Research Article

Effects of Feeding Multi-Strain Probiotics and Multi-Enzymes to Broilers on Growth Performance, Intestinal Morphology and Cost Effectiveness of Production

Anguara Khatun[1]*, Sachchidananda Das Chowdhury[1], Bibek Chandra Roy[2], S.M. Shafikul Gani[3], Bipul Chandra Ray[1], Tanvir Ahmed[1]

[1]Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [2]Central Poultry Farm, Mirpur, Dhaka, Bangladesh; [3]SMG Animal Health Co. Ltd., Dhaka, Bangladesh.

Abstract | The experiment was carried out to investigate the effects of feeding multi-strain probiotics and multi-enzymes on growth performance, intestinal morphology, and cost-effectiveness in commercial broiler production. Four hundred eighty Cobb 500 straight run broiler chicks were randomly allocated to three dietary treatments each of eight replications having 20 birds each. The basal diets were corn-soya broiler starter and broiler grower diets. Starter diet was fed up to 21 days and grower diet during 22-35 days. In treatment 1, chicks were fed a basal diet (Diet 1), in treatment 2, chicks received a basal diet plus a multi-strain probiotics @100g/100kg feed (Diet 2) and in the third treatment, the basal diet was supplemented with multi-enzymes @100g/100kg feed (Diet 3). The birds were reared following identical care and management. Growth performance data were recorded weekly. Samples of intestinal segments were collected and examined for intestinal morphology at the end of the feeding trial (35 days). Data were analyzed using SAS Computer Package Program (version 9.1, SAS, 2009). Results showed that the addition of multi-strain probiotics and multi-enzymes to diets independently improved body weight, feed conversion ratio, and decreased feed intake as compared to control. Multi-strain probiotics significantly increased duodenum villi height, width and reduced crypt depth ($p<0.01$). The jejunum and ileum villi height and width were significantly increased in the multi-enzymes supplemented group but showed a reduction in crypt depth ($p<0.01$). Feeding multi-strain probiotics to broilers were more cost-effective. In conclusion, multi-strain probiotics and multi enzymes in diet enhances growth performance, improves feed efficiency, intestinal morphology, and increases profit.

Keywords | Probiotics, Enzymes, Broiler performance, Morphology, Cost-benefit

Received | August 01, 2021; **Accepted** | December 09, 2021; **Published** | January 10, 2022
***Correspondence** | Anguara Khatun, Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; **Email:** anjuarabau@gmail.com
Citation | Khatun A, Chowdhury SD, Roy BC, Gani SMS, Ray BC, Ahmed T (2022). Effects of feeding multi-strain probiotics and multi-enzymes to broilers on growth performance, intestinal morphology and cost effectiveness of production. Adv. Anim. Vet. Sci. 10(2): 389-396.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.2.389.396
ISSN (Online) | 2307-8316

INTRODUCTION

A number of feed additives are being considered for inclusion in poultry ration with the objectives of enhancing feed quality, growth performance, palatability and productivity, as well as for preventing animals from different kinds of environmental stresses (McDonald et al., 2010). Probiotics, phytobiotics and enzymes are manufactured globally not only to enhance productivity but also to explore their advantages as viable alternatives to antibiotics (Chahal et al., 2008). The notable advantage of probiotics is that it does not keep any residue in food products of animal origin and exerts no resistance against antibiotics. The promising effects of both probiotics and enzymes for improving birds' performance is well documented (Chowdhury et al., 2020; Kirkpinar et al., 2018).

Probiotics are 'direct fed beneficial' live microorganisms comprised of either single or multi-strains bacteria/yeast. Well known probiotics contain spore and non-spore forming bacteria like *Bacillus subtilis, Bacillus licheniformis, Saccharomyces cerevisiae, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacteriumbifidum,* and *Streptococcus thermophilus*. Reports generated from research stated that multi-strain probiotics are more potential than single strain probiotics and are able to enhance growth performance, better feed efficiency, and healthy gut in chicken by stabilizing the intestinal microflora (Yirga, 2015; Mountzouris et al., 2010).

Following ingestion of probiotics, the useful bacteria start colonization and multiplication in the environment of the intestine. Probiotics block the receptor sites thereby preventing the attachment of harmful bacteria such as *E. coli* and/or *Salmonella*. Probiotics decrease the risk of infections and intestinal disorders (Sartor, 2004). After establishment in the gut, the probiotic organisms produce some substances with bactericidal properties such as lactoferrin, organic acids, lysozyme, etc. (Tejero-Sarinena et al., 2012). These substances lower the intestinal pH. Lower pH reduces the secretion of hydrochloric acid in the gut. By competitive exclusion (CE) method, the number of pathogenic microorganisms is decreased due to competition for energy and nutrients between beneficial and harmful pathogenic bacteria (Dhama and Singh, 2010). Supplementation of beneficial bacteria in the diet alters the gut environment, improve birds' immune system and increases the number of favorable bacteria by multiplication (Lee et al., 2010). It has been reported that broiler performance, gut microbiota modulation, pathogen suppression, and immunomodulation have all been shown to benefit from probiotic species (Higgins et al., 2007; Khaksefidi and Ghoorchi, 2006).

