



## **Study on the Comparative Efficacy of Natural Growth Promoter (AV/AGP/10) with Antibiotic Supplements on Overall Growth Performance and Intestinal Micrometry of Broiler Birds**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author SM designed and coordinated the study. Author AT wrote the draft manuscript. Authors MJS and KR designed, advised, evaluated the data and finalized the manuscript for publication. Authors ABK and VKM performed and executed the study. Authors MAK and PNT managed and assisted in statistical analyses. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The study was conducted to compare the efficacy of natural growth promoter AV/AGP/10 with antibiotic supplements on overall growth performance and intestinal micrometry of broiler birds.

**Study Design:** Total of 150 healthy day old Vencob broiler chicks of nearly similar live body weight were equally divided into 5 groups of 30 birds each with three replicates in each group. All the groups were fed with basal diet. Group-I was positive control without any supplement, Group- II was supplemented with AV/AGP/10 @ 250g/ton of feed, Group-III supplemented with AV/AGP/10@500g/ton of feed, Group-IV supplemented with Bacitracin Methylene Dicycylate @100g/ton of feed and Group-V supplemented with Oregostim @ 250g/ton of feed.

**Place and Duration of Study:** the study was conducted in the department of Animal Nutrition, College of Veterinary and Animal sciences, Udgir, Dist. Latur, Maharashtra, India

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during the month of April- June 2012 for 42 days. The mean maximum daily temperature recorded at the time of trial was  $41 \pm 2^\circ\text{C}$  and relative humidity (RH)  $80.57 \pm 1.50\%$ .

**Methodology:** the efficacy of the products was assessed on the basis of feed consumption, body weight gain, feed conversion ratio (FCR), metabolic trial / nutrient retention trial, intestinal micrometry and carcass yield / dressing percentage.

**Results:** at the end of sixth week, significantly higher live body weight (1874.19, 1921.51, 1720.39 and 1673.58) with more economical FCR (1.74, 1.71, 1.78 and 1.78) along with marked improvement in digestibility of nutrients from supplementation of herbal growth promoter with equal competence as that of synthetic antibiotic was observed. The intestinal micrometry at day 21 and 42 also revealed better results with natural growth promoter as compared to synthetic growth promoter and control group in terms of villous height, width and crypts depth.

**Conclusion:** Considering the overall trial results and harmful effects of antibiotic growth promoter such as bacterial resistance or undesired residues in animal products, the natural product AV/AGP/10 is better option as growth promoter and performance enhancers in broiler birds.

*Keywords: Antibiotic; growth promoter; metabolic; micrometry; natural, resistance; safe.*

## 1. INTRODUCTION

The poultry farming plays a major role in Indian economy. Extensive efforts are made world over to improve the overall performance of the poultry in terms of growth and production to improve the economy of poultry production. Growth promoters are chemical and biological substances which are added to swine & poultry feed with the aim to improve the growth, fattening, improve the utilization of food and in this way realize better production and financial results.

Growth promoters are generally liver tonics which optimize hepatic functions of the birds. They help in better feeding and synthesis of amino acids [1,2], better appetite, improved feed conversion, stimulation of the immune system and increased vitality, regulation of the intestinal micro-flora and improves the protein content [3] etc. In this 'No Chemical Era', the knowledge of herbs and their medicinal properties had made forays in the poultry industry for maximizing better production without residual toxicity as side effects on consumer.

The World Health Organization estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components. Those plants and their components are perceived as "natural" and "safe" by consumers; however, we now understand that certain materials also have added technical benefits that may be exploited to maintain animal performance. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, polyphenols, carotenoids, coumarins, saponins and plant sterols have been identified [4]. Addition of these substances to the feeds and water improved feed intake, feed conversion ratio and carcass yield [5,6,7]. Recently, the use of antibiotics as a growth promoter in chicken has got lot of criticism due to adverse and unwanted results [8] such as undesired residue in animal products viz. Meat, milk or eggs, residua in tissues, long withdrawal period, and development of resistance in microorganisms, allergies, genotoxicity and harmful effects on human health by development of microbial resistance to specific products. Herbs have been used as food and for medicinal purposes for centuries. Keeping

the above facts in view the present study was designed to compare the efficacy of natural growth promoter AV/AGP/10 (supplied by M/S Ayurved Limited, Baddi, H.P., India) with antibiotic supplement on overall performance and intestinal micrometry of broiler birds.

