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Influence of using probiotic on the productivity and morphometry of the organs in the visceral cavity of broiler chicks

Ziad Ahmad Alabdallah[1,2*], Aleksandr Alekseevich Nikishov[2], Cueva Jimenez Jhonn Lenon[2], Mario Leonardo Ortiz Manzano[3]

1. Department of Anatomy-Histology and Embryology, Al Furat University, Deirez-Zor, College of Veterinary, Syria
2. Peoples Friendship University of Russia (RUDN University) Moscow, Miklukho-Maklaya Street, Russia
3. University of Armed Forces – ESPE, Ecuador

**Corresponding author's Email: Zead3133@gmail.com*

ABSTRACT
Probiotics are feed additives that have gained popularity in poultry. They are one of the more universal feed additives and can be easily combined with other additives. This study was designed to evaluate the impact of including recommended doses of probiotics and growth-promoting antibiotics in concentrated foods on productivity and gut health in broilers. Six hundred one-day-old unsexed broiler chicks (Ross 308 strain) were used in this study. They were divided into three groups (40 chicks per group). Group T_0 received balanced feed with growth stimulating antibiotic (Zinc Bacitracin), while group T_1 received balanced diet (no growth stimulating antibiotic) + probiotic (*Bacillus subtilis* sp.; 1.6×10^9 CFU g^{-1}) at an inclusion rate of 500 g ton^{-1}. Group T^2 was fed the basal balanced diet with a growth-stimulating antibiotic (Zinc Bacitracin) and a probiotic (*Bacillus subtilis* sp.). Statistical analysis and processing the material were performed using the data analysis package "MS Excel 2010" and the program "Statistics for Windows". In this study, we assessed the effect of supplementation of probiotics and acidifiers as well as their combination on broiler live body weight, net carcass, along with dressing percentage, the weight of the internal organs, relative bowel length (small intestine length, large intestine length, caecum length, and fabric bag length) and weight of broiler cuts breast. The outcomes revealed a difference between the control and other treatment groups which was statistically significant ($p < 0.05$). The ratio of villus height to crypt depth and villus height in the duodenum and ileum were both raised ($p < 0.05$) by the addition of either probiotics or synbiotics.

Keywords: Probiotics, Chicks, Internal organs, Breast cuts, Bowel length.
Article type: Research Article.

INTRODUCTION
Being one of the most highly productive branches of animal husbandry, bird breeding creates good opportunities for quickly and effectively solving the problem of protein nutrition for the population of the whole world (Alabdallah *et al.* 2021; Alabdallah *et al.* 2021; Angel Daniel *et al.* 2022). Currently, many drugs have been developed that affect the microbiocenosis of the bird's digestive tract: antibiotics, symbiotics, prebiotics, and probiotics. Antibiotics as part of feed in most countries are no longer used in poultry diets (in the EU since 2006), as the resistance of pathogenic bacteria has increased and the number of beneficial normal flora is decreasing. As a result, antibiotics also accumulate in meat and can affect human microflora (Abd El Hack *et al.* 2020). Therefore, it is not the development of new antibiotics that is more relevant now, but the search for probiotic preparations. Probiotics usually include live microorganisms belonging to the normoflora. Probiotic preparations stimulate nonspecific immunity, act suppressive on pathogenic microflora, and contribute to the formation of normal microbiocenosis of the digestive tract. Once adding probiotics to the feed, the work of the gastrointestinal tract of the bird is corrected (Adhikari *et al.* 2019; BC *et al.* 2022). The effectiveness of the multifunctional feed additive