Proteins that operate as biological catalysts are known as enzymes. Catalysts help to speed up chemical reactions. Substrates are the molecules on which enzymes can function, and the enzyme changes the substrates into various molecules called products (Stryer et al., 2002). Enzymes are manufactured by living microorganisms e.g. bacteria (*Bacillus lentus, B. subtilis, B. stearothermophils* and *B. amyloliquifaciens*), yeasts (*Sacharomyces cerevisiae*) and Fungi (*Asperigillusniger, A. oryzae* and *Triochoderma longibrachiatum*) (Wallis, 1996). Similar to probiotics, enzymes may be of either single or multi-enzymes. Multi-enzymes are a combination of different types of enzymes like xylanase, glucanase, protease, amylase, etc. Recently, nutritionists prefer to include exogenous enzymes in commercial broiler feeds (Alagawany et al., 2018). In cereal-based diets, the exogenous enzyme is claimed to break down non-starch polysaccharide (NSP) which is beneficial for improving utilization of nutrients, reducing the viscosity of gut content resulting in increased nutrient availability to poultry birds that contribute to an improvement in overall growth performance (Wang et al., 2005). In addition, supplementation of multi-enzymes in feed improves the environment of poultry shed by reducing nitrogen content in feces thus has less impact on the environment (Alagawany et al., 2018).

Current trends in broiler production are the use of multi-strain probiotics and multi-enzymes in the feed manufacturing process rather than single strain probiotic and single enzyme. To the authors' knowledge, experiments reported so far involving the comparative study of these two vital feed additives are limited. Moreover, economic data in relation to this study are not so extensive. Therefore, this study was designed to investigate the effects of feeding mostly available multi-strain probiotics and multi-enzymes independently to explore their effects on growth performance and intestinal morphology. The economic feasibility of such effects on productivity was also considered.

MATERIALS AND METHODS

Ethical approval

All protocols and experimental designs were approved by the Animal Welfare and Ethical Committee, Bangladesh Agricultural University, Mymensingh-2202. (No. AWEC/BAU/2021/20).

Experimental birds, site and house

A total of 480 days old Cobb 500 straight-run commercial broiler chicks were considered for this experiment. The experiment was conducted at Bangladesh Agricultural University Poultry Farm for a period of 35 days. Before the arrival of the chicks, the house was properly cleaned, washed, disinfected, and dried. Rice husk was used as litter materials at a depth of 5.0 cm. The area of each pen was approx. 28 square meters. Each pen was partitioned into 12 equal small pens by using a wire net and bamboo materials.

Experimental diet and management system

A corn-soya based mash diet was formulated to meet the nutrient requirements of Cobb -500 commercial broiler (Cobb Commercial Broiler Management Guide, 2012). The chicks were divided into three dietary treatments having eight sub-groups. Each group had 20 chicks. The first group received Diet 1: Basal diet (control), the second group was maintained on Diet 2: control diet supplemented with multi-strain probiotics (*Bacillus* spp., *Saccharomyces* spp., *Lactobacillus* spp.) @100g/100kg feed and the third group received Diet 3: control diet supplemented with multi-enzymes (amylase, protease, β- glucanase, xylanase, lipase,

and cellulase) @100g/100kg feed. Diets were offered to the experimental birds in two different phases, the first one was as broiler starter from 0- 21 days and the second one was as broiler grower from 22-35 days. The ingredients and nutrient composition of the diets are available in Table 1. After the arrival of day-old chicks, Vit-C enriched glucose saline was supplied for providing instant energy and reduce transportation stress. Brooding of chicks was carried out for only 7 days. The experimental chicks were exposed to 23 hr. of lighting and 1hr. dark period daily. Feeds and drinking water was supplied without any restriction by using tube feeder and round drinker, respectively. To reduce ammonia and other harmful gases as well as dampness, litter was stirred at least twice daily. Birds were vaccinated against Newcastle and Infectious bursal disease by Clone 30 and 228E, respectively. ND vaccine (Clone 30) was given on the 5th day and followed by a booster dose of same on 21st days of age. Infectious bursal disease vaccine (228E) was administered at 10 days and a booster dose at 17 days of age. All vaccines were collected from local representatives of Intervet International, BV, Boxmeer. The Netherlands and administered according to manufacturer's instructions. Proper biosecurity was maintained to prevent the outbreak of disease. All equipment in the experimental house was kept clean by using glutaraldehyde and ammonium chloride containing TH4^{+} disinfectant (Manufactured by THESEO, France).

Data collection and record-keeping

All growth performance (body weight, feed intake (FI), and feed conversion ratio (FCR)) were calculated at 7 days intervals. Temperature and humidity were recorded four times daily (7 AM, 12 AM, 5 PM, and 10 PM) by a digital thermo-hygrometer.

