



Comparative Evaluation of Herbal Growth Promoter (Auctus) and Antibiotic Growth Promoter (Enramycin) in Broiler Chickens

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ABSTRACT

The present study aimed to evaluate the efficacy of a herbal growth promoter (Auctus) as an alternative to an antibiotic growth promoter (AGP) in broilers. A total of 180 one-day-old VenCobb 430 Y chicks were randomly assigned to three groups: T0 (basal diet), T1 (basal diet + 100 g/MT Enramycin), and T2 (basal diet + 500 g/MT Auctus), with four replicates per group. The trial duration was over 42 days. Results showed a significant improvement ($P \leq 0.05$) in body weight, FCR, and European Production Efficiency Factor (EPEF) in both T1 and T2 groups compared to the control. Histomorphometry analysis revealed significantly higher ($P < 0.05$) villus height in the duodenum, jejunum, and ileum in the T2 group. The cecal microbial analysis indicated a significant reduction in *E. coli* counts in T2 group and an increase ($P < 0.001$) in *Lactobacilli* in the T1 and T2 groups. Immune response analysis showed a significantly higher ($P < 0.001$) antibody titer against Newcastle disease virus in the T1 and T2 groups. Furthermore, there was a significant ($P < 0.05$) increase in the bursa weight of broilers in the T2 group compared to the control group. Herbal growth promoter fed broilers (T2) showed comparable growth performance and immune responses to AGP fed broilers (T1) but had lower *E. coli* levels in the gut and longer small intestine villi. These findings suggest that Auctus can effectively replace AGPs, supporting broiler performance while addressing concerns related to antibiotic use and human health.

HIGHLIGHTS

- Auctus supplementation significantly improved growth performance in broilers, comparable to antibiotic growth promoter.
- Broilers fed Auctus exhibited reduced *E. coli* and increased *Lactobacilli* populations in the cecum.

Keywords: Antibiotics, Broiler, *E. coli*, Growth Promoter, Herbal

The intensification of poultry production systems has driven the widespread use of antibiotics to improve animal health and productivity. In-feed antibiotics have been a well-established practice at sub-therapeutic doses in the animal industry, enhancing productivity and supporting the intensification of modern poultry production systems. However, extensive use of sub-therapeutic levels of antibiotics in intensive animals has raised scientific concerns regarding the development of antimicrobial

resistance, because of extended withdrawal periods, and presence of antibiotic residues in animal products which represents a significant public health threat (Lillehoj *et al.*, 2018). The emergence of antibiotic-resistant bacterial

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strains is closely linked to the excessive use of sub-therapeutic doses of antibiotics. This overuse accelerates the development of antimicrobial resistance within the microbiota, posing significant challenges to both animal and human health.

Therefore, there is an increasing need to identify viable and alternative antibiotic strategies to promote growth and enhance animal production performance. The available classes of antibiotic alternatives designed to enhance productivity and enable poultry to achieve their genetic potential under commercial conditions include probiotics, organic acids, prebiotics, enzymes, antimicrobial peptides, and bacteriophages. The beneficial effects of these alternatives have been addressing limited aspects of animal health and performance.

Phytogenics, or phytogetic feed additives, are plant-derived compounds encompassing a wide range of substances, including essential oils, herbs, spices, plant extracts or part of plants (roots, leaves, stems, flowers, bark, roots, seeds and fruits) (Seidavi *et al.*, 2021). The use of these plant-based additives, often referred to as phytobiotics, has been practiced since ancient times when herbs were utilized for both the prevention and treatment of diseases in humans and animals.

Herbal plants are a source of diverse bioactive compounds that exert synergistic effects to enhance health and performance in animals. These compounds support gut health by inhibiting the growth of pathogenic bacteria and promoting the growth of beneficial bacteria, thereby maintaining a balanced microbiota. They also stimulate the activity of digestive enzymes, facilitating more efficient feed utilization. Furthermore, herbal plants enhance immune function, enabling animals to better resist infections while their antioxidant and anti-inflammatory activities help mitigate oxidative stress and inflammation, ultimately contributing to improved productivity and overall physiological well-being (Putritamara *et al.*, 2021). Phytobiotics are increasingly recognized as potential alternatives to antibiotic growth promoters due to their natural origin, non-toxic nature, easy availability, and residue-free (Krauze, 2021).

