Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.3 / 001-008

http://dx.doi.org/10.1590/1806-9061-2022-1620

Original Article

A Comparative Study of the Effect of Non-Antibiotic Feed Additives on Experimental Colonization of Salmonella Enterica Serovar Enteridis and Intestinal Pathomorphology in Broiler Chickens

■Author(s)

Raffie AR*[I] https://orcid.org/0000-0003-1585-3850
Aslam A*[I] https://orcid.org/0000-0003-0383-6930
Tipu MY*[I] https://orcid.org/0000-0002-6996-7258
Altaf I*[I] https://orcid.org/0000-0002-0157-4239
Mustafa A*[II,III] https://orcid.org/0000-0002-8790-1121
Imran MS[I] https://orcid.org/0000-0003-4353-6107
Farooq MZ[IV] https://orcid.org/0000-0002-7309-9553
Abbas G[II] https://orcid.org/0000-0002-2741-994X
Goswami N[III] https://orcid.org/0000-0002-6710-7721
Mohsin M[V] https://orcid.org/0000-0003-3826-7352
Khan AA[II] https://orcid.org/0000-0001-8972-8855
Aslam S[II] https://orcid.org/0009-0002-3712-4616
Abdul K[VI] https://orcid.org/0000-0002-5524-6492

[I] Department of pathology, University of Veterinary and animal Sciences, Lahore, Pakistan.
[II] Riphah college of Veterinary Sciences, Riphah International University, Lahore, Pakistan.
[III] Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, PR China.
[IV] Department of Animal Science, University of Veterinary and Animal Science (Jhang Campus) Lahore, Pakistan.
[V] Shantou University Medical College, Shantou, PR China.
[VI] Shandong Sinder Technology Co., Ltd, Qingdao, Shandong, PR China.

*These authors contributed equally to this work.

■Mail Address

Corresponding author e-mail address
Ahsan Mustafa
Department of Animal Production, Riphah College of Veterinary Sciences, Lahore, Pakistan.
Phone: +92-333-4453486
Email: dr.ahsan.mustafa@gmail.com

■Keywords

Non-Antibiotic Feed Additives, Prebiotics, Salmonella, essential oils, organic acids.

Submitted: 10/January/2022
Approved: 11/July/2023

ABSTRACT

The objective of this study was to evaluate the effect of eubiotics on the intestinal morphology of broilers. For this purpose, 125 birds were divided into six groups with two replicates each (10 birds in each replicate). Group A was given a Basal diet. All groups except group A were challenged with *Salmonella enterica serovar Enteritidis*. Group B was provided the basal diet, group C was fed a Probiotic-added diet; group D was fed a Prebiotics-based diet; group E was given essential oils plus the basal diet; and group F was provided with organic acids plus the basal diet. Two separate experiments were carried out for *Salmonella* recovery, checking the cecal tonsils and conducting an intestinal pathomorphic evaluation. Villus length, villus width, villus surface area, and crypt depth were measured by micrometry. There was an overall improvement ($p<0.05$) in intestinal morphometric parameters for all the treatment groups except for the negative control group, which showed the lowest villus height and villus depth values. Maximum villus height ($p<0.05$) of the duodenum was achieved by group E, which was fed a diet containing essential oils, whereas a maximum villus surface area index ($p<0.05$) was recorded for the birds of Group D, which were fed a diet containing prebiotics. Maximum villus height ($p<0.05$) and surface area index in ileum mucosa was recorded ($p<0.05$) in the birds of group D (treated with prebiotics). It is concluded that there is an overall increase in the gut histology of broilers fed non-antibiotic based feed.

INTRODUCTION:

The use of antimicrobial agents as a preventive measure has been questioned, given the extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria. Non-antibiotic feed additives (probiotics, prebiotics, essential oils, and organic acids) are being considered to fill this gap and a few farmers in the country are already using them with good results (Abbas *et al.,* 2018; Mustafa *et al.,* 2021a). On the other hand, the practicality of antimicrobial mediators as a prophylactic measure has been strongly questioned due to the risk of selection and emergence of antimicrobial resistance amid pathogenic microorganisms (Batool *et al.,* 2020). Therefore, the use of antibiotics as growth promoters for poultry and the fear of residual impacts of their consumption as therapeutic mediators have created an atmosphere of debate and consumer reluctance, causing a search for alternatives (Abbas *et al.,* 2018). The use of natural products such as plant extracts, essential oils, organic acids, prebiotics, phytochemicals, spices, and probiotics has been acknowledged and is currently suggested both for antibiotic replacement in farms and animal nourishment among smallholders (Ogbuewu *et al.,* 2022). Such non-antibiotic feedstuff (herbs and additives) are being considered capable of bridging this

gap, as some agronomists used to utilize these instead of antibiotics for poultry (Nava *et al.,* 2005; Jong *et al.,* 2020; Mustafa *et al.,* 2021a; Abbas *et al.,* 2022a; Mohamed *et al.,* 2022).