## 2. MATERIALS AND METHODS

The study was conducted in the department of Animal Nutrition, College of Veterinary and Animal Sciences, Udgir, Dist. Latur, Maharashtra, India during the month of April- June 2012 after approval from Committee for the purpose of control and supervision of experimentation on animals (CPCSEA). The mean maximum daily temperature recorded at the time of trial was  $41 \pm 2^\circ\text{C}$  and relative humidity (RH)  $80.57 \pm 1.50\%$ . Before *in vivo* trial on birds the herbal test product was subjected to toxicity trial as per the research guidelines given by the World Health Organization, WHO in order to assess its safety of administration. The product was found safe with no harmful effects on animal health and environment.

### 2.1 Experimental Design

Total of 150 healthy day old Vencob broiler chicks of nearly similar live body weight were obtained from authorized supplier and were equally divided into five groups of thirty birds each with three replicates in each group. All the groups were fed basal diet.

Group-I: positive control without any supplement

Group- II: test group supplemented with AV/AGP/10 @ 250g/ton of feed.

Group-III: test group supplemented with AV/AGP/10 @ 500g/ton of feed.

Group-IV: test group supplemented with synthetic growth promoter Bacitracin Methylene Dicycylate @ 100g/ton of feed.

Group-V: test group supplemented with synthetic growth promoter Oregostim @ 250g/ton of feed.

AV/AGP/10 is a phytoadditive that is a 'herbal growth promoter with essential oils', added to feed of poultry & swine and possesses a number of beneficial effects, including: rapid development of a healthy gut microflora, stabilization of digestion, increased growth performance, stimulation and rapid maturation of the immune system & many more. The product comprises of oil of herbs viz. *Allium sativum*, *Zingiber officinale*, *Trigonella foenum graecum*, *Eruca sativa* & many others in a fixed concentration.

The chicks of all the five groups were housed separately under similar environmental conditions and maintained on *ad libitum* broiler starter and finisher ration (composition Table 1) and clean drinking water throughout the experiment. The chicks were vaccinated for Lasota and IBD vaccines on 7<sup>th</sup> and 14<sup>th</sup> day of age, respectively. The booster dose of IBD vaccine was given on 21<sup>st</sup> day.

**Table 1. Composition of broiler starter and finisher ration**

<b>Ingredient (%)</b>	<b>Starter</b>	<b>Finisher</b>	<b>Nutrient</b>	<b>Starter</b>	<b>Finisher</b>
Maize	55.32	56.47	Crude protein (%)	22.27	21.13
Soybean meal	38.00	35.00	ME (kcal/kg)	2985	3117
Lime stone	1.00	1.00	C:P ratio	134.04 : 1	147.51 : 1
Dicalcium phosphate	2.10	2.00	Crude fibre (%)	3.95	3.81
Oil	2.00	4.00	Ether extract (%)	4.07	5.98
Salt	0.35	0.35	Calcium (%)	1.09	0.96
DL-methionine	0.26	0.26	A. Phosphorus (%)	0.49	0.47
L-lysine	0.22	0.22	Lysine (%)	1.38	1.30
Sodium bicarbonate	0.15	0.10	Methionine (%)	0.60	0.59
Vitamin / mineral premix	0.60	0.60			
Total	100	100			

## **2.2 Parameters Estimated**

### **2.1.1 Feed consumption**

Measured quantity of feed was fed to chicks every day and the feed in balance was recorded after 24 hrs. The difference between the feed offered and balanced feed was worked out to know the actual feed consumed by each group on a particular day and expressed as g/day/group. In the similar manner, feed consumption as g/week/group was computed and at the end total feed consumption was calculated for 42 days.

### **2.1.2 Live body weight and body weight gain**

Ten chicks from each group were weighed individually on day 0 and at weekly intervals thereafter. Mean live body weight (g/chick/week) was computed at weekly intervals from 1<sup>st</sup> week to 6<sup>th</sup> week of study.