The objective of the present study was to assess the efficacy of a herbal growth promoter (Auctus), as an alternative to antibiotic growth promoter (Enramycin). The study focused on evaluating its impact on the

performance, immune response, gut health parameters, and histomorphometry of small intestine in commercial broiler chickens. Auctus is a polyherbal feed supplement from PhyGeno a division of Avitech Nutrition Pvt. Ltd., Haryana, India. It contains bioactive compounds like tannins, saponins, glycosides, terpenoids, diterpenoids, flavonoids and other phytogetic compounds.

MATERIALS AND METHODS

Experiment design

A total of 180, VenCobb 430Y strain day old broiler chicks were procured and wing banded. The study included three groups: T0 (control group receiving a basal diet), T1 (basal diet supplemented with Enramycin 10% at 100 g/MT of feed), and T2 (basal diet supplemented with Auctus at 0.50 kg/MT of feed). The experimental design consisted of four replicates per group, with each replicate containing 15 birds (60 birds per group). The study was conducted over 42-day period at the Veterinary College in Bidar, Karnataka Veterinary, Animal, and Fisheries Sciences University (KVAFSU), Karnataka, India. All the procedures followed during the trial were approved by the Institutional Animal Ethical Committee of Veterinary College Bidar, Karnataka under project proposal No: VCB/IAEC/LPM-09/2023-24.

Experimental Diets, Feeding and Management

The experimental diet, outlined in Table 1, was formulated to fulfill all nutrient requirements specified in the VenCobb 430 Y recommendations. The birds were fed with broiler pre-starter (0-7 d) diet and starter diet (08 -21 d) and the finisher diet (22 -42 d). An ad libitum feed was offered to all the three group birds throughout the experimental period.

All treatment were fed iso-caloric and iso-nitrogenous diets. The birds were reared under deep litter system considering all the standard management practices.

Parameters studied

Growth performance

Weekly live body weight changes, feed consumption, feed conversion ratio, and European Production Efficiency

Factor (EPEF) were recorded. Mortality and weight of dead birds were recorded as and when occurred to calculate the mortality corrected feed conversion ratio. EPEF were calculated taking into account of feed conversion, mortality and average daily gain in live weight using the following formula: $EPEF = (\text{Average grams gained/day} \times \text{livability \%}) / (\text{FCR} \times \text{age of slaughter})$.

Table 1: Per cent ingredient and nutrient composition of basal experimental diet

Ingredient	Pre starter	Starter	Finisher
Maize	52.34	55.46	57.56
SBM	38.98	36.57	33.5
Vegetable Oil	4.2	4.3	6
Lysin	0.3	0.2	0.1
DL- Methionine	0.35	0.32	0.11
DCP ¹	1.9	1.22	0.5
Salt	0.4	0.4	0.4
Trace Minerals Premix ²	0.1	0.1	0.1
Vitamin Premix ³	0.1	0.2	0.5
Shell grit	1.1	1	1
Hepatocare	0.1	0.1	0.1
Biobantox	0.1	0.1	0.1
Coccidiostat	0.03	0.03	0.03
Total	100	100	100
Nutrient Composition			
Nutrient	Pre starter	Starter	Finisher
Crude protein (%) ^a	22.50	21.12	19.20
Metabolizable energy (Kcal/Kg) ^b	3005.26	3119	3224
Calcium (%) ^b	0.94	0.92	0.88
Phosphorus (%) ^b	0.452	0.425	0.402
Lysine (%) ^b	1.421	1.274	1.141
Methionine (%) ^b	0.624	0.584	0.542

¹Provides per kg: Calcium – 230g and phosphorus – 180g; ²Provides per kg: Mn – 80mg, Zn – 80mg, I – 3.29mg, Fe – 32mg and Se – 0.1mg; ³Provides per kg: A – 12375 IU, D₃ – 1800 IU, B₂ – 7.5 mg, K – 1.5mg, B₁ – 4mg, B₆ – 8mg, B₁₂ – 40 µg, E – 20mg, Niacin – 60 mg and Calcium pantothenate – 12.5 mg; ^aAnalysed values; ^b Calculated values.

