Evaluation of Ashwagandha and Wild Mint as herbal feed additives on intestinal histomorphology of Cobb Chicks under thermal stress

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Abstract

The present study was designed to investigate the effect of ashwagandha and wild mint and combination of ashwagandha and wild mint as dietary supplement to assess histomorphometry of small intestines in Cobb strain broilers under normal and heat stress condition. The experiment was conducted on 300 one-day-old Cobb chicks, housed under a deep litter system for 42 days. Birds were divided into five groups: control (C1–normal temp, C2–heat stress) and treatments (T1–Ashwagandha, T2–Wild mint, T3–combination). Diets were formulated as per NRC (1994) in three phases (pre-starter, starter, finisher). Heat stress (up to 48°C) was applied from day 4–17. At day 42, intestinal tissue samples were collected and processed for histomorphometric analysis (villus height, width, epithelial height, lamina muscularis). All micrometrical parameters exhibited a significant ($P<0.05$) increase in the duodenum, jejunum, and ileum across all experimental groups (C1, C2, T1, T2, and T3) with advancing 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: Ashwagandha; wild mint; 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 neem (Gobezie, 2022) turmeric, fenugreek, cumin (Yesuf et al., 2017), *Moringa oleifera* (Vaghamashi et al., 2025), ashwagandha, aloe vera, , 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).

Ashwagandha (*Withania somnifera*) have been utilized as indigenous medicine in ayurvedic treatment. *Withania somnifera* leaves and roots are used as drugs in many human and animal medicines (Ansari et al. 2013). Apart from its therapeutic attributes, it possesses numerous other benefits such as antioxidative, antistress, antilipidemic, anticoccidial, and immunomodulatory effect. *Mentha longifolia* (Wild mint) is another locally found plant near most of the water bodies. It is an important herbal remedy for domestic use as an antiseptic agent and for its antioxidant properties (Iqbal et al. 2013). The essential oil of *Mentha longifolia* has shown antimicrobial activity against *Mucor ramamnianus* (Gibriel et al. 2011).

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 Ashwagandha and Wild mint 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, one day old Cobb strain chicks were obtained from Venky's Private Limited, Satwari, Jammu. Before the start of the experiment, the shed was prepared by thorough cleaning and fumigation. The experimental birds were kept in deep litter system and were bedded on rice hulls with a depth of 8 cm. The study was conducted from the mid-May to the end of June that is a period of 42 days. After the initial 2 days i.e. the period given for acclimatization, the birds were individually weighed before being randomly assigned to 16 pens with 20 birds per pen. The ration composition and calculation were done for three phases. The pre starter phase that is from day 1 to day 14, the starter phase from day 14 to day 21 and finisher phase from day 21 to day 42. During the 1st week the chicks were kept in light intensity of 20 lux for 23 hours of light and 1 hour of darkness with a floor area @ 0.5 sq ft or 450 cm2 per chick. In the Second week, the chicks were exposed to a light intensity of 15 lux and for 19 hours of light and 5 hours of darkness. During 3rd and 4th week the floor area was increased @ 1.2 sq ft or 1000 cm2 per chick with light intensity of 10 lux for duration of 19 hours of light and 5 hours of darkness. Later from 5th week onwards the birds were given a light intensity of 5 lux for a duration of 22 hours of light and 2 hours of darkness.

Dietary regimen

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	Prestarter ration (%)	Starter ration (%)	Finisher ration (%)
Maize	52.8	60.88	67.5
Soybean meal	39.8	30.37	24.5
MBM	5	5	5
Salt	0.25	0.25	0.25
Sodium bicarbonate	0.01	0.01	0.01
Soyabean meal	1.5	2.7	2.2
Methionine	0.15	0.13	0.09
LSP	0.18	0.59	0.29
Vitamin supplement	0.05	0.05	0.05
Trace mineral mixture	0.1	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.

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

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

Treatment 1(T1): Along with the basal diet the birds were supplemented with ashwagandha at a rate of 0.5% of diet that is 5grams in 1kg of feed.

Treatment 2(T2): Along with the basal diet the birds were supplemented with wild mint @150mg/kg of the feed.

Treatment 3(T3): Along with the basal diet the birds were given 0.5% of ashwagandha and 150 mg/kg of wild mint in combination.

Application of heat stress

For three days following their arrival, the chicks were given a period for acclimatization. From day four onwards until the conclusion of experiment, heat stress was imposed on the C2, T1, T2, and T3 groups. The birds were exposed to heat stress from day 4 onwards till day 17. During this period the temperature was gradually raised up to 48°C and then it was maintained for the remaining days.

