

## PRODUCTION AND ASSESSMENT OF SUPERWORMS BASED POSTBIOTIC IN MEAT TYPE CHICKENS.

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### ABSTRACT

This experiment was conducted to develop super worm-based postbiotics and examine its response on growth performance, nutrients digestibility, immunity, gut microbiota, apparent metabolizable energy, intestinal histology and economics of meat type chickens. Already developed cultures of *Lactobacillus acidophilus* in the laboratory of microbiology confirmed through DNA sequence analysis and preserved at  $-20^{\circ}\text{C}$  were used. The bacterial culture was rejuvenated and kept at  $-20^{\circ}\text{C}$  in deMan, Sharpe and Rogosa (MRS) medium (Darmstadt, Merck,) inclusion with 20 percent (Sharpe V/V) glycerol. Sterile 0.8% (W/V) sodium chloride (Merck, Darmstadt) solution was used to wash the active bacterial cells and adjusted to  $3 \times 10^9$  CfU per ml to prepare inoculums and stored at  $-20^{\circ}\text{C}$  until mixed with wheat bran. The super worm's cultures were kept in environmentally controlled chamber and prepared spray of *Lactobacillus acidophilus* was applied @ of  $3 \times 10^9$  CfU per ml per kg of substrate per week. Full fat super worms produced in wheat bran supplemented with the mentioned bacterial culture till harvesting was used for its evaluation on broilers. For this purpose, a total hundred and fifty, one day old healthy meat type chicks were purchased from local commercial hatchery and were assigned to five experimental groups. Chicks in group A were provided with basal ration having no postbiotics and was maintained as control while birds in the other four groups were provided the same ration mixed with the super worm-based postbiotics at the dose level of 0.2%, 0.4%, 0.6% and 0.8%, respectively. Feed consumption, weight gain and feed conversion ratio among experimental groups were found significantly improved in treated groups provided with different levels of super worm-based postbiotics. Microbial count of *Lactobacillus acidophilus* was significantly ( $p < 0.05$ ) increased while that of *Salmonella* and *E. coli* was significantly decreased in Pos-Bio 0.8% as compared to control and other dietary groups. Nutrients digestibility of different nutrients and apparent metabolizable energy was increased in treated groups as compared to negative control group. Similarly, carcass yield and economic return was also increased in the treatments Pos-Bio 0.6% and 0.8% as compared to non-treated group. Intestinal morphologic features (length and weight of villus and crypt depth) were increased in probiotics supplemented groups 0.6% and 0.8% as compared to control. Immune boosting effect in the form ( $p < 0.05$ ) high antibodies titer against IB, IBD and ND was observed in chicks provided with 0.6%, 0.8%, 0.4% 0.2% postbiotics as compared chicks maintained without postbiotics. Based on present data we concluded that the use of super worm-based postbiotics improved the productive performance of broilers through its positive effect on nutrient digestibility, metabolizable energy, Intestinal morphologic features, robust growth of gut commensals, reduced gut pathogens count and boosted immune response against prevalent viral diseases. Regular use of super worm-based postbiotics at the dose rate of 0.8% in the diet of broilers chicks is recommended for best performance and high economic return.

**Keywords:** super worm, postbiotics, nutrient digestibility, apparent metabolizable energy, immunity, gut microbiota, growth performance

## INTRODUCTION

The application of antibiotics in poultry farming has elevated massive community health concerns. These consist of the existence of drug residue in poultry products, appearance and multiplication of antibiotics resistant strains of bacteria (Nhung *et al.*, 2017). Furthermore, increasing community awareness of the need to consume harmless and vigorous foods particularly of animal sources, has led to the ban of the utilization of antibiotics growth promoters in 2005 in the EU (EPC, 2005) and succeeding restriction in USA and other kingdoms (Editors, 2017).

Moderately postbiotic is an innovative expression also considered as either - simply metabolites or cell free supernatant (CSF), biogenic and metabiotics. Postbiotic are described as "non-viable bacterial products or metabolic by-products produced from probiotic microorganisms that have biologic activity in the host" (Patel and Denning 2013). Furthermore, postbiotics have a number of inspiring characteristics including typical shelf-life longevity, chemical structures and safety dose (Tomar *et al.*, 2015). Postbiotics have also revealed encouraging distribution, absorption, and secretion abilities, which could certainly impact a wide range of host tissues and organs and in this manner exerting different biological activities (Shenderov, 2013). Trails carried out on postbiotics stated their encouraging consequences on the modulation of intestinal microbiota, anti-inflammatory effect, stimulation of immune system and pathogen antagonism on birds and animals (Compare *et al.*, 2017). Additionally, postbiotics were helpful against the salmonella pathological responses in the mice intestinal mucosa (Tsilingiri *et al.*, 2012) and in people with post-infectious bowel syndrome (Compare *et al.*, 2017). This designates that postbiotics have an immune stimulation consequence just like probiotic (Rine *et al.*, 2021).

Postbiotics have a parallel mechanism of action and capability as probiotic (Thanh *et al.*, 2009). Since these microbial by products consisting antimicrobial metabolites, like as bacteriocins and organic acid, they can decrease the gut pH and slow down the propagation of opportunistic bacteria in the gut and feed of birds and animals (Aguilar-Toalá *et al.*, 2018). The use of postbiotics as a ration additive in livestock encourages the growth performance and health of piglets, layers and broilers (Kareem *et al.*, 2016; Loh *et al.*, 2014 & Loh *et al.*, 2013), as well as improving rumen fermentations and health in ruminants (Izuddin *et al.*, 2019).

## Objectives

1. Production and assessment of insect-based postbiotics in meat type chicken.
2. To work out economics of using insect-based postbiotics in the production of meat type Chicken.
3. To find out the health status of meat type chicken using insect-based postbiotics

## METHODOLOGY

### Preparation of Postbiotic

Lactobacillus strains isolated in the department of microbiology; the University of Agriculture Peshawar were properly identified through DNA sequence analysis. Using the procedure (Foe *et al.*, 2003) the bacterial culture was rejuvenated and kept at  $-20\text{ }^{\circ}\text{C}$  in deMan, Sharpe and Rogosa (MRS) medium (Darmstadt, Merck,) inclusion with 20 percent (Sharpe V/V) glycerol. Sterile 0.8% (W/V) sodium chloride (Merck, Darmstadt) solution was used to wash the active bacterial cells and adjusted to  $3\times 10^9$  Cfu per ml to prepare inoculums and stored at  $-20\text{ }^{\circ}\text{C}$  until mixed with wheat bran.

