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Efficacy of a phytogenic formulation as a replacer of antibiotic growth promoters at improving growth, performance, carcass traits and intestinal morphology in broiler chicken

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Abstract
The current study aimed to investigate the effects of dietary supplementation of broiler chicken with a phytogenic formulation as a replacer of antibiotic growth promoters on their growth, performance, carcass traits and intestinal morphology. Two hundred and ten (210) one-day-old, straight run Cobb chicks were randomly assigned to either of seven equal groups (T0-T6), each having three replicates, and reared for 42 days in deep-litter system at Institutional Poultry Farm, Gannavaram (16.54° N, 80.80° E) during February to March, 2018 without environmental control (mean ambient temperature 26 °C, relative humidity 73%). T0, receiving a standard basal diet without any growth promoting, antimicrobial or anti-coccidial agents, served as the negative control. T1 received a phytogenic growth promoter, Nbiotic™ (Ayurvet Limited, India) at 500 ppm in feed, T2 received Brand A (amoxycillin 50%) at 250 ppm, T3 received Brand B (ciprofloxacin 25%) at 500 ppm, T4 received Brand C (neomycin 10% + doxycycline 10%) at 500 ppm, T5 received Brand D (oxytetracycline 50%) at 500 ppm, and T6 received Brand E (tiamulin 10%) at 500 ppm of feed. Different parameters pertaining to growth, performance, carcass traits and intestinal morphology, including ultrastructural studies, were recorded in the birds and, unless stated otherwise, the statistical significance of the differences between group mean values were ascertained by one-way analysis of variance (ANOVA) at $p<0.05$. Besides significantly better FCR than groups T0, T3, T5 and T6, and comparable FCR to groups T2 and T4, group T1 showed significantly better weight gain than group T0 and comparable growth to all other groups. Group T1 showed significantly higher values of serum albumin, serum total protein, height of ileal villi and depth of ileal crypts as compared to the unsupplemented control and the antibiotic-supplemented groups. Group T1 also showed significant improvements in body weight gain, muscle protein content, ileal villous width, jejunal villous height and width, jejunal crypt depth, and blood glucose over the supplemented control T0 and most of the antibiotic-supplemented groups. It could be concluded that the phytogenic formulation Nbiotic™ is an efficacious natural growth promoter for broiler chicken and that it could be used to successfully replace antibiotic growth promoters in broiler feeds for improving growth, performance, carcass traits and intestinal morphology.

Keywords: Antibiotic, broiler chicken, growth promoter, nbiotic™, phytogenic

Introduction
World population by 2050 it increase by 10 billion and to cater the needs the feed or foodstuffs that are available will be scanty unless we shift to animal protein ("How to Feed the World in 2050,") [14] Among meat poultry meat is cheaper and nutritious. When compared to other diets, meat and the products generated from it supply significant amounts of vital elements. The amount of nutrients found in an animal's musculature does not differ much between species, but the proportion of fat to muscle mass in the edible region does. Even though recent research has revealed that certain farming practises (organic, free range) might affect some compositional elements of poultry meat, the quality of animal fat and the levels of nutrients mostly depend on the diet or genetic makeup of the animal (Marangoni *et al*.,). In the early days to augment meat production antibiotics were used. Since then, a number of antibiotics have been used to encourage farm animals' growth. These drugs were introduced at a time when intensive animal rearing was occurring. These antibiotics increased feed conversion, boosted animal growth, and decreased morbidity and mortality from clinical and subclinical illnesses. Feed utilisation increased by 2 to 5 percent, and the average growth improvement was predicted to be between 4 and 8 percent. (Ewing and Cole, 1994) [10]. In this connection few.

Studies also reported usage of Amoxycillin antibiotic has improved feed efficiency as well as carcass traits (Swaroop *et al*., 2021) [28]. Nonetheless excess antibiotics usage leads to antimicrobial resistance. To combat this feeding of antibiotics are restricted (Love *et al*., 2011; Marshall and Levy, 2011; Sapkota *et al*., 2011) [18, 19 26]. Alternatively, organic acids or phytogenic growth promoters superseded antibiotics. Usage of these promoters have markedly improved animal meat production. Due to their capacity to enhance performance by maintaining a healthy intestinal environment, phytogenic feed additives (PFA) have recently attracted a lot of interest. (Murugesan *et al*., 2015; Oso *et al*., 2019) [22, 24] PFA are regarded as sensory and flavorful substances that are mostly made of plant extracts (essential oils, oleoresins, and flavonoids) and their active components (Mountzouris *et al*., 2011) [21]. It has been proposed that the essential oils found in PFA, which contain the majority of the plant's active ingredients, can improve gastrointestinal health (Giannenas *et al*., 2003; Isabel and Santos, 2009) [13, 15], nutrient digestibility (Jamroz *et al*., 2005) [16], and growth performance (McReynolds *et al*., 2009) [20]. PFA's many advantageous properties are primarily come from its bioactive compounds, which include carvacrol, thymol, capsaicin, cineole, etc. These characteristics of PFA position them as viable alternatives to AGP.

Potential infections are controlled, and the gut flora is benefited by PFA's main modes of action. It is well known that a number of plant extracts exhibit antibacterial, antiviral, anticoccidial, fungicidal, and/or antioxidant properties (Applegate *et al*., 2010) [3]. In view of the above benefits present study has been designed to understand the effect of phytogenic growth promoter in broiler feed conversion efficiency, carcass traits, ileal morphology and stereology through scanning electron microscope.

Objective

"Efficacy evaluation of supplementation of natural growth promoter as a replacement of antibiotic growth promoter in improving growth, performance, carcass traits and intestinal morphometry and stereology in Broilers".

Methodology

Localization

The experiment was carried out in the Department of Animal Nutrition and Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram. Lab analysis was carried out at Departments of Animal Nutrition, Veterinary Biochemistry in the college and a few of the parameters were out sourced. Feed ingredients like maize, soybean meal, and vegetable oil for preparation of experimental diets were procured from the local market. Natural growth promoter was supplied by *M/S Ayurvet Limited,* Baddi, H.P., India and Antibiotics were purchased from local market.

Chemical Analysis

Feed ingredients were analyzed for their proximate composition (AOAC, 2005). The basal diets (control, Table 3, 4) of broilers for starter and finisher phases were prepared as per the nutrient requirements of poultry (BIS,1992).