Immunological Response

Antibody Titers against Newcastle Disease and Infectious Bursal Disease

To acquire the titer of anti-NDV and anti IBD-antibodies in the serum of the experimental birds, whole blood samples

were collected at end of experiment from the wing veins of birds (two bird was selected randomly from each of the replicate pens under each treatment).

Antibody titers against Newcastle Disease Virus (NDV) were estimated using the HA-HI test (Allan and Gough, 1974), while antibody titers against Infectious Bursal Disease Virus (IBDV) were measured using an indirect ELISA kit.

Lymphoid organ weight

The weights of lymphoid organs, including the spleen, bursa of Fabricius, and thymus, were measured at the termination of the experiment (42nd day) during the slaughter of the birds and expressed as a percentage of live body weight.

Intestinal microbial count

On day 42, intestinal contents were collected from eight birds per dietary treatment after slaughtering. Total viable counts of *Escherichia coli* and *Lactobacillus* spp. were assessed using Eosin Methylene Blue (EMB) agar and MRS agar, respectively. Intestinal samples (~1 g) were homogenized in phosphate buffer solution (PBS, pH 7.4) and serially diluted up to 10⁶. From each dilution, 1 mL was plated on selective media. Aerobic *E. coli* plates were incubated at 37°C for 24 hours, while anaerobic *Lactobacillus* plates were incubated in an anaerobic jar with a gas pack system at 37°C for 24 hours. Bacterial colonies were counted using a colony counter, and results were expressed as log CFU colony-forming units (CFU).

Histomorphology examination of small intestine

Histomorphological examination of the small intestine was performed following the humane slaughter of birds at the experiment conclusion and analyzed by the procedure of Song *et al.* 2014. A 2 cm segment each of the duodenum, jejunum, and ileum was collected, rinsed with normal saline, and fixed in 10% neutral buffered formalin. Histological sections were examined at 2X magnification and analyzed using OLYMPUS cell Sens Standard software. Villus length and crypt depth were measured in 20 well-oriented crypt-villus units per bird, and mean lengths were calculated. Villus length was measured

from the tip to the base, and crypt depth from the valley to the basal membrane. Histological technique involves processes like fixation of tissue, dehydration, clearing, embedding, cutting and staining.

STATISTICAL ANALYSIS

The data generated were statistically analyzed (Snedcor and Cochran, 1994) using SPSS software. Mean values were compared using one-way ANOVA. Statistical significance among the treatment means was determined using Duncan's multiple range test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The weekly body weight gain of broiler chickens recorded during the experimental period is presented in Table 2. During the first week of the experimental period, the weekly body weights for T0, T1, and T2 were 86.15 ± 1.24 g, 89.47 ± 1.13 g, and 86.97 ± 1.69 g, respectively, and no significant differences were observed among the groups. Significant ($P < 0.05$) differences in weekly body weights were observed in the T1 (enramycin) group compared to other treatment groups during the second, and third weeks

of the experiment. Significant ($P < 0.001$) higher weekly body weights in the final week for Auctus fed birds (T2) were 2200.18 ± 47.71 g, followed by Enramycin fed group (2124.07 ± 29.29 g) compared to control group (1995.50 ± 35.41 g). These findings align with previous reports highlighting the growth-promoting effects of phytogetic feed additives. For instance, Youssef *et al.* (2021) observed that broiler birds receiving diets containing saponins exhibited significantly ($P < 0.05$) higher body weights compared to the control group. Similarly, Prihambodo *et al.* (2020) reported that the addition of flavonoids significantly ($P < 0.05$) enhanced the average daily gain (ADG) of broilers during the finisher phase. Choi *et al.* (2020) highlighted that tannins have been classified as anti-nutritional factors due to their potential to interfere with nutrient utilization also possess the potential to improve growth performance in poultry.

