 ^{cb} ±0.94
C2	65.39 ^{aA} ±0.91	327.44 ^{bA} ±0.89	332.49 ^{cA} ±0.98
T1	79.48 ^{ac} ±0.91	520.46 ^{bc} ±0.89	534.52 ^{cc} ±0.94
T2	81.52 ^{ac} ±0.92	538.52 ^{bd} ±0.89	547.59 ^{cd} ±0.96
T3	84.64 ^{ad} ±0.91	552.53 ^{be} ±0.92	564.79 ^{ce} ±0.98

Mean±SE with different superscripts in row wise (a, b, c) and column wise (A, B, C, D, E) differ significantly ($P<0.05$).

Villus Width

The villus width of the duodenum, jejunum and ileum of Cobb strain broiler chicken in different treatment and control groups in their growing stages are depicted in Table 4. With the advancement of age, a significant increase ($P<0.05$) of villus width of all the three segments of small intestine was observed in all the experimental groups.

In duodenum, significantly higher ($P<0.05$) values were recorded in T1 and T2 groups on day 3 as compared to other groups. On day 28 and 42, significantly higher ($P>0.05$) villus width was recorded in T3. When comparing the values between control groups, significantly higher ($P<0.05$) values were found in C1.

While comparing villus width of jejunum among groups, significantly higher ($P>0.05$) values were recorded in treatment groups as compared to control groups. Between the treatment groups, significantly higher ($P>0.05$) values were recorded in T3 group on day 28 (138.60 ±0.93µm) and day 42 (157.51 ±0.92 µm); however no significant variation were observed between T1 and T2 groups on day 28 and 42 of study period.

In the ileum among different experimental groups, significantly higher ($P<0.05$) values were recorded in T2 and T3 groups, followed by T1, than that of C1 and C2 on day 28.

On day 42, significantly higher ($P<0.05$) values were recorded in T3 group (194.51±0.90µm), followed by T2 (186.43 ±0.91 µm) and least in C2 group (114.50±0.90 µm).

Table 4: Effect of lemongrass and aloe vera supplementation on villus width of small intestine (Mean±SE) in Cobb strain broilers under normal and heat stress conditions

Groups	Days of Experiment		
	03	28	42
Duodenum(µm)			
C1	52.42 ^{aA} ±0.96	127.49 ^{bB} ±0.89	132.49 ^{cB} ±0.88
C2	53.47 ^{aA} ±0.89	97.53 ^{bA} ±0.88	100.53 ^{cA} ±0.90
T1	57.52 ^{ab} ±0.96	135.47 ^{bc} ±0.87	149.43 ^{cc} ±0.93
T2	59.38 ^{ab} ±0.95	138.33 ^{bd} ±0.33	150.45 ^{cc} ±0.89
T3	54.56 ^{aA} ±0.89	148.57 ^{bE} ±0.90	154.47 ^{cE} ±0.89
Jejunum(µm)			
C1	57.52 ^{aA} ±0.92	99.45 ^{bB} ±0.94	128.45 ^{cB} ±0.88
C2	56.57 ^{aA} ±0.95	92.49 ^{bA} ±0.89	118.45 ^{cA} ±0.88
T1	58.46 ^{aB} ±0.90	122.48 ^{bc} ±0.96	133.50 ^{cc} ±0.90
T2	60.49 ^{ab} ±0.87	124.11 ^{bc} ±0.79	134.70 ^{cc} ±0.94
T3	56.49 ^{aA} ±0.88	138.60 ^{bd} ±0.93	157.51 ^{cE} ±0.92
Ileum(µm)			
C1	50.52 ^{aA} ±0.89	141.49 ^{bB} ±0.98	153.55 ^{cB} ±0.92
C2	52.54 ^{aA} ±0.92	99.55 ^{bA} ±0.92	114.50 ^{cA} ±0.90
T1	51.56 ^{aA} ±0.97	153.59 ^{bc} ±0.90	183.48 ^{cc} ±0.87
T2	55.56 ^{ab} ±0.92	158.51 ^{bd} ±0.92	186.43 ^{cd} ±0.91
T3	55.57 ^{ab} ±0.94	158.57 ^{bd} ±0.91	194.51 ^{cE} ±0.90

Mean±SE with different superscripts in row wise (a, b, c) and column wise (A, B, C, D, E) differ significantly ($P<0.05$).

Width of Muscularis Laminae

The width of muscular laminae of all three segments of small intestine of Cobb strain broiler chicken in different treatment and control groups in their growth period are depicted in Table 5. With the advancement of age, the values were significantly ($P<0.05$) increased in all the experimental groups from day 3 to 42.

Among different trial groups, significantly higher ($P<0.05$) values of width of muscularis laminae of duodenum were observed in treatment groups as compared to control groups (C1 and C2) on day 28 and 42 of study period. Significantly lower ($P<0.05$) values were recorded in heat-stressed control (C2) as compared to birds reared in normal control temperature (C1) on day 42 of the experiment.

In jejunum, significantly higher ($P<0.05$) values were observed in treatment groups than control groups on day 28 and 42. Between control groups, significantly higher ($P<0.05$) values were recorded in normal control (C1) group.