### Production of super worm

Plastic containers of 10 inches x 22 inches and 10 inches deep were used for the production of super worms. After thorough cleaning and drying the containers were kept in warm and relatively dark environment. Sterilized wheat bran was added to the boxes and spread to a depth of 02-03 inches. The known amount of minute Super worm larvae were added to the boxes and kept in proper environment (Chamber) maintaining the temperature  $28\text{ }^{\circ}\text{C}$  and humidity 65%. Wheat bran used as substrate was sprayed with bacterial culture at the rate of one ml per kg of the substrate per week till harvesting the meal worm larvae on week 8th.

Larvae after attaining the required age was collected from boxes and frizzed for 24 hrs. The frozen larvae were kept in oven for drying purpose for 04 hours at  $60\text{ }^{\circ}\text{C}$  and grinded to make a fine powder. The powder was mixed in ration and offered to the broilers on the prescribed protocol.

## Birds Husbandry and Experimental Plan

For this experiment 150 birds at week second were separated into five groups with each group having three replicates and each replicate having 10 birds. The diets were expressed as 0% (Pos-D-1) 0.2% (Pos-D2), 0.4% (Pos-D3)0.6% (Pos-D4) and 0.8% (Pos-D-5) where commercial ration was supplemented with meal worm postbiotic @ 0.0, 0.2, 0.4, 0.6 and 0.8 %.

**Table 01. Experimental layout for assessment of Super worm as Postbiotic in meat type chicks**

S. No.	Super worm based postbiotics	Replicates		
		R1	R2	R3
1	(Pos-D-1) 0	10	10	10
2	(Pos-D-2) 0.2 %	10	10	10
3	(Pos-D-3) 0.4 %	10	10	10
4	(Pos-D-4) 0.6 %	10	10	10
5	(Pos-D-5) 0.8 %	10	10	10

### Production traits

Feed intake, body weight gain, feed conversion ratio (FCR), dressing percentage, HI antibody titer against ND, digestibility, apparent metabolizable energy, bacterial count and economics was determined. Data were statistically analyzed using standard procedure of ANOVA in a completely randomized design using Statistical package SAS (1998). Least significant difference test (LSD) was used for separation of means (Jan *et al.*, 2009).

## Results

### 1. Production parameters of meat type chicken.

Table. 02 specifies the computed and analyzed values of experimental ration of various treatment groups. It was perceived after the statistical analysis of documented data, that the inclusion of super worm-based postbiotics had substantial consequences on overall consumption of ration, statistically maximum and same intake were computed for (Pos-Bio 0.2 % = 3274.5<sup>a</sup>±7 gm), (Pos-Bio 0.6 % = 3286.5<sup>a</sup>±8 g) and for (Pos-Bio 0.8 % = 3260.7<sup>a</sup>±8 g) while the minimum and same feed intake was recorded for (Control = 3275.6<sup>b</sup> ±9 g) and for (Pos Bio 0.4 % = 3255.07<sup>b</sup>±5g) groups. Similarly in case of weight gain the collected data showed non-significant results ( $p>0.05$ ) on birds' weight gain on weekly basis however the total weight gain was statistically maximum for (Pos-Bio 0.8 % = 1901<sup>a</sup>±1) while the minimum weight was computed for non-treated group and (Pos-Bio 0.2 % = 1865<sup>c</sup>±5.6). incase of feed conversion ratio and dressing percentage of meat type chickens statistically no variation was noted among the different groups.

**Table 02: Impact of various inclusion levels of super worm-based postbiotics on Production parameters of meat type chicken.**

Parameters	Treatments					P value
	Control	Pos-Bio 0.2%	Pos-Bio 0.4%	Pos-Bio0.6%	PosBio0.8%	
	Mean+ SD	Mean+ SD	Mean+ SD	Mean+ SD	Mean+ SD	
Feed Intake(gm)	3275.6 <sup>a</sup> ±9	3274.5 <sup>a</sup> ±7	3255.07 <sup>b</sup> ±5	3286.5 <sup>a</sup> ±8	3260.7 <sup>b</sup> ±8	0.003
Weight gain	1869 <sup>c</sup> ±7	1865 <sup>c</sup> ±5	1889 <sup>b</sup> ±7	1894 <sup>ab</sup> ±3	1901 <sup>a</sup> ±1	0.005
FCR	1.75±1	1.75±1	1.72±1	1.73±1	1.71±1	0.929
Dressing%	65± 2.	71± 4	67± 2	69± 8	72± 2	0.390

Means with non-similar superscripts differs significantly at  $\alpha$  0.05

Pos-Bio represent Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

## 02. Antibody Titer & Apparent Metabolizable Energy

Table 03. summarized the antibody titer and apparent metabolizable energy of meat type chickens served with different levels of super worm-based postbiotics. The inclusion of super worm-based postbiotics in diet of meat type chicken inconsequentially influences the antibody titer of birds. However, the maximum apparent metabolizable energy of various experimental treatments was computed for (Pos-Bio 0.8 % = 3064.05<sup>a</sup>±4.03) followed by (Pos-Bio 0.6 % = 3037.76<sup>b</sup>±5.06), (Pos-Bio 0.2 % = 3016.25<sup>c</sup>±6.15), (Pos-Bio 0.4 % = 2997.13<sup>d</sup>±7.01) and minimum for control group as (Control = 2980.4<sup>e</sup>±10.2).

**Table 03: Effect of Super worm based Postbiotics on Antibodies titers and Apparent Metabolizable Energy (AME)**

Groups	Antibodies titer Mean ± SE	Apparent Metabolizable Energy (AME) kcal/Kg Mean ± SE
Control	4.84±0.84	2980.4 <sup>e</sup> ±10.2
Pos-Bio 0.2 %	4.70±0.70	3016.25 <sup>c</sup> ±6.15
Pos-Bio 0.4 %	4.94±0.44	2997.13 <sup>d</sup> ±7.01
Pos-Bio 0.6 %	5.10±0.10	3037.76 <sup>b</sup> ±5.06
Pos-Bio 0.8 %	5.20±0.20	3064.05 <sup>a</sup> ±4.03
<b>P- value</b>	<b>0.793</b>	<b>0.003</b>

Means with non-similar superscripts differs significantly at  $\alpha$  0.05

Pos-Bio represent Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

## 03: Nutrients Digestibility

The overall nutrients digestibility of dry matter, crude fiber, crude protein, calcium, phosphorus, and ash of various treatments are described in Table 04. The digestibility of crude protein, calcium and phosphorus was increased in treated groups with increased substitution of super worm based postbiotics.