Probiotics exhibit numerous significant modes of action, such as an antagonistic action against pathogenic microbes by altering gut pH; having an antimicrobial effect by the excretion of products inhibiting their expansion, such as bacteriocins, hydrogen peroxide, and organic acids; the intestinal production of short-chain fatty acids (SCFA); host immune system regulation; regularization of intestinal microbiota, along with diverse metabolic results (Vamanu *et al.,* 2010; Ferreira *et al.,* 2011). Favorable effects of probiotic addition may be seen in amplified abdominal enzyme creation, reduction of phenol and ammonia yields, and increased resistance against the pathogenic microbe's propagation in the abdomen by the mean of competitive exclusion (CE; Yusrizal & Chen, 2003). CE is a beneficial strategy consisting of adding specific probiotics (culture of non-pathogenic bacteria) to the gastrointestinal tract (GIT), which ultimately hinders the colonization of pathogenic bacteria, and produces subsequent competition for accessible nutrients and growth elements (Patterson & Burkholder, 2003; Konieczka *et al.,* 2022).

Prebiotics are particular fermented components that allow specific variations in all the configurations and actions of the gastrointestinal microbiota and ultimately favor host health (FAO/WHO, 2002). Prebiotics comprising galactose, glucose, mannose, fructose, and xylose has been largely studied and seem to be predominantly positive (Gibson & Roberfroid, 1995; Patterson & Burkholder, 2003). Most of them have been demonstrated to improve defense against *Salmonella*, since prebiotics physically imitate their binding sites and prevent their adhesion to intestinal epithelial cells (Ferreira *et al.,* 2011). Essential oils, commonly known as ethereal or volatile oils, are aromatic greasy fluids obtained from plants. They have a favorable effect on the metabolism of lipids, anti-microbial and anti-oxidant characteristics, anti-inflammatory properties, and work as digestive stimulants (Rota *et al.,* 2004; Acamovic *et al.,* 2005). Owing to their antimicrobial characteristics, essential oils are regarded as potential feed additives (Dibner & Buttin, 2002; Lee *et al.,* 2004). Short-chain fatty acids (SCF) and medium-chain fatty acids (MCFA) in animal feed have been widely proclaimed as anti-microbial agents. Their anti-microbial characteristics are due to their ability to lower pH due to the dissociation of carboxyl groups, which can infiltrate bacterial cells and ultimately cause cell death. Due to their antimicrobial characteristics, organic acids are considered substitutes for synthetic growth promoters (Cherrington *et al.,* 1991; Dibner & Buttin, 2002).

Customer refusal of artificial food extracts and antimicrobial growth promoters (AGP) has increased due to the devastating stated effects of these harmful chemicals. Therefore, the present research focuses on the potential advantageous results of non-antibiotic feed additives for an improved understanding and awareness of the topic.

MATERIALS AND METHODS

Source of birds

A total of 125 Hubbard classic A-grade (38.5 gm) day-old broiler chicks were procured commercially were raised in the experimental open-sided poultry house of the Pathology Department, University of Veterinary and Animal Sciences, Lahore. All bird handling procedures are in line with International Animal Care.

Housing Conditions

Washing of the shed and utensils was done with high-pressure water, followed by washing and scrubbing with surf and phenol, with subsequent sun drying. Later on, the shed was whitewashed with limestone, and formalin was sprayed on the walls and floor. Finally, fumigation was performed after putting in the utensils and litter. Chicks were housed in pens of identical size (1×2 m^2) in a deep litter system with rice husk. On the first day, the room temperature was set at 32 °C and lowered stepwise to 24°C for the rest of the experiment. Feed (pre-starter and starter) in the form of crumbs was purchased commercially and formulations were made according to NRC (1994) guidelines. Birds were reared under the same management and environmental conditions. A LED light of 30 lx was used for 22 hours for the first 3 days, for 20 hours for the first week, and the duration and intensity were further reduced thereafter with the age of the birds.