Experimental Procedure

Two hundred and ten (210) day old chicks were procured and divided randomly into 7 groups of 30 chicks each and each group consisting of three replicates with each replicate comprising of 10 chicks. Group T_0 is control & group T1, T2, T3, T4, T5 & T6 are treatments. Control Group T_0 was fed standard basal ration without any antibiotic/natural growth promoter added to it. Treatment group T_1 was supplemented with natural growth promoter AV/AGP/10@250gm/ton of feed from 0-42 days. Treatment group T_2 was supplemented with Antibiotic growth promoter Brand A @250 g/ton of feed along with commercial basal diet form 0-42 days. Treatment group T_3 was supplemented with Antibiotic growth promoter Brand B@500 g/ton of feed along with commercial basal diet form 0-42 days. Treatment group T_4 was supplemented with Antibiotic growth promoter Brand C@500 g/ton of feed along with commercial basal diet form 0-42 days. Treatment group T_5 was supplemented with Antibiotic growth promoter Brand D@500 g/ton of feed along with commercial basal diet form 0-42 days. Treatment group T_6 was supplemented with Antibiotic growth promoter Brand E@500 g/ton of feed along with commercial basal diet form 0-42 days (Table 1, 2)

Duration

Experiment duration was for 6 weeks. Sufficient Waterers and feeders were facilitated for easy access of birds throughout the experimental period. Fresh and clean potable drinking water was provided throughout the experimental period.

Observations

Following parameters were studied to find out the efficacy of the N biotic among other antibiotics. Individual body weight of the birds were recorded upto six weeks of age, daily feed intake (were recoded replicate wise and feed efficiency was calculate weekly) final body weight, weekly body weight, feed consumption and left over feed recorded daily and finally feed efficiency ratio was calculated by calculating feed intake to body weight while excluding the mortality of chicks.

Feed intake and feed Efficiency

In each treatment, the amount of feed consumed on a weekly basis was noted, and the feed efficiency was computed in accordance with this information.

Body weight gain

Body weight of individual birds was recorded at weekly interval up to 6 weeks of age. From this average weekly body weight gain per bird was calculated in all the replicates of the seven treatments.

At the end of the trial, the birds were held for 10-12h without food and water prior to the determining of final body weights. Each bird was weighed live, slaughtered and allowed to bleed for 180s. The bird then processes by removing the head, neck shanks and feets, and was eviscerated by cutting around the vent removing the viscera without disturbing the fat pad along the abdominal wall. The heart, liver and gizzard were dissected from the viscera and gizzard was cut open and rinsed of its content (Brake *et al*., 1993) [4].

Intestinal morphometry

The segments of the small intestine (duodenum, jejunum and Ileum) were separated by dissection and were externally and internally washed with 0.9% NaCl to remove the intestinal contents individually then they were transferred to jars containing 10% buffered formalin for fixation. After a 12-24 h fixation period, samples were embedded in paraffin, sectioned to a 2-5 µm thickness, mounted on glass slides, and stained with haematoxylin - eosin. Villi height, width and

crypt depth were then measured using stereoscopic microscope. The villus height (measured from the tip to the base, excluding the intestinal crypt), the villus width (measured halfway between the base and the tip) (Nain *et al*., 2012) [23], the crypt depth (measured from the base upward to the region of transition between the crypt and villi) (Brudnicki *et al*., 2017) [5]. The surface area of the villus was calculated as the product of the height multiplied by the width. The villus height: crypt depth ratio was then calculated.

Scanning Electron microscopic Examination
Slice of tissue from the middle portion of the duodenum, jejunum and ileum were fixed in 4% gluteraldehyde. Tissue samples for SEM were processed as described previously (Yamauchi *et al*., 2007) [32]. In brief, tissue samples were opened and washed with 0-1 M phosphate buffered saline at pH 7-4. Tissue samples were pinned out within a fixative containing paraformaldehyde (40 g/1) in 0-1 M phosphate buffer (pH 7-4), kept at room temperature for 30 min and cut into 10X10 mm squares for SEM. All segments were fixed in glutaraldehyde (30 ml/1) and paraformaldehyde (40 g/1) in 0-1 M cacodylate buffer (pH 7'4) for 2 h at room temperature. After washing in the same buffer all specimens were postfixed in osmium tetroxide (10 g/1) in 0-1 M ice-cold cacodylate buffer (pH 7-4) for 1 h. Specimens were washed in distilled deionised water and dehydrated in graded ethanol solutions (from 500 to 1000 ml/1 for 1 h each). The specimens for SEM were kept in isoamyl acetate and dried in a critical point drying apparatus (Hitachi HCP-1) using liquid carbon dioxide as the medium. The dried specimens were mounted on aluminum stubs with electrically conducting cement (silver paste), sputter coated with platinum (RMC-Eiko RE vacuum coater) at 100 millitorr, 7 milliamperes for 15 min and examined with a Hitachi S-800 SEM at 8 kV. Ultrathin sections were stained with lead citrate.

Serum biochemical properties
The blood samples were collected from each bird after slaughter and serum was separated. The separated serum was then made clear by centrifuging at 3000 RPM for 10 minutes and transferred to dry, clean Eppendorf tubes and stored in a refrigerator at (-20 °c) for estimation of serum parameters (Serum total protein, Serum albumin, Serum globulin & SGPT, calcium and phosphorous). All these tests were performed as per protocol described by Erba kits, Company, India.

Carcass characteristics
Carcass traits were studied (Study carried out on representative 3 birds/per replicate or 9 birds per group at 6th week) Liver, heart, gizzard, and abdominal fat were promptly weighed after the defeathering and evisceration of the carcasses. Clean carcasses (without the head, neck, and feet) and parts (breast, thighs with the drumsticks, and wings) were then weighed. All values were then reported as a % of live body weight (Rosa *et al*., 2007) [25].

Statistical analysis
Statistical tests were performed using one way Anova with multiple parameters comparing groups means and SEM between columns and control at significant level (p<0.05). Analysis was performed by using Graph pad prism 9.4.1 version software.

Results

Effect of supplementation of growth promoters on Broiler performance

Feed intake
Significant differences were observed in feed intake of broilers in all the weeks or during all the periods i.e., starter and finisher periods. During overall period the feed intake in T0, T1, T2, T3, T4, T5 and T6 were 3749.34, 3436.00, 3479.00, 3499.34, 3522.34, 3431.00 and 3683.34 g, respectively. However feed intake in T1-Nbiotic group and T5-TM-200 group are significantly lower.

Body weight gain during Starter period (0-28 days)
The body weight gain during starter period (0-28 days) was presented in the Table 3. Body weight gains in T0, T1, T2, T3, T4, T5 and T6 were 739.00, 909.44, 867.90, 790.57, 824.50, 822.04 and 824.74, g, respectively. Significantly higher (p<0.05) body weight gain (909.44g) was observed in broilers fed with antibiotic growth promoter Nbiotic and T2-Amox, T4-Megadox, T5 -TM-200 and T6- Tiamulin groups when compared to control group.