**Table 04: Effect of Super worm based Postbiotics on Broiler Nutrient Digestibility**

Groups	DM Mean $\pm$ SE	CP Mean $\pm$ SE	CF Mean $\pm$ SE	Ca Mean $\pm$ SE	P Mean $\pm$ SE	Ash Mean $\pm$ SE
Control	73.08 $\pm$ 4.0	78.61 <sup>ab</sup> $\pm$ 3.6	81.66 $\pm$ 2.0	29.13 <sup>b</sup> $\pm$ 6.1	41.78 <sup>ab</sup> $\pm$ 2.7	48.10 $\pm$ 6.1
Pos-Bio 0.2 %	74.16 $\pm$ 8.1	76.33 <sup>b</sup> $\pm$ 4.3	79.97 $\pm$ 5.0	33.11 <sup>ab</sup> $\pm$ 5.1	43.32 <sup>ab</sup> $\pm$ 3.3	47.36 $\pm$ 3.3
Pos-Bio 0.4 %	70.22 $\pm$ 3.2	81.61 <sup>ab</sup> $\pm$ 5.6	82.50 $\pm$ 3.0	37.17 <sup>ab</sup> $\pm$ 7.1	36.11 <sup>b</sup> $\pm$ 6.1	52.17 $\pm$ 5.1
Pos-Bio 0.6 %	71.33 $\pm$ 8.3	80.17 <sup>ab</sup> $\pm$ 2.1	80.28 $\pm$ 4.0	31.61 <sup>ab</sup> $\pm$ 4.6	40.56 <sup>ab</sup> $\pm$ 2.5	54.12 $\pm$ 6.1
Pos-Bio 0.8 %	75.30 $\pm$ 5.3	84.89 <sup>a</sup> $\pm$ 4.0	82.95 $\pm$ 6.0	39.22 <sup>a</sup> $\pm$ 3.2	44.69 <sup>a</sup> $\pm$ 4.5	55.96 $\pm$ 1.4
P. Value	0.849	0.197	0.999	0.220	0.185	0.719

Means with non-similar superscripts differs significantly at  $\alpha$  0.05

Pos-Bio represent Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

#### 4. Determination of Bacterial load

During finisher phase *Salmonella* counts were noted minimum for (Pos-Bio 0.4 %) and (Pos-Bio 0.8 %) while statistically same and higher numbers were recorded for non-treated group and (Pos-Bio 0.2 %) supplemented group (Table:5). Similarly lower number of *E. coli* was computed for (Pos-Bio 0.8 %) group followed by statistically similar and high number for non-treated and (Pos-Bio 0.2 %) groups. On the other hand, the *lactobacillus acidophilus* was found maximum for (Pos-Bio 0.8 %) and (Pos-Bio 0.6 %) followed by Pos-Bio 0.4% and non-treated groups while the minimum number of *lactobacillus acidophilus* were counted for (Pos-Bio 0.2%) group.

**Table 5: Effect of Super worm based Postbiotics on Gut microbial load (CFU/g) of meat type Chicks.**

Gut bacterial load	Groups					P-value
	Control Mean±SE	Pos-Bio0.2 % Mean±SE	Pos-Bio0.4% Mean±SE	Pos-Bio0.6% Mean±SE	Pos-Bio 0.8 % Mean±SE	
Salmonella	6.12 <sup>a</sup> ±0.06	6.14 <sup>a</sup> ±0.14	5.44 <sup>b</sup> ± 0.44	5.51 <sup>ab</sup> ±0.44	5.31 <sup>b</sup> ±0.52	0.035
E. coli	6.13 <sup>a</sup> ±0.03	6.05 <sup>a</sup> ±0.6	5.29 <sup>ab</sup> ±0.4	5.81 <sup>ab</sup> ±0.04	5.12 <sup>b</sup> ±0.7	0.01
Lactobacillus acidophilus	7.66 <sup>b</sup> ±0.05	7.11 <sup>c</sup> ±0.40	8.02 <sup>b</sup> ± 0.52	8.81 <sup>a</sup> ±0.30	9.11 <sup>a</sup> ±0.26	0.003

Means with non-similar superscripts differs significantly at  $\alpha$  0.05.

Pos-Bio represents Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

## 5. Intestinal Histology:

At the end of experiment the inclusion of super worms based postbiotics notably ( $P < 0.05$ ) affected the intestinal histological characteristics (table 6). The maximum villus length and villus height to crypt depth ratio in small intestine (duodenum) was recorded in the treatment provided the basal feed with 0.8% super worms based postbiotics as compared to control and other treatment groups.

## 06. Economics:

The economic was computed on the basis of cost acquired on the processing of super worm-based postbiotics and ration constituent utilized for the formulation of ration Table: 07. On an overall basis the net profit of treated groups was high as compared to the control group. The values from the table demonstrated maximum profit return for treatment (Pos-Bio 0.8 % = 18.1<sup>a</sup>±5.0) while minimum revenue was noted for control group.



