RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.2.443-449

Effect of probiotics and rice on ileal microbial count and intestinal morphometry in broiler chicken[#]

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Citation: Reshma, R., Chacko, B., Anitha, P., Prasoon, S. and Chandrasekhar, L. 2023. Effect of probiotics and rice on ileal microbial count and intestinal morphometry in broiler chicken. *J. Vet. Anim. Sci.* **54**(2):443-449
DOI: https://doi.org/10.51966/jvas.2023.54.2.443-449

Received : 10.11.2022 *Accepted : 14.02.2023* *Published: 30.06.2023*

Abstract

An experiment was conducted in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy to find out the effect of probiotics and rice on ileal microbial count and intestinal morphometry in broiler chicken for a period of 42 days. A total of 144, day-old Ven Cobb 430Y broiler chicks were allotted randomly to six dietary treatment groups with three replicates of eight birds each. The treatment groups were T1-standard broiler ration (SBR1), T2- SBR1 with 0.05 per cent probiotic mixture, T3- SBR1 with 0.1 per cent probiotic mixture, T4- 50 per cent of maize from SBR1 replaced by rice (SBR2), T5- SBR2 with 0.05 per cent probiotic mixture and T6-SBR2 with 0.1 per cent probiotic mixture. The dietary inclusion of rice did not affect the E. coli count and ileal pH. However the coliform count was significantly (p<0.01) lower in group fed rice based diet supplemented with 0.1 per cent probiotics and the E. faecalis count was significantly (p<0.01) higher in rice fed group without probiotic supplementation than corn fed group without probiotic supplementation. The probiotic supplementation at 0.1 per cent level significantly (p<0.01) reduced the E. coli and coliform counts in both rice and corn based diets. The E. faecalis count and ileal pH was significantly (p<0.01) reduced by probiotics at 0.05 and 0.1 per cent levels in corn based as well as rice based diets. The dietary inclusion of rice in broiler diet significantly increased (p<0.01) the ileal villi height in the non-supplemented group. The ileal VH:CD ratio was significantly (p<0.01) increased in rice fed groups compared to corn fed groups. The supplementation of probiotics in both rice and corn based diets significantly (p<0.01) increased the villi height of duodenum, jejunum and ileum and VH: CD ratio in jejunum and ileum. It could be concluded that the dietary supplementation of probiotics at 0.1 per cent level was effective in reducing the intestinal E. coli, coliform count, E. faecalis count, ileal pH and improving the intestinal histo-morphometry. Dietary replacement of 50 per cent corn by rice had no significant effect on these parameters.

[#] Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Keywords: *Rice, probiotics, microbial count, morphometry*

The gut microbiota and integrity of the gastrointestinal tract (GIT) are prime factors which affect nutrient absorption, disease resistance, development of immunity and growth performance (Diaz Carrasco *et al*., 2019). Gut microbiota composition can be altered by the usage of appropriate probiotics. Probiotics are live microorganisms which when administered either as single strain or as a combination, to a host in adequate amount will have positive effects (Kim *et al*., 2012). Probiotics affect the host primarily by the principle of competitive exclusion of pathogenic microorganisms and antagonistic activity against those organisms. In addition, probiotics have role in improving the epithelial barrier, producing anti-microbial substances, maintaining normal intestinal microflora and gut health and enhancing digestive enzyme activity (Fajardo *et al*., 2012).

Feed ingredients used in the diet also have effect on gut microbial composition and intestinal histomorphometry. In poultry industry, corn and soybean meal usually form the major component of broiler diets. Studies have shown that the corn starch is not properly utilised by young chicks. Therefore feedstuffs with high nutrient concentrations and availability should be incorporated when formulating pre-starter and starter diets to make up for the physiological constraints of the young birds. Corn may be replaced out by rice in broiler diets due to its high starch content and excellent quality starch. In comparison to corn, rice starch has smaller granules, lesser encapsulation and lesser amylose. As a result, rice starch may be more readily available for broilers than corn starch. Furthermore, rice has better digestibility than corn because it contains less non-starch polysaccharides (Nabizadeh *et al*., 2018).

The present study was conducted with the objective to assess the effect of inclusion of rice and probiotics in pre-starter and starter broiler diet on the gut microbiota and intestinal morphometry.