Treatments

The probiotic used contained spores of Bacillus subtilis at cfu/g, and was mixed in the feed at the rate of 10 g /t. Prebiotic, a refined yeast-based mannan oligosaccharides preparation, was added at the rate of 400g/ton in the feed. Essential oils, a water-soluble concentrate containing essential oils of Eucalyptus, Menthol, and Saponins was added to drinking

water at 0.25ml/liter. The organic acids used were a synergistic combination of formic, lactic, and propionic acid or their salts, and a surfactant. It was added to the drinking water at 0.5 ml/liter. The recommended vaccination schedule for broilers issued by the National Disease Control Committee of the Pakistan Poultry Association as of 24th May 2016 was followed. Upon arrival, five chicks were randomly selected and checked for Salmonella (presence/absence) in the ceca-cecal tonsils and were found to be negative. Trade names of the products are not displayed due to the commercial impact of the results.

Bacterial Stain and culture conditions

The challenge organism used in all experiments was a poultry isolate of *Salmonella enterica serovar Enteritidis* (SE), tested against antiserum. SE was cultured in Tetrathionate broth. Post-incubation, bacterial cells were collected, reconstituted in saline, quantified by total viable count, and diluted to an approximate concentration of 4 × CFU per 0.25 milliliter (Prado-Rebolledo *et al.,* 2017). Concentrations of the isolate were further verified by serial dilution and plating on *Salmonella*-Shigella agar for enumeration of the actual CFU used to challenge the chickens.

Experimental Design

The chicks were divided randomly into six groups (A, B, C, D, E, and F), with 20 chicks per group and two replicates in each group. The treatments were given to the respective groups after experimental infection upon arrival and were continued during the whole experiment. The treatments were as follows: Group A was given a basal diet (The negative control group). Group B Challenge + Basal diet (Positive control group). Group C: Probiotic + Challenge + Basal diet. Group D: Prebiotic + Challenge + Basal diet. Group E: Essential oils + Challenge + Basal diet. Group F: Organic acids + Challenge + Basal diet. Two separate experiments were carried out for *Salmonella* recovery by checking cecal tonsils and conducting an intestinal pathomorphic evaluation. Villus length, villus width, villus surface area, and crypt depth were measured by micrometry.

Experiment 1

This experiment involves evaluating *Salmonella* establishment and colonization in ceca-cecal tonsils. Groups B, C, D, E, and F were challenged with SE at 4 × CFU/0.25 mL per bird through oral gavage after arrival. Group A was given sterile normal saline as a vehicle through oral gavage.

Salmonella Recovery

Four birds were nominated indiscriminately from all the designed groups, seventy-two hours post-challenge, and were slaughtered humanely. Ceca-cecal tonsils were harvested aseptically after performing *post-mortem*. After harvesting the particular tissue, the ceca-cecal tonsils underwent homogenization and enrichment aseptically in tetrathionate broth. They were diluted with saline and serial dilutions post-enrichment and were plated on *Salmonella*-Shigella agar to enumerate the cfu/g of ceca-cecal tonsils and to check for positive H_2S, non-lactose fermenting, colorless clear transparent colonies with dark black mid-points to ascertain the presence or absence of *Salmonella* (Prado-Rebolledo *et al.,* 2017).

Experiment 2

Intestinal Morphology Evaluation

For the intestine morphometric analysis, at 72 hours, 7 days, and 14 days, two birds from all the groups were chosen randomly and slaughtered humanely. Two different intestinal sections of approximately 1cm, one from the lower ileum and the other from the center of the duodenum, were aseptically collected from each slaughtered bird, after carefully washing the tissue with normal saline for the removal of any intestinal content residues.

Salmonella presence and colonization

Table 1 – Results of this experiment are given as the total number of birds found positive for *Salmonella* presence out of the total number of birds tested, and the Log10 *Salmonella* Enteritidis /g of ceca-cecal tonsils at day 3.

Treatment group	Samlonella presence	Log10 Salmonella Enteritidis/g of ceca-cecal tonsils
1. Group A (-ve control)	0/4	Negative control group
2. Group B (+ve control)	4/4	4.73±0.45^{c}
3. Group C (PRO)	4/4	1.83±0.67a
4. Group D (PRE)	4/4	3.27±0.35b
5. Group E (EO)	4/4	3.46±0.87b
6. Group F (OA)	4/4	1.98±0.61a

a–c Values within columns with no common superscript differ significantly at $p<0.05$. Results for Log10 Salmonella Enteritidis /g of ceca-cecal tonsils demonstrated a significant decrease in the recoverable count of Salmonella only by groups C and F.

Standards techniques routinely practiced in the UVAS pathology lab were used for the histopathological examinations of the collected tissue samples. The steps comprise the fixation of the tissue, dehydration of the sample, clearing, sectioning post embedding, and lastly the careful staining of the sectioned tissue (Athanassopoulou *et al.,* 1999).