Morphological study

Sample collection

At the end of the trial, 6 broilers, two broilers from each treatment close to average weight were randomly taken, weighed and sacrificed for intestinal morphological study. From each part, 2cm length of the duodenum (at midpoint region of the duodenum), jejunum (midpoint between the bile duct entry and Meckel's diverticulum) and ileum (at the distal end of lower ileum) were cut to measure villus height, width, and crypt depth (Sharifi et al., 2012).

Sample preparation

Samples for morphological study of intestinal segments were prepared by following steps followed by Luna (1968). Collected tissue samples were flushed with fresh water and immediately preserved in a 10% neutral formaldehyde solution. Then 10% neutral buffered formalin was added to a plastic container (10 folds of the tissue size and weight) and the tissue was fixed for 5 days. The tissues were then trimmed into a thin section and washed overnight in running tape water to remove formalin. The tissues were dehydrated by a series of ascending ethanol (50, 70, 80, 95, and 100%) to prevent shrinkage. Impregnation was done in melted paraffin at 56-60°C for 3 hours. Then the tissues were sectioned with a microtome at 5-µm thickness. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The sections were allowed to spread on a warm water bath at 40-42°C. Then the sections were taken on grease-free clear slides. The slides containing sections were air-dried and kept in a cool place. The sectioned tissues were stained by eosin. The section was differentiated and dehydrated in 95% alcohol. Then, tissues were mounted with a coverslip by using DPX. The slides were dried at room temperature and examined under low (10X) and high (40X) power objectives.

Table 1: Ingredients and nutrient contents of control diet (kg/100kg).

Ingredients (%)	Starter diet (0-21 days)	Grower diet (22-35 days)
Corn	58.17	62.20
Soybean meal (44%)	28.00	25.75
Protein concentrate (62%)	8.00	5.75
Soybean oil	3.00	3.60
Limestone	1.20	1.00
Di calcium phosphate	0.40	0.50
Salt	0.25	0.30
Sodium-bi-carbonate	0.10	0.08
Broiler premix(1)	0.15	0.15
DL-Methionine	0.28	0.22
Blend acidifier(2)	0.15	0.15
L-lysine	0.10	0.10
Shark liver oil	0.10	0.10
Choline chloride-60	0.10	0.10
Nutrient composition (%)		
ME, kcal/kg	3000	3100
CP	22.0	20.0
Lysine	1.36	1.07
Cystine	0.40	0.31
Methionine	0.50	0.45
Methionine+cystine	0.90	0.76
Calcium	0.90	0.90
Available phosphorus	0.45	0.45

ME: Metabolizable energy; kcal: kilo calorie; kg: kilogram; CP: crude protein; protein concentrate: CP-62.30%, ME (kcal/kg)-2865 kcal/kg. (1)Broiler premix contained Vitamin A 12.50 MIU, vitamin D3 2.50 MIU, vitamin E 25g vitamin K 4g, Iron 24g, Zinc 40g, Manganese 48g, Selenium 0.12 g, and Cobalt 0.30g. (2) Blend acidifier** contained formic acid 65%, sodium 32%, silica 3%.

Image capture and villi measurement

The measurement for villus and crypts dimensions were carried out using an Olympus CX41 Laboratory Microscope (Serial no. 2031313, Olympus Corporation, Tokyo, Japan) at 40 x magnification. Pictures of villus and crypts were obtained with a video camera (JVC TK 99 1085E), connected to a monitor screen (Dell), and computer, with measurements made using the Spot Basic imaging software (Diagnostic Instruments, Inc., Sterling Height, MI). Fifteen well-oriented villi and crypts from the duodenum, ileum, and caecum were measured along with their length (height and depth, respectively) and width. The villus height (VH) was measured from the crypt-villus junction to the brush border at the tip. Villus width (VW) was measured parallel to the adjoining villus. The crypt depth (CD) was measured from the base near the lamina propria to the crypt-villus junction. The VH, VW, and CD were measured by Image J software.

Methods of economic analysis

Cost of production was calculated based on some specific items such as the cost of chicks, feed, vaccine, test materials, casual labor, and other inputs. However, the total production cost per bird and per kg broiler and profit earned were calculated.

Statistical analysis

Data of growth performance and intestinal morphology were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) employing statistical computer package program (SAS, 2009). Duncan's Multiple Range Test (DMRT) was performed to compare the differences in mean values.