Among the different experimental groups, on day 28 and 42, significantly higher ($P<0.05$) values of muscularis laminae of ileum were recorded in T3 group as compared to T1 and T2 groups. T1 and T2 groups showed significant ($P<0.05$) difference only on day 42. Treatment groups values were significantly higher ($P<0.05$) than those of control group on day 28 and 42 of experiment. Values of width of muscularis laminae in birds reared in normal elevated temperature (C1) was found significantly higher ($P<0.05$) than that of heat stressed birds (C2 group).

Table 5: Effect of lemongrass and aloe vera supplementation on width of muscularis laminae of small intestine (Mean± SE: μm) in Cobb strain broilers under normal and heat stress conditions

Groups	Days of Experiment		
	03	28	42
Duodenum(μm)			
C1	77.56 ^a ±0.91	155.04 ^{ba} ±0.79	203.49 ^{cb} ±0.93
C2	79.56 ^a ±7.95	178.40 ^{bb} ±5.91	184.53 ^{ba} ±0.86
T1	72.54 ^a ±0.85	234.11 ^{bc} ±0.75	276.45 ^{cc} ±0.88
T2	79.59 ^a ±0.87	237.47 ^{bc} ±0.86	279.46 ^{cc} ±0.90
T3	81.62 ^a ±0.82	257.49 ^{bd} ±0.88	286.49 ^{cc} ±0.35
Jejunum(μm)			
C1	30.54 ^{ab} ±0.92	67.43 ^{bb} ±2.90	82.42 ^{cb} ±0.92
C2	27.43 ^{aA} ±0.92	58.53 ^{bA} ±0.92	73.48 ^{cA} ±0.94
T1	31.70 ^{ab} ±0.82	84.51 ^{bc} ±0.90	98.50 ^{cc} ±0.90
T2	30.55 ^{ab} ±0.90	87.42 ^{bd} ±0.89	104.72 ^{cd} ±0.88
T3	35.08 ^{ac} ±0.84	89.50 ^{bd} ±0.93	119.64 ^{ce} ±0.97
Ileum(μm)			
C1	88.18 ^a ±0.83	577.84 ^{bb} ±0.18	600.55 ^{cb} ±0.92
C2	90.49 ^a ±0.92	496.40 ^{bA} ±0.19	519.53 ^{cA} ±0.94
T1	92.55 ^a ±0.94	612.34 ^{bc} ±0.88	673.47 ^{cc} ±0.86
T2	91.50 ^a ±0.89	614.43 ^{bc} ±0.93	677.41 ^{cd} ±0.87
T3	98.57 ^a ±0.86	726.69 ^{bd} ±0.78	765.44 ^{ce} ±0.88

Mean± SE with different superscripts in row wise (a, b, c) and column wise (A, B, C, D, E) differ significantly ($P<0.05$).

Discussion

Height of epithelial cells

The present investigation showed a significant increase ($P<0.05$) in the height of epithelial cells of duodenum, jejunum and ileum portion of small intestine in all the experimental groups (C1, C2, T1, T2, and T3) with the advancement of age. Intestinal mucosa development is directly and indirectly influenced by lipid transfer to the egg and an older breeders deposit more lipids in the egg yolk because they produce less eggs (Applegate *et al* 1999). In breeder flock, age affects the intestinal histology; therefore the older breeder flocks had greater villus surface area, increased villus length and width in all small intestinal segments (Schaefer *et al* 2004). Our finding corroborated with the finding of Patil *et al* (2004) who observed that the epithelial height of different portions of small intestines increased with the age. The histomorphological features of broiler intestines illustrate the absorptive capacity which ultimately defines the growth performance of broilers (Grande *et al* 2020).

As reflected in results, a significant increased ($P<0.05$) height of epithelial cells in all the three segments of small intestine i.e., duodenum, jejunum and ileum in control (C1) and lemongrass and aloe vera supplemented groups (T1, T2 and T3) as compared with the heat stressed (C2) group of vencobb strain of broiler. The histological improvement observed in the chickens supplemented with essential oils can be explained by the reduction of toxins in the intestine as the lemongrass essential oil is beneficial in modifying the microbial content of the intestine, which increases the absorption of nutrients to improve the histological structure of the intestine (Park and Kim 2018). Similarly, Alagawany *et al.* (2015) found an increase in the height of epithelial cells in broilers supplemented with lemongrass essential oil leading to an enhanced broiler performance. The height of epithelial cells broilers fed with 2% and 2.5% of aloe vera gel in their diet have increased height of epithelial cells as compared to the control group (Xu *et al* 2003).

Villus height

As inferred from the results, a significant increase ($P<0.05$) of villus height of duodenum, jejunum and ileum portion of small intestine of Cobb strain broiler chicks in all the experimental groups (C1, C2, T1, T2 and T3) was noticed with the advancement of age.