**Table 6: Effect of Super worms based Postbiotics on intestinal health of meat type chicks**

Gut Portion	Treatments						P. Value
	Control	Pos-Bio 0.2 %	Pos-Bio 0.4 %	Pos-Bio 0.6 %	Pos-Bio 0.8 %		
Duodenum	VH( $\mu\text{m}$ )	998.4 <sup>d</sup> ±5.2	1042.1 <sup>b</sup> ±3.0	1026.1 <sup>c</sup> ±5.8	1048.7 <sup>b</sup> ±8.2	1062.1 <sup>a</sup> ±3.0	0.000
	CD( $\mu\text{m}$ )	204.2 <sup>bc</sup> ±2.02	209.8 <sup>b</sup> ±0.43	198.2 <sup>c</sup> ± 2.2	223.17 <sup>a</sup> ±5.05	195.17 <sup>d</sup> ±2.5	0.021
	VCR	4.88± 1.13	4.96±2.13	5.17± 2.5	4.69±0.10	5.44±2.01	0.988
Jejunum	VH( $\mu\text{m}$ )	1005.1 <sup>d</sup> ±4.1	1011.1 <sup>d</sup> ±2.1	1023.8 <sup>c</sup> ±4.4	1039.4 <sup>b</sup> ±2.4	1070.2 <sup>a</sup> ±5.1	0.000
	CD( $\mu\text{m}$ )	207.1 <sup>c</sup> ±3.06	215.12 <sup>b</sup> ±1.04	205.25 <sup>c</sup> ±6.03	229.37 <sup>a</sup> ±2.06	211.4 <sup>b</sup> ±2.30	0.000
	VCR	4.85±2.2	4.70±1.2	4.98±1.0	4.53± 1.1	5.06±2.2	0.237
Ileum	VH( $\mu\text{m}$ )	1021.24 <sup>c</sup> ±3.1	999.34 <sup>d</sup> ±5.17	1024.11 <sup>c</sup> ±3.01	1035.37 <sup>b</sup> ±1.06	1051.11 <sup>a</sup> ±1.01	0.000
	CD( $\mu\text{m}$ )	107.25 <sup>c</sup> ±2.05	111.21 <sup>b</sup> ±1.10	117.21 <sup>a</sup> ±2.10	108.40 <sup>bc</sup> ±1.30	104.32 <sup>d</sup> ±2.04	0.003
	VCR	9.52 <sup>b</sup> ±0.12	8.98 <sup>c</sup> ±0.12	8.73 <sup>d</sup> ±0.17	9.55 <sup>b</sup> ±0.18	10.07 <sup>a</sup> ±0.09	0.008

Means with non-similar superscripts differs significantly at  $\alpha$  0.05.

Pos-Bio represents Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

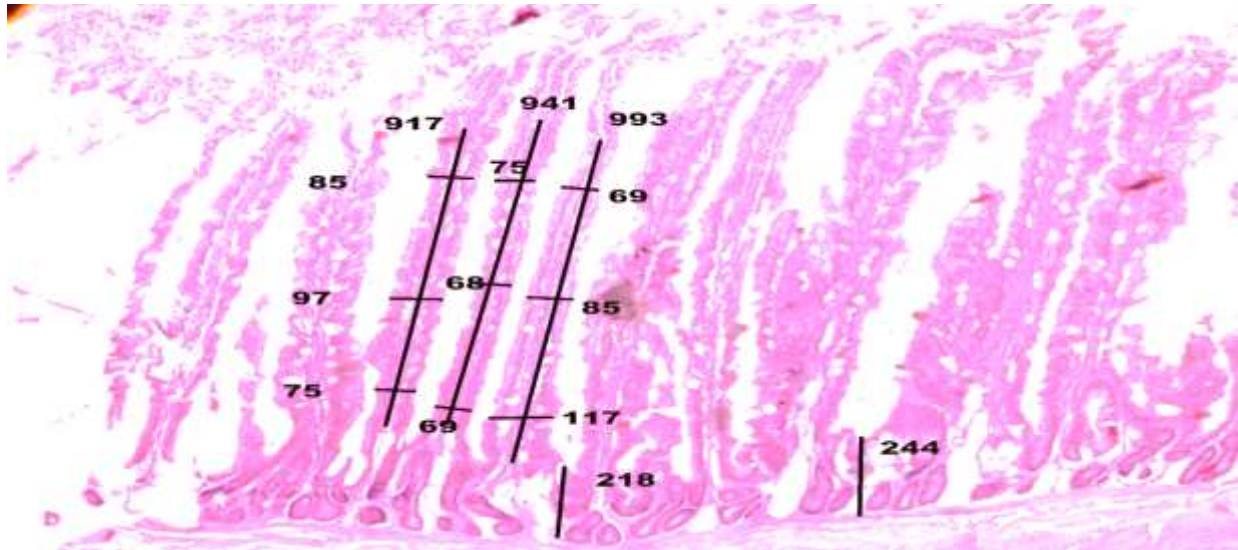


Fig. 01 Normal stature of villi were seen in control.

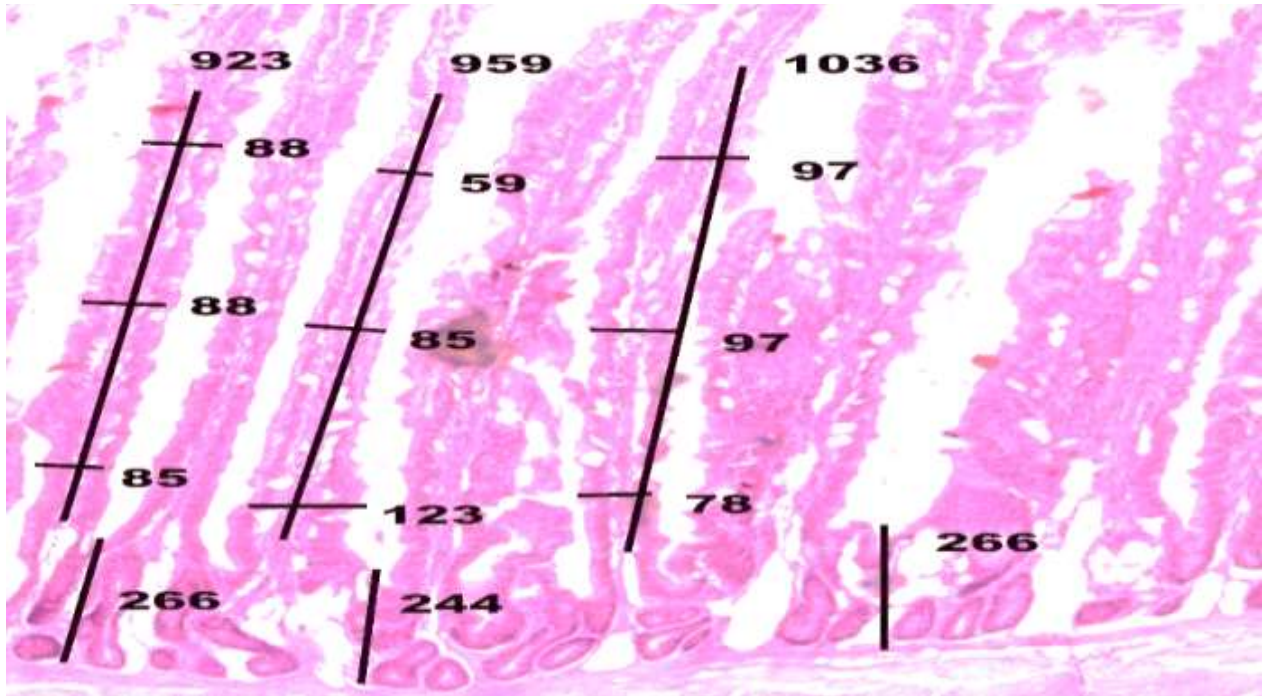


Fig. 02 Mild changes were seen in group post-Bio 0.2 %. Length of villi and width of villi indicating mild changes in the villi were found. hematoxylin-Eosin.