RESULTS

Growth performance

The effect of feeding multi-strain probiotics and multi-enzymes on the growth performance of broilers is shown in Figure 1. At the end of 35 days, the highest final body weight (FBW) was found in multi-strain probiotics treated birds (1618.33g/bird). This was followed by broiler receiving multi-enzymes (1573.33g/bird) and control diet (1462.25 g/bird) respectively. An increase of 10.67% body weight was observed in the group fed multi-strain probiotic when compared with the control group. This was followed by a 7.6% increase in multi-enzymes treated birds. Weight differences (body weight and body weight gain) between birds fed multi-strain probiotics and multi-enzymes differed significantly from those receiving the control diet. There was no significant effect on FI due to supplementation of multi-strain probiotics and multi-enzymes. Lowest FCR (1.55) was found in multi-strain probiotics supplemented group whereas, multi-enzymes treated birds showed intermediate FCR (1.61) but lower than the control group (1.74). Although supplementation of multi-strain probiotics and multi-enzymes improved FCR, the difference between those two dietary treatments was not significant. Satisfactory survivability was noted in all dietary groups.

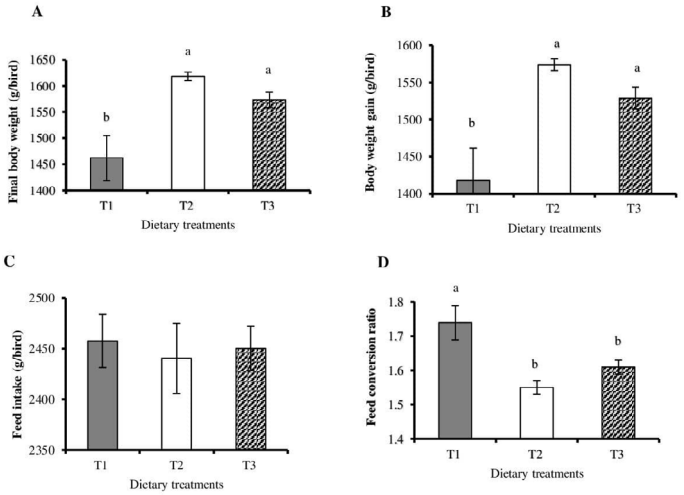

Figure 1: Growth performance of broiler chicken (0-35 days). T_1= Basal diet (control); T_2= Basal diet +multi strain probiotics (100g/100kg of feed); T_3= Basal diet + multi enzymes (100g/100kg of feed); Final body weight (A), body weight gain (B), feed intake (C) and feed conversion ratio (D). a, b = means bearing dissimilar superscript differ significantly ($p<0.05$). Bar indicates standard error of mean (SEM).

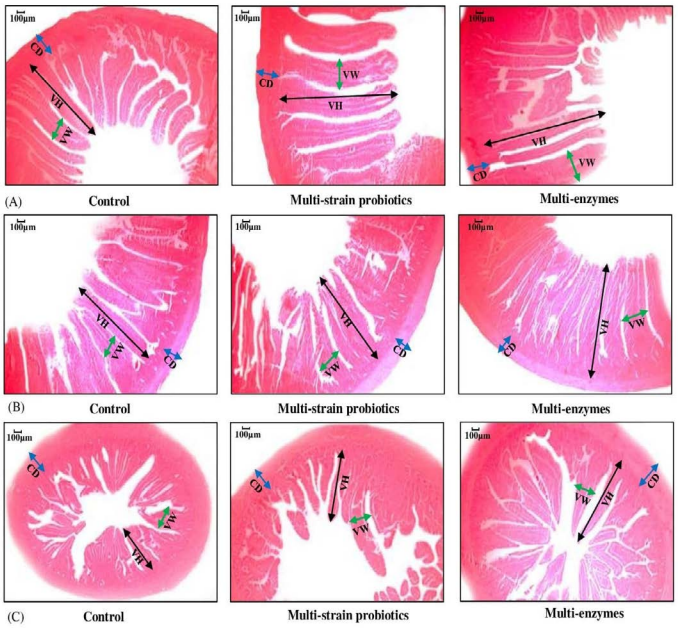

Figure 2: Morphological structure (40X) of intestine of birds receiving different diets. Duodenum (A), jejunum (B) and ileum (C). Control (basal diet), multi strain probiotics (100g/100kg of feed); multi enzymes (100g/100kg of feed); villi height (VH)↔; villi width (VW) ↔; crypt depth (CD) ↔.

Intestinal morphology

Results of morphological study of experimental birds are shown in Table 2 and Figure 2. VH of duodenum, jejunum, and ileum was significantly ($p<0.01$) increased in treated groups after the inclusion of multi-strain probiotics and multi-enzymes. In the duodenum, greater villus was observed in multi-strain probiotics and multi-enzymes treated group. In the jejunum, VH was significantly ($p<0.01$) improved in treated groups. The longest jejunum villus was observed in multi- enzymes treated birds and the shortest villus was found in multi-strain probiotics and control group. In the case of the ileum, VH also showed significant ($p<0.01$) variation among the treatments. In this segment, the tallest VH was seen in the multi-enzymes fed group and the shortest villi were found in the control group. In duodenum, jejunum, and ileum, VW demonstrated significant ($p<0.01$) difference among the dietary groups. The broaden VW was found in the duodenum segment of the multi-strain probiotics fed group and the lowest VW was observed in the ileum part of the control group.

