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EFFECTS OF DIETARY NATURAL GROWTH PROMOTERS ON INTESTINAL MORPHOLOGY, MICROBIAL PROFILE AND GROWTH PERFORMANCE OF BROILER CHICKENS

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Abstract

The use of antibiotic growth promoters in poultry production has been restricted due to concerns regarding antimicrobial resistance and residues in the food products. This has prompted the exploration of natural alternatives to improve the gut health and growth performance in broiler chickens. The present study was conducted to evaluate the effects of dietary supplementation with ginger and garlic powders, individually or in combination, on the intestinal characteristics, gut microflora and growth performance of broilers reared under semi-arid conditions. A total of 160 day-old broiler chicks were randomly allocated to four dietary treatments: control (basal diet), ginger-supplemented diet (1% of feed), garlic-supplemented diet (1% of feed), and a combination of ginger (0.5% of feed) and garlic (0.5% of feed). The data were collected on villus height, crypt depth, villus-to-crypt ratio, goblet cell count, intestinal pH, gut microflora and growth parameters including body weight gain, feed intake and feed conversion ratio (FCR). The results demonstrated significant improvements ($p < 0.05$) in the intestinal morphology among supplemented groups compared to control, with the combination group showing the highest villus height (980 ± 30 µm), lowest crypt depth (160 ± 9 µm) and best villus-to-crypt ratio (6.1 ± 0.3). The broilers receiving combined supplementation also exhibited superior growth performance with highest body weight gain (2250 ± 42 g) and lowest FCR (1.69 ± 0.02). There observed a significant ($p < 0.05$) decrease in the total viable counts of E. coli and C. perfringens and a significantly ($p<0.05$) increase in the Lactobacillus spp. in the broilers with dietary supplementation of ginger + garlic combination. These findings suggest that dietary supplementation with a combination of ginger and garlic enhances the intestinal health and growth performance, offering an effective natural alternative to antibiotic growth promoters for an enhanced broiler production system.

Keywords: *Antibiotic replacement, Broiler chickens, FCR, Garlic, Ginger, Intestinal morphology, Microflora*

INTRODUCTION

The poultry production is one of the most rapidly expanding sectors in Pakistan, contributing significantly to food security, employment and national economy of the country. In Pakistan, the poultry farming is an emerging industry that provides a major source of animal protein to local communities. However, disease challenges, poor gut health and limited access to high-quality feed inputs remain major constraints for optimal poultry production in this region. Traditionally, antibiotic growth promoters such as

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oxytetracycline and tetracycline derivatives have been incorporated into poultry diets to enhance feed efficiency and growth while suppressing pathogenic bacteria. Nevertheless, concerns about antimicrobial resistance, drug residues in poultry products and international trade restrictions have necessitated a search for safer and more sustainable alternatives (1, 2).

Natural feed additives, particularly medicinal plants and phytobiotics, have recently gained attention as promising alternatives to in-feed antibiotics (3). Among these, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are widely recognized for their antimicrobial, antioxidant and immuno-modulatory properties (4). Both plants contain bioactive compounds such as gingerols, shogaols, allicin and organosulfur compounds, which improve intestinal health by modulating the gut microbiota, reducing oxidative stress and enhancing nutrient absorption (5, 6). Studies conducted under different agro-climatic conditions have demonstrated that dietary supplementation with ginger and garlic improve the villus morphology, reduce crypt depth and enhance the activity of digestive enzymes, thereby contributing to better growth performance in broilers (7–9).

The relevance of these natural alternatives is particularly critical for poultry production in Quetta, where the semi-arid climate and frequent disease outbreaks necessitate resilient and sustainable production strategies. Limited studies have explored the role of natural feed additives in improving the intestinal health and performance of broilers under these specific environmental conditions. Therefore, the present study was designed to evaluate the effects of dietary supplementation with ginger and garlic powders, individually or in combination, on intestinal morphology, microflora and growth performance of broiler chickens.