Sample collection

Two chicks from each replicate, a total six birds in each group, were kept for histomorphometric parameters until the 42nd day, when they were sacrificed and tissue samples from the different parts of small intestine were collected and fixed in 10% neutral buffered formalin (NBF) solution and processed as per standard procedure (Luna 1968). Tissue sections with a thickness of 5 µ were cut out on clean glass slides and stained with Haematoxylin and Eosin. Using a microscope (Magnus Microscope with camera, MX21i-TrLED), various micrometrical parameters i.e. epithelial height, villus height, villus width and width of the lamina muscularis were examined in the sections of stained tissue.

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 and discussion

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. Our results were consistent with Patil et al. (2019), who observed that the epithelial height of different portions of small intestines increased with the age.

There was a significant increase ($P<0.05$) in the height of epithelial cells in all three segments of the small intestine (duodenum, jejunum, and ileum) in the control (C1) and ashwagandha and wild mint supplemented (T1, T2, and T3) groups. As stated by Santos et al. (2019) the thermal stress leads to mucosal and villus damage of the small intestine, which can be attributed to the compromised digestive and absorptive functions of chickens. Jyotsna et al. (2019) had also reported that supplementation of ashwagandha in diet of poultry birds improves body weight gain and intestinal morphometry. According to Eman et al. (2020), improved gut health integrity was observed, especially in *Withania somnifera* 5% supplemented group in terms of increased villous height, the width of lamina propria in all parts of the intestine.

Table 2: Effect of ashwagandha and wild mint supplementation on height of epithelial cells 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	$28.49^{Ba} \pm 0.74$	29.22 ± 1.65^{Aa}	41.24 ± 1.05^{Bb}
C2	$26.92^{Aa} \pm 0.65$	28.28 ± 0.78^{Ab}	36.34 ± 1.78^{Ac}
T1	$28.48^{Ba} \pm 0.63$	30.05 ± 1.15^{Bb}	45.81 ± 0.67^{Cc}
T2	$28.66^{Ba} \pm 0.68$	39.90 ± 2.19^{Cb}	48.02 ± 1.35^{Dc}
T3	$26.83^{Aa} \pm 0.67$	35.99 ± 1.30^{Cb}	50.07 ± 0.48^{Dc}
Jejunum(µm)			
C1	26.97 ± 1.36^{Aa}	65.31 ± 0.18^{Bb}	80.52 ± 0.27^{Ac}
C2	26.55 ± 1.03^{Aa}	61.97 ± 0.22^{Ab}	79.52 ± 0.39^{Ac}
T1	33.33 ± 0.79^{Ba}	82.29 ± 0.13^{Cb}	94.86 ± 0.38^{Bc}
T2	27.40 ± 0.89^{Aa}	84.51 ± 0.23^{Db}	95.10 ± 0.65^{Bb}
T3	30.83 ± 0.26^{Ba}	83.82 ± 0.37^{Db}	96.96 ± 0.23^{Cc}
Ileum(µm)			
C1	$19.35^{a} \pm 0.75$	$45.32^{Bb} \pm 2.43$	$82.03^{Ac} \pm 0.52$
C2	$19.21^{a} \pm 0.84$	$35.43^{Ab} \pm 1.79$	$80.90^{Ac} \pm 0.68$
T1	$19.88^{a} \pm 0.43$	$66.28^{Cb} \pm 2.19$	$83.62^{Bc} \pm 2.51$
T2	$20.47^{a} \pm 0.42$	$74.39^{Db} \pm 0.28$	$96.20^{Cc} \pm 0.28$
T3	$22.17^{a} \pm 2.23$	$73.41^{Db} \pm 0.46$	$95.58^{Cc} \pm 0.31$