Duodenum CD value also showed significant ($p<0.01$) differences among the dietary treatments. The deepest CD value was noticed in the control group that differed from multi-strain probiotics and multi-enzymes fed birds ($p<0.01$). In the jejunum, the CD value also revealed a significant ($p<0.01$) difference among the treatments. The tallest CD value was recorded in the control group and the shortest value was observed in the multi-enzymes supplemented group. Ileum data also revealed the lowest CD value.

Table 2: Effect of multi-strain probiotics and multi-enzymes on intestinal morphology.

Variables	Control	Multi-strain probiotics	Multi-enzymes	P-Value
Duodenum				
Villi height (µm)	$612.63^{c}\pm1.32$	$936.36^{a}\pm2.29$	$856.68^{b}\pm1.45$	0.01
Villi width(µm)	$79.08^{c}\pm1.73$	$192.43^{a}\pm1.09$	$140.51^{b}\pm2.92$	0.01
Crypt depth (µm)	$86.65^{a}\pm1.27$	$76.33^{b}\pm1.53$	$80.33^{b}\pm2.54$	0.01
Jejunum				
Villi height (µm)	$625.63^{b}\pm2.87$	$565.49^{c}\pm2.85$	$753.42^{a}\pm1.55$	0.01
Villi width (µm)	$168.63^{a}\pm2.25$	$157.66^{b}\pm1.93$	$151.59^{b}\pm1.69$	0.01
Crypt depth (µm)	$77.52^{a}\pm1.74$	$58.72^{b}\pm2.50$	$45.53^{c}\pm1.70$	0.01
Ileum				
Villi height (µm)	$326.76^{c}\pm1.23$	$341.71^{b}\pm1.78$	$532.57^{a}\pm1.86$	0.01
Villi width(µm)	$47.37^{c}\pm1.71$	$113.69^{b}\pm1.68$	$153.24^{a}\pm2.18$	0.01
Crypt depth (µm)	$59.55^{a}\pm1.71$	$32.62^{b}\pm1.63$	$26.47^{c}\pm1.23$	0.01

Control (basal diet), multi-strain probiotics (100g/100kg of feed); multi-enzymes (100g/100kg of feed), µm= micro meter. a, b, c= means bearing dissimilar superscript differ significantly ($p<0.01$). (±) indicates standard error of mean (SEM).

Table 3: Expenses of production and return over investment (ROI) in different dietary treatments.

Items	Control	Multi-strain probiotics	Multi-enzymes	p-values
(a) Feed cost (Tk./bird)	97.6±1.04	96.9±1.38	97.2±0.87	0.89
(b) Test materials cost[(1)] (Tk./bird)	0^{c}	$1.09^{a}\pm0.0$	$0.65^{b}\pm0.0$	0.01
(c) Chick cost (Tk./bird)	35.0	35.0	35.0	-
(d) Other costs (vaccines, litter, disinfectants, transport, labor etc.) (Tk./bird)	35.0	35.0	35.0	-
(e) Total cost (Tk./bird) (a+b+c+d)	167.6± 1.04	168.0±1.38	167.8±0.87	0.97
(f) Total cost (Tk./kg body weight)	$115.1^{a}\pm3.32$	$103.8^{b}\pm0.93$	$106.8^{b}\pm1.38$	0.05
(g) Sale price (Tk. /bird @ BDT 130/kg live wt.)	$190.1^{b}\pm5.60$	$210.4^{a}\pm1.06$	$204.5^{a}\pm1.94$	0.02
(h) Profit (Tk./bird) (g-e)	$22.5^{b}\pm5.30$	$42.4^{a}\pm1.59$	$36.7^{a}\pm2.50$	0.03
(i) Profit (Tk./kg)	$14.9^{b}\pm3.32$	$26.2^{a}\pm0.93$	$23.3^{a}\pm1.38$	0.05
(j) Profit over control (Tk./bird)	-	19.93	14.23	-
(j) Profit over control (Tk./kg)	-	11.29	8.38	-

[(1)]Multi-strain probiotics @ BDT Tk. 450/kg and multi-enzymes @ BDT Tk. 265/kg; Control (basal diet), multi-strain probiotics (100g/100kg of feed); multi-enzymes (100g/100kg of feed). BDT=Bangladeshi taka; a, b, c= means bearing dissimilar superscript differ significantly ($p<0.05$). (±) indicates standard error of mean (SEM).

Economic appraisal

The cost-benefit analysis of this experiment is shown in Table 3. The highest feed cost per bird was found in the control group and lowest in the multi-strain probiotics supplemented group. The total cost of production per kg live weight was highest in the control group which differed significantly ($p<0.05$) from multi-strain probiotics and multi-enzymes groups. The sale price per bird were more in multi-strain probiotics and multi-enzymes groups which showed significant ($p<0.05$) differences when compared to the control group. In terms of per bird and per kg body weight, the highest profits were obtained in the multi-strain probiotics group compared to the control group which also significantly differed ($p<0.05$). Multi-enzymes fed group also showed better profit but less than the multi-strain probiotics fed group.