The results on weekly cumulative feed consumption (g/bird/week) as influenced by supplementation groups in broilers from the first to the sixth week of age are presented in Table 3. The statistical analysis of data revealed that feed consumption during first, second, third, fifth and sixth week of age remained significantly ($P \leq 0.05$) different in Auctus fed birds (T2), and enramycin fed birds (T1) when compared to the control group (T0). These results align with Demir *et al.* (2005), who found a significant ($P \leq 0.05$)

Table 2: Effects of herbal and antibiotic growth promoter on weekly body weight of broilers at 42-day age

Treatment	Weekly cumulative body weight					
	I	II	III	IV	V	VI
T0	86.15 ± 1.244	275.18 ± 6.240^b	587.03 ± 13.225^b	1110.18 ± 24.411^b	1763.87 ± 33.934^b	1995.50 ± 35.413^b
T1	89.47 ± 1.318	301.20 ± 4.812^a	645.80 ± 14.539^a	1183.05 ± 15.495^a	1885.67 ± 28.170^a	2124.07 ± 29.295^a
T2	86.97 ± 1.695	279.10 ± 7.255^b	604.05 ± 15.455^b	1120.55 ± 29.164^{ab}	1790.40 ± 38.501^b	2200.18 ± 47.712^a

a, b Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 3: Effects of herbal and antibiotic growth promoter on weekly feed consumption of broilers at 42-day age

Treatment	Weekly cumulative feed consumption					
	I	II	III	IV	V	VI
T0	112.13 ± 1.488^b	395.43 ± 05.035^b	939.93 ± 3.405^b	1777.30 ± 12.497	2773.66 ± 14201^{ab}	3587.10 ± 22.905^b
T1	122.70 ± 5.126^a	419.58 ± 8.349^{ab}	956.60 ± 7.957^a	1784.46 ± 14.238	2749.83 ± 25.876^b	3684.20 ± 29.262^a
T2	130.83 ± 1.150^a	430.73 ± 10.969^a	959.13 ± 4.106^a	1761.20 ± 11.895	2826.66 ± 29.941^a	3743.58 ± 14.461^a

a, b, Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 4: Effects of herbal and antibiotic growth promoter on weekly FCR of broilers at 42-day age

Treatment	Weekly cumulative feed conversion ratio					
	I	II	III	IV	V	VI
T0	1.296±0.035 ^c	1.437±0.017 ^b	1.605±0.024 ^a	1.605±0.018 ^a	1.576±0.011 ^a	1.802±0.022 ^a
T1	1.397±0.015 ^b	1.425±0.015 ^b	1.529±0.028 ^c	1.511±0.037 ^b	1.461±0.037 ^b	1.715±0.025 ^b
T2	1.506±0.024 ^a	1.600±0.002 ^a	1.591±0.025 ^{ab}	1.572±0.015 ^{ab}	1.579±0.013 ^a	1.703±0.007 ^b

a,b,c Means in the same column with different superscripts differ significantly (P<0.05).

difference in broiler feed intake with antibiotic growth promoters and natural feed additives at 0–14 days.

However, the FCR (Table 4) was also significantly different in the supplemented group at the end of the experiment compared to the control (T0). Priambodo *et al.*, (2020) also observed that the addition of flavonoids reduced (p<0.01) the FCR of broilers. European production efficiency factor (EPEF) and survival percentage during 0-42 days of age have been listed in Table 5. The highest value of EPEF was observed in T2 group (300.00) supplemented with Auctus, followed by T1 group (298.47) fed Enramycin and significantly differed (P≤0.001) compared to the control. Mahanta *et al.* (2017) reported a similar finding when broilers were fed herbal growth promoters. Survival percentage ranged from 98 to 99 % in different groups.

Table 5: Effects of herbal and antibiotic growth promoter on EPEF and survival % of broilers at 42-day age

Group	EPEF	Survival %
T0	257.41±7.30 ^b	98
T1	298.47±4.17 ^a	99
T2	300.00±1.41 ^a	98

a,b Means in the same column with different superscripts differ significantly (P<0.05).