Intestinal Morphology Analysis

The stained slides were then examined under 4X magnification and pictures were taken and analyzed by PixelPro software by Labomed Inc. Villus length was measured from the top of the villus to the top of the lamina propria (Mustafa *et al.,* 2021b). Crypt depth was measured from the base upward to the region of transition between the crypt and villus (Aptekmann *et al.,* 2001). Villus width was measured at the widest area of each villus, while the villus : crypt ratio was determined as the ratio of villus height to crypt depth. Villus surface area was calculated using the formula (2π) (VW/2) (VL), where VW = villus width and VL = villus length (Sakamoto *et al.,* 2000).

Statistical Analysis of the Data

The collected data from both experiments were statistically analyzed using a completely randomized design and means were compared by ANOVA (Analysis of variance) through SPSS 16.0.

RESULTS

The results of experiment 1 consist of the total number of birds found positive for *Salmonella* presence out of the total number of birds tested and the Log10 of *Salmonella* Enteritidis /g of ceca-cecal tonsils. 72 hours post-challenge, four birds were selected randomly from each of the groups, humanely slaughtered, and checked for enumeration of *Salmonella* CFU/g. Results of this experiment are given as the total number of birds found positive for *Salmonella* presence out of the total number of birds tested, and the Log10 *Salmonella* Enteritidis/g of ceca-cecal tonsils (Table 1). The result indicates all birds were found positive in all treatments except the control group. Moreover, the lowest prevalence (*Salmonella* CFU/g) was observed in groups C and F which were fed probiotic and organic acid, respectively. Experiment 2 consisted of an enteric morphometric analysis: two birds from each of the groups were humanely slaughtered on the designated evaluation day (3rd day, 7th day, and 14th day). Segments of the midpoint of the duodenum and the distal end of the lower ileum from each bird were collected and processed for histopathological examination (Figures 1 & 2). For this experiment, results are given as the average value calculated from 5 sections of both the duodenum and ileum of 2 birds per group at the designated evaluation days. All values are expressed as Mean ± Standard deviation (Tables 2 & 3).

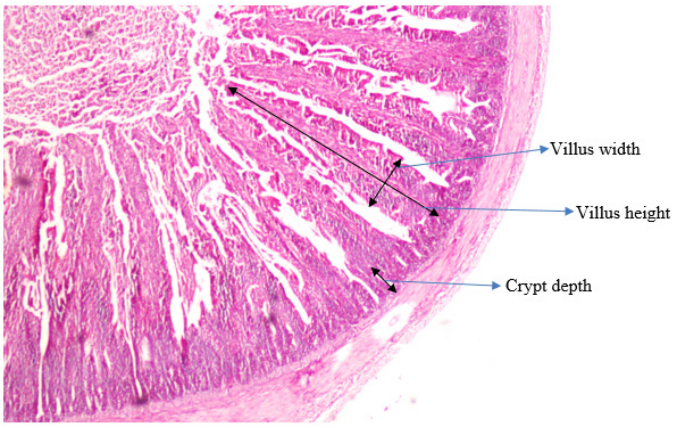

Figure 1 – Morphometric analysis of Villi of Duodenal mucosa at 40x.

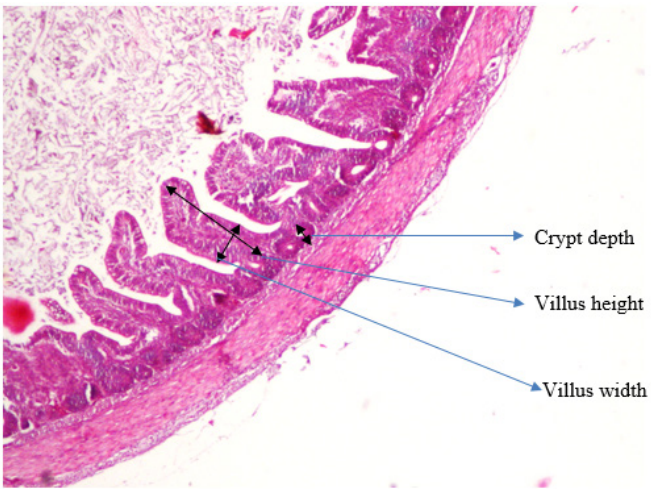

Figure 2 – Morphometric Analysis of Ileal Mucosa at 40x.

On the 3rd day of the study, there was a significant difference in the mean of all the parameters of morphometric analysis of duodenal mucosa between all six groups. Group F, which was fed with organic acids, showed the highest villus height of 1060.4±87.76 µm, crypt depth of 143.07±9.80 µm, and villus surface area index of 477.72±64.94 mm^2 in the duodenum. On the other hand, the result of iliac mucosa showed that the prebiotic group had improved villus height, crypt width, villus dept, and villus surface area index as compared to other groups. On the 7th day, the result of duodenal mucosa indicated that group E had improved crypt width and villus surface area index. However, organic acids showed a pronounced effect on the iliac mucosa.