Finisher period (29-42 days)
Body weight gains in T0, T1, T2, T3, T4, T5 and T6 were 1053.97, 1120.70, 1169.04, 1118.94, 1167.04, 1046.57 and 1200.74g, respectively. No Significant difference noticed (p>0.05) in finisher period (Table 5).

Overall period (0-42 days)
During the overall period, the body weight gain in T0, T1, T2, T3, T4, T5 and T6 were 1792.97, 2030.14, 2036.94, 1909.50, 1991.54, 1868.60 and 2025.47g, respectively (Table 3). All the treatments differed significantly with the control in terms of total body weight (p<0.05) and treatments Nbiotic and T2-Amox performed relatively high.

Feed efficiency

Starter period (0-28 days)
During starter period significantly higher (p<0.05) feed efficiency was observed in broilers fed with Nbiotic, T2-AMOX followed by T3- CPRO, T4-MEG, T5 TM-200) di*et all* the treatments were significantly (p<0.05) differed with control.

Finisher period (29-42 days)
No Significant difference between various treatments under observation noticed *et al* (p<0.05) level. However relatively better feed efficiency than control were noted in Nbiotic, T2-Amox, T4-Meg and T6 Tiamulin groups (Table 5).

Total FCR (0-6 wks)
Significantly higher (p<0.05) feed efficiency was observed in broilers fed with Nbiotic, T2-Amox and T4-Megadox (Table 6; Figure 2a)**.**

Carcass characteristics Groups fed with Nbiotic showed a significant difference (p<0.05) in dressed weight and ready to cook yield compared to other groups (Table 7; Figure 2i)

Biochemical parameters
Glucose is significantly different (p<0.5) among different treatments group fed with T1-Nbiotic have lesser glucose levels to control, however no significant difference observed in levels of calcium and phosphorous (Table 8, Figure 2e, 2f).

But there were significant difference (p<0.05) in the levels of Albumin, Globulin and Total protein. Albumin levels are considerably higher in T1-Nbiotic compared to T5-TM-200. On contrast Globulin and Total protein levels are higher in T5-TM-200 followed by T1-Nbiotic (Table 8, Figure 2g, 2h).SGPT liver function enzyme is significantly higher in T5-TM-200 group followed by T2-Nbiotic (Table 10; Figure 2i, 2j).

Intestinal Morphometry

Villar length is significantly more in T5-TM-200 compared to Nbiotic group and control, in contrast, crypt length is more in T1-Nbiotic compared to control and other groups. Tunica muscularis no treatment showed difference except control group (Table 9 Figure 2c).

Scanning Electron microscopy examination

On SEM examination villi of ileum in groups T1- Nbiotic showed a significant difference among others. However villi of jejunum are significantly (p<0.05) are measured high in TM-200 group followed by T1-Nbiotic group (Table 11).

Carcass characteristics Groups fed with Nbiotic showed a significant difference (p<0.05) in dressed weight and ready to cook yield compared to other groups (Figure 2i)

Table 1: Group distribution and treatment plan followed during experiment

S.No	Group Name	No. of Birds/Group	Group/Treatment
1.	T_0	30	Standard basal diet(Control diet) 0-42 days
2.	T_1	30	Standard basal diet+ AV/AGP/10@500 g/ton of feed
3.	T_2	30	Standard basal diet+ Brand A*
4.	T_3	30	Standard basal diet+ Brand B*
5.	T_4	30	Standard basal diet+ Brand C*
6.	T_5	30	Standard basal diet+ Brand D*
7.	T_6	30	Standard basal diet+ Brand E*

Table 2: Brand names and composition and dosage of N biotic and Antibiotics

S.No		Trade name	Drug	Dose
1	Brand A	Amox-50	Amoxycillin	250 gms/ton
2	Brand B	Ciprofloxacin	Ciprofloxacin	500 gms/ton
3	Brand C	Megadox NFS	Neomycin and doxy cycline	1kg/ton
4	Brand D	TM 200	Oxy tetracycline	1kg/ton of feed
5	Brand E	Dynamutin	Tiamulin	500gms/ton

Table 3: Ingredients of the basal diet.

Feed Ingredients and additives	Starter	Finisher	*Starter	*Finisher	*Starter	*Finisher
			CP%	CP%	ME Kcal/Kg	ME Kcal/Kg
Maize	57	60.6	4.96	5.36	1881	2032.8
Vegetable oil	2	2.4	0	0	180	216
DORB	0	0	0	0	0	0
Soybean meal	39	35	17.16	15.4	1050.66	942.9
DCP	1	1	0	0	0	0
Shell grit	0	0	0	0	0	0
Trace Min. Mix	0.2	0.2	0	0	0	0
Salt	0.3	0.3	0	0	0	0
Lysine	0.13	0.13	0	0	0	0
DL-Methionine	0.1	0.1	0	0	0	0
Vit AB2D3	0.02	0.02	0	0	0	0
Choline Chloride	0.2	0.2	0	0	0	0
Coccidiostat	0.04	0.04	0	0	0	0
Antibiotic	0.01	0.01	0	0	0	0
Sub Total	100	100	22.12	20.76	3111.66	3191.7

Table 4: Proximate principles of the starter diet vs finisher diet

Nutrient	Starter	Finisher
Dry matter	90.48	92.04
Organic matter	92.07	93.61
Crude protein	22.32	19.46
Ether extract	6.16	7.57
Crude fibre	4.66	5.49
NFE	57.46	62.71
Total Ash	7.93	6.39
AIA	1.45	0.76
Calcium (%)	1.27	1.12
Phosphorus (%)	0.83	0.72
NDF	33.58	40.03
ADF	8.85	6.86
Hemi-cellulose	24.73	33.17

Cellulose	7.05	5.14
ADL	1.17	1.08
Silica	0.59	0.35

Table 5: Effect of treatment on feed intake and body weight during starter, finisher stage and overall