### **2.1.3 Feed conversion ratio (FCR)**

The values of FCR of each group were calculated at weekly intervals on the basis of weekly live weights and weekly feed consumption.

### **2.1.4 Metabolic trial / nutrient retention trial**

Three birds from each group (one bird from each replicate) were selected and kept in a separate metabolic cage for nutrient retention studies. Daily feed consumption as well as faecal output from each bird was collected, measured and preserved for proximate analysis studies. The feed and faecal samples were subjected for analysis of dry matter (DM), crude protein (CP), crude fibre (CF), nitrogen free extract (NFE) and ether extract (EE). The nutrient digestibility was calculated after analysis of nutrient content in feed consumed and feces voided by the selected birds. Weende's system of proximate analysis was adopted for estimation of chemical composition of feed and feces.

### **2.1.5 Intestinal micrometry**

The samples were preserved in 10 % neutral formal saline and sections were cut at 3 to 5  $\mu$  thickness and were stained with Mayer's Haematoxyline and eosin for microscopic

examination for histopathological changes. Histological parameters villous height, width and crypt depth of duodenum, jejunum, ileum were measured at 21<sup>st</sup> and 42<sup>nd</sup> day.

### **2.1.6 Carcass yield / dressing percentage**

Live weights, dressed weight (carcass yield) and dressing percentage of experimental birds of all groups were determined at the end of experiment, as under.

- 1) Dressed weight = Live weight-weights of (Head+ skin + feathers + legs).
- 2) Dressing % = (Carcass yield / Live weight) x 100

### **2.3 Statistical Analyses**

All the results were analyzed statistically by analysis of variance to determine the means and standard error as per the methods described by Snedecor and Cochran [9].

## **3. RESULTS AND DISCUSSION**

The records were observed weekly for six weeks duration with regards to body weight gain and FCR. The intestinal samples were collected at 21<sup>st</sup> and 42<sup>nd</sup> day period for morphological studies. At the end of sixth week carcass yield and dressing percentage were calculated.

### **3.1 Feed Consumption**

The average values of voluntary feed intake of all the groups during entire period were found to be significant from 1<sup>st</sup> to 6<sup>th</sup> week period. The total feed intake of treatment groups (3256.80, 3288.60, 3073.02 and 2993.42g for group II, III, IV and V respectively) was significantly different from the control group (2749.71 g). However, the highest feed intake was seen in group III followed by group II fed with herbal growth promoter @ 500 and 250 g/ton of feed respectively. Abdel [10] and Guo et al. [11] reported similar results when *Trigonella foenum graecum* was added to broiler feed as growth promoter. Similarly, Sultan et al. [12] also reported increase in feed intake of broiler chicken supplemented with *Allium sativum* and Rajav et al. [13] observed increase in feed intake of broiler chicken when fed with aqueous extract of *Zingiber officinale*. The mode of action of these feed additives is not completely clear. They have antimicrobial, antiviral, antioxidant and many other biological activities [14,15]. They act as a digestibility enhancers, stimulating the secretion of endogenous digestive enzymes [16].

### **3.2 Live Body Weight and Body Weight Gain**

The mean values of body weight gain (g/bird/week) in different groups at different interval of study are shown in Table 2. At the end of sixth week, significantly higher live body weight was observed. The average values of body weight gain of all the five groups were found significantly different (P<0.05) from each other during scheduled intervals of study period with the highest gain in live body weight seen in group III and II and lowest in group I during 1<sup>st</sup> to 6<sup>th</sup> week period of study