Effects of early feeding in combination with probiotics

There was an overall increase in all the parameters of intestinal morphometric analysis for all the treatment groups, except for the control negative group which showed the lowest values. The maximum villus height

Table 2 – Morphological analysis of duodenal mucosa (Mean ± SD).

Treatment	Villus height (µm)	Crypt width (µm)	Villus depth ratio	Villus: crypt index (mm^2)	Villus surface area
3 day					
A. Negative control group	867.63±25.27^{ab}	111.13±10.42^{a}	114.62±6.29^{b}	7.59±0.58^{ab}	302.71±29.24^{ab}
B. Positive control group	760.33±74.42^{a}	101.17±8.69^{a}	84.31±5.95^{a}	9.07±1.32^{b}	241.68±33.56^{a}
C. PRO group	1035.6±70.79^{c}	102.57±14.58^{a}	160.27±20.43^{c}	6.52±0.74^{a}	334.56±57.83^{b}
D. PRE group	895.78±16.59^{b}	114.45±10.66^{a}	127.92±15.63^{b}	7.09±0.86^{a}	321.65±27.09^{b}
E. EO group	781.53±60.84^{a}	134.73±13.08^{b}	105.10±12.64^{ab}	7.58±1.58^{ab}	329.74±10.10^{b}
F. OA acid group	1060.4±87.76^{c}	143.07±9.80^{b}	156.57±17.08^{c}	6.79±0.44^{a}	477.72±64.94^{c}
7 day					
A. Negative control group	1066.4±27.11^{ab}	199.98±12.53^{ab}	150.62±22.78^{a}	7.21±1.11^{b}	669.33±40.31^{a}
B. Positive control group	976.28±40.19^{a}	176.95±18.79^{a}	132.32±20.41^{a}	7.54±1.33^{b}	543.28±71.24^{a}
C. PRO group	1513.8±56.09e	221.37±21.18^{b}	204.95±25.32^{b}	7.45±0.64^{b}	1051.11±92.96^{c}
D. PRE group	1382.2±71.13de	196.52±10.69^{ab}	209.12±30.25^{b}	6.73±1.02^{b}	852.75±60.99^{b}
E. EO group	1329.2±92.93^{c}d	258.00±23.45^{c}	197.67±14.87^{b}	6.75±0.69^{b}	1071.40±42.80^{c}
F. OA acid group	1190.1±164.18^{bc}	228.70±23.99^{bc}	278.40±17.44^{c}	4.27±0.46^{a}	860.92±188.5^{b}
14 day					
A. Negative control group	1436.7±88.15^{a}	247.65±31.56^{ab}	227.45±28.76^{c}	6.36±0.47^{a}	1114.6±136.92^{ab}
B. Positive control group	1348.7±46.26^{a}	194.62±33.58^{a}	110.47±10.42^{a}	12.29±1.18^{b}	825.64±154.34^{a}
C. PRO group	1548.4±43.24^{b}	250.38±33.67^{ab}	244.25±25.02^{c}	6.39±0.73^{a}	1220.5±192.22^{b}
D. PRE group	1767.0±37.76^{c}	300.12±72.65^{b}	162.08±34.48^{b}	1.39±2.79^{b}	1662.6±389.16^{c}
E. EO group	1794.2±63.96^{c}	221.08±36.95^{a}	216.23±26.03^{c}	8.42±1.27^{a}	1242.4±187.47^{b}
F. OA acid group	1601.2±37.12^{b}	255.70±23.00^{ab}	246.30±25.52^{c}	6.57±0.78^{a}	1285.7±120.73^{bc}

$^{a–c}$ Values within columns with no common superscript differ significantly at $p<0.05$.

Table 3 – Morphological analysis of iliac mucosa (Mean ± SD).