DISCUSSION

Growth performance

The increased body weight in multi-strain probiotics the group fed might be due to the presence of beneficial bacteria that aided better digestion, absorption and utilization of dietary nutrients by creating a suitable environment in the intestine (Olukosi and Cowieson, 2007). Non-starch polysaccharide (NSP) was probably broken down due to the presence of some exogenous enzymes like xylanase, β-glucanase, mannanase, cellulase, etc. that helped in nutrient utilization and reduced gut viscosity and consequently the birds gained an increase in body weight. Using multi-strain probiotics (*Lactobacillus acidophilus, Bacillus subtilis)* in a broiler diet caused highest body weight compared to control (Zhang and Kim, 2014). In a recent study, it was found that the inclusion of *Saccharomyces* spp., *Lactobacillus* spp., and *Bacillus* spp., containing probiotics in the diet significantly enhanced body weight (Thorat et al., 2015), which is also in line with our results. In another study, it was reported that multi-strain probiotic (*Lactobacillus* spp., and *Bacillus* spp.) and multi-enzymes (amylase, xylanase, pectinase, cellulose and glucanase) supplemented diets significantly increased growth and gut health of broiler chickens (Hossain et al., 2015; Rosin et al., 2007; Vidyarthi et al., 2007), which coincided with our results.

Higher amount of feed was consumed by birds of the control group. A different strains of probiotics (*Lactobacillus* spp., *Bifidobacterium* spp., *Bacillus* spp.) and multi-enzymes (amylase, lipase, xylanase, etc.) might help in the multiplication of beneficial micro-flora in the gut and secreted additional enzymes in addition to endogenous enzymes which already established in the gut resulting in better digestion and utilization of nutrients and reduced feed wastage. Better digestion, absorption, and assimilation of nutrients might have occurred due to the combined effect of endogenous and exogenous enzymes. Zakaria et al. (2010) showed an evidence that protease, pectinage, amylase, containing multi-enzymes additives in the broiler diet reduced FI which coincided with our findings. Jin et al. (1996) reported that the inclusion of multi-strain probiotics (*Lactobacillus* spp. and *Bacillus subtilis*) in the diet increased the number of intestinal beneficial microflora and created a healthy gut environment and consequently decreased FI and improved feed efficiency. Improvement in FCR is a clear indication of the effects of multi-strain probiotics and multi-enzymes. Anjum et al. (2005) reported that multi-strain probiotics supplemented diet improved FCR but our result showed a similar trend although it was not significant. The findings of the present study are also in agreement with the results of other researchers (Khaksefidi and Rahimi, 2005) where they found that supplementation of *Lactobacillus* spp., *Bifidobacteriumbifidum, Streptococcus faecium* containing multi-strain probiotics in the diet significantly improved FCR. Multi-enzymes treated group showed better FCR in broilers as reported by several authors (Hossain et al., 2015; Swain et al., 2014) which were in agreement with our results. The survivability was more in multi-strain probiotics and multi-enzymes fed group as compared to control. The higher trends in survivability, although not significant, agreed well with previous studies (Olukosi and Cowieson, 2007; Cmiljanic et al., 2001).

Intestinal morphology

Application of multi-strain probiotics and multi-enzymes treated diets had positive effects on duodenal, jejunal, and ileal villi. Longer villi of ileum indicate higher absorption of amino acids vitamins and minerals that resulted in higher body weight of these groups. It has been reported that longer villus exhibits excellent gut health, maximum absorption, and good intestinal tract (Alfaro et al., 2007). A few authors (Salim et al., 2013; Sen et al., 2012) reported that the use of *Lactobacillus* spp. and *Bacillus* spp. containing probiotics in broiler chicken diet resulted in an increased intestinal VH which resembled our results. Our findings were similar to those obtained in another study (Balamurugan et al., 2011) where it was found that multi-enzymes (cellulase, xylanase, and pectinase) treated group significantly ($p<0.01$) increased VH compared to that of the control group.

Duodenum segment of multi-strain probiotics fed group showed extended VW. The formation of extended villus might be a result of greater nutrient retention in the body. Our results were similar to results of another study (Salim et al., 2013) who found taller VH in probiotics fed group. Researchers (Sen et al., 2012) showed that the widest villi were obtained due to the inclusion of multi-strain probiotics and multi-enzymes in diet which was consistent

with our results. Our findings also resembled the results of another study (Balamurugan et al., 2011) where it was found that villi width significantly increased in the multi-enzymes treated group. In multi-enzymes supplemented group, VH and VW were increased in a manner similar to our findings (Thavasiappan et al., 2016). The deepest CD value in the control group might have occurred due to poor nutrient digestion, absorption, and utilization of the gastrointestinal tract (Xu et al., 2003). On the other hand, multi-strain probiotics treated birds showed the shortest CD because probiotics helped in the digestion and absorption of nutrients properly. In our research, we used *Bacillus, Lactobacillus and Saccharomyces* containing multi-strain probiotics whereas Sen et al. (2012) used *Bacillus* spp. containing probiotics and found that it reduced CD in the duodenum, jejunum, and ileum segment of the intestine.