**Table 2. Mean (+ SE) values of Gain in weight at weekly intervals of study**

Gr.	Initial Av live body wt.	Intervals of study (weeks)						Final live body wt.
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	
I	48.70	78.03 <sup>d</sup> ±	197.12 <sup>c</sup> ±	183.49 <sup>c</sup> ±	264.43 <sup>c</sup> ±	330.01 <sup>d</sup> ±	417.28 <sup>abc</sup> ±	1519.05 <sup>a</sup> ±
		1.20	3.97	4.50	11.62	14.08	10.55	8.65
II	50.17	85.69 <sup>ab</sup> ±	236.08 <sup>a</sup> ±	271.38 <sup>a</sup> ±	380.58 <sup>ab</sup> ±	420.51 <sup>ab</sup> ±	429.77 <sup>ab</sup> ±	1874.19 <sup>b</sup> ±
		1.16	2.78	10.80	12.51	12.28	10.58	8.92
III	48.43	87.68 <sup>a</sup> ±	242.32 <sup>a</sup> ±	267.72 <sup>a</sup> ±	399.87 <sup>a</sup> ±	428.48 <sup>a</sup> ±	447.00 <sup>a</sup> ±	1921.51 <sup>c</sup> ±
		1.43	3.21	9.08	10.61	13.83	9.49	4.41
IV	49.15	82.70 <sup>bc</sup> ±	208.15 <sup>b</sup> ±	230.20 <sup>b</sup> ±	367.83 <sup>ab</sup> ±	372.75 <sup>c</sup> ±	409.61 <sup>bc</sup> ±	1720.39 <sup>d</sup> ±
		1.52	3.34	13.38	18.97	9.50	10.49	10.68
V	49.02	79.88 <sup>cd</sup> ±	207.35 <sup>b</sup> ±	201.96 <sup>c</sup> ±	359.86 <sup>b</sup> ±	389.52 <sup>bc</sup> ±	386.00 <sup>c</sup> ±	1673.58 <sup>e</sup> ±
		0.75	3.70	11.26	12.20	14.06	13.48	9.68

Values with different superscripts differ significantly ( $P < 0.05$ ) in a column.

Though there was significant increase in the body weight, but the mean body weight is comparatively lower in all the groups than that is attained under standard managerial conditions due to higher mean ambient temperature and humidity ( $41 \pm 2^\circ\text{C}$  and  $80.57 \pm 1.50\%$ ) the birds were under environmental and physiological stress.

The results of present study are in agreement with the findings of Farman et al. [17] who observed gain in the body weight of broilers chicken when fed with extract of *Trigonella foenum graecum*. These results are also in line with the findings of Sultana et al. [12] who reported higher weight gain in broilers fed on rations supplemented with *Allium sativum*. The improvement in weight gain may be due to the action of allicin (an antibiotic substance found in garlic), which inhibits the growth of pathogenic bacteria and aflatoxin producing fungi.

### 3.3 Feed Conversion Ratio (FCR)

The mean values of feed conversion ratio (FCR) in different groups at different interval of study are shown in Table 3. The treatment groups (II, III, IV and V) have shown significant difference ( $P < 0.05$ ) in the FCR values during the entire study period. Better mean FCR value (1.71) was found in group III, which indicates that test group fed with the basal diet supplemented with test feed AV/AGP/10 @ 500g/ton of feed gained more weight for per Kg of feed consumed as compared to other groups. These results agree with the findings of Abdel [11] who also reported increase in feed conversion efficiency of broiler chicken when fed with *Trigonella foenum graecum* supplemented diet and Rajab et al. [13] with *Zingiber officinale* and Ademola et al. [18] with *Allium sativa* and *Zingiber officinale*.

**Table 3. Mean (+ SE) values of Feed Conversion Ratio at weekly intervals of study**

Gr.	Intervals of study (weeks)						Mean FCR (Total feed intake/final body wt.)
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	
I	1.12 <sup>ab</sup> ± 0.01	1.32 <sup>a</sup> ± 0.01	1.55 <sup>a</sup> ± 0.01	1.61 <sup>a</sup> ± 0.01	1.81 <sup>a</sup> ± 0.01	1.87 <sup>a</sup> ± 0.01	1.81
II	1.11 <sup>ab</sup> ± 0.01	1.28 <sup>bc</sup> ± 0.01	1.38 <sup>c</sup> ± 0.01	1.54 <sup>bc</sup> ± 0.01	1.71 <sup>c</sup> ± 0.01	1.79 <sup>b</sup> ± 0.01	1.74
III	1.07 <sup>b</sup> ± 0.02	1.26 <sup>c</sup> ± 0.01	1.36 <sup>c</sup> ± 0.01	1.52 <sup>c</sup> ± 0.01	1.69 <sup>c</sup> ± 0.01	1.76 <sup>b</sup> ± 0.01	1.71
IV	1.14 <sup>a</sup> ± 0.01	1.30 <sup>ab</sup> ± 0.01	1.44 <sup>b</sup> ± 0.02	1.58 <sup>ab</sup> ± 0.02	1.76 <sup>b</sup> ± 0.01	1.84 <sup>a</sup> ± 0.01	1.78
V	1.14 <sup>a</sup> ± 0.01	1.31 <sup>a</sup> ± 0.01	1.47 <sup>b</sup> ± 0.02	1.59 <sup>a</sup> ± 0.01	1.76 <sup>b</sup> ± 0.01	1.84 <sup>a</sup> ± 0.01	1.78