Treatment	Villus height (µm)	Crypt width (µm)	Villus depth ratio	Villus: crypt index (mm2)	Villus surface area
3 day					
A. Negative control group	340.52±32.73^{b}	79.46±10.93^{b}	74.60±13.05^{ab}	4.69±1.02*	85.15±16.63^{b}
B. Positive control group	244.68±34.05^{a}	55.93±12.59^{a}	60.06±13.47^{a}	4.26±1.18	43.37±13.09^{a}
C. PRO group	354.18±25.21^{bc}	108.45±6.83^{c}	72.26±12.72^{ab}	5.02±0.89	120.87±14.49^{c}
D. PRE group	387.52±21.01^{c}	188.50±10.69e	93.80±10.43^{b}	4.18±0.59	229.73±22.90e
E. EO group	271.27±19.38^{a}	128.77±14.97^{cd}	55.31±13.97^{a}	5.16±1.26	109.51±13.36^{bc}
F. OA acid group	374.98±13.46^{bc}	143.02±19.63^{d}	89.73±17.77^{b}	4.31±0.82	168.79±27.23^{d}
7 day					
A. Negative control group	388.72±26.61^{a}	174.15±19.69^{c}	94.05±11.72^{ab}	4.17±0.50^{b}	212.33±26.50^{b}
B. Positive control group	353.82±25.68^{a}	114.55±12.21^{a}	80.55±8.66^{a}	4.41±0.42^{b}	126.84±11.54^{a}
C. PRO group	638.52±25.40^{c}	253.83±14.41^{d}	102.60±13.03^{ab}	6.30±0.85^{c}	508.30±21.72^{d}
D. PRE group	738.03±30.78^{d}	131.35±10.78^{ab}	114.00±21.53^{b}	6.67±1.27^{c}	304.51±29.13^{c}
E. EO group	450.22±18.64^{b}	161.42±34.89^{bc}	102.55±10.68^{ab}	4.43±0.50^{b}	228.07±48.74^{b}
F. OA acid group	437.92±24.11^{b}	254.07±23.56^{d}	159.57±18.29^{c}	2.76±0.26^{a}	350.71±51.78^{c}
14 day					
A. Negative control group	630.27±41.87^{c}	158.12±31.09^{b}	190.52±17.19^{c}	3.32±0.35^{a}	312.76±61.17^{b}
B. Positive control group	507.22±23.46^{a}	121.97±22.12^{a}	144.82±25.78^{b}	3.58±0.59^{a}	195.28±44.07^{a}
C. PRO group	561.70±32.88^{b}	161.33±19.34^{bc}	105.32±17.20^{a}	5.46±0.97^{bc}	285.33±43.71^{b}
D. PRE group	940.35±23.96^{d}	192.65±10.60^{cd}	144.92±20.89^{b}	6.60±0.94^{c}	568.92±36.27^{d}
E. EO group	672.92±27.99^{c}	208.05±13.91^{d}	184.68±20.27^{c}	3.67±0.38^{a}	440.10±41.99^{c}
F. OA acid group	676.68±31.00^{c}	218.08±12.67^{d}	159.77±18.99^{bc}	4.28±0.52^{ab}	463.47±35.93^{c}

$^{a–c}$ Values within columns with no common superscript differ significantly at $p<0.05$.

of 1794.2±63.96 µm in the duodenum was achieved by group E, which was fed essential oils, whereas the maximum villus surface area index of 1662.6±389.16 mm^2 was recorded in group D, which was treated with prebiotics. The maximum villus height of 940.35±23.96 µm and surface area index of 568.92±36.27 mm^2 in the ileum mucosa were recorded in group D, treated with prebiotics.

Final results show that there is an overall increase in histological parameters of the mucosa of the

duodenum and ileum in the groups fed non-antibiotic feed additives as compared with positive and negative controls. Prebiotics showed the maximum positive effects. Therefore, this study suggests that a combination of non-antibiotic feed additives will be beneficial for the intestinal health of broiler chickens, but there is a need for more research on the combinations of non-antibiotic feed addition.

DISCUSSION

Growth promoters are used in poultry feed to enhance the microflora of intestines and to develop the immune system to ultimately improve performance. However, antimicrobial agents as prophylactic means have been strongly questioned due to the advancement of antimicrobial resistance amidst pathogenic microorganisms. Consequently, the possibility of antibiotics not being used as progression growth drugs for poultry anymore and the apprehension about the secondary results of their consumption as therapeutic mediators has formed an atmosphere in which consumers and manufacturers are equally seeking alternatives. In the current study, treatment with probiotics improved the morphology of the intestine as described by a study performed by Biloni *et al.* (2013), in which a combination of Early Bird and FloraMax-B11 supplementation (an encouraging probiotic supplement) was used. Researchers deter-mined that a mix of Early Bird FloraMax enhanced gut morphology with the reduced recoverable amount of *Salmonella,* while also increasing poultry mass in comparison with controls separately by each product. Till today, a list of products comprising plant extracts, essential oils, organic acids, prebiotics, spices, and probiotics have been acknowledged and suggested equally for antibiotic substitutions in farms and smallholder animal nourishment. Such non-antibiotic feedstuff herbs and additives are being studied to bridge this gap, as some agronomists use to utilize these instead of antibiotics for poultry (Griggs & Jacob, 2005; Nava *et al.,* 2005). The final results of this study show an increase in intestinal health of broiler chicks that were treated with non-antibiotic feed additives in the presence of the challenge strain *Salmonella enterica serovar Enteritidis*. There is an overall improvement in intestinal health as measured through the micrometry technique. Villus height, villus width, crypt depth, and villus surface area index increased in groups given non-antibiotic feed additives treatments such as probiotics, prebiotics, essential oils, and organic acids (Abbas *et al.,* 2022b). The results of prebiotics on the ileum mucosa were the highest, and essential oils and prebiotics both contributed to the highest results for the improvement in duodenal morphology. When compared with a negative control group and a positive control group, all the non-antibiotics showed gut morphology improvement. In this trial, the increase in gut morphology by treating with prebiotics can be supported by a study conducted by Sultan *et al.* (2015) who analyzed the effects of a particular strain of yeast-derived-carbohydrate-fractions (Actigen) at various stages on the performance of the broiler and gut histo-morphology.