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

Table 3: Effect of ashwagandha and wild mint 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	$1123.2^{a} \pm 28.25$	$1194.8^{bA} \pm 17.35$	$1395.3^{cAB} \pm 43.72$
C2	$1117.3^{a} \pm 29.76$	$1150.6^{aA} \pm 12.71$	$1355.2^{BA} \pm 43.57$
T1	$1108.9^{a} \pm 81.17$	$1320.1^{bB} \pm 71.41$	$1508.1^{cB} \pm 39.94$
T2	$1112.7^{a} \pm 30.05$	$1378.4^{bB} \pm 16.23$	$1524.4^{cB} \pm 45.68$
T3	$1111.7^{a} \pm 72.57$	$1331.7^{bB} \pm 55.01$	$1536.1^{cB} \pm 48.05$
Jejunum (µm)			
C1	$413.9^{a} \pm 8.69$	$598.2^{b} \pm 17.67$	$683.2^{Bc} \pm 2.61$
C2	$399.7^{a} \pm 13.71$	$592.3^{b} \pm 17.93$	$652.8^{Ac} \pm 1.68$
T1	$406.8^{a} \pm 20.76$	$621.5^{b} \pm 13.96$	$853.3^{Cc} \pm 6.61$
T2	$414.6^{a} \pm 8.23$	$623.5^{b} \pm 13.98$	$856.4^{Cc} \pm 6.35$
T3	$408.7^{a} \pm 14.12$	$615.5^{b} \pm 17.08$	$863.4^{Cc} \pm 5.57$
Ileum (µm)			
C1	$277.5^{a} \pm 4.41$	$400.3^{Bb} \pm 3.81$	$511.5^{c} \pm 11.38$
C2	$280.12^{a} \pm 3.86$	$385.8^{Ab} \pm 2.77$	$502.6^{c} \pm 10.09$
T1	$266.32^{a} \pm 10.12$	$416.4^{Cb} \pm 6.77$	$526.12^{c} \pm 18.12$
T2	$279.73^{a} \pm 5.23$	$426.9^{Cb} \pm 6.82$	$529.8^{c} \pm 14.11$
T3	$273.72^{a} \pm 7.30$	$420.07^{Cb} \pm 6.11$	$554.2^{c} \pm 27.47$

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 height

The villus height of the duodenum, jejunum, and ileum of Cobb strain broiler chicks increased significantly ($p<0.05$) in all experimental groups with the advancement of age. The villus increases the absorptive area of the small intestine for digestion and absorption of nutrients (Kondrashina et al. 2023). Intestinal villus height is a key indicator of the effective absorption area of the small intestine. A larger absorption area provides a greater capacity to take up nutrients (Segú et al. 2022). Therefore, the height of intestinal villus directly affects the absorption of nutrients in the small intestine (Wang et al. 2025).

As depicted from the present investigation (Table 3), a significant ($P<0.05$) increase in the villus height was observed in all the three segments of intestines in experimental groups (C1, T1, T2 and T3) as compared to C2 (heat stress) group. The supplementation of powdered ashwagandha root increased villi height significantly ($P<0.05$). According to Segú et al. (2022), an increase in villi height may be a sign of an increase in surface area available for nutrient absorption. The villus height-to-crypt depth ratio is key index of intestinal morphology and is commonly used to estimate the digestive capacity of the small intestine as well as to reflect the rate of mucosal turnover (Alagbe et al. 2023). According to Olmez and Yoruk (2021), the duodenum villus lengths were found to be enhanced by the addition of pennyroyal (*Mentha pulegium L.*) in varying doses to the diet.

Villus Width

Table 4 indicated a significantly higher ($P<0.05$) villus width of the duodenum, jejunum, and ileum in each of the experimental groups from day 3 to day 42 which was similar to the findings of Sozcu and Ipex (2015). A significantly higher ($P<0.05$) villus width of intestinal segments was observed in the supplemented groups (T1, T2 and T3) and in C1 group (normal temperature) as compared to group C2 (heat stress). According to Jyotsna et al. (2019), *Withania somnifera* supplemented chicks displayed the highest villlus width (160.07μm) as compared to the unsupplemented chicks. Therefore, adding ashwagandha root powder could improve the broiler chickens' overall growth, gut microbiota, and morphometry (Jyotsna et al. 2019).

Width of Muscularis Laminae

With the advancement of age, significantly higher ($p<0.05$) villus width of muscularis laminae of duodenum, jejunum, and ileum segments of small intestine was observed in all groups studied (Table 5). The intestine's histological features show how well it can absorb nutrients, which determines how well growing birds perform in terms of growth (Wen et al. 2018). Levkut et al. (2017) further observed that the intestinal villi are quickly and continuously adjusted as a response to conditions in the lumen of the intestine which reflects to the dynamic environment present inside the gut.