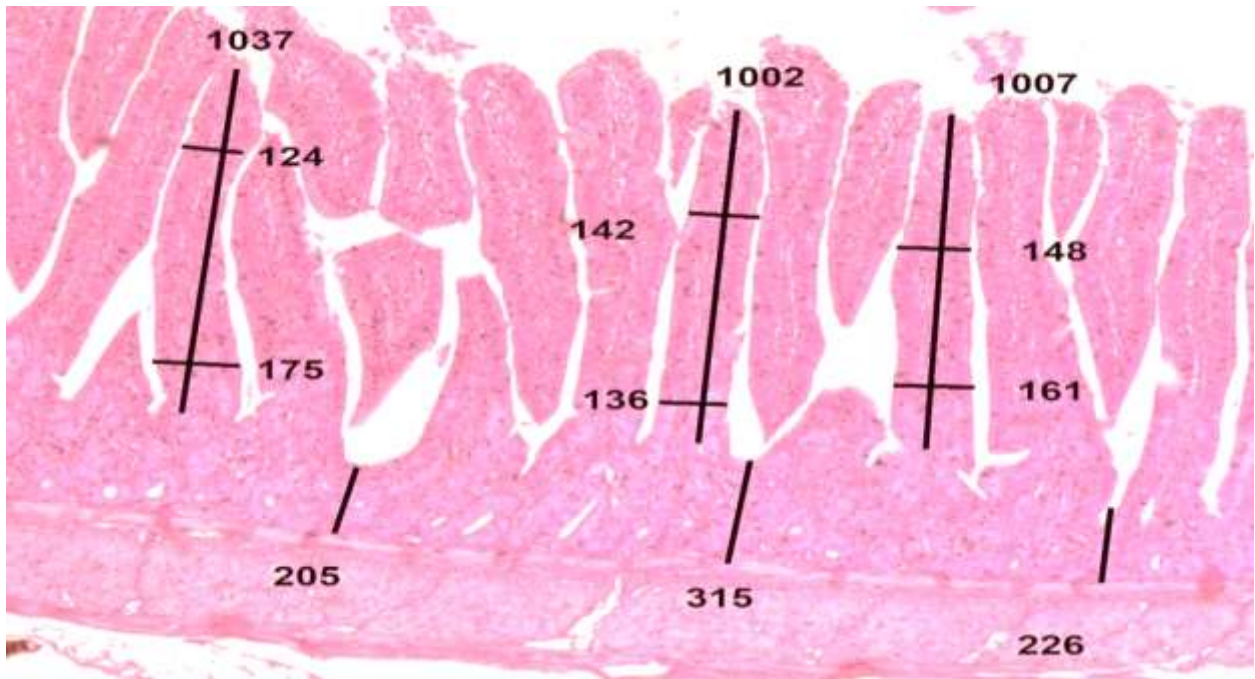


Fig.03 Slight changes appear in villis from group post-Bio 0.4 %. Length of villi, width of villi and Surface area of villi increase in size. Haematoxylin-Eosin.

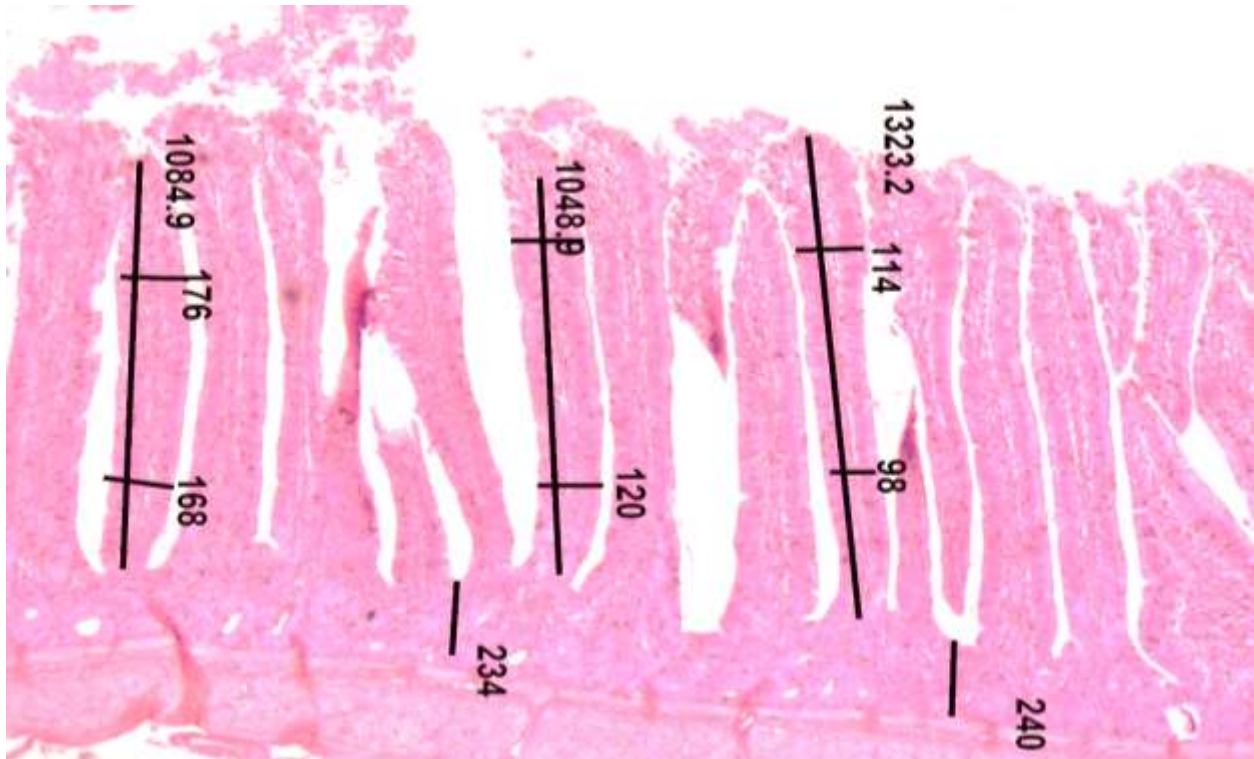


Fig.04 increase changes appear in villus height from group post-Bio 0.6 %. Length of villi, width of villi and Surface area of villi increase in size. Hematoxylin-Eosin.