	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
Feed intake (Starter Phase)	1639.67±41.79^{c}	1451.00±36.16^{ab}	1458.34±26.02^{b}	1420.67±25.52^{b}	1491.00±37.69^{b}	1355.67±23.52^{a}	1655.67±25.07^{c}
Feed Intake (Finisher Phase)	2109.67±24.59^{c}	1985.00±10.98^{a}	2020.67±14.75^{a}	2078.67±10.22^{bc}	2031.00±26.84^{ab}	2075.34±7.76^{bc}	2027.67±10.22^{ab}
Feed intake (Total period)	3749.34±65.75^{b}	3436.00±29.84^{a}	3479.00±34.71^{a}	3499.34±16.59^{a}	3522.34±32.52^{a}	3431.00±15.77^{a}	3683.34±27.93^{b}
Body Wt.gain (Starter Phase)	739.00±8.60^{a}	909.44±27.56^{c}	867.90±49.78^{bc}	790.57±30.71^{ab}	824.50±25.25^{abc}	822.04±5.20^{abc}	824.74±14.28^{abc}
Body Wt.gain (Finisher Phase)	1053.97±72.19	1120.70±116.07	1169.04±93.01	1118.94±55.06	1167.04±63.03	1046.57±35.49	1200.74±26.08
Body Wt. (Total Period)	1792.97±65.02^{a}	2030.14±92.06^{b}	2036.94±43.33^{b}	1909.50±25.24^{ab}	1991.54±45.76^{b}	1868.60±30.38^{ab}	2025.47±25.56^{b}

^{abc} Values in column bearing different superscripts differ significantly * (p<0.05)

Table 6: Effect of treatment on FCR

		T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
FCR	(Starter Phase)	2.23±0.07^{c}	1.60±0.06^{a}	1.69±0.10^{a}	1.82±0.14^{ab}	1.81±0.04^{ab}	1.66±0.09^{a}	2.01±0.05^{bc}
FCR	(Finisher Phase)	2.01±0.09	1.79±0.10	1.77±0.19	1.89±0.16	1.75±0.08	2.00±0.13	1.70±0.06
FCR (Total)		2.10±0.03^{d}	1.70±0.03^{a}	1.72±0.07^{ab}	1.84±0.03^{c}	1.77±0.04^{abc}	1.84±0.04^{c}	1.82±0.02^{bc}

^{abc} Values in column bearing different superscripts differ significantly * (p<0.05)

Table 7: Effect of treatments on dressing (as raw weight)

	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
Live Wt. (gm)	1755.17±74.09^{a}	2042.34±68.57^{b}	1901.17±55.59^{ab}	1829.84±90.67^{a}	2078.50±89.93^{b}	1914.34±19.98^{ab}	1716.67±30.29^{a}
Dressed Wt. (gm)	965.67±5.19^{a}	1176.50±18.12^{d}	1233.34±4.39^{e}	1147.34±15.99^{d}	1109.50±15.31^{c}	1069.67±3.42^{b}	1140.00±13.07^{cd}
RCY (gm)	1032.50±4.51^{a}	1265.84±19.25^{d}	1325.00±4.80^{e}	1236.34±16.02^{cd}	1197.67±15.20^{c}	1151.50±2.76^{b}	1233.50±14.31^{cd}
Gizzard (gm)	35.17±2.92	41.17±2.69	41.84±1.73	38.17±2.58	37.17±2.99	34.84±2.38	39.84±0.97
Liver (gm)	32.84±1.65^{a}	37.34±1.23^{ab}	40.17±2.36^{b}	40.00±2.00^{b}	39.34±2.45^{b}	37.67±2.37^{ab}	43.00±0.86^{b}
Heart (gm)	10.67±0.95	10.84±0.96	9.67±0.71	10.84±0.72	11.67±0.50	9.34±0.92	10.67±0.30
Spleen (gm)	4.34±0.31	5.17±0.34	5.67±0.48	5.17±0.61	5.17±0.61	4.84±0.57	5.50±0.18

^{abc} Values in column bearing different superscripts differ significantly * (p<0.05)

Table 8: Effect of treatments on dressing (as % of dressed weight)

Dressing (%)	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
	55.05±2.31^{a}	58.03±2.56^{ab}	65.29±1.74^{c}	62.97±2.14^{b}	53.82±1.89^{a}	56.31±0.68^{a}	66.45±0.99^{c}
RCY (%)	58.89±2.50^{a}	62.44±2.91^{ab}	70.18±1.97^{c}	67.87±2.33^{b}	58.11±2.04^{a}	60.62±0.81^{a}	71.91±1.10^{c}
Gizzard (% dressed wt.)	3.63±0.24	3.50±0.22	3.41±0.13	3.32±0.30	3.42±0.32	3.28±0.23	3.53±0.09
Liver (% dressed wt.)	3.47±0.18	3.24±0.12	3.49±0.30	3.45±0.20	3.66±0.22	3.52±0.29	3.61±0.08
Heart (% dressed wt.)	1.11±0.09^{c}	0.93±0.09^{abc}	0.79±0.07^{a}	0.95±0.06^{abc}	1.05±0.05^{bc}	0.88±0.09^{ab}	0.94±0.03^{abc}
Spleen (% dressed wt.)	0.45±0.03	0.44±0.03	0.46±0.05	0.46±0.06	0.47±0.06	0.46±0.06	0.49±0.02
Crude Protein in muscle (%)	25.09±0.34^{abc}	27.63±0.16^{bc}	27.18±1.54^{bc}	24.90±0.18^{ab}	27.89±1.18^{c}	25.74±1.18^{abc}	23.83±0.40^{a}

^{abc} Values in column bearing different superscripts differ significantly * (p<0.05)

Table 9: Effect of Treatments on Ileum and Jejunum (Scanning Electron Microscope)

	Treatment	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200
ILEUM (Villi)	Height um	294.33±2.33	624±2.4	481±4.4	442±2.3	512±1.15	405±3.71
	Width um	121±2.72	152±5.7	126±3.52	122±5.23	153±5.45	123±6.35
	Crypt depth	179±3.84	196±6.69	194±2.72	186±2.64	184±6.00	176.66±3.92
JEJUNUM (Villi)	Height um	989.33±2.33	1140±3.71	1220±1.6	803±3.21	750±1.15	1004±1.76
	Width um	168±1.7	190±2.4	191.66±2.4	120±1.76	176.3±4.25	136.33±5.78
	Crypt depth	180±2.3	193±2.4	200±2.02	183±3.28	187±5.23	176±1.73