The marginal improvement in production performance observed in the Auctus-supplemented group can be attributed to the presence of phytochemicals with antibacterial properties, which demonstrated efficacy against pathogenic bacteria in the gastrointestinal tract. By reducing the microbial load, these phytochemicals likely enhance nutrient absorption and reduce competition for nutrients in the gut environment. Additionally, their immune-modulatory effects may contribute to improved health and overall performance in broilers (Srividya *et al.*, 2010; Youssef *et al.*, 2021; Choi *et al.*, 2020).

Immunological parameters

Antibody titer

Antibody titers against Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV) in different treatment groups are presented in Table 6. The mean log HI titer values for NDV in T0, T1, and T2 groups were 1.52 ± 0.04, 1.76 ± 0.04, and 1.80 ± 0.03, respectively. Significantly higher (P<0.001) HI titers for NDV were observed in the T1 and T2 groups compared to the control group (T0). However, no significant differences were observed in the mean antibody titer values for IBDV among the treatment groups. These results of the present study agree with the (Mathivanan and Kalairasi 2007; Astuti *et al.*, 2023; and Omidiwura *et al.*, 2023).

Table 6: Effects of herbal and antibiotic growth promoter on immune response of broilers at 28-day age

Treatment	ND	IBD
T0	1.522±0.044 ^b	1445.50±11.976
T1	1.760±0.036 ^a	1449.25±0.6169
T2	1.810±0.032 ^a	1420.25±04.442

a,b Means in the same column with different superscripts differ significantly (P<0.001).

Lymphoid organs

The weights of lymphoid organs, including the bursa of Fabricius and thymus, measured at the end of the experiment, are presented in Table 7. The results showed a significant (P<0.05) increase in bursa weight in broilers supplemented with Auctus compared to the control group. These results of the present study are in agreement with the immunomodulating activity of herbal growth promoters as

reported by Lonkar *et al.* (2008); the weight of bursa of fabricus was increased by the inclusion of garlic powder, neem seed cake and their combination in broiler ration.

Table 7: Effects of herbal and antibiotic growth promoter on lymphoid organ weight (percentage of live body weight)

Treatment	Bursa	Thymus
T0	0.106±0.014 ^b	0.126±0.025
T1	0.133±0.006 ^{ab}	0.098±0.006
T2	0.180±0.021 ^a	0.137±0.047

a,b Means in the same column with different superscripts differ significantly (P<0.05).

Intestinal microbial count

The results on the *Escherichia coli* (*E. coli*) and *Lactobacillus spp.* counts (expressed in log₁₀ CFU/mL) in the caeca at the 42nd day of age are presented in Table 08. The cecal microbiota of modern poultry plays a vital role in energy harvesting from the diet, as highlighted by Marimuthu *et al.*, (2019). Results demonstrated a significant reduction (P<0.001) in the caecal population of *E. Coli* in broilers when supplemented with Auctus (4.55±0.23), compared to groups supplemented with Enramycin (4.71±0.13) and the control group (4.96±0.19). Similarly, a significant increase (P<0.001) in the caecal *Lactobacilli* population was observed among broilers that had Auctus and Enramycin in their diet, compared to the control group.

Table 8: Effects of herbal and antibiotic growth promoter on microbial enumeration (log cfu/g) of cecal digesta of broilers at 42-day age

Group	<i>E. coli</i> (log CFU/ g)	<i>Lactobacilli</i> (logCFU/g)
T0	4.96±0.19 ^a	7.77±0.07 ^b
T1	4.71±0.13 ^b	8.15±0.09 ^a
T2	4.55±0.23 ^c	8.23±0.19 ^a

a,b,c Means in the same column with different superscripts differ significantly.

These findings align with the observations of Iqbal *et al.* (2020), who reported that tannins promote the growth of *Lactobacilli* while inhibiting pathogenic microorganisms.