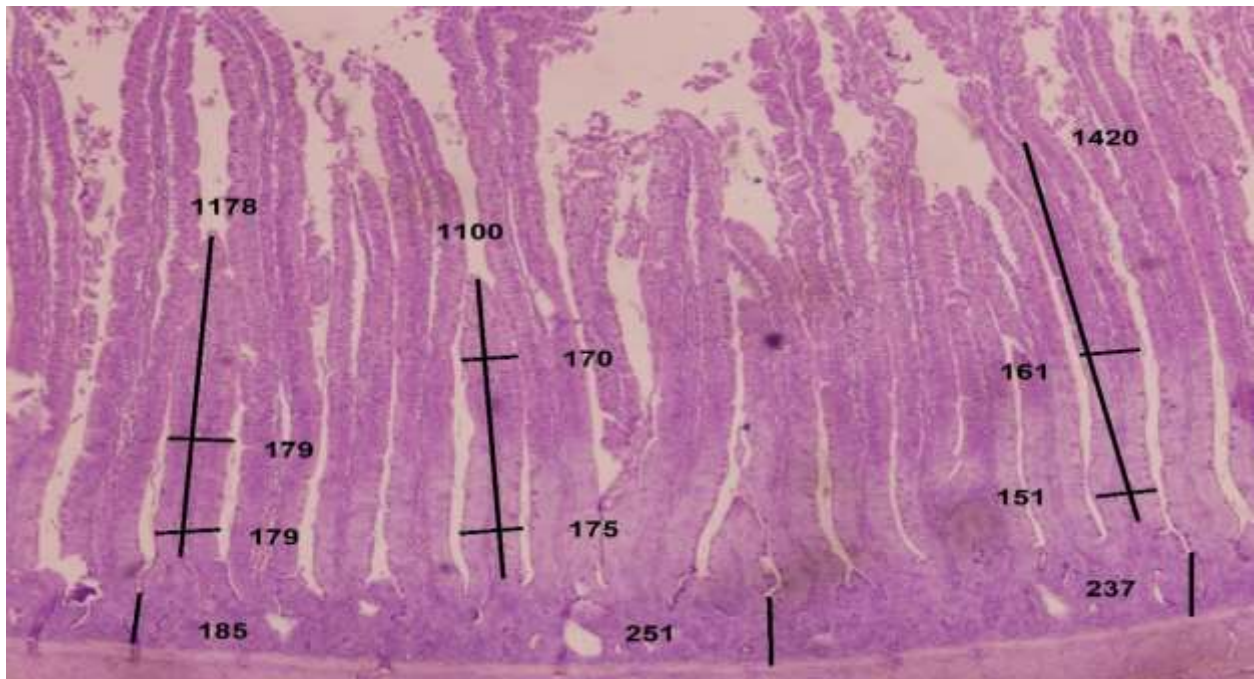


Fig.05 maximum changes appear in villus height from group post-Bio 0.8 %. Length of villi, width of villi and Surface area of villi increase in size. hematoxylin-Eosin.

**Table 07: Effect of Super worm based Postbiotics on economics of meat type chickens.**

Groups	Cost /kg Ration Mean ±SE	Super worm Pos-Bio cost Mean ±SE	Running cost Mean ±SE	Total cost Mean ±SE	Return Mean ±SE	Profit/kg Mean ±SE
Control	126.3±2.30	00 <sup>d</sup> ±0.0	95±3.7	221.3 <sup>bc</sup> ±3.3	229.8 <sup>c</sup> ±2.4	8.5 <sup>b</sup> ±1.2
Pos-Bio 0.2 %	122.7±4.35	1.4 <sup>c</sup> ±0.4	95±1.5	219.1 <sup>c</sup> ±6.3	231.5 <sup>bc</sup> ±5.3	12.4 <sup>ab</sup> ±3.0
Pos-Bio 0.4 %	128.8±1.41	2.8 <sup>b</sup> ±0.1	95±4.4	226.6 <sup>a</sup> ±2.2	241.9 <sup>a</sup> ±2.0	15.3 <sup>ab</sup> ±1.2
Pos-Bio 0.6 %	123.1±3.00	4.2 <sup>a</sup> ±0.8	95±4.8	225 <sup>abc</sup> ±1.0	238.3 <sup>ab</sup> ±5.1	16 <sup>ab</sup> ±3.2
Pos-Bio 0.8 %	125.1±5.00	5.6 <sup>a</sup> ±0.3	95±1.8	227.7 <sup>ab</sup> ±3.8	243.8 <sup>a</sup> ±3.4	18.1 <sup>a</sup> ±5.0
<b>P. Value</b>	<b>0.276</b>	<b>0.000</b>	<b>1.000</b>	<b>0.054</b>	<b>0.004</b>	<b>0.120</b>

Means with non-similar superscripts differs significantly at  $\alpha$  0.05

Pos-Bio represent Super worm-based Postbiotic: Pos- Bio 0.2 %, represent 0.2% supplementation of super worm-based postbiotic. Pos-Bio 0.4 % represents 0.4% supplementation of super worm-based postbiotic. Pos-Bio 0.6 %, represent 0.6% supplementation of super worm-based postbiotic. Pos-Bio 0.8 %, represent 0.8% supplementation of super worm-based postbiotic.

## Discussion

### 1. Performance parameters

Postbiotics augmented the growth performance through a number of hypothesized methods when added to the bird's ration. The bacteriocins and short chains fatty acids in the postbiotics exerted bactericidal and bacteriostatic characteristics against harmful bacteria in the gastrointestinal region. Furthermore, the acidic nature of short chains fatty acids declined the pH of gut, which has an unhelpful correspondence with the production of low acidic tolerant diseases causing bacteria like as *E. coli*, *Salmonella* and *Enterobacteriaceae* (Loh *et al.*, 2021).