The overall improvement in gut morphology caused by non-antibiotic feed additives shows the need for these products to be used in combination rather than alone. Such combinations may help farmers to overcome the issue of a ban on antibiotics and antibiotic resistance.

CONCLUSION

This experiment aimed to evaluate the antimicrobial and growth promoter efficiency of probiotics, prebiotics, synbiotics, essential oils, and organic acids. These non-antibiotic antimicrobial agents have gained popularity due to their positive effects on growth, gut health, metabolism, and immunity. Dietary use of prebiotics and probiotics is a pre-requisite for regulating the micro-flora to promote better health and prevent diseases. Based upon the results of the present research, it can be concluded that the dietary addition of non-antibiotic antimicrobial chemicals such as probiotics, prebiotics, organic acids, and short-chain fatty acids in poultry feed may have good and safe antibiotic effects without having any residual or side effects on body organs, especially gut histomorphology. However, further research is required to clearly understand the mechanism of action of these chemicals at the cellular level.

STATEMENT OF ANIMAL RIGHTS

All animals were handled according to international, national, and institutional guidelines for the care and use of animals.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

Abbas G, Iqbal MA, Riaz M, *et al.* Comparative Effect of Different Levels of Probiotics (Protexin) on Hemato-chemical Profile in Broilers. Advances in Zoology and Botany 2018;6(3):84-7. https://doi.org/10.13189/azb.2018.060302

Abbas G, Arshad M, Saeed M, *et al.* An update on the promising role of organic acids in broiler and layer production. Journal of Animal Health and Production 2022a;10(3):273-286. https://doi.org/10.1080/09712119.2015.1079527

Abbas G, Arshad M, Tanveer AJ, *et al.* Combating heat stress in laying hens a review. Pakistan Journal of Science 2022b;73(4):633-55.

Acamovic T, Brooker J. Biochemistry of plant secondary metabolites and their effects in animals. Proceedings of the Nutrition Society 2005;64(3):403-12. https://doi.org/10.1079/PNS2005449

Aptekmann K, Artoni SB, Stefanini M, *et al.* Morphometric analysis of the intestine of domestic quails (Coturnix coturnix japonica) treated with different levels of dietary calcium. Anatomia, Histologia, Embryologia 2001;30(5):277-80. https://doi.org/10.1046/j.1439-0264.2001.00331.x

Athanassopoulou F, Prapas T, Rodger H. Diseases of Puntazzo puntazzo Cuvier in marine aquaculture systems in Greece. Journal of Fish Diseases 1999;22(3):215-8. https://doi.org/10.1046/j.1365-2761.1999.00151.x

Batool SA, Ahsan F, Nawaz M, *et al.* Study of in-vitro probiotic properties and antibiotic resistance in lactobacilli isolated from commercial probiotic products in Pakistan. Pakistan Journal of Science 2020;72(1):1-6.

Biloni A, Quintana C, Menconi A, *et al.* Evaluation of effects of EarlyBird associated with FloraMax-B11 on *Salmonella* Enteritidis, intestinal morphology, and performance of broiler chickens. Poultry Science 2013;92(9):2337-46. https://doi.org/10.3382/ps.2013-03279.

Charalampopoulos D, Rastall RA. Prebiotics and probiotics science and technology. New York: Springer Science & Business Media; 2009.

Cherrington C, Hinton M, Mead G, *et al.* Organic acids:chemistry, antibacterial activity and practical applications. Advances in Microbial Physiology 1991;32:87-108. https://doi.org/10.1016/S0065-2911(08)60006-5.

Dibner J, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research 2002;11(4):453-63. https://doi.org/10.1093/japr/11.4.453

FAO/WHO. Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Rome; 2002.