Values with different superscripts differ significantly ( $P < 0.05$ ) in a column

### 3.4 Nutrient Retention / Metabolic Trial

The mean values of digestibility coefficients of various nutrients of different groups are shown in Table 4. The statistical analysis of digestibility coefficient values of DM, CP, CF, EE, and NFE revealed significant ( $P < 0.05$ ) difference in all the experimental groups. The highest values were observed in group III followed by group II, group IV, group V and lowest in control group I.

**Table 4. Mean (+ SE) values of digestibility coefficient (%) of various nutrients**

Gr.	Digestibility Coefficient (%) of Nutrients				
	DM	CP	CF	EE	NFE
I	68.92 <sup>c</sup> ± 0.45	69.71 <sup>c</sup> ± 0.64	63.24 <sup>c</sup> ± 0.38	66.42 <sup>c</sup> ± 0.53	66.63 <sup>c</sup> ± 0.64
II	74.34 <sup>a</sup> ± 0.46	80.59 <sup>a</sup> ± 1.05	67.71 <sup>a</sup> ± 0.25	72.70 <sup>a</sup> ± 0.58	75.23 <sup>a</sup> ± 0.27
III	75.26 <sup>a</sup> ± 0.55	82.08 <sup>a</sup> ± 0.95	68.46 <sup>a</sup> ± 0.52	74.21 <sup>a</sup> ± 0.38	76.08 <sup>a</sup> ± 0.10
IV	72.08 <sup>b</sup> ± 0.10	76.40 <sup>b</sup> ± 0.11	66.00 <sup>b</sup> ± 0.31	70.54 <sup>b</sup> ± 0.49	71.07 <sup>b</sup> ± 0.65
V	71.56 <sup>b</sup> ± 0.37	75.28 <sup>b</sup> ± 0.55	65.21 <sup>b</sup> ± 0.23	69.65 <sup>b</sup> ± 0.37	69.95 <sup>b</sup> ± 0.21

Values with different superscripts differ significantly ( $P < 0.05$ ) in a column

### 3.5 Carcass Yield / Dressing Percentage

The carcass yield and dressing percentage of different groups is represented in table 5. The statistical analyses revealed highly significant ( $P < 0.05$ ) difference in all the five groups. However, the treatment group III has shown highest dressing percentage ( $76.29 \pm 0.52$ ) followed by group II ( $73.29 \pm 0.88$ ) and these groups have shown significant difference among themselves and also different from group IV ( $68.33 \pm 0.68$ ), group V ( $68.54 \pm 0.67$ ) and control group I ( $64.45 \pm 0.95$ ). Similarly the mean values of carcass yield were found significantly higher in group III ( $1465.90 \pm 10.21$ ) followed by group II ( $1373.10 \pm 11.15$ ) as compared to other treatment groups. The increase in the carcass yield of the broiler chicken was also observed by Abdel [10] in case of *Trigonella foenum graecum*, Sultan et al. [12] in case of *Allium sativum* and Javed et al. [19] reported that carcass characteristics and yield improved in broilers fed with different levels of powder/aqueous extract of *Zingiber officinale*. Zeinab [20] also reported increase in carcass yield of broilers when fed with *Eruca sativa* supplemented diet.