Resembling to our findings (Kareem *et al.*, 2020) applied postbiotics at various ratio and noted insignificant differences among the control and experimental groups for feed consumption. The findings of (Siadati *et al.*, 2017) also concluded that the inclusion of lactobacillus could not manipulate the ration intake in Japanese quail which are in line with our results. The feed intake of egg type hens was not influenced by the inclusion of insect meal; conversely the individual egg

production of hens was affected by using a combination of 4.7% larvae meal and 3% fish meal (Agunbiade *et al.*, 2007). In research executed by (Gariglio *et al.*, 2019) broiler chicks fed with 9.0% of black soldiers fly larva meal, in replacement of gluten meal and could not lead to any variation in feed consumption throughout different feeding stages. No clear differences on growth performance were documented by (Schiavone *et al.*, 2018) who supplemented broilers ration with 3.40 and 6.85% of black soldier fly fat through partial or complete replacement of soybeans oil.

Conversely, our results disagreed with the findings of (Liu *et al.*, 2012) who stated that prebiotics probiotics and postbiotics appreciably enhance the intake of ration in comparison with the non-treated groups. Despite *Lactobacillus strain* postbiotic from *B. subtilis* also exhibited favorable consequences in broilers and egg type hens, including feed consumption and quality of eggs (Zhu *et al.*, 2020).

One feasible cause for the improved overall body weight is possibly due to the existence of beneficial bacteria (*Lactobacillus*) vitamins, bacteriocin and organic acids in postbiotics (Loh *et al.*, 2006; Sampath *et al.*, 2021). The observations of (Loh *et al.*, 2003a & Foo *et al.*, 2003a, b) are in line with our current findings who pointed that nourishing *Lactobacilli* species improved growth performance in post weaning mice. Similarly in previous findings (Kareem *et al.*, 2016a) also described significantly maximum body weight gain for meat type chickens fed on postbiotics. Bacteriocins produced by *L. plantarum* F1 enhanced the ratio of growth in meat type chickens (Ogunbanwo *et al.*, 2004). Contrarily to our findings (Cakir *et al.*, 2008) demonstrated that there were no variations for weekly body weight and overall body weight gains of experimental birds by addition of postbiotics when compared with the non-treated birds. Correspondingly (Rosyidah *et al.*, 2011) could not scrutinize noticeable variation in body weight and final body weight of birds nourished with postbiotics and a mixture of postbiotics and acidifier groups. Similarly opposing to our results several authors reported that inclusion of postbiotics had no results on broilers and layers performance (Chen *et al.*, 2012; Józefiak *et al.*, 2012; Guo *et al.*, 2012 & Ogunbanwo *et al.*, 2004).

As declared earlier, that the high consumption of nutrients in post biotic treated birds may be due to up regulation of nutrients genes expressions lead to augmentation of the ration consumption and diet conversion ratio of broilers birds (Kalavathy *et al.*, 2003). Supporting our findings (Humam *et al.*, 2019) conducted an experiment and used antioxidant (vitamin E) and postbiotics with the

control diet and stated that the result on total body weight and diet conversion ratio of antioxidant was not as recorded for postbiotic. Similarly (Sampath *et al.*, 2021) investigated the experimental trail by using 0.1% *L. plantarum* supplement in broiler feed and noted no effect on ration conversion ratio and weight gain throughout the experimental duration. A group of researchers had also revealed no negative effect on ration conversion ratio of meat type chickens provided diets added with a combination of different lactobacillus species (Kalavathy *et al.*, 2008; Saminathan *et al.*, 2014; Kalavathy *et al.*, 2006; Kalavathy *et al.*, 2003 & Timmerman *et al.*, 2006) or with combinations of lactobacillus and other species of bacteria (Nayebpor *et al.*, 2007).

Conversely to our findings (Yu *et al.*, 2007) stated that addition of *L. reuteri* based postbiotics enhanced 05% ration conversion ratio in broilers birds. Likewise, Thu *et al.* (2011) conducted an experiment by using postbiotics and noted poor ration conversion ratio comparable to those of negative controls. In the same way (Peng *et al.*, 2016) pointed out that the dietary addition of 0.5% *L. plantarum* inclusion has increased the feed conversion ratio of meat type chickens.

## **2. Dressing percentage, Digestibility, Metabolizable energy and Gut microbiota count**

Statistically non-significant effect on dressing percentage and mortality was recorded when super worm based postbiotics were used in the feed of meat type chickens. Supporting our results (Hussein *et al.*, 2020) also noted no noticeable variation in the outcomes of paraprobiotic and postbiotic on the carcass yield of the broilers birds. In the same way (Humam *et al.*, 2019) provided postbiotics to birds under summer stress condition and at the end of experiment did not note positive outcomes on their carcass yield. Opposing to our results, the addition of 10% and 15% of defatted black soldiers fly larvae in the feed of growing female quail (from 1<sup>st</sup> to 4<sup>th</sup> week of age) led to best production performances and carcass characteristics with those of quails fed commercial soybeans meal (Cullere *et al.*, 2016).

Benzertiha *et al.* (2019) summarized that adding of minute quantity of insects-based meal can improve poultry health and nutrients digestibility such as 0.2% to 0.3% inclusion of full fat mealworms diet have not any adverse result on ileal digestibility and declined the prospective diseases causing bacteria in poultry. Feeding black soldier fly (*H. illucens*) to birds potentially enhanced the nutrient digestibility and absorption (Schivone *et al.*, 2017 & Elwert *et al.*, 2010). Insect defatting had important consequences on coefficient of apparent digestibility (ADC) as completely defatted insects had inferior ADC values than incompletely defatted insects (Schivone *et al.*, 2017). The findings of (Thanh *et al.*, 2010) is almost similar to our results they suggested

that increase in digestibility and consumption of nutrients due to the addition of postbiotics may supply greater metabolizable energy and protein to the lambs leading to superior growth performance. In contradiction (Sampath *et al.*, 2021) reported that the addition of 0.1% *L. plantarum* to the broilers diet could not affect the digestibility of dry matter and ether. in the same way, (Chen *et al.*, 2006) also pointed that growing pigs fed *Bacillus* based postbiotics addition has no considerable variations on digestibility of crude protein, nitrogen, and dry matter. Furthermore, (Shin *et al.*, 2013) also stated that finishing pig fed 0.20% *Lactobacillus* combination ( $1 \times 10^9$  Cfu per kg) ration could not influence the total tract digestibility of nitrogen and dry matter.

The statistics values achieved from the experiment illustrated that the number of lactobacilli was increased statistically in broilers fed with various doses of postbiotics in contrast to the non-treated group. Similarly (Gong *et al.*, 2014) also noted that all treatments which fed on postbiotics reduced population of *E. coli* in contrast with control treatment. Furthermore, increased fermentation action and elevated concentration of the volatile fatty acids (VFAs) is associated with a decline pH, which is correlated with an inhibition of harmful bacteria and enhanced solubility of many nutrients (Józefiak *et al.*, 2011).