Table 10: Effect of treatment on biochemical profile of birds

	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
Glucose	274.87±9.21^{bc}	245.40±5.44^{a}	296.96±12.82^{c}	251.20±3.22^{ab}	234.40±14.09^{a}	259.91±8.43^{ab}	245.24±6.59^{a}
Albumin	0.57±0.03^{a}	1.53±0.02^{g}	1.03±0.02^{d}	1.18±0.01^{e}	0.93±0.04^{c}	1.38±0.03^{f}	0.86±0.01^{b}
Globulin	0.46±0.02^{a}	1.04±0.08^{d}	0.98±0.05^{d}	1.15±0.01^{e}	0.86±0.01^{c}	1.33±0.02^{f}	0.70±0.02^{b}
Total Protein	1.04±0.01^{a}	2.56±0.03^{e}	1.79±0.02^{c}	2.38±0.02^{d}	1.84±0.02^{c}	2.56±0.12^{e}	1.57±0.02^{b}
SGPT (IU)	72.67±0.99^{a}	75.00±0.26^{b}	77.00±0.58^{c}	72.34±0.50^{a}	72.34±0.56^{a}	72.34±0.50^{a}	71.50±0.50^{a}
Serum Cholesterol	109.92±6.76^{ab}	126.92±2.62^{b}	146.70±10.30^{c}	116.49±3.39^{ab}	115.02±3.06^{ab}	118.33±4.09^{ab}	104.14±2.41^{a}
Calcium	12.36±0.83	13.61±0.42	13.83±1.39	14.90±1.07	14.31±0.68	13.68±0.56	12.91±0.84
Phosphorous	13.55±1.12	13.96±0.51	13.15±1.08	13.74±0.52	14.43±1.74	13.95±0.71	13.07±2.31

Table 11: Effect of treatment on intestinal histology

	T0- CONTROL	T1 AV/GMP	T2-AMOX	T3- CPRO	T4-MEG	T5 TM-200	T6 TIAMLUN
Villar length	70.50±5.71^{ab}	68.34±2.48^{ab}	64.67±3.76^{ab}	66.67±1.67^{ab}	72.50±8.83^{ab}	102.50±4.96c	60.84±0.84a
Villar width	5.34±0.56^{a}	7.50±1.12^{abc}	8.00±0.00^{bc}	5.34±0.22^{a}	7.34±0.99^{abc}	8.84±0.84c	6.34±0.72ab
Crypt length	20.17±1.76^{b}	30.50±4.97^{c}	14.00±0.64^{ab}	16.67±0.96^{ab}	33.00±2.24^{c}	17.50±1.71ab	12.84±1.11a
Crypt width	3.84±0.17^{a}	6.17±0.41^{c}	5.67±0.43^{c}	7.00±0.97^{c}	5.84±0.17^{bc}	5.84±0.41bc	4.00±0.00a
Tun muscularis	23.34±1.06^{d}	9.67±1.18^{ab}	8.00±0.52^{a}	10.67±1.59^{ab}	8.67±1.31^{a}	13.00±1.30b	19.84±1.20c

Supplementary Figure 1Scanning Electron microscope images a) Ileum T0 Control group showing villi heigsht is 294.33±2.33 b) villi width 121.66±2.77 and c) Ileum T1 group SEM images showing ileum villi height is 624.33±2.4 d) villi width 152±5.77 e) Ileum T2 Control group SEM images showing ileum villi height is 481.66± 4.4 f) villi width 126.66±3.52 g) Ileum T3 Control group SEM images showing ileum villi height is 442±2.3 h) villi width 122.33±5.23 i) Ileum T4 Control group SEM images showing ileum villi height is 512± 1.15 j) villi width 153.66±5.45 k) Ileum T5 Control group SEM images showing ileum villi height is 405±3.71 l) villi width 123.33±6.35.

Supplementary Figure 2 a) line graph showing effects of treatments on FCR starter, finisher and total, FCR total ratio in Nbiotic group is significantly lower than control and other groups. b) Tukey test on FCR depicting significant difference between Nbiotic and control, and Nbiotic with Amoxycillin, Megadox, Tiamulin c) Line graph showing effects of treatments on intestinal Histology; villar length and villar length are significantly differ in Nbiotic. D) Line graph showing SEM analysis of ileum and Jejunum; it shows significant difference in ileum villi height and jejunum villi height e) Line graph showing different biochemical parameters Glucose, Cholesterol, phosphorous and calcium with other antibiotics. f) Tukey tests showing n ignificant difference in Glucose, Cholesterol, phosphorous and calcium between treatments. g) line graphs showing effects of treatments on Albumin, Globulin and Total protein. h) Tukey test showing significant difference between control and Nbiotic, Nbiotic and Amoxicillin in Albumin, Globulin and Total protein. i) line graphs showing effects of treatments on live weights, dressing weights and Ready to cook yield j) Tukey test showing significant difference between control and Nbiotic, Nbiotic and Amoxicillin in dressing percentage.

