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RESEARCH ARTICLE
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Synergistic effect of non starch polysaccharide enzymes, synbiotics and phytase on performance, nutrient utilization and gut health in broilers fed with sub-optimal energy diets

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Abstract

Aim: In view of the ban on antibiotic growth promoters (AGPs), an attempt was made to explore the possibility of harnessing synergistic effect of non starch polysaccharide (NSP) enzymes, synbiotics and phytase on performance, nutrient retention, gut health and histology of broilers fed with corn-soybean meal based low calorie diets.

Materials and Methods: A total of 150 a day-old broiler chicks were weighed, wing banded and randomly distributed into five experimental groups, six replicates per group and five birds per replicate and raised in electrically heated battery brooders. Evaluated the synergistic effect of the NSP enzymes, synbiotics and phytase alone or in combination, supplemented to corn-soybean meal based broiler diet, low in energy concentration (Basal diet (BD)) (-225 kcal lower metabolizable energy than standard diet (SD), on performance, nutrient retention, carcass traits, gut conditions and cost per kg live weight gain.

Results: The body weight gain in broiler chicks fed with BD supplemented with NSP enzymes, synbiotics and phytase was significantly (P<0.01) higher. Supplementation of NSP enzymes, synbiotics and phytase alone or in combination had significant effect on feed intake. Synergistic effect of NSP enzyme, synbiotics and phytase was observed on overall feed conversion ratio (1.86), which improved (P<0.05) in comparison to BD (2.06) and SD (2.02), respectively. The supplementation of NSP enzymes, synbiotics and phytase to BD improved (P<0.05) utilization of organic matter (OM), crude protein (CP), nitrogen free extract (NFE), gross energy (GE), phosphorus and the tibia ash compared to BD, whereas no effect on retention of DM and CF was observed. Intestinal viscosity and *E. coli* count significantly (P<0.01) reduced with addition of NSP enzymes, synbiotics plus phytase or combination of all. The supplementation of NSP enzymes, synbiotics plus phytase had no effect on intestinal histology. The cost of feeding was lower (P<0.01) in BD. Addition of these feed additives to BD did not increase the feeding cost and was comparable to unsupplemented ones and lower (P<0.01) than SD. Similarly, the feed cost per kg live weight gain during various phases of broiler production was reduced (P<0.01) due to supplementation of all the feed additives compared to SD and BD.

Conclusions: It can be concluded from the above experiment that supplementing sub-optimal energy diets with NSP enzymes along with synbiotics and phytase improved body weight gain, FCR, nutrient retention, tibia ash and reduced the cost of production considerably.

Keywords: gut health, histology, live weight gain, NSP enzymes, nutrient retention, synbiotics, phytase,

Introduction

The productivity of broilers has improved significantly, through genetic improvements. Increased rearing density has concentrated and increased disease challenges making birds more susceptible to various pathogens especially enteropathic microbes such as *Escherichia coli, Salmonella* spp., *Clostridium perfringens* and *Campyobacter* spp. This increased susceptibility has resulted in the use of antimicrobial growth promoters which are primarily used to enhance gut health and control sub-clinical challenges. With increasing public health concerns about bacterial resistance to antibiotics, the use of antibiotics in therapeutic or sub-therapeutic doses in poultry feed has been severely limited or eliminated in many countries. In view of the above circumstances, there has long been interest in finding alternatives to antibiotics for poultry production. Resident microbes in the birds' digestive tract have a profound effect on some of the physiological processes of their host. It is important to understand the dynamics of the intestinal microbial ecology of the chicken to find alternatives to antibiotics. Under normal circumstances there is a delicate balance of beneficial and pathogenic bacteria in the gastrointestinal tract (GIT). This is influenced by symbiotic and competitive interactions and relationships. The microbial communities will not only protect the GIT but also enhance productivity in the host. NSP enzymes degrade NSP and by this improve gut motility and nutrient (mainly energy) availability [1]. Prebiotics are non-digestible substances, mainly oligo-and polysaccharides, lowering pH in the gut and by this inhibit colonization of pathogenic microorganisms, stimulate immunity and neutrale toxins. Probiotics act by competitive exclusion, lower gut pH, produce bacteriocins, lysozyme and

Table-1. Details of experimental diets

Diet no.	Diet
1	Standard diet (SD)
2	Basal diet (BD)
3	BD + NSP enzymes
4	BD +synbiotics + Phytase
5	BD + NSP enzymes+synbiotics + Phytase

Table-2. Ingredient composition of Basal diet (BD)

Ingredient (g/kg)	prestarter	starter	finisher
Maize	522.8	597.2	657.5
Soybean meal	380.2	342.6	305.0
De oiled rice bran	59.2	23.7	0.00
Oil (vegetable)	0.0	0.0	3.1
Salt	4.5	4.5	4.5
DL-methionine	2.2	2.1	1.8
Di-Calcium phosphate	18.3	17.7	16.5
Shell grit	7.7	7.0	6.8
Trace mineral mixture[1]	1.2	1.2	1.2
Vitamin premix[2]	0.40	0.40	0.40
Choline Chloride (50%)	0.6	0.60	0.60
Toxin Binder	2.0	2.0	2.0
Antibiotic	0.5	0.50	0.50
Coccidiostat	0.5	0.50	0.50
Total	1000	1000	
Nutrient Composition (calculated)			
ME (kcal/kg)	2725.0	2825.0	2925.0
Protein (%)	22.5	21.00	19.50
Calcium (%)	0.90	0.85	0.80
Available phosphorus (%)	0.45	0.43	0.40
Lysine (%)	1.23	1.13	1.03
Methionine (%)	0.55	0.52	0.48
Crude fibre (%)	4.37	3.82	3.39

1. Trace mineral provided per kg diet: Manganese 120mg; Zinc 80mg; Iron 25mg; Copper 10mg; Iodine 1mg and Selenium 0.1mg.
2. Vitamin premix provided per kg diet: Vitamin A 20000IU; Vitamin D3 3000IU; Vitamin E 10mg; Vitamin K 2mg; Riboflavin 25mg; VitaminB1 1mg; Vitamin B6 2mg; Vitamin B12 40mcg and Niacin 15mg.

Table-3. Ingredient composition of standard diets

Ingredient (g/kg)	pre-starter	starter	finisher
Maize	542.0	572.8	