**Table 5. Mean (+ SE) values of carcass yield (g) and dressing percentage of experimental Birds of different groups at day 42**

Group	Carcass yield (g)	Dressing %
I	978.41 <sup>d</sup> ± 10.20	64.45 <sup>d</sup> ± 0.95
II	1373.10 <sup>b</sup> ± 11.15	73.29 <sup>b</sup> ± 0.88
III	1465.90 <sup>a</sup> ± 10.21	76.29 <sup>a</sup> ± 0.52
IV	1175.12 <sup>c</sup> ± 9.26	68.33 <sup>c</sup> ± 0.68
V	1146.73 <sup>c</sup> ± 9.35	68.54 <sup>c</sup> ± 0.67

Values with different superscripts differ significantly ( $P < 0.05$ ) in a column

### 3.6 Intestinal Micrometry

The product significantly affected the intestinal morphology. Villous height and width and crypt depth ( $\mu\text{m}$ ) of duodenum on day 21<sup>st</sup> and 42<sup>nd</sup> are represented in Table 6. The statistical analyses of the data revealed that villous height, width and crypt depth ( $\mu\text{m}$ ) of duodenum on day 21 as well as day 42 were significantly better in group III followed by group II as compared to other groups (Fig. 1). Similar results were seen on micrometry of ileum and jejunum in case of all the groups at day 21 and day 42 in which villous height and width and crypts depth showed better results in group III as compared to other groups. Similar trend of the results were observed by Saki et al. [21], Gunal et al. [22] and Peric et al. [23]. The short

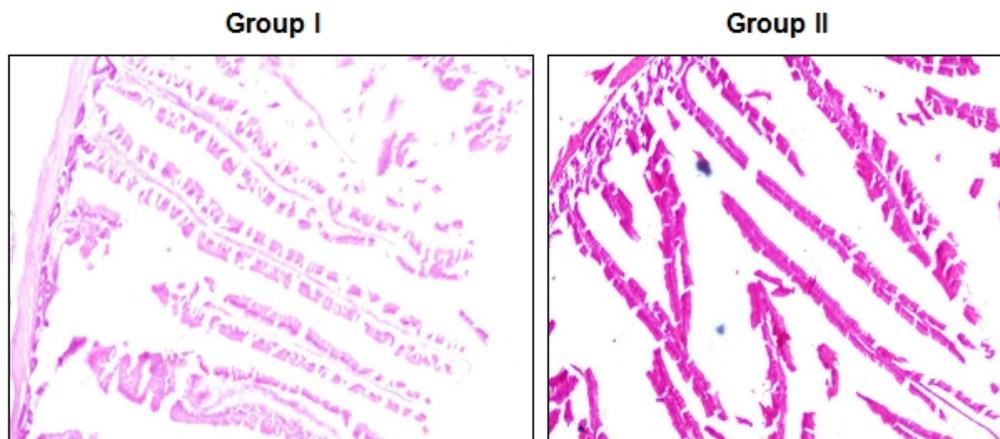
chain fatty acids which are by products of bacterial fermentation stimulate the proliferation of epithelial cells of the bowel [24].