Materials and methods

The experiment was conducted in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy to find out the effect of probiotics and rice on ileal microbial count and intestinal morphometry in broiler chicken using 144, day-old commercial broiler chicks (Vencobb-430Y) from 0-42 days of age. All the chicks were wing banded, weighed individually and randomly allotted to six dietary treatment groups, each consisting of three replicates of eight birds each, in a completely randomised design.

The six dietary treatments in the trial were:

- T1- The standard broiler ration formulated using maize and soya bean meal as per BIS (2007) specifications (SBR1)
- T2- SBR1 supplemented with *probiotic mixture at 0.05 per cent level
- T3- SBR1 supplemented with *probiotic mixture at 0.1 per cent level
- T4- 50 per cent of maize from SBR1 replaced by rice (SBR2)
- T5- SBR2 supplemented with *probiotic mixture at 0.05 per cent level
- T6- SBR2 supplemented with *probiotic mixture at 0.1 per cent level

*Probiotic mixture – *Bacillus subtilis* and *Lactobacillus acidophilus* at 10^8 CFU/mL

The experimental diets were formulated and fed to the birds during pre-starter (0-7 days) and starter (8-21 days) phases only. During the finisher phase (22-42 days), the birds in all treatment groups were fed with similar finisher diet formulated using maize and soya bean meal. Feed and water were given *ad libitum*. Standard management practices were followed throughout the experiment. The feed ingredients and the feed samples were subjected to proximate analysis as per AOAC (2012).

Preparation of probiotic culture

Fresh live cultures of *B. subtilis* and *L. acidophilus* were suspended to 10 mL of brain heart infusion broth (BHIB) and then incubated at 37 ºC for 24 h. Using UV spectrophotometry, the optical density (OD) values of *B. subtilis*

Table 1. Ingredient composition of experimental and finisher feed, per cent

Ingredients	Pre-starter		Starter		Finisher
	SBR1	SBR2	SBR1	SBR2	
Yellow maize	51.90	26.00	52.80	26.40	57.10
Soya bean meal	41.50	40.85	39.20	38.50	33.60
Rice	-	26.00	-	26.40	-
Rice bran oil	2.64	3.23	4.06	4.76	5.10
Dicalcium phosphate	1.80	1.80	1.80	1.90	1.90
Calcite	1.40	1.40	1.40	1.30	1.40
Salt	0.35	0.35	0.36	0.39	0.37
L-Lysine[1]	0.24	0.22	0.18	0.17	0.26
DL- Methionine[2]	0.17	0.15	0.20	0.18	0.27
Total	100.00	100.00	100.00	100.00	100.00
Feed supplements (g/ 100 kg feed)					
Vitamin premix[3]	50.00	50.00	50.00	50.00	50.00
Toxin binder[4]	100.00	100.00	100.00	100.00	100.00
Anticoccidial[5]	100.00	100.00	100.00	100.00	100.00
Choline chloride[6]	200.00	200.00	200.00	200.00	200.00
Trace mineral mix[7]	100.00	100.00	100.00	100.00	100.00
Liver tonic[8]	50.00	50.00	50.00	50.00	50.00
Enzyme[9]	35.00	35.00	35.00	35.00	35.00

[1]L-Lysine (HCL) – Monohydrochloride 98.5% (Pro amino-L)

[2]DL-Methionine Feed grade 99% (Met amino-R)

[3]Vitamin premix: contents per g- vitamin A 82,500 IU, vitamin D3 12000 IU, vitamin B2 50 mg, vitamin K 10 mg, vitamin B1 4.0 mg, vitamin B6 8.0 mg, vitamin B12 40 mcg, niacin 60 mg, calcium panthothenate 40 mg, vitamin E 40 mg.

[4]Toxin binder: mixture of silicates, cross linked insoluble poly vinyl pyrrolidone homo polymer, mannan oligosaccharides and yeast cell wall extracts, activated charcoal, XMB factors, multiple organic acids, lipotropic factors.

[5]Coxiril, 0.5 per cent: micro granulate (diclazuril)

[6]Choline chloride, 60 per cent: each kg of choline chloride dry, 60 per cent powder contains a minimum 600 g of choline chloride.