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Profort combines the qualities of an enzyme and a probiotic. It includes two strains of bacteria that synthesize lactate and vitamin B_{12} (Odefemi 2016). The drug promotes the breakdown of cellulose, hemicellulose, and pectin, and the resulting lactic acid provides regeneration of the intestinal epithelium and rapid recovery of antioxidants (Krysiak *et al.* 2021). Studies on broilers revealed that as the composition of the normoflora developed and the activity of pathogenic microflora upraised, the weight of the bird increased. The number of goblet cells in the mucous membrane of the blind processes declined, the length of the duodenal villi elevated, and the small intestine's overall enzymatic activity increased, all led to greater food digestion and assimilation (Stęczny & Kokoszyński 2020). The introduction of the biologically-active probiotic supplement into the diet of broiler chickens results in an increase in productivity of 9.1-13.4%, a decrease in mortality, an increase in livestock safety of 6.5%, and an increase in feed conversion. Vetlaktoflor is a liquid probiotic containing a strain of *Lactobacillus acidophylus* EP 317/402 "Narine" highly antagonistic to pathogenic microorganisms. *L. acidophylus* also inhibits the growth and reproduction of opportunistic microorganisms and stimulates the development of the normal flora of the bird's digestive tract with their secretions (Rehman *et al.* 2020). According to experiments, the commercial probiotic FloraMax-B11, which was based on inactivated strains of *L. acidophilus*, *Pediococcus acidilacticii*, and *Saccharomyces cerevisiae*, increased the live weight of cockerels by 7.1% compared to the birds in the control group and upraised the live weight of hens by 6.8% compared to the control. The body weight difference between males and females was 8.8%, according to the authors. It may be a result of the genetic potential that males were able to express more fully due to diets which were nutrient-optimized for better body growth (María *et al.* 2012). Evaluation of the effect of a native probiotic developed on the basis of *L. acidophilus* and *Bacillus subtilis* on the gastrointestinal tract of Ross 308 cross broilers showed an increase in the live weight of the bird (2710 g versus 2586.7 g), a decline in feed conversion (1.78 versus 1.92), and a reduction in the mortality rate (2.69% vs. 5.5%) compared to controls. Laboratory analyses did not reveal gastrointestinal parasites, *Escherichia coli*, *Eimeria* sp. and *Salmonella* sp. in the examined bird (González *et al.* 2016). Ortiz [2013], analyzing the effect of the probiotic ECOBIOL with the inclusion of *Bacillus amyloliquefaciens* CECT 5940, reported that the final weight of 2575 g was obtained 2.5 days earlier than in the control group. Feed conversion was 1.98 vs. 2.06, and mortality reduction was 6.28% vs. 6.77%. In this study, the effects of feeding broilers concentrated meals containing prescribed doses of probiotics and antibiotics that promote growth on productivity and gut health, were analyzed. Magnification, a variety of zootechnical characteristics, and carcass yield were measured. On days 21 and 42, we evaluated the morphometric characteristics of the visceral cavity organs of broilers. The length, width, and depth (in mL) of the crypt of the intestinal villi in broiler chickens on day 42 were also measured.

MATERIALS AND METHODS

Experimental Birds

The experimental part of the work was carried out in the Poultry Workshop of the Agricultural Engineering Career of the University of the Armed Forces ESPE, Ecuador, in the period from 2020 to 2022. Six hundred one-day-old unsexed broiler chicks (Ross 308 strain) were purchased from the local commercial company during the winter season. The chicks were divided into three groups with five replicates (40 chicks per group). Birds were raised in a deep litter system under the same management conditions. Continuous lighting was provided throughout the entire duration of the experiment. Feed and water were provided ad libitum. Vaccination was strictly adhered to as recommended for broiler chicks in the tropical environment.

Experimental diets

The experimental diets were formulated from local ingredients except for the imported super concentrate. The birds were allowed free access to feed and water during the rearing period, which was divided into two phases: starter (1–21 days) and finishing (2–42 days), in which the birds fed on starter and finisher diets, respectively. Group T_0 received balanced feed with growth stimulating antibiotic (Zinc Bacitracin; control); group T_1 received balanced diet (no growth stimulating antibiotic) + probiotic (*Bacillus subtilis*; 1.6 × 109 CFU g^{-1}) at an inclusion rate of 500 g ton^{-1}. Group T_2 was fed the basal balanced diet with a growth-stimulating antibiotic (Zinc Bacitracin) and a probiotic (*B. subtilis*). The formulation and calculation of the experimental diets (starter and finisher) were done according to the guidelines provided by (NRC 1994).

Data Collection

The reported minimum and maximum temperature and relative humidity were recorded throughout the experimental period to be (17.2 ºC–36.8 ºC and 15.2%–43.3%, respectively). At the end of the experiment (day

42), the birds were fasted overnight except for water. Two birds from each replicate were randomly selected, then their legs were banded and they were individually weighed. Birds were then manually slaughtered without stunning, scalded, and their feathers manually plucked and washed. Afterwards, the head was removed, and the feet as well as the shanks were dissociated at the hock joints. Evisceration was accomplished by ventral cuts for complete removal of viscera. Thereafter, weighing was performed on the internal organs, including the heart, liver, spleen, muscular stomach, glandular stomach, pancreas, goiter, gutted carcass, chicken breast, then we measured their lengths including small intestine, large intestine, cecum length, and bags of Fabric.

Morphometry of the intestinal villi of broiler chickens

Random selection of five birds from each experimental group (T_0, T_1, and T_2) samples from each experimental group consisted of a 3-cm duodenal loop section. Histopathological analysis and morphometry of the intestinal villi including length and width of the villi and the depth of the crypts (in mL) were also performed.