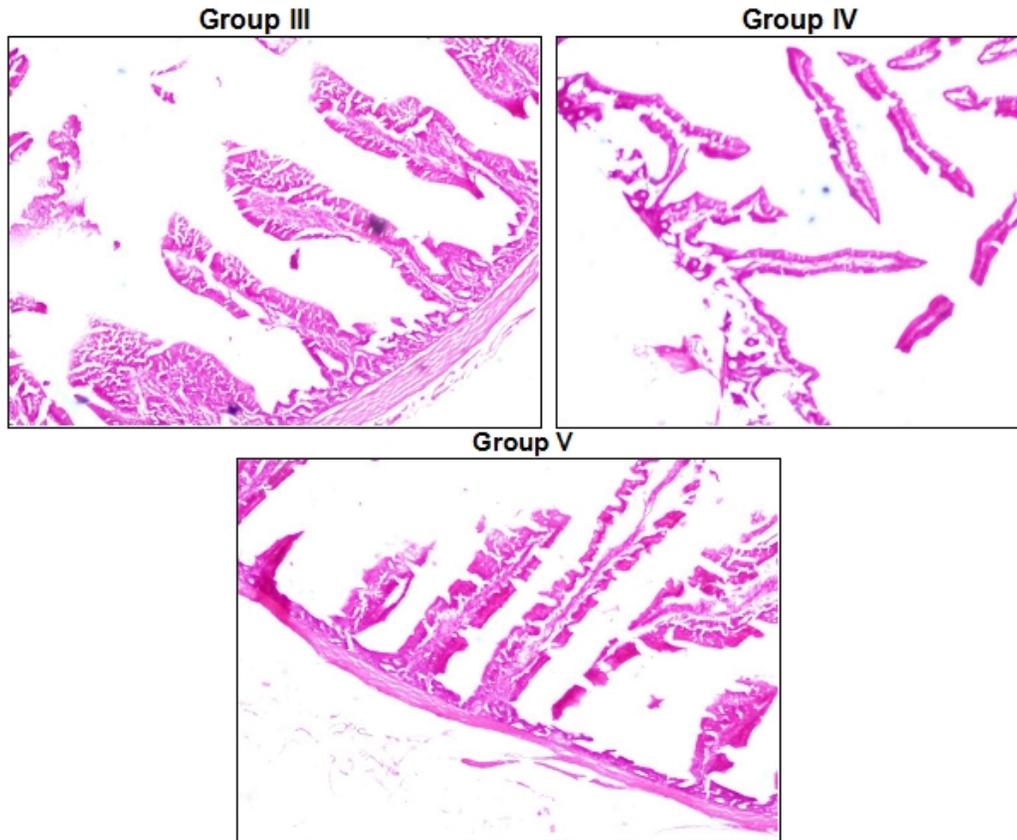
**Table 6. Mean values of Intestinal morphology ( $\mu\text{m}$ ) of duodenum of experimental Birds of different groups at day 21 and 42 of study period**

Groups	Day 21			Day 42		
	Villous Ht.	Villous width	Crypt depth	Villous Ht	Villous width	Crypt depth
<b>Grp I</b>	976.38	112.97	172.62	817.82	87.71	146.08
<b>Grp II</b>	1137.21 <sup>a</sup>	118.16 <sup>a</sup>	173.01	1009.34 <sup>a</sup>	97.87 <sup>a</sup>	150.84 <sup>a</sup>
<b>Grp III</b>	1210.51 <sup>b</sup>	120.06 <sup>b</sup>	177.02 <sup>a</sup>	1016.80 <sup>b</sup>	102.57 <sup>b</sup>	153.13 <sup>b</sup>
<b>Grp IV</b>	965.30	108.97	168.45	999.30	81.40	139.19
<b>Grp V</b>	955.04	106.16	170.63	994.75	84.40	142.28

*Values with different superscripts differ significantly ( $P < 0.05$ ) in a column*

The morphology of intestinal villi and crypts has been associated in chickens with intestinal function and growth. Adverse changes in the content of the digesta, such as high population of pathogenic bacteria, parasites or damaging substances, could lead to changes in the surface of intestinal mucosa, because of their close proximity. A lower villous height/crypt depth ratio has been associated with the presence of toxins, poor nutrient absorption, and increased secretion in the gastrointestinal tract, diarrhoea, reduced disease resistance and lower overall performance. A large crypt indicates a fast tissue turnover and a high demand for new tissue [25].





**Fig. 1. Histo-morphometrical slides of Duodenum of experimental Birds in different groups depicting changes in villi and crypts at day 21 of study period**

#### **4. CONCLUSION**

In this study, the overall trial results of natural growth promoter (AV/AGP/10) was found to be efficacious in promoting growth and improving performance, feed efficiency, dressing percentage, intestinal micrometrical development and overall nutrient digestibility as compared to Oregostim and Bacitracin Methylene Dicycylate. The product can successfully replace antibiotic growth promoter in poultry feed. The main advantage of natural or herbal growth promoters over antibiotic is that they usually do not bear any risk regarding bacterial resistance or undesired residues in animal products such as meat, milk or eggs and can be used as feed additives alternative to antibiotics. The efficacy of AV/AGP/10 as a bacteriostatic herbal growth promoter and gut function modulator may be attributed to the constituent herbs of the product namely *Allium sativum*, *Trigonella foenum graecum*, *Zingiber officinale*, *Eruca sativa* and many more. AV/AGP/10 can result in production of better quality chicken meat for human consumption.

#### **ETHICAL APPROVAL**

The study was approved from committee for the purpose of control and supervision of experimentation on animals (CPCSEA).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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