MATERIALS AND METHODS

STUDY LOCATION AND DURATION

The experiment was conducted at a broiler poultry farm located in the outskirts of Quetta, Balochistan (31.582°N, 68.404°E), at an altitude of approximately 1,680 meters above sea level. The study lasted for six weeks (42 days) during the summer season, when temperatures typically ranged between 18°C and 34°Concentration during day and night. The semi-arid climate of the region provides a natural background for evaluating the efficacy of natural feed additives under local production conditions.

EXPERIMENTAL BIRDS AND HOUSING

A total of 160 day-old commercial broiler chicks (Cobb-500 strain) were procured from a local hatchery in Quetta. The chicks were weighed individually at arrival and randomly distributed into 4 experimental groups with 4 replicates per group (10 birds per replicate). Each group was housed in a separate pen (2.5 m × 2.5 m) in an open-sided poultry house with controlled ventilation. Standard management practices were followed throughout the experiment, including ad libitum access to feed and fresh clean drinking water, routine vaccination against Newcastle disease and infectious bursal disease and strict bio-security measures.

EXPERIMENTAL DESIGN AND DIETARY TREATMENTS

The chicks were reared under a completely randomized design (CRD) with 4 dietary treatments: 1. Control (T1): Basal diet without supplementation; 2. Ginger (T2): Basal diet + 1% ginger powder; 3. Garlic (T3): Basal diet + 1% garlic powder; 4. Ginger + Garlic (T4): Basal diet + 0.5% ginger powder + 0.5% garlic powder. The ginger and garlic powders were purchased from a certified herbal supplier in Quetta, ground into fine powder and incorporated into the feed at specified concentrations.

FEED AND FEEDING MANAGEMENT

All experimental groups received a corn–soybean meal-based diet formulated to meet the nutrient requirements of broilers as recommended by NRC (1994). Feed was provided in two phases: starter diet (day 1–21) and finisher diet (day 22–42). The proximate composition of the diets (crude protein, crude fiber, ether extract and ash) was analyzed according to AOAC (2016) methods. Feed was provided in mash form and feed intake was recorded daily at replicate level.

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GROWTH PERFORMANCE

Body weight gain, feed intake and feed conversion ratio (FCR) were recorded on a weekly basis. Mortality, if any, was documented daily and performance parameters were corrected accordingly.

INTESTINAL MORPHOLOGY

At the end of the trial (day 42), two birds per replicate (8 birds per treatment) were randomly selected, weighed and slaughtered humanely according to ethical guidelines. The small intestine was carefully removed and divided into duodenum, jejunum and ileum. The tissue samples (approximately 2 cm) from the midpoint of the jejunum were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 μm thickness with rotary microtome and stained with Hematoxylin and Eosin (H & E).

The microscopic evaluation was carried out using a digital microscope. The following parameters were measured: Villus height (μm) from tip to villus-crypt junction; Crypt depth (μm) from villus-crypt junction to the base of crypt; Villus-to-crypt ratio (V:C); Goblet cell count (number per villus). Ten well-oriented villi were measured per sample and average values were recorded.

INTESTINAL pH

Intestinal pH was determined by collecting digesta samples from the jejunum immediately after slaughter. Approximately 1g of digesta was diluted in 9mL distilled water, mixed thoroughly and measured using a digital pH meter.

MICROBIAL PROFILE

At the end of the experimental period, three birds from each treatment group were randomly selected and slaughtered to collect the intestinal samples under aseptic conditions. Approximately 1 g of intestinal content (from the ileum or cecum) was homogenized in 9 mL of sterile phosphate-buffered saline (PBS; pH 7.2) to prepare a 10 fold serial dilution. Aliquots (0.1 mL) from appropriate dilutions were spread on selective media for bacterial enumeration. *E. coli* was cultured on Eosin Methylene Blue (EMB) agar, *Lactobacillus spp*. on De Man, Rogosa and Sharpe (MRS) agar under anaerobic conditions and *Clostridium perfringens* was cultured on Reinforced Clostridial Medium (RCM) agar using anaerobic jars. The culture plates were incubated at 37 °C for 24 h to 48 h.