Economic appraisal

Although a number of mode of action of multi-strain probiotics and multi-enzymes are similar, supplementation of multi-strain probiotics supplemented group was more economic and multi-enzymes treated group also showed a better profit. The addition of multi-strain probiotics in the diet increased profit, similar to the findings of previous researchers (Anjum et al., 2005). A mixture of probiotics and a multi-enzyme (amylase, protease, cellulase, lipase containing enzymes) also showed a profit in a previous study (Swain and Chakarkur, 2009).

CONCLUSIONS AND RECOMMENDATIONS

Supplementation of multi-strain probiotics and multi-enzymes in the diet of broilers enhances growth performance and improves feed conversion by increasing villi length and villi width. Both multi-strain probiotics and multi-enzymes increases profit, but feeding the former was most cost-effective. It is therefore recommended that diets of broiler may be supplemented with multi-strain probiotics and multi-enzymes for good results'.

ACKNOWLEDGEMENTS

The research was conducted with the financial support of Grants for Advanced Research of the Ministry of Education, Government of Bangladesh and SMG Animal Health Co. Ltd, Dhaka. The authors would like to express gratitude to the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University for their support in preparing the intestinal samples.

NOVELTY STATEMENT

The results of the study is promising and therefore will help the Bangladesh poultry industry.

AUTHOR'S CONTRIBUTION

AK, SDC, BCR, BCR and SMSG designed and executed the experiment. All authors were involved with the preparation of initial draft and revision of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alagawany M, Elnesr SS, Farag MR (2018). The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. Iran. J. Vet. Res., 19(3): 157–164.

Alfaro DM, Silva AVF, Borges SA, Maiorka FA (2007). Use of Yucca schidigera extract in broiler diets and its effects on performance results obtained with different coccidiosis control methods. J. App. Poult. Res., 16(2): 248-254. https://doi.org/10.1093/japr/16.2.248

Anjum MI, Khan AG, Azim A, Afzal M (2005). Effect of dietary supplementation of multi-strain probiotic on broiler growth performance. Pak. Vet. J., 25(1): 25-29.

Balamurugan R, Chandrasekaran D, Kirubakaran A (2011). Effects of multi-enzyme supplementation on gut morphology and histomorphology in broilers. Ind. J. Sci. Technol., 4(1): 15-18. https://doi.org/10.17485/ijst/2011/v4i1.13

Chahal US, Niranjan PS, Kumar S (2008). Handbook of General Animal Nutrition. International Book Distributing Co., India.

Chowdhury SD, Ray BC, Khatun A, Redoy MR, Afsana AS (2020). Application of probiotics in commercial layer diets: A review. Bangladesh J. Anim. Sci., 49(1): 1-12. https://doi.org/10.3329/bjas.v49i1.49372

Cmiljanic R, Lukic M, Trenkovski S (2001). The effect of paciflorc probiotic on gain, feed conversion and mortality of fattening chicks. Biotec. Anim. Husb., 17: 33-38.

Cobb Breeder Management Guide (2012). Available from: https://www.cobb-vantress.com

Dhama K, Singh SD (2010). Probiotics improving poultry health and production: An overview. Poult. Punch., 26(3): 41-46.

Higgins JP, Higgins SE, Vicente JL, Wolfenden AD, Tellez G, Hargis BM (2007). Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. Poult. Sci., 86: 1662–1666. https://doi.org/10.1093/ps/86.8.1662

Hossain MM, Begum M, Kim IH (2015). Effect of Bacillus subtilis, Clostridium butyricumand Lactobacillus acidophilus endospores on growthperformance, nutrient digestibility, meat quality, relative organ weight, microbial shedding and excretanoxious gas emission in broilers. Vet. Med., 60(2): 77–86. https://doi.org/10.17221/7981-VETMED

Jin LZ, Ho YW, Abdullah N, Jalaludin S (1996). Influence of dried Bacillus subtilis and lactobacilli cultures on intestinal microflora and performance in broilers. Asian-Austral. J. Anim. Sci., 9(4): 397-404. https://doi.org/10.5713/ajas.1996.397

Khaksefidi A, Ghoorchi T (2006). Effect of probiotic on performance and immunocompetence in broiler chicks. J. Poult. Sci., 43: 296–300. https://doi.org/10.2141/jpsa.43.296

Khaksefidi A, Rahimi SH (2005). Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. Asian-Austral. J. Anim. Sci., 18(8): 1153-1156. https://doi.org/10.5713/ajas.2005.1153

Kirkpinar F, Açikgöz Z, Mert S, Işik O (2018). Effects of dietary probiotic, prebiotic and enzyme mixture supplementation on performance, carcase, organs, ileal ph and viscosity of broilers. J. Anim. Prod., 59(2): 1-9. https://doi.org/10.29185/hayuretim.469862

Lee K, Lillehoj HS, Siragusa GR (2010). Direct-fed microbials and their impacts on the intestinal microflora and immune system of chickens. J. Poult. Sci., 47(2): 106-114. https://doi.org/10.2141/jpsa.009096

Luna LG (1968). In: Manual of histologic staining methods of the armed forces institute of pathology. 3rd ed. McGraw Hill Book Co., New York, USA.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG (2010). Animal Nutrition. 7th ed. Prentice Hall.