Similarly in swine, an increase in the villus height was recorded with the advancement of age (Cera 1988). Increased villus height and villus surface area were associated with the absorption of the available nutrients (Awad *et al* 2008), in fact long villus were correlated with better gut health (Baurhoo *et al* 2008). Reduced villus height could also indicate presence of toxins, reduced absorption of nutrients, increased secretion in gastrointestinal tract, reduced disease resistance and lower overall performance (Xu *et al* 2003). The histomorphological features of broiler intestines showed the efficiency of the absorptive capacity which defines the growth performance of broilers (Grande *et al* 2020).

As reflected from the present investigation, a significant ($P<0.05$) increase was observed in the villus height of all the three segments (duodenum, jejunum and ileum) of intestines in experimental groups (C1, T1, T2 and T3) as compared to C2 (heat stress) group. Heat stress affects the integrity of gastrointestinal epithelium in broilers (Kulkarni and Newberry 2019). Similarly, Lin (2005) observed an increase in the villus height of the intestinal segments of broilers fed with lemongrass essential oil when compared with the control group. Hong *et al.* (2012) also observed that lemongrass essential oil supplemented birds had longer duodenum villi, while jejunal and ileal villous height as well as cryptal depth were similar among all groups in day-old-Arbor Acres broiler. Yason *et al.* (1987) recorded that the broilers fed with aloe vera at different levels had an increase in the villus height of the intestinal segment which was similar to the present findings. Diet mixed with 2 % of aloe vera gel had the largest villus height to crypt depth ratio as compared to the antibiotic virginiamycin group (Darabighane *et al* 2011). Similarly, Ghanzafari *et al.* (2015) stated that aloe vera (1%) supplementation in vanaraja chickens had higher villi height as compared to the group supplemented with the combination of aloe vera and neem.

Villus Width

Present results revealed a significantly higher ($P<0.05$) villus width of intestinal segment (duodenum, jejunum and ileum) in all the experimental groups from day 3 to day 42. A positive relationship existed between the intestinal development along villus development with the advancement of age (Sozcu and Ipek 2015). Similar study was found by Batal and Parsons (2002) who concluded improvement in the morphological characteristics changes with the nutrient digestibility on advancement of age.

A significantly higher ($P<0.05$) villus width of intestinal segment (duodenum, jejunum and ileum) in the supplemented (T1, T2 and T3) groups and in C1 (normal temperature) as compared to C2 (heat stress) group is referred in Table 4. The results of present study are in agreement with the report of Homan *et al.* (2013) who showed significantly higher ($P<0.05$) villus width in the commercial broilers as compared to the red jungle fowl for all intestinal segments when fed with lemongrass essential oil. Darabighane *et al.* (2011) mentioned that diet mixed with 2 % aloe vera gel group had the largest villus height and the greatest villus height to crypt depth ratio as compared to the antibiotic virginiamycin group. Longer villi are essential to animal development because it would result in an increased surface area for absorption of nutrients (Kadhim *et al* 2012). Similarly, Moghaddam and Alizadeh-Ghamsari (2013) recorded significantly higher ratio of villus height: Crypt depth which indicated that the aloe vera supplementation had made the gut

environment free of microbial toxins, which was also reflected in our study. In addition, lower crypt depth with aloe vera supplementation indicated for slow tissue turnover preventing the pathogens from tissue destruction in the gut (Ghazanfari *et al* 2015).

Width of Muscularis Laminae

Results revealed significantly higher ($P < 0.05$) villus width of muscularis laminae of all intestinal segments in the all groups studied with the advancement of age. The histological features of intestine illustrate its absorptive capacity which defines the growth performance in the growing birds. The thickness of muscularis mucosae is directly associated with an increase in the absorptive capacity of the intestine (De Grande *et al* 2020).

As reflected from the present study, significantly higher ($P < 0.05$) values of width of muscularis laminae of the three segments of small intestine (duodenum, jejunum and ileum) were observed in the lemongrass and aloe vera and combined lemongrass and aloe vera supplemented groups as compared with the control (C1 and C2) groups. Significantly lower ($P < 0.05$) values were recorded in heat-stressed control group (C2) when compared to birds reared in normal control temperature (C1). Heat stress affected the intestinal integrity in broilers (Zhang *et al*, 2018). Radhika *et al.* (2023) stated significantly higher ($P < 0.05$) crypt depth and villus height in the broilers supplemented with lemongrass essential oil when compared to group supplemented with antibiotic growth promoters. Similarly, significantly higher ($P < 0.05$) crypts depth and villus height was observed in the broiler chicks fed with lemongrass essential oil. Deeper intestinal villi crypts is an indication of rapid metabolism of tissue in order to allow renewal of intestinal villi (Hamedi *et al* 2011). In another study Xu *et al* (2003) recorded increased villus height in broilers supplemented with aloe vera when compared with virginiamycin group.

Conclusion

The current study illustrated that the inclusion of lemongrass and aloe vera in feed significantly enhanced histomorphometric parameters of small intestine in Cobb strain broiler chicks when compared to heat-stressed group. The birds fed with combination of lemongrass and aloe vera @ 15 mg + 1.5 mg/kg i.e. T3 group showed better results than the birds fed aloe vera alone @ 1.5 mg/kg (T2 group) as compared to the birds fed lemongrass alone @ 15 mg/kg (T1 group).

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