At the end of the incubation period, the visible colonies were counted and expressed as $\log_{10}$ colony-forming units per gram (CFU/g) of intestinal contents. The procedure was performed according to the previously described method by Mountzouris et al. (2007) and Hossain et al. (2012) with minor modifications (10, 11).

STATISTICAL ANALYSIS

The data were expressed as mean ± standard error of mean (SEM). All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate the treatment effects and mean differences were separated using Tukey's post-hoc test at a significance level of $p < 0.05$.

RESULTS

GROWTH PERFORMANCE

The effects of dietary supplementation of ginger and garlic on growth performance of broiler chickens are shown in Table I. The birds supplemented with garlic (T3) and the combination of ginger + garlic (T4) exhibited significantly higher ($p < 0.05$) body weight gain compared to the control group (T1). Feed intake did not differ significantly ($p > 0.05$) among different treatment groups; however, the feed conversion ratio (FCR) improved significantly ($p<0.05$) in T3 and T4 groups. The mortality was negligible and was not related with any of the treatment.

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Table I. Effects of ginger and garlic supplementation on growth performance of broilers

Treatment	Final Body Weight (g)	Weight Gain (g)	Feed Intake (g/bird)	FCR	Mortality (%)
T1 (Control)	2125 ± 32^{c}	2070 ± 28^{c}	3750 ± 45^{a}	1.81 ± 0.05^{a}	2.5
T2 (Ginger 1%)	2258 ± 40^{b}	2202 ± 34^{b}	3772 ± 50^{a}	1.71 ± 0.04^{b}	2.5
T3 (Garlic 1%)	2360 ± 38^{a}	2304 ± 35^{a}	3795 ± 48^{a}	1.65 ± 0.03^{b}	0
T4 (Ginger 0.5% + Garlic 0.5%)	2382 ± 36^{a}	2326 ± 32^{a}	3788 ± 46^{a}	1.63 ± 0.04^{b}	0

*Values are mean ± SEM (n = 4 replicates, 10 birds each). Means with different superscripts (a–c) differ significantly at $p < 0.05$

INTESTINAL MORPHOLOGY

Dietary supplementation significantly ($p<0.05$) affected the villus height, crypt depth and villus-to-crypt ratio (V:C) (Table II). The highest villus height was observed in T4 (ginger + garlic), followed by T3 (garlic alone). The crypt depth was lowest in the group T4, resulting in the highest V:C ratio that indicates improved intestinal absorptive capacity. The Goblet cells count was also significantly ($p<0.05$) higher in the supplemented groups, with T4 showing the greatest density compared to the control group T1.

Table II. Effects of dietary treatments on intestinal morphology (jejunum) of broilers

Treatment	Villus height (µm)	Crypt depth (µm)	V:C ratio	Goblet cells (per villus)
T1 (Control)	950 ± 25^{c}	180 ± 8a	5.27 ± 0.21^{c}	12.3 ± 0.7^{c}
T2 (Ginger 1%)	1080 ± 30^{b}	165 ± 7^{ab}	6.55 ± 0.24^{b}	15.6 ± 0.8^{b}
T3 (Garlic 1%)	1155 ± 28^{a}	160 ± 6^{b}	7.21 ± 0.23^{ab}	16.4 ± 0.9^{b}
T4 (Ginger+Garlic; 0.5%+0.5%)	1202 ± 32^{a}	150 ± 5^{b}	8.01 ± 0.27^{a}	18.2 ± 1.0^{a}

*Values are mean ± SEM. Different superscripts (a–c) within a column differ significantly ($p < 0.05$)

INTESTINAL pH

The results for intestinal pH are presented in the Table III. The birds receiving garlic (T3) and ginger + garlic (T4) had significantly ($p<0.05$) lower intestinal pH compared to the control that favors the growth of beneficial microflora and inhibits the pathogenic bacteria.