Ferreira CL, Salminen S, Grzeskowiak L, *et al.* Terminology concepts of probiotic and prebiotic and their role in human and animal health. Revista de Salud Animal 2011;33(3):137-46.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition 1995;125(6):1401-12. https://doi.org/10.1093/jn/125.6.1401. https://doi.org/10.1093/jn/125.6.1401.

Griggs J, Jacob JP. Alternatives to antibiotics for organic poultry production. Journal of Applied Poultry Research 2005;14(4):750-6.

Jong MC, Boers I, van Wietmarschen H, *et al.* Development of an evidence-based decision aid on complementary and alternative medicine (CAM) and pain for parents of children with cancer. Supportive Care in Cancer 2020;28(5):2415-29. https://doi.org/10.1007/s00520-019-05058-8.

Konieczka P, Sandvang D, Kinsner M, *et al.* Bacillus-based probiotics affect gut barrier integrity in different ways in chickens subjected to optimal or challenge conditions. Veterinary Microbiology 2022;265:109323. https://doi.org/10.1016/j.vetmic.2021.109323

Lee K, Everts H, Kappert H, *et al.* Growth performance of broiler chickens fed a carboxymethyl cellulose containing diet with supplemental carvacrol and/or cinnamaldehyde. International Journal of Poultry Science 2004;3(9):619-22. https://doi.org/10.3923/ijps.2004.619.622.

Mourey A, Canillac N. Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control 2002;13(4-5):289-92. https://doi.org/10.1016/S0956-7135(02)00026-9

Mohamed SIA, Shehata SAM, Bassiony SM, *et al.* Does the use of different types of probiotics possess detoxification properties against aflatoxins contamination in rabbit diets?. Probiotics & Antimicro. Proteins; 2022. https://doi.org/10.1007/s12602-022-09990-w.

Mustafa A, Bai S, Zeng Q, *et al.* Effect of organic acids on growth performance, intestinal morphology, and immunity of broiler chickens with and without coccidial challenge. AMB Express 2021a;11(1):140. https://doi.org/10.1186/s13568-021-01299-1

Mustafa A, Bai S, Zeng Q, *et al.* Limitation and potential effects of different levels of aging corn on performance, antioxidative capacity, intestinal health, and microbiota in broiler chickens. Animals 2021b;11(10):2832. https://doi.org/10.3390/ani11102832

Nava G, Bielke L, Callaway T, *et al.* Probiotic alternatives to reduce gastrointestinal infections:the poultry experience. Animal Health Research Reviews 2005;6(1):105-18. https://doi.org/10.1079/ahr2005103.

NRC - National Research Council. Nutrient requirements of poultry. Washington: National Academy Press; 1994.

Nychas G. Natural antimicrobials from plants. In: Goul G. New methods of food preservation. New York: Springer; 1995. p.58-89. ISBN: 978-1-4613-5876-3

Ogbuewu IP, Mabelebele M, Sebola NA *et al.* Bacillus probiotics as alternatives to in-feed antibiotics and its influence on growth, serum chemistry, antioxidant status, intestinal histomorphology, and lesion scores in disease-challenged broiler chickens. Frontiers in Veterinary Sciences 2022;9:876725. https://doi.org/10.3389/fvets.2022.876725

Patterson J, Burkholder K. Application of prebiotics and probiotics in poultry production. Poultry Science 2003;82(4):627-31. https://doi.org/10.1093/ps/82.4.627.

Prado-Rebolledo OF, Delgado-Machuca JdJ, Macedo-Barragan RJ, *et al.* Evaluation of a selected lactic acid bacteria-based probiotic on *Salmonella* enterica serovar Enteritidis colonization and intestinal permeability in broiler chickens. Avian pathology 2017;46(1):90-4. https://doi.org/10.1080/03079457.2016.1222808.

Rota C, Carraminana J, Burillo J, Herrera A. In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. Journal of Food Protection 2004;67(6):1252-6.

Sakamoto K, Hirose H, Onizuka A, *et al.* Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. Journal of Surgical Research 2000;94(2):99-106. https://doi.org/10.1006/jsre.2000.5937.

Sultan A, Uddin I, Khan S, *et al.* Effect of yeast derived carbohydrate fraction on growth performance, apparent metabolizable energy, mineral retention and gut histomorphology of broilers during starter phase. Pakistan Veterinary Journal 2015;35(4).

Vamanu E, Vamanu A. The influence of prebiotics on bacteriocin synthesis using the strain Lactobacillus paracasei CMGB16. African Journal of Microbiology Research 2010;4(7):534-7.

Yusrizal Y, Chen T. Effect of adding chicory fructans in feed on fecal and intestinal microflora and excreta volatile ammonia. International Journal of Poultry Science 2003;2(3):188-94. https://doi.org/10.3923/ijps.2003.188.194.