Table 4: Effect of ashwagandha and wild mint 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	$101.99^{a} \pm 5.26$	$119.38^{Bb} \pm 0.66$	$125.48^{Bb} \pm 0.71$
C2	$100.96^{a} \pm 4.53$	$115.56^{Ab} \pm 0.71$	$123.06^{Ab} \pm 0.41$
T1	$100.80^{a} \pm 1.55$	$120.74^{Bb} \pm 0.61$	$156.31^{Cc} \pm 0.58$
T2	$103.11^{a} \pm 3.71$	$132.46^{Db} \pm 0.75$	160.54Dc ± 0.60
T3	$96.10^{a} \pm 4.35$	$126.57^{Cb} \pm 0.68$	159.58Dc ± 0.66
Jejunum(μm)			
C1	$67.73^{Aa} \pm 7.28$	$85.87^{b} \pm 1.11$	$109.30^{Bb} \pm 4.29$
C2	$70.77^{Ba} \pm 7.01$	$82.88^{b} \pm 2.04$	$100.32^{Ac} \pm 3.77$
T1	$70.71^{Ba} \pm 5.63$	$90.79^{b} \pm 1.08$	$119.65^{Cc} \pm 3.51$
T2	$77.59^{Ba} \pm 5.31$	$92.25^{b} \pm 2.40$	$120.85^{Cc} \pm 3.46$
T3	$70.72^{Ba} \pm 9.24$	$88.91^{b} \pm 1.52$	$121.48^{Cc} \pm 2.58$
Ileum(μm)			
C1	$55.57^{a} \pm 3.39$	$117.74^{Ab} \pm 4.95$	$138.99^{Ac} \pm 3.86$
C2	$60.74^{a} \pm 2.82$	$112.78^{Ab} \pm 4.31$	$130.60^{Ac} \pm 1.67$
T1	$63.00^{a} \pm 5.67$	$119.68^{Ab} \pm 7.99$	$157.46^{Bc} \pm 6.01$
T2	$63.62^{a} \pm 4.31$	$141.36^{Bb} \pm 5.76$	$164.77^{Bc} \pm 6.73$
T3	$65.08^{a} \pm 5.24$	$150.74^{Bb} \pm 6.08$	$166.86^{Bb} \pm 4.26$

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

Table 5: Effect of ashwagandha and wild mint 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	12.53^{a} ± 0.72	13.65^{Aab} ± 0.86	16.12^{Bb} ± 1.03
C2	12.02^{a} ± 3.32	13.76^{Aa} ± 1.18	14.38^{Aa} ± 0.94
T1	14.17^{a} ± 2.39	14.91^{Aa} ± 1.21	20.27^{Bcb} ± 0.52
T2	13.79^{a} ± 1.68	15.60^{Aa} ± 1.03	22.09^{Cb} ± 0.64
T3	14.59^{a} ± 0.55	15.20^{Ba} ± 0.77	23.45^{Cb} ± 0.87
Jejunum(µm)			
C1	13.50^{A} ± 0.60	14.87^{a} ± 0.31	18.46^{Ab} ± 0.34
C2	14.16 ± 0.42	14.56^{a} ± 0.31	17.42^{Ab} ± 0.26
T1	14.78 ± 0.79	16.51^{a} ± 0.73	20.12^{Bb} ± 0.33
T2	11.88 ± 0.27	14.62^{b} ± 0.81	21.48^{Cc} ± 0.38
T3	11.89 ± 0.47	15.23^{b} ± 1.25	21.93^{Cc} ± 0.52
Ileum(µm)			
C1	18.28^{a} ± 1.04	19.76^{Aab} ± 1.39	22.80^{Bb} ± 0.21
C2	18.70^{a} ± 0.72	18.56^{A} ± 1.43	20.19^{A} ± 0.97
T1	17.1^{a} ± 0.89	18.56^{Aa} ± 1.43	23.95^{Bb} ± 0.44
T2	16.99^{a} ± 0.72	15.78^{Aa} ± 0.40	24.43^{Cb} ± 0.29
T3	15.59^{a} ± 0.71	24.54^{Bb} ± 1.45	25.16^{Cb} ± 0.28

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

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 ashwagandha supplemented, wild mint supplemented and combined ashwagandha and wild mint supplemented groups as compared with the control groups (C1 and C2). Significantly lower (P<0.05) values were recorded in control C2 (heat stressed) when compared to birds reared in control C1 (normal temperature). As per Zhang et al. (2017), heat stress also affected the intestinal integrity in broilers.

Conclusion

The current study illustrated that the inclusion of ashwagandha and wild mint in feed had a significant impact on the morphometry of the small intestine in Cobb strain chicks. Supplementation of combination of ashwagandha and wild mint @ 0.5% and 150g/kg (T3) showed better result than the group supplemented with wild mint @ 150g/kg (T2) or the group supplemented with ashwagandha @ 0.5% (T1). Supplementation with Ashwagandha and Wild Mint may serve as effective natural feed additives to promote intestinal development and enhance resilience in broiler chickens, particularly under environmental stress as heat exposure. Further research is recommended to explore their mechanisms of action and to evaluate performance, immune response, and overall health outcomes.

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