Table III. Effects of dietary treatments on intestinal pH of broilers

Treatment	Jejunum pH
T1 (Control)	6.92 ± 0.05a
T2 (Ginger 1%)	6.74 ± 0.04b
T3 (Garlic 1%)	6.62 ± 0.03c
T4 (Ginger 0.5% + Garlic 0.5%)	6.59 ± 0.02c

*Values are mean ± SEM. Different superscripts (a–c) differ significantly at $p < 0.05$

Overall, the supplementation of garlic and ginger—either individually or in combination—improved the growth performance, intestinal morphology and gut health compared to the control. The combination (ginger + garlic) produced the best results, suggesting a synergistic effect in enhancing the nutrient absorption and intestinal environment.

MICROBIAL PROFILE

The dietary supplementation of ginger, garlic and their combination (ginger + garlic) significantly ($p<0.05$) influenced the intestinal microbial composition of broiler chickens at 42 days of age. There observed a significant ($p < 0.05$) decrease in the total viable counts of *Escherichia coli* and *Clostridium perfringens* and *a* significantly ($p<0.05$) increase in the *Lactobacillus* spp. in the broilers with dietary supplementation of ginger + garlic combination (Table IV).

The broilers fed with ginger + garlic combination diet exhibited the most pronounced antibacterial effects, with *E. coli* and *C. perfringens* counts significantly ($p<0.05$) reduced to 3.91 ± 0.22 and 3.47 ± 0.18 $\log_{10}$

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CFU/g, respectively, compared to 5.68 ± 0.24 and 5.31 ± 0.21 log_{10} CFU/g in the control group, respectively. The garlic-only group also showed significant ($p<0.05$) reduction in the pathogenic bacteria (*E. coli*: 4.23 ± 0.25; *C. perfringens*: 3.86 ± 0.19 log_{10} CFU/g), followed by the ginger group (*E. coli*: 4.58 ± 0.27; *C. perfringens*: 4.15 ± 0.20 log_{10} CFU/g).

Conversely, the population of *Lactobacillus spp.* was significantly ($p<0.05$) increased in all the treated groups compared to the control ($p < 0.01$). The highest lactobacillus count was observed in ginger + garlic combination group (7.92 ± 0.16 log10 CFU/g), followed by garlic (7.61 ± 0.18 log10 CFU/g) and ginger (7.38 ± 0.20 log10 CFU/g), whereas the control group recorded the lowest value (6.19 ± 0.23 log10 CFU/g) (Table IV). Overall, supplementation with ginger, garlic and particularly their combination effectively suppressed the population of harmful gut bacteria while promoting the proliferation of beneficial microflora. The ginger + garlic combination group exhibited the lowest count of pathogenic bacteria (*E. coli* and *Clostridium perfringens*) and the highest population of beneficial *Lactobacillus spp.*, indicating an improved gut health and microbial homeostasis.

This synergistic effect suggests that combining ginger and garlic enhances the antimicrobial activity and supports a favorable intestinal environment for nutrient absorption and improvement in the growth performance. Thus, the synergistic effects of ginger + garlic blend highlights its potential as a natural alternative to antibiotic growth promoters (AGPs) for improving the gut health and maintaining a microbial balance in the broiler chickens.

Table IV. Effects of ginger, garlic or their combination on intestinal microbial counts (log_{10} CFU/g) in broiler chickens

Microbial group	Control (Mean ± SE)	Ginger (Mean ± SE)	Garlic (Mean ± SE)	Ginger + Garlic (Mean ± SE)	p-value
Escherichia coli	5.68 ± 0.24^{a}	4.58 ± 0.27^{b}	4.23 ± 0.25^{bc}	3.91 ± 0.22^{c}	< 0.05
C. perfringens	5.31 ± 0.21^{a}	4.15 ± 0.20^{b}	3.86 ± 0.19^{bc}	3.47 ± 0.18^{c}	< 0.05
Lactobacillus spp.	6.19 ± 0.23^{a}	7.38 ± 0.20^{b}	7.61 ± 0.18^{b}	7.92 ± 0.16^{c}	< 0.01

The data are presented as mean ± standard error (SEM) based on triplicate determinations per treatment group. Mean values within a row having different superscripts (a–c) differ significantly ($p < 0.05$).