SEM Pictures of Villi of Ileum

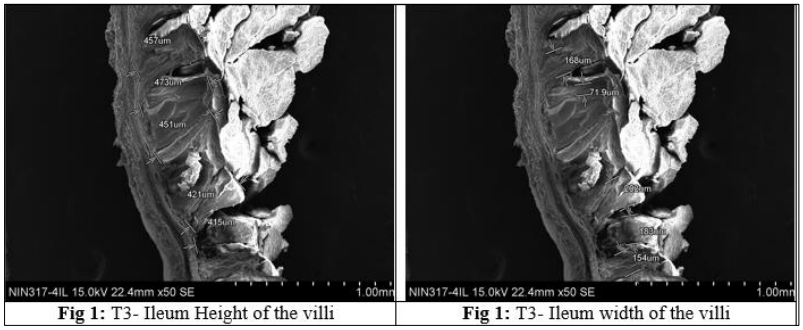


Fig 1: T3- Ileum Height of the villi

Fig 1: T3- Ileum width of the villi

Fig 1: T4- Ileum Height of the villi

Fig 1: T4- Ileum width of the villi

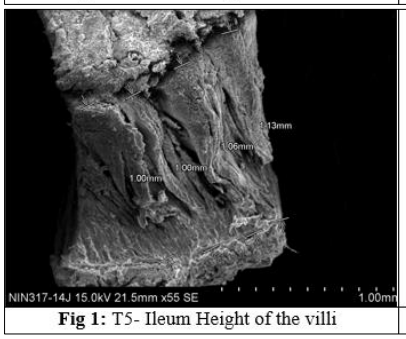


Fig 1: T5- Ileum Height of the villi

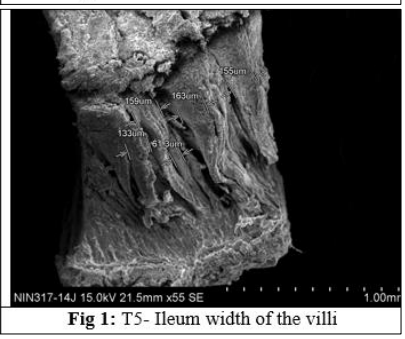


Fig 1: T5- Ileum width of the villi

SEM Pictures of Villi of Jejunum

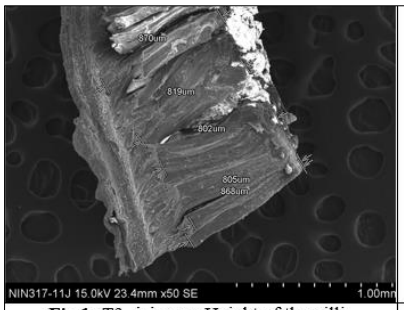


Fig 1: T3- jejunum Height of the villi

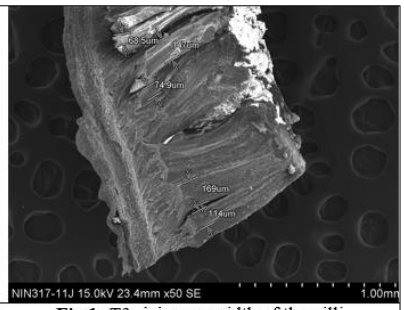


Fig 1: T3- jejunum width of the villi

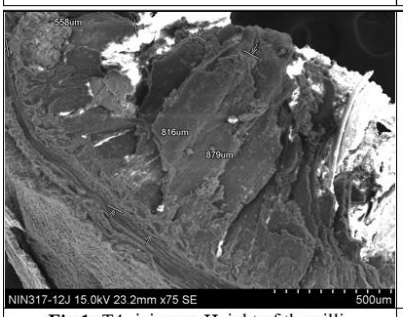


Fig 1: T4- jejunum Height of the villi

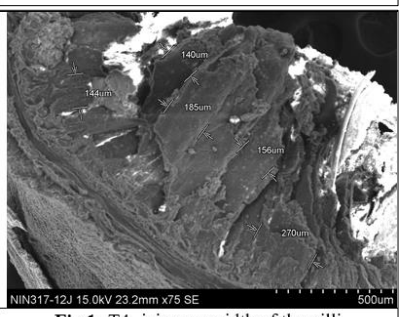


Fig 1: T4- jejunum width of the villi

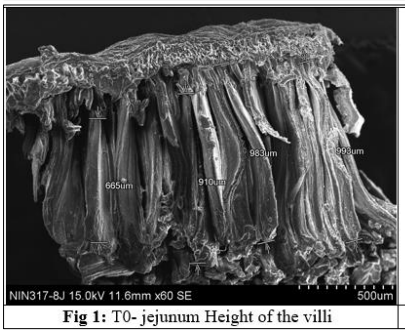


Fig 1: T0- jejunum Height of the villi

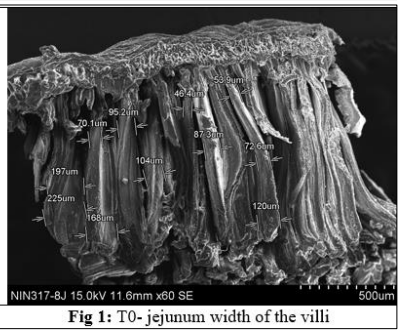


Fig 1: T0- jejunum width of the villi