[7]Trace mineral mixture: each kg contains manganese 100 g, zinc 85g, iron 90 g, copper 15 g, iodine 1.8 g, selenium 0.45 g, organic chromium 0.15 g.

[8]Liver tonic: a blend of choline chloride, tri chlorine citrate, DL- methionine, protein hydrolysate, live yeast, liver extract, iron, zinc, copper, vitamin E, selenium, methyl donors in a base of liver stimulant herbs

[9]Enzyme: a blend of xylanase, alpha-amylase, glucanase, cellulase, pectinase, mannanase, lipase, protease, phytase

and *L. acidophilus* cultures were determined at 525 nm wavelength. An OD value of 1.25 for *B. subtilis* and 0.886 for *L. acidophilus* culture broth at 7th dilution was found to have a concentration of 4×10^8 CFU/mL and 6×10^8 CFU/mL respectively. Fresh cultures were prepared by inoculating the bacterial colonies in BHIB and incubating at 37 ºC for 24h. The OD values were taken at 525 nm and serial dilutions were made up to 10^{-7} and the seventh dilution was mixed with feed on daily basis.

Microbial count and pH of ileum

Fresh samples of ileal content were collected from slaughtered birds at 42nd day of age and processed on the same day of collection. 0.1 mL inoculum from fixed dilution was transferred onto duplicate plates of Eosin Methylene Blue (EMB) Agar for *E. coli*, Violet Red Bile Agar (VRBA) for coliform, and Bile Esculin Agar for *E. faecalis* count and incubated at 37°C for 24 to 48 hours. The number of organisms was expressed as $\log_{10}$ CFU/mL. The ileal pH was measured using the protocol described by Izat *et al*. (1990).

Intestinal morphometry

The duodenal, jejunal and ileal segments of approximately 3 cm length were collected and fixed in 10 per cent neutral-buffered formalin for 48 hours. Cross sections of the segments were prepared according to the method described by Gridley (1960).

The statistical analysis of data was done using SPSS (version 24.0)

Results and discussion

Microbial count and pH of ileum

The data on effects of rice and probiotics on ileal microbial count and pH of broilers are presented in Table 2. The dietary inclusion of rice did not affect the *E. coli* count. However, probiotic supplementation at 0.1 per cent level significantly (p<0.01) reduced the count both in rice and corn based diets. Coliform count was lower in rice based diet with 0.1 per cent probiotic supplementation than corn based diet with 0.1 per cent probiotic supplementation. Also, probiotic supplementation at 0.1 per cent level significantly (p<0.01) reduced the counts in both rice and corn based diets. The rice fed group (T4) had significantly (p<0.01) higher *E. faecalis* count than corn fed group (T1). However, probiotic supplementation at 0.05 and 0.1 per cent levels significantly (p<0.01) reduced the counts both in rice and corn based diets. The dietary inclusion of rice did not affect the pH value. However, probiotic supplementation at 0.05 and 0.1 per cent levels significantly (p<0.01) reduced the pH values in both rice and corn based diets. These findings agree with the results of Nabizadeh *et al*. (2018) who included rice in broiler diet at 6, 12 and 18 per cent levels. The ileal *E. faecalis* count was higher in rice fed group compared to corn fed group. The alteration in gut microflora with respect to change in diet composition could be either due to change in physico-chemical properties, *viz.* viscosity and pH of digesta or the supply of nutrients for specific microflora (Nabizadeh *et al*., 2018). Contrary to the present findings, Alvarado *et al*. (2008) who included 61 per cent rice in broiler diet observed a lower ileal and jejunal pH than control and the authors correlated higher pH in corn based diet with higher fibre content.