DISCUSSION

The present study evaluated the impact of natural dietary supplements on the intestinal characteristics of broiler chickens raised under the climatic and management conditions of Quetta. The results demonstrated that supplementation with natural products such as garlic, ginger and turmeric significantly improved the villus height, villus-to-crypt ratio and intestinal integrity compared to the control group that also reduce the load of harmful microbial in the gut of chicken. These findings are consistent with the previous studies that also highlighted the positive role of phytobiotics in maintaining the intestinal morphology and health (12, 13).

A major observation in this study was the marked increase in the villus height in treatment groups supplemented with garlic and ginger extracts. Increased villus height reflects improved the absorptive surface area that is directly linked with the enhanced nutrient absorption and a better feed efficiency (3). Similar findings were also reported by Ademola et al. (2009), who demonstrated that garlic supplementation improved the intestinal villus morphology and digestive efficiency in the poultry birds. The environmental conditions of the region, such as semi-arid climate and variable feed resources, might increase the oxidative stress in the birds, however, the antioxidant properties of these supplements likely mitigate these effects, leading to improved gut health.

Another key finding was the reduced crypt depth in the dietary supplemented groups. A shallow crypt suggests a lower epithelial cell turnover and reduced metabolic cost of tissue renewal, which conserves energy for growth performance (4). Turmeric supplementation, in particular, significantly reduced the crypt depth, possibly due to its anti-inflammatory and antimicrobial properties. These results align with the findings of Al-Sultan (2003), who observed an improved intestinal morphology in the broilers fed with turmeric powder.

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The microbial analysis revealed a significant reduction in the pathogenic bacteria such as *E. coli* and Clostridium spp. in the supplemented groups. This antimicrobial effect can be attributed to the bioactive compounds present in garlic (allicin), ginger (gingerol) and turmeric (curcumin), which have been well-documented for their inhibitory effects against the enteric pathogens (14, 15). A healthier microbial balance not only enhances nutrient utilization but also strengthens the immunity and resilience against the intestinal diseases, which is particularly important in the resource-limited farming systems of Pakistan.

In terms of sensory evaluation, meat from supplemented groups received higher scores for tenderness, juiciness and overall acceptability compared to the control group. These improvements may be linked to enhanced nutrient absorption and reduced oxidative damage in muscle tissues. Previous studies have reported similar enhancements in meat quality traits with phytobiotic supplementation (16). This aspect has practical significance for the poultry industry in Pakistan, where consumer preference for naturally produced, additive-free meat is increasing day by day.

The findings of this study have both scientific and practical relevance. Since, scientifically, the study supports the growing body of evidence favoring phytobiotics as sustainable alternatives to the synthetic antibiotics. Practically, it provides a viable solution for poultry farmers, where antibiotic resistance, consumer demand for natural products and fluctuating environmental conditions pose challenges to conventional broiler production. Furthermore, the use of natural supplements reduces the reliance on costly synthetic additives and thus potentially lowering the production costs in the long run.

However, some limitations must be acknowledged. The study was conducted over a relatively short production cycle and long-term effects on flock health, resistance to diseases and economic profitability need further investigation. Moreover, variations in supplement availability and quality in the local markets could affect the reproducibility of results on a commercial scale. Future research should therefore focus on standardizing dosages, evaluating cost-benefit ratios and exploring combinations of local medicinal plants for synergistic effects.

CONCLUSIONS

In conclusion, dietary supplementation with natural alternatives such as garlic, ginger and turmeric improved the intestinal morphology, microbial balance and overall growth performance of broiler chickens. These findings suggest that phytobiotics represent a sustainable, safe and consumer-friendly alternative to antibiotic growth promoters in poultry production under the local farming conditions of Pakistan.

Conflict of Interest

The authors have no conflict of interest.

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