Statistical analysis

The statistical processing of the obtained data was carried out in accordance with the guidelines for the presentation of the results of measurement materials and algorithms for processing the material using the data analysis package "MS Excel 2010" and the program "Statistics for Windows" (Alabdallah *et al.* 2021).

RESULTS AND DISCUSSION

Mass of broiler chickens

Live body weight and carcass characteristics: Table 1 shows how adding probiotics and acidifiers to a diet affected broiler live body weight, net carcass weight, and dressing percentage (Adhikari *et al.* 2019; Abd El Hack *et al.* 2020). The outcomes reveal a difference between the control and other treatment groups that is statistically significant ($p < 0.05$). This outcome is consistent with the findings of (Mahajan *et al*. 1999; Kabir *et al.* 2004; Awad *et al.* 2009; Odefemi 2016; Rehman *et al.* 2020; Stęczny & Kokoszyński 2020; Reuben *et al.* 2021), who found a significant impact of probiotic supplementation on broiler performance and carcass yield. This outcome conflicts with the findings of (Maiorka *et al.* 2001; Corrêa *et al.* 2003; Malik *et al.* 2018; Nam *et al.* 2022) who found no appreciable impact on broiler performance or carcass yield in response to probiotic supplementation. These findings conflict with those of (Adil *et al.* 2011), who claimed that there were no discernible differences between different treatments in terms of the carcass features of broiler chickens fed diets supplemented with organic acids ($p > 0.05$). Nevertheless, (Kabir *et al.* 2004) and (Falaki *et al.* 2010) discovered that probiotics enhance carcass yield significantly ($p < 0.05$) in both vaccinated and unvaccinated broiler chicks.

Table 1. Weight of broiler chickens on different days (g).

Age, days:	Groups		
	T_0	T_1	T_2
1	50.5 ± 0.307	51.27 ± 0.463	50.66 ± 0.682
7	204.2 ± 3.476	205.2 ± 2.958	209 ± 3.037*
14	387.14 ± 9.387	396.35 ± 5.161*	391.46 ± 3.177
21	993.9 ± 12.072*	1013.8 ± 7.077	1032.06 ± 5.958
28	1578.6 ± 15.717*	1699.9 ± 16.056	1718.6 ± 10.183
35	2329.51 ± 25.767*	2482.06 ± 8.566	2462 ± 15.821
42	3043.6 ± 18.159*	3170.6 ± 22.493	3161.2 ± 31.856

Table 2. Broiler live body weight, net carcass, and dressing percentage in different treatments.

Parameters	Treatments		
	T_0	T_1	T_2
Live Weight (g)	3043.6 ± 18.159	3170.6 ± 22.493*	3161.2 ± 31.856
Net Carcass (g)	2558.4 ± 74.77	2654.4 ± 87.93*	2490.8 ± 46.56
Dressing (%)	84.06	83.72	78.79*

Morphometry of the organs of the visceral cavity of broiler chickens

Internal organs. The relative weight of internal organs (heart, liver, spleen, gizzard, glandular stomach, pancreas, and goiter) in the treated groups of broilers is presented in Table 3. The results demonstrated a significant ($p < 0.05$) difference among the different experimental groups. This indicated that the inclusion of probiotics and acidifiers and their combination had an effect on the weight of the internal organs. This result is in agreement with (Al Sultan *et al.* 2016; Stęczny & Kokoszyński 2020; Reuben *et al.* 2021) while is not in agreement with (Zamanzad *et al.* 2011; Odefemi 2016; Malik *et al.* 2018; Rehman *et al.* 2020; Krysiak *et al.* 2021; Nam *et al.* 2022) who found that the edible inner organs (liver and abdominal fat) were not affected by probiotic supplementation. However, (Mahajan *et al.* 1999) reported that the mean values of giblets are significantly higher in probiotic-fed broilers.

Table 3. Weight of internal organs of broilers treated groups.