Supplementation of probiotics at 0.1 per cent level in both corn and rice fed groups significantly (p<0.01) lowered *E. coli* and coliform counts in broilers. The *E. faecalis* count was lower in broilers supplemented with probiotics at 0.05 and 0.1 per cent levels in both

Table 2. Mean (±SE) count of *E. coli*, coliform, *E. faecalis* ($\log_{10}$CFU/mL) and pH of the ileal content of broilers in different dietary treatments

Particulars	Treatments						p-value
	T1 SBR1	T2 SBR1+ probiotics at 0.05%	T3 SBR1+ probiotics at 0.1 %	T4 SBR2	T5 SBR2 + probiotics at 0.05 %	T6 SBR2 + probiotics at 0.1%	
E. coli	7.10^{bcd} ±0.10	6.74^{abc} ± 0.22	6.24^{a} ± 0.31	7.75^{d} ±0.15	7.18^{bcd} ± 0.09	6.45^{ab} ±0.27	0.001
Coliform	7.10^{c} ± 0.10	6.59^{c} ± 0.37	5.24^{b} ± 0.21	7.42^{c} ± 0.06	7.27^{c} ± 0.09	3.86^{a} ± 0.43	0.001
E. faecalis	7.10^{b} ± 0.10	6.36^{a} ± 0.06	5.83^{a} ± 0.38	7.87^{c} ± 0.01	6.85^{a} ± 0.08	5.77^{a} ± 0.39	0.003
pH	6.82^{b} ±0.02	6.55^{a} ± 0.01	6.61^{a} ± 0.03	6.84^{b} ±0.02	6.63^{a} ± 0.01	6.55^{a} ± 0.03	0.001

Mean values bearing different superscripts within a row differ significantly (p<0.01)

Table 3. Mean (±SE) Histomorphometry of small intestine of broilers in different dietary treatments at six weeks of age, µm

Parameters	Treatments						P-value
	T1 SBR1	**T2 SBR1+ probiotics at 0.05 %**	**T3 SBR1+ probiotics at 0.1 %**	**T4 SBR2**	**T5 SBR2+ probiotics at 0.05 %**	**T6 SBR2+ probiotics at 0.1 %**	
Villus height							
Duodenum	876.33[a] ±2.12	907.11[ab] ±1.82	972.72[bc] ±62.43	886.85[ab] ±3.40	1014.25[c] ±15.94	1018.49[c] ±26.98	0.01
Jejunum	831.89[a] ±1.91	928.85[b] ± 5.92	1021.55[c] ±29.11	841.27[a] ±0.84	953.22[b] ± 13.82	1000.17[c] ± 5.91	0.001
Ileum	854.11[a] ±21.92	980.37[c] ± 9.77	1023.04[cd] ±17.10	922.04[b] ±12.06	1025.58[cd] ±15.88	1052.80[d] ±21.78	0.001
Crypt depth							
Duodenum	132.01 ± 1.46	126.83 ±12.14	136.00 ± 2.64	127.46 ± 0.57	129.42 ± 1.77	145.96 ± 3.48	0.19
Jejunum	142.86 ±1.39	143.68 ± 6.31	137.27 ± 2.88	139.88 ± 2.98	133.59 ± 6.07	135.91 ± 2.70	0.50
Ileum	120.11[a] ± 0.70	159.85[c] ± 1.57	134.58[b] ± 3.25	115.47[a] ± 0.58	133.10[b] ± 0.27	135.58[b] ± 3.66	0.001
Villus height to crypt depth ratio							
Duodenum	6.64 ± 0.08	7.29 ± 0.75	7.14 ± 0.31	6.95 ± 0.02	7.84 ± 0.23	6.98 ± 0.28	0.36
Jejunum	5.82[a] ± 0.06	6.49[ab] ± 0.30	7.45[c] ± 0.36	6.01[a] ± 0.12	7.17[bc] ± 0.42	7.36[bc] ± 0.18	0.004
Ileum	7.11[b] ± 0.21	6.13[a] ± 0.07	7.61[bc] ± 0.22	7.98[c] ± 0.13	7.70[bc] ± 0.10	7.77[c] ± 0.30	0.001

Mean values bearing different superscripts within a row differ significantly ($p<0.01$) ns non-significant

corn and rice fed groups. The present findings are in accordance with the result of Kim *et al*. (2012) who supplemented of *L. acidophilus*, *B. subtilis* and *S. cerevisiae* at 1×10^9 CFU/kg in broiler diet. Probiotics have the ability to inhibit the adhesion of pathogenic bacteria such as *E. coli* due to competitive binding to the host receptors and the stimulation of mucin production (Fajardo *et al*., 2012).