Mountzouris KC, Tsitrsikos P, Palamidi I, Arvaniti A (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobins and cecal microflora composition. Poult. Sci., 89(1): 58–67. https://doi.org/10.3382/ps.2009-00308

Olukosi OA, Cowieson AJ (2007). Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult. Sci., 86(1): 77-86. https://doi.org/10.1093/ps/86.1.77

Rosin EA, Blank G, Slominski BA, Holley RA (2007). Enzyme supplements in broiler chicken diets: in vitro and vivo effects on bacterial growth. J. Sci. Food Agric., 87(6): 1009-1020. https://doi.org/10.1002/jsfa.2797

Salim HM, Kang KH, Akter N, Kim DW (2013). Supplementation of direct-fed microbial as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. J. Poult. Sci., 92(8): 2084-2090. https://doi.org/10.3382/ps.2012-02947

Sartor RB (2004). Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastro, 126(6): 1620-1633. https://doi.org/10.1053/j.gastro.2004.03.024

SAS Statistical Package (2009). SAS Institute. Version 9.1. Available from: http:// supports.sas.com

Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakare JD (2012). Effect of supplementation of Bacillus subtilis LS1-2 to broiler diet on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. Res. Vet. Sci., 93(1): 264–268. https://doi.org/10.1016/j.rvsc.2011.05.021

Sharifi SD, Dibamehr A, Lotfollahian H, Baurhoo B (2012). Effects of flavomycin and probiotic supplementation to diets containing different sources of fat on growth performance, intestinal morphology, apparent metabolizable energy, and fat digestibility in broiler chickens. Poult. Sci., 91(4): 918-927. https://doi.org/10.3382/ps.2011-01844

Stryer L, Berg JM, Tymoczko JL (2002). Biochemistry (5th ed.). San Francisco: W.H. Freeman. ISBN 0-7167-4955-6

Swain BK, Chakurkar EB (2009). Assessment of problend supplementation on the performance and economics of production of vanaraja chickens. J. App. Anim. Res., 36(2): 203-206. https://doi.org/10.1080/09712119.2009.9707060

Swain BK, Naik PK, Sing NP (2014). Performance, carcass characteristics and economics of production of broiler chickens fed diet supplemented with multi-enzyme. Int. J. Poult. Sci., 49(2): 163-166. http://www.indianjournals.com/ijor.as

Tejero-Sarinena S, Barlow J, Costabile A, Gibson GR, Rowland I (2012). *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acid. Anaerobe, 18(5): 530-538. https://doi.org/10.1016/j.anaerobe.2012.08.004

Thavasiappan V, Selvaraj P, Jayachandran S, Visha P, Paramasivan S, Naik BR (2016). Influence of enzyme supplementation on intestinal morphology in broiler chicken. J. Agric. Vet. Sci., 9(1): 25-28.

Thorat SG, Panwar VS, Dahiya DS, Tewatia BS (2015). Efficacy of probiotics, prebiotics and enzymes as growth promoters on the performance of broiler chicken. Haryana Vet. J., 54(1): 75-78. http://www.luvas.edu.in/.../22.pdf

Vidyarthi VK, Zuyie R, Savino N, Nizamuddin CR, Sharma VB (2007). Effect of exogenous enzyme supplementation on the economics of broiler chicken in Nagaland. In: Proceedings of Tropical Animal Nutrition Conference, October 4-7, Karnal, Haryana, India.

Wallis I (1996). Enzymes in poultry nutrition. Technical Note, SAC. West Mains road, Edinburgh.

Wang ZR, Qiao SY, Lu WQ, Li DF (2005). Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci., 84(6): 875-888. https://doi.org/10.1093/ps/84.6.875

Xu Z, Hu C, Xia M, Zhan X, Wang M (2003). Effects of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci., 82(6): 1030-1036. https://doi.org/10.1093/ps/82.6.1030

Yirga H (2015). The use of probiotics in animal nutrition. J. Prod. Health, 3(2): 132-142. https://doi.org/10.4172/2329-8901.1000132

Zakaria HAH, Jalal MAR, Ishmais MAA (2010). The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality of broiler chickens. Int. J. Poult. Sci., 9(2): 126-133. https://doi.org/10.3923/ijps.2010.126.133

Zhang ZF, Kim IH (2014). Effects of multi-strain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. Poult. Sci., 93(2): 364-370. https://doi.org/10.3382/ps.2013-03314