Age, days	Parameters	Groups		
		T0	T1	T2
21	Mass of heart (g)	5 ± 0.32	5.6 ± 0.4	6.4 ± 0.51
42		16.2 ± 0.92	21 ± 0.55*	19.8 ± 0.97
21	Weight of liver (g)	23.2 ± 1.39	22.6 ± 0.68	24.6 ± 1.08
42		71.8 ± 5.11	79.6 ± 1.5*	76 ± 6.23
21	Mass of the spleen (g)	1 ± 0.0	1 ± 0.0	1 ± 0.0
42		3.6 ± 1.14	4.2 ± 1.1	5 ± 0.71*
21	Mass of muscular stomach (g)	16.8 ± 1.16	21.4 ± 0.81	21.8 ± 1.36*
42		52 ± 1.58	59.4 ± 3.87*	58.4 ± 4.8
21	Mass of the glandular stomach (g)	4 ± 0.32	4.2 ± 0.37	4.4 ± 0.24*
42		10 ± 0.32	12.6 ± 0.4*	12 ± 0.89
21	Pancreas weight (g)	2.8 ± 0.2	3.2 ± 0.2	3.4 ± 0.24*
42		7.2 ± 0.86	8 ± 0.63	8 ± 0.84
21	Goiter weight (g)	6.8 ± 0.73	6.4 ± 0.81	7.8 ± 0.37*
42		15.6 ± 0.93	20 ± 1.38*	17.2 ± 0.92

Relative bowel length (small intestinal length, large intestinal length, caecum length, and fabric bag length) of broilers treated groups was presented in Table 4. The addition of acidifiers affects the intestinal length, however, this result is not in agreement with (Malik *et al.* 2018; Reuben *et al.* 2021; Nam *et al.* 2022), albeit is in agreement with what has been reported by (Denli *et al.* 2003; Adil *et al.* 2011; Stęczny & Kokoszyński 2020), who illustrated that chicks fed diets supplemented with organic acids exhibited a significant increase in the length and weight of the small intestine when compared to the control group. This might be attributed to the fact that organic acids have a direct stimulatory effect on gastrointestinal cell proliferation.

Table 4. Relative bowel length of broilers treated groups.

Age, days	Parameters	Groups		
		T0	T1	T2
21	Small Intestine Length (cm)	158.2 ± 4.79	138 ± 4.3	153.5 ± 3.1
42		218.4 ± 6.	243.8 ± 6.92	234 ± 4.46
21	Large Intestine Length (cm)	5.28 ± 0.19	6.4 ± 0.43	6.8 ± 0.64
42		8.9 ± 0.81	10.22 ± 0.95	11.1 ± 0.64
21	Caecum Length (cm)	11.7 ± 0.7	12.8 ± 0.8	12.14 ± 0.6
42		21.6 ± 1.29	25 ± 0.63	23 ± 1.18
21	Fabric Bag Length (cm)	1.9 ± 0.04	2.3 ± 0.16	2 ± 0.04
42		2.5 ± 0.11	3 ± 0.05	2.4 ± 0.14
42	weight of eviscerated carcass (g)	2558.4 ± 74.77	2654.4 ± 87.93	2490.8 ± 46.56
42	weight of chicken breast (g)	899.2 ± 30.63	919.6 ± 45.41*	887.8 ± 27.19

The relative weight of a broiler's breast is shown in Table 4. The results demonstrated a significant ($p < 0.05$) difference in breast weight among the experimental groups. Birds fed a probiotic-supplemented diet showed significantly ($p < 0.05$) heavier breasts versus those fed a diet supplemented with acidifiers. However, birds fed the control diet showed no significant ($p > 0.05$) difference when compared to other dietary treatments. This result is not in agreement with (Odefemi 2016; Rehman *et al.* 2020), however, is in agreement with what has been reported by (Kabir *et al.* 2004; Malik *et al.* 2018; Nam *et al.* 2022). Breast is one of the most important economic primal cuts in chickens; the increase in this cut might be due to the great retention of nutrients caused by probiotics. This result is in contrast to (Zamanzad *et al.* 2011), who noted that the probiotic-supplemented group has a higher breast percentage compared to the control group. Intestinal villus morphometry, shown in Table 5, moreover, food modifications affected the villi of the small intestine's histomorphological parameters. The ratio of villus height to crypt depth and villus height in the duodenum and ileum were both raised ($p < 0.05$) by the addition of either probiotics or synbiotics (Fig. 1). The depth of the duodenal crypt was unaltered ($p > 0.05$; Fig. 2). However, nutritional supplements reduced the depth of the ileal crypt when compared to the control, which is consistent with [30]'s reports (Fig. 3).

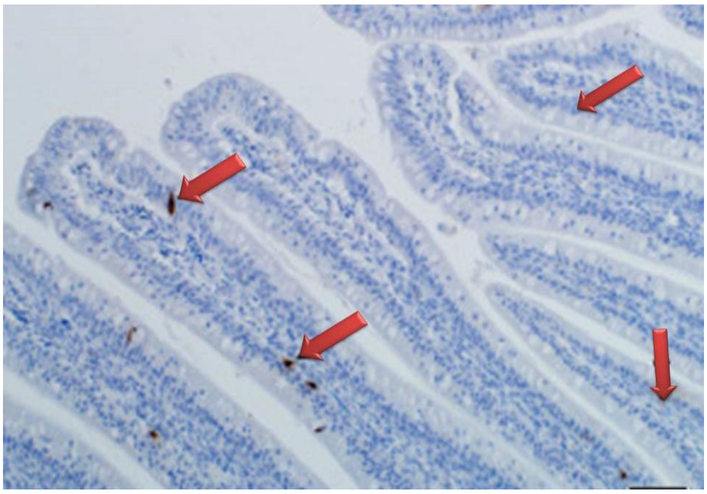

Fig. 1. Light photomicrograph of the duodenum in a broiler chick 42 days showing the epithelia of villi (V).