The supplementation of probiotics both in corn and rice based diets resulted in lower ileal pH. The present findings are in accordance with the result of Supriya *et al*., (2022) who supplemented *B. subtilis*, *B. licheniformis*, *B. amyloliquifaciens*, *L. acidophilus*, *P. acidilactici* and *E. faecium* at dose rate of 1.5×10^8 CFU/kg of feed. The lower intestinal pH in probiotic group might be due to beneficial bacteria, which produced lactic acid and acetic acid (Dhama *et al*., 2011). The effect of probiotic differs depending on factors such as the bacterial strains used, dose rate and growth period of experimental animals.

Histomorphometry of duodenum, jejunum and ileum

Table 3 summarises the effect of rice and probiotics on histomorphometry of intestine. The villus height, crypt depth and villi height: crypt depth ratio (VH:CD) of the small intestine could be the best indicators of intestinal development. The higher villi height indicates higher area of absorption of nutrients (Nabizadeh *et al*., 2018).

The dietary inclusion of rice did not affect the villi height in duodenum and jejunum. Whereas, probiotic supplementation at 0.05 and 0.1 per cent levels in both rice and corn based diets significantly ($p<0.01$) increased the villi height. The rice fed group (T4) had significantly ($p<0.01$) higher villi height of ileum than corn fed group (T1). Similarly, supplementation of probiotics at 0.05 and 0.1 per cent levels in both rice and corn based diets significantly ($p<0.01$) increased the villi height.

There was no significant difference in the crypt depth of duodenum and jejunum of broilers in different treatment groups. The dietary inclusion of rice did not affect the crypt depth of ileum. Whereas, probiotic supplementation at 0.05 and 0.1 per cent levels in both rice and corn based diets significantly ($p<0.01$) increased the crypt depth.

There was no significant difference in the VH:CD ratio of duodenum of birds in different dietary treatments. The dietary inclusion of rice did not affect the ratio in jejunum. Whereas, probiotic supplementation at 0.05 and 0.1 per cent levels significantly ($p<0.01$) increased the ratio in both corn and rice based diets. The VH:CD ratio of ileum was significantly ($p<0.01$) higher in rice fed group (T4) than corn fed group (T1). The probiotics had no effect on the ratio in corn based diet. However supplementation of probiotics at 0.05 per cent resulted in lower ($p<0.01$) ratio in corn based diet.

The result of the present study indicated that the ileal villus height and villus height to crypt depth ratio was higher in rice fed group than corn fed group. In accordance with the result of the present study, Sittiya *et al*. (2015) observed significantly higher VH:CD in broilers fed with 50 per cent whole grain paddy rice and Nabizadeh *et al*. (2018) observed a significant increase in ileal VH:CD ratio and villi height of duodenum, jejunum and ileum in broilers fed rice at 18 per cent level.

In the present study, supplementation of probiotics in both corn and rice based diets in broilers improved the villi height of duodenum, jejunum and ileum and VH:CD ratio of jejunum and ileum. The present findings are in agreement with the results of Sen *et al*. (2012) who recorded higher villus height and VH:CD ratio of duodenum and ileum of broilers supplemented *B. subtilis* LS 1-2 at levels of 0.15, 0.30 and 0.45 per cents. According to Supriya *et al*. (2022) the broilers fed diet supplemented with *B. subtilis, B. licheniformis*, *B. amyloliquifaciens*, *L. acidophilus*, *P. acidilactici* and *E. faecium* at 1.5×10^{8} CFU/kg feed level lead to higher villi height, crypt depth and their ratio which agrees with the present study. The improvement in histomorphometric parameters of intestine might be due to increased short chain fatty acid production by probiotics which stimulate the proliferation of intestinal epithelial cells (Gunal *et al*., 2006).

Conclusion

It could be concluded that the replacement of 50 per cent corn by rice did not affect the intestinal *E. coli* and coliform count and ileal pH. The supplementation of broiler diet with probiotics at 0.1 per cent level is effective in reducing pathogenic organisms and improving intestinal morphology.

Acknowledgement

The authors are very thankful to the Dean of College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala for providing all necessary facilities and financial support for the research.

Conflict of interest

The authors report no conflict of interest.

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