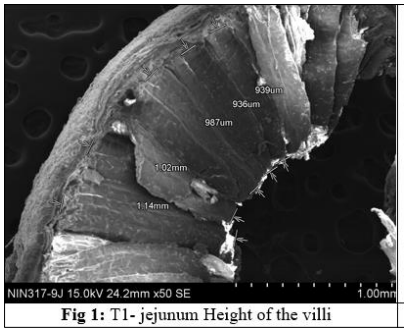


Fig 1: T1- jejunum Height of the villi

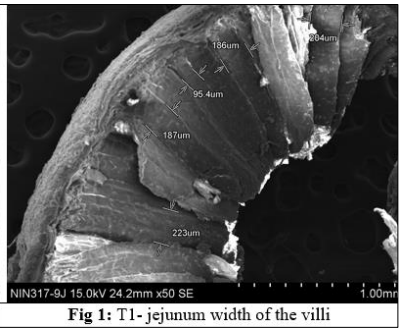


Fig 1: T1- jejunum width of the villi

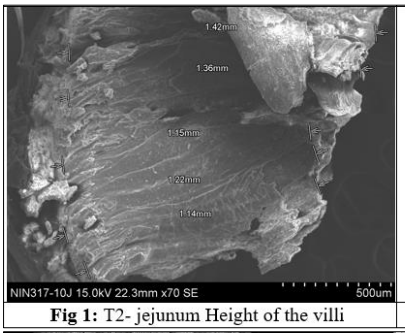


Fig 1: T2- jejunum Height of the villi

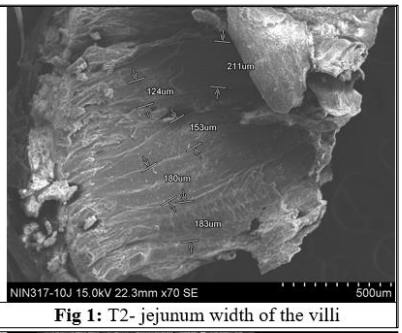


Fig 1: T2- jejunum width of the villi

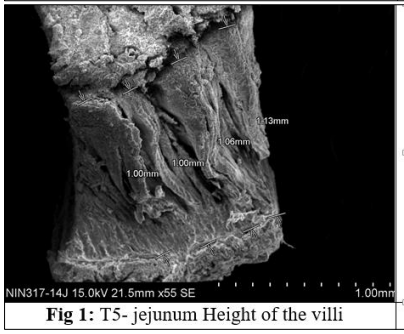


Fig 1: T5- jejunum Height of the villi

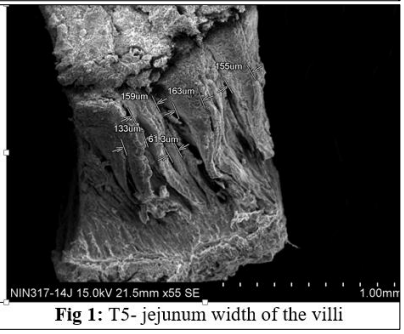


Fig 1: T5- jejunum width of the villi

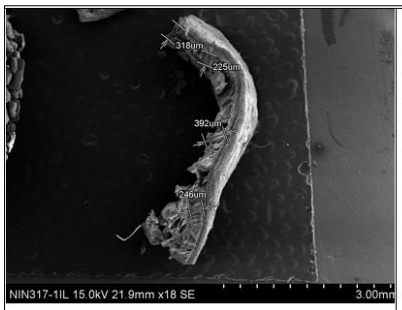


Fig 1: T0- Ileum Height of the villi

Fig 2: T0- Ileum width of the villi

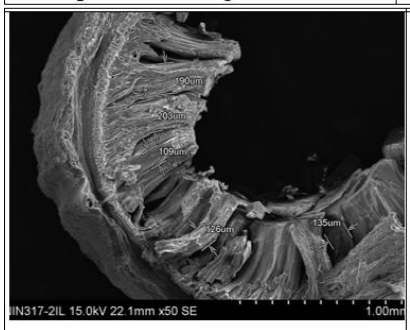


Fig 1: T1- Ileum Height of the villi

Fig 1: T1- Ileum width of the villi

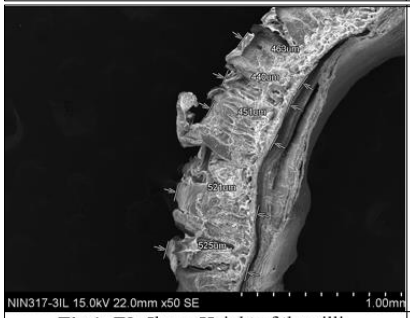
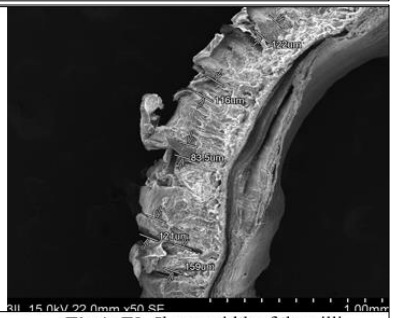


Fig 1: T2- Ileum Height of the villi



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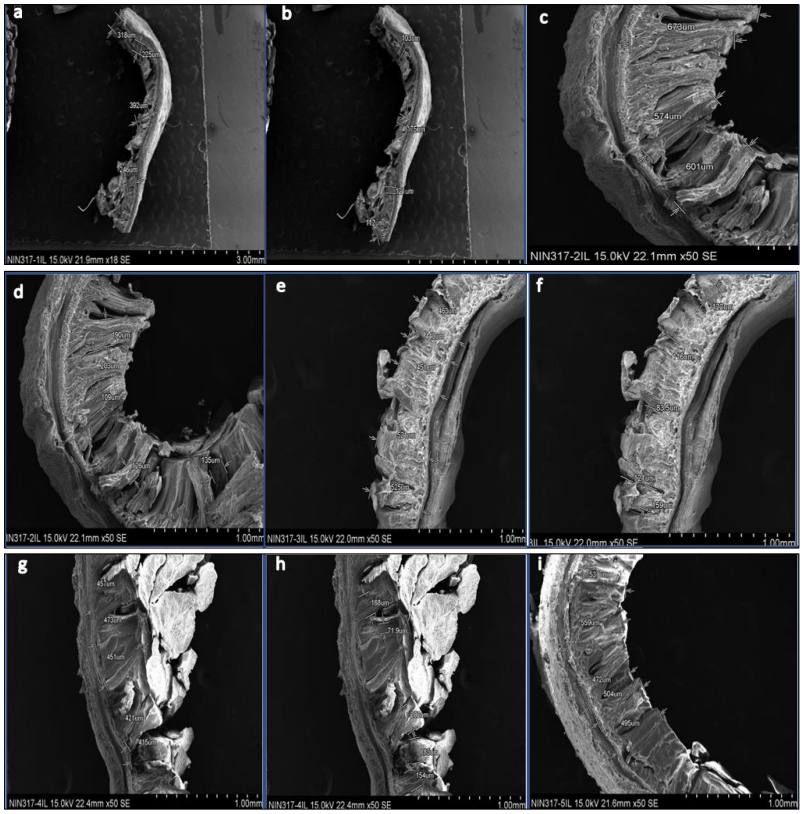

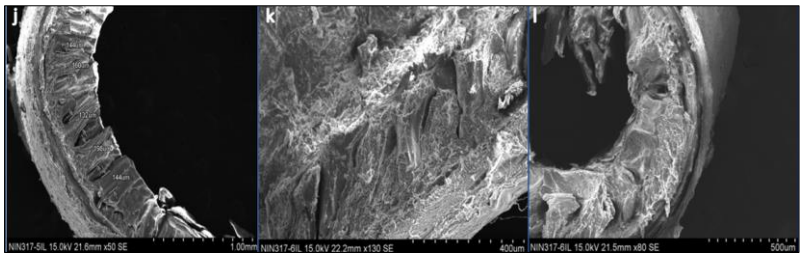
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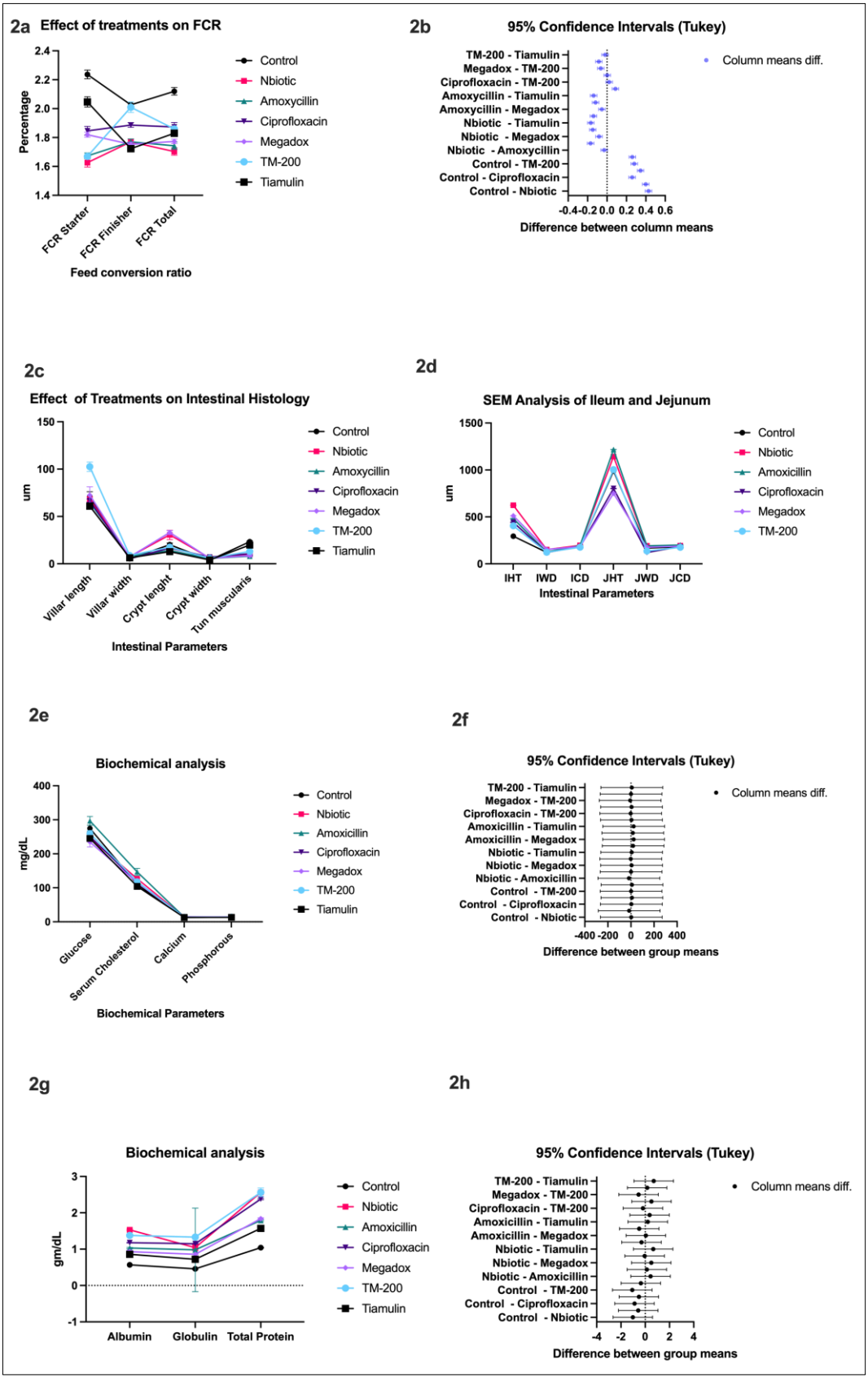
2a
Effect of treatments on FCR
Percentage
Feed conversion ratio
FCR Starter
FCR Finisher
FCR Total
Control
Nbiotic
Amoxycillin
Ciprofloxacin
Megadox
TM-200
Tiamulin
2b
95% Confidence Intervals (Tukey)
TM-200 - Tiamulin
Megadox - TM-200
Ciprofloxacin - TM-200
Amoxycillin - Tiamulin
Amoxycillin - Megadox
Nbiotic - Tiamulin
Nbiotic - Megadox
Nbiotic - Amoxycillin
Control - TM-200
Control - Ciprofloxacin
Control - Nbiotic
Column means diff.
Difference between column means
2c
Effect of Treatments on Intestinal Histology
um
Villar length
Villar width
Crypt lenght
Crypt width
Tun muscularis
Intestinal Parameters
2d
SEM Analysis of Ileum and Jejunum
IHT
IWD
ICD
JHT
JWD
JCD
Intestinal Parameters
2e
Biochemical analysis
mg/dL
Glucose
Serum Cholesterol
Calcium
Phosphorous
Biochemical Parameters
Amoxicillin
2f
95% Confidence Intervals (Tukey)
Difference between group means
2g
Biochemical analysis
gm/dL
Albumin
Globulin
Total Protein
2h
95% Confidence Intervals (Tukey)
Difference between group means

Discussion

Feed consumption is significantly l**ower** in T 1, T2 and T5 when compared to other antibiotic group except T6. The phytogenic growth promoters' leads to activation of hypothalamic AgRP which is critical for nutrient utilization and metabolic efficiency coupled to weightgain ("Use of herbs and spices and their extracts in animal nutrition." Kumar *et al*., 2014; Cavalcanti-de-Albuquerque *et al*., 2019) [17, 6]. As result there is reduced feed intake (Erhan *et al*., 2012) [9]. It is evident that probiotics promotes synergistic microorganisms growth thus promote less feed consumption.

Though there were no reports on feeding of phytogenic growth promoter will improve body weight gains Our study reported a significant improvement in body weight gain in all phytogenic and antibiotics fed growth promoters except groups treated with Tiamulin.

Feed efficiency during starter and finisher and feed efficiency is more in Nbiotic group compared to control however Amoxycillin T2, T4, T6 groups were also significantly different. The reason phytogenic group showed more significance is due to modulation of intermediary lipid and protein metabolism signaling path ways (Flees *et al*., 2021) [11]. The stimulating impact of the phytogenic feed additive on endogenous digestive enzymes and on an expanded absorption surface area in the ileum may be the other factor. (Amad *et al*., 2011) [2] Additionally, it was shown that phytogenic feed additives induced intestinal mucus secretion in broilers, which was thought to inhibit pathogen adherence and stabilise the microbial EUBIOSIS in the animals' guts (Windisch *et al*., 2008) [31].

Carcass traits and relative internal organs of broiler chickens

Results on carcass traits and other internal organs were significant in Nbiotic fed group compared to control. Earlier reports suggest that phytogens exert positive influences on the development of carcass traits, internal organs, and meat qualities of broiler chickens (Adu *et al*., 2020) [1]. Similar findings were observed by studies on broilers fed with clove powder (S.I. Al-Mufarrej *et al*., 2019) [27]. However some studies didn't report any improvement in weight of internal organs when fed with other phytogens like clove powder at 0.5% (Tariq *et al*., 2015) [29].

Intestinal morphometry

Villar width, crypt length and crypt width were significantly different in Nbiotic fed group than control group. These findings justify the reports that villi height would directly affect the capacity of the intestine to absorb nutrients since it would increase the absorptive and surface area (Czernichow *et al*., 1996; Fuller, 2004) [8, 12]. On Scanning electron microscopy it is clearly evident that ileum height, width, and crypt depth were increased in Nbiotic fed group to control and other treatments. These findings second previous reports that Intestinal crypts are invaginations of the epithelium around the villi, and are lined by epithelial cells which secrete enzymes. The base of the crypts is constantly dividing to maintain the structure of the villi. Therefore, an increase in crypt depth would produce more developed villi. In general, supplementation of Nbiotic is able to improve the development of the gut as a whole (Chwen *et al*., 2013) [7].

Summary

Phytogenic growth promoter Nbiotic, when compared to controls and other antibiotics, generally shown a notable difference. Nbiotic has been shown to be more cost-effective and superior feed additives for broilers in terms of growth, feed efficiency absorption, and biochemical parameters, even though a few parameters were inferior to other groups.

Conclusion

Overall, the research presented here proved the value of using PFA and underlines how critical it is to take PFA into account as a possible replacement for AGP in chicken diets.

Acknowledgement

Authors are grateful to Ayurvet Pvt. Ltd for financial assistance and National Institute of Nutrition Hyderabad, for Scanning Electron microscope facility and finally Sri Venkateshwara Veterinary University, Tirupati, Andhra Pradesh for experimental facilities.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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