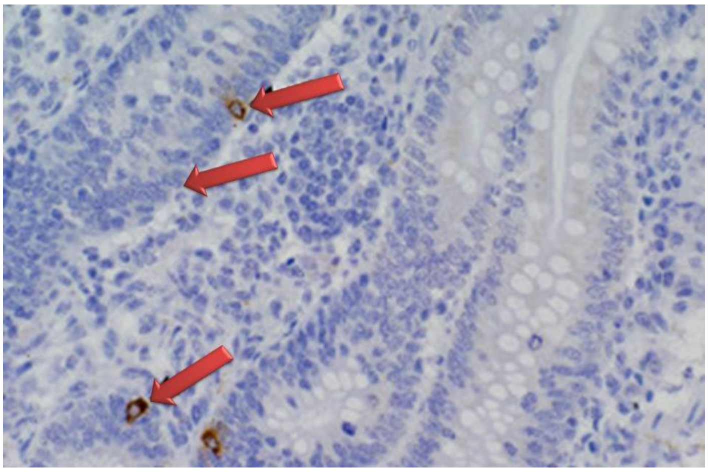

Fig. 2. Light photomicrograph of the duodenum in a broiler chick 42 days showing the epithelial lining of intestinal crypts.

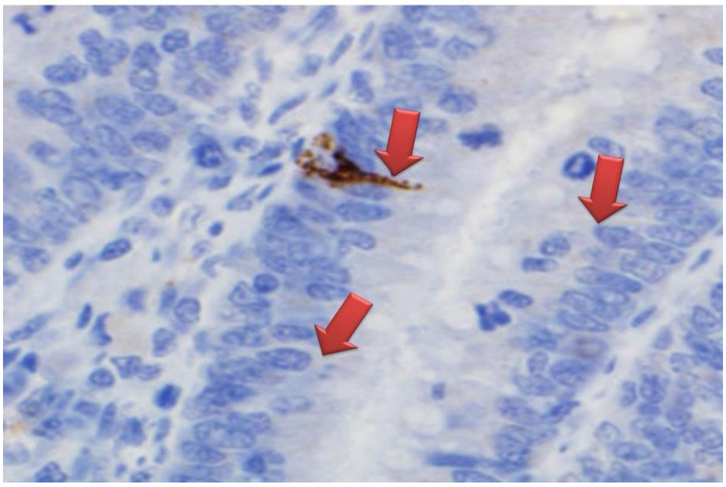

Fig. 3. Longitudinal section of the ileum in a broiler chick 42 days showing a long apical cytoplasmic process extending toward the lumen of the intestinal crypt.

Table 5. Morphometry of intestinal villi in broiler chicks (Day 42).

Groups	Morphometry of intestinal villi (Day 42)		
	Length	Width	Depth
T0	3425.9 ± 364.42	3425.9 ± 364.42	752.4 ± 59.47
T1	3654.5 ± 251.22	3654.5 ± 251.22	1125.6 ± 365.40*
T2	5235.6 ± 1784.55*	5235.6 ± 1784.56*	656.9 ± 62.49

Broiler growth boosters, either synbiotics or probiotics, worked better. Moreover, the broilers' intestinal mucosa's villus height and crypt depth increased as a result of the nutritional supplements. Improvements in growth performance for both synbiotics and probiotics were correlated with increases in villus height and the villus height/crypt depth ratio. This suggests that synbiotics and probiotics can be employed in broiler diets as a growth booster and can enhance gut health. As pressure to eliminate the use of growth-promoting antibiotics grows, these compounds exhibit encouraging effects as antibiotic replacements (Al Sultan *et al.* 2016).

CONCLUSION

Concluding, using probiotic preparations exhibited a positive effect on the body weight, total intestinal length, and length and diameters of intestinal segments in 42-day-old Ross 308 chickens. The probiotics had a significant ($p < 0.05$) effect on live body weight, net carcass, dressing percentage, weight of internal organs, total intestine length, and intestinal villus morphometry.

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