Similarly, Choi *et al.* (2020) suggested that tannins may exhibit prebiotic-like effects by stimulating the proliferation of beneficial bacteria, thereby improving gut health. Furthermore, Shehata *et al.* (2022) highlighted the role of plant-derived compounds, such as flavonoid and glycosides, in modulating the gut microbiota. This modulation may contribute to the improved gut ecosystem observed in Auctus-fed broilers.

Histomorphology examination of small intestine (villus height and crypt depth)

The gut morphometry parameters, including villus length (VL) and crypt depth (CD) of the small intestine (duodenum, jejunum, and ileum), were evaluated in broilers at 42nd day of age and are presented in Table 9. A healthier gut environment, characterized by a reduced pathogenic load, promotes villus growth and regeneration, leading to enhanced nutrient absorption (Prihambodo *et al.*, 2020).

The mean duodenal villus lengths for the Control (T0), Enramycin (T1), and Auctus (T2) groups were 1150.40±8.99 µm, 1219.07±14.21 µm, and 1406.70±11.15 µm, respectively. The Auctus-supplemented group demonstrated a significantly higher (P<0.05) duodenal villus length and a shorter crypt depth (161.73 ± 1.50 µm) among the treatment groups.

The jejunum and ileum villus lengths in the Auctus-supplemented group were 1261.0±9.59 µm and 913.24±14.97 µm, respectively, which were significantly higher (P<0.05) than those observed in the Control and Enramycin (T1) groups.

This is consistent with findings by Prihambodo *et al.* (2020), who reported that higher doses of flavonoids significantly (P<0.01) increased villus height in the duodenum. Similarly, Youssef *et al.* (2021) observed that diets supplemented with saponins resulted in significant (P<0.05) increases in villus height, reaching values of 1877.3±2.12 µm, 1026.2±1.07 µm, and 702.2±1.12 µm in the duodenum, jejunum, and ileum, respectively. Increased villus height, as observed in this study, is indicative of better intestinal health and improved nutrients absorptive capacity, further supported by the findings of Abolfathi *et al.* (2019). The phytogetic compounds in Auctus may also stimulate mucus secretion, providing better

Table 9: Effects of herbal and antibiotic growth promoter on histomorphometry of small intestine of broilers at 42-day age

Treatment	Duodenum		Jejunum		Ileum	
	Villi length (µm)	Crypt depth (µm)	Villi length (µm)	Crypt depth (µm)	Villi length (µm)	Crypt depth (µm)
T0	1150.40±08.98 ^c	172.75±1.40 ^a	1020.78±24.24 ^c	157.62±0.91 ^b	702.18±10.08 ^c	158.26±2.73
T1	1219.06±14.21 ^b	171.91±2.15 ^a	1134.83±11.52 ^b	151.97±0.92 ^c	805.33±12.53 ^b	161.45±2.84
T2	1406.69±11.15 ^a	161.73±1.50 ^b	1261.05±09.59 ^a	164.83±1.57 ^a	913.24±14.97 ^a	157.32±1.98

a,b,c Means in the same column with different superscripts differ significantly (P<0.05).

villus protection and supporting the growth of beneficial probiotic bacteria in the intestine (Daramola, 2019).

Furthermore, a significant reduction in ileal crypt depth was observed in the Auctus group, aligning with findings by Demir *et al.* (2005), who reported that dietary inclusion of garlic and thyme reduced crypt depth compared to diets supplemented with antibiotic growth promoters (P<0.05). Crypts, which are considered “villi factories,” play a crucial role in tissue regeneration. Deeper crypts indicate higher tissue turnover and increased nutrient demands for maintenance, as highlighted by Savage *et al.* (1997). Therefore, reduced crypt depth in the T2 group suggests improved intestinal efficiency with lower energy demands for tissue renewal.

CONCLUSION

The study demonstrated that the broilers supplemented with Auctus in feed had similar performance (body weight, FCR and EPEF) and immune responses as those which were supplemented with Enramycin. However, broilers supplemented with Auctus significantly exhibited lower *E. coli* population within gut and had longer villi in the small intestine compared to the Enramycin. Auctus has the potential to replace antibiotic growth promoters in broiler farming thereby playing a critical role in mitigating the development of antibiotic resistance in both animal and human population.

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