Our recent results are also in agreement with the outcomes of (Kareem *et al.*, 2016b) who disclosed that various doses of postbiotic leads to boost the population of *lactobacillus* and simultaneously reduce the number of diseases causing bacteria like enterobacteriaceae and *E. coli* in large intestine of broiler's and recommended that using of postbiotics as a novel ration additive having key hindering results on harmful bacteria in the quail gut microbiota. The combination of inulin and postbiotics prevented proliferation of diseases causing bacteria like as *Salmonella enterica*, vancomycin resistant *Enterococci*, *Listeria monocytogenes* and *Escherichia coli* (Loh *et al.*, 2010). Our findings are in agreement with the results of (Paul *et al.*, 2007) who pointed that postbiotics with their significant elements organic acid and bacteriocins prevent growth of several harmful bacteria by reducing the population of bacteria in the epithelium and lead to decreasing infection and inflammation at the mucosa of intestine.

On the contrary, to our results (Siriken *et al.*, 2003) documented that the inclusion of postbiotics could not affect bacterial population in large intestine of quails. The results of (Vali *et al.*, 2013) are also opposed our findings who stated that inclusion of postbiotics in broilers feed had no satisfactory effect on *lactobacillus* population.

### 3. Immunity and intestinal histology

The destructive results of birds colibacillosis are predominantly common in the poultry production, as well as poor husbandry and biosecurity practices, particularly in newly emerging countries, generate a complicated paradigm for enhancement without some easily applied intercession policies (Ebrahimi-Nik *et al.*, 2018). In worldwide poultry industry, the disease provokes considerable financial annual losses (Roth *et al.*, 2019). For this reason, it was compulsory to uncover feasible substitute assets to shield levels of production and still sustain the birds health (Seal *et al.*, 2013). One of these alternatives is biotic feed additives like as prebiotic, probiotic and postbiotic (Klemashevich *et al.*, 2014). Similar to our findings, the postbiotics obtained from *L. plantarum* included several advantageous compounds like as organic acids, enzymes, bacteriocins and antioxidant compounds; these compounds have beneficial effect on health status of birds (Mohamad *et al.*, 2020 & Chiang *et al.*, 2010). Accordingly, the postbiotics transform the immune responses, encourage the propagation of beneficial bacteria in the GIT, fight against harmful diseases and enhance poultry and livestock production and growth performance (Humam *et al.*, 2020; foo *et al.*, 2003 & Humam *et al.*, 2019). The production of plantaricin W and plantaricin EF bacteriocins, in postbiotics as well as saturated fatty acids like as lactic acid, caproic acid and acetic acid are well familiar for their wide range of anti-inflammatory and antimicrobial actions, concerned in intestinal health, mainly tight junction and production of mucous (Lewis *et al.*, 2010 & Pelaseyed *et al.*, 2014). Shojadoost *et al.* (2022) also documented that inoculation of four different species of *Lactobacilli* in meat type chickens decreased severity of necrotic-enteritis, notably altered the immune responses of broilers by adaptation of interferon- $\gamma$ , IL-2, IL-12p35, IL-17 and IL-1 $\beta$  and modulating the transcription of growth factor beta gene in the intestine, improved production of CD8 + T cells and B cells in the cecal tonsil, and transform the intestinal microbiota composition.

The intestinal morphology measurement can enhance the absorption of nutrients when there is maximum height of villus, small crypt depth and increased villus height crypt depth ratio (Jha *et al.*, 2020). When, *Lactobacillus* supplement go through the intestinal region it can convert carbohydrate into lactic acids and enhance the actions of various digestive enzymes like lipase, amylase, trypsin and total proteolytic enzymes to enlarge the villus length and reduce the crypt



depth, which is advantageous for the digestion and absorption of feed nutrient by the poultry (Högberg and Lindberg, 2006).

Like our findings the earlier results of (Kareem *et al.*, 2016) pointed that feeding poultry birds with postbiotic caused development of intestinal histomorphology through augmented height of villus in the ileum and duodenum regions. The prebiotics and postbiotics changed the mucosal structural design in terms of maximum villus height and amplified birds' performance (Abdel-Raheem *et al.*, 2012; Yang *et al.*, 2007 & Thanh *et al.*, 2009). The results of our study agreed with the results of (Samanya and Yamauchi 2002 & Miles *et al.*, 2006) who published a large increase in height of villus of ileum and duodenum in four-week-old birds fed with *Bacillus subtilis*. Recognized confirmation disclosed that postbiotics could develop architecture of the small intestines by rising beneficial microbiota like as *Lactobacillus*, which may cause a decline in the threat of villi damage resulted by harmful microbes of the gut. (Thu *et al.*, 2011; Thanh *et al.*, 2009 & Loh *et al.*, 2010). The enlarged villus height and villus height to crypt depth proportion is associated with augmented epithelial cells turnover claimed by (Foo *et al.*, 2014).

#### 4. Economics

The study reported a high return from the birds fed on diet containing supplementary number of super worms based postbiotic, at the same time as the minimum revenue was computed from birds fed with the non-treated diet. Supporting our results regarding economics of super worm based postbiotics (Jang *et al.*, 2019) stated that the substitution of insect-based protein to bird's ration enhances the production characteristics and economics of ducks. Similarly in earlier study (Jang *et al.*, 2017) documented that insect feeding increases profitability by escalating the weight gain and declining intake of feed and feed demand of livestock. In the same way (David *et al.*, 2013) provided 100% fish meal and insect-based protein diet to growing pigs and point out that insect-based protein is not only important constituent of pigs diet from a performance point of view but also from an economic perspectives.

#### Conclusion:

It can be concluded that postbiotics derived from *Lactobacillus acidophilus* can improve performance and promote bird's health by modulating gut microbiota. The supplementation of super worm based postbiotic to broilers through feed can be used as an alternative to antibiotics to balance gut microbiota. They can replace antibiotics without compromising the growth performance, carcass yield and immune status of broiler chickens.

**Recommendations:**

Following recommendations are made on the bases of current research findings.

1. Regular use of super worm-based postbiotics at the dose rate of 0.8% in the diet of broilers chicks is recommended for best performance and high economic return.
2. Further studies are needed to inspect the role and exact mode of action with different strains and doses of super worms-based postbiotic in the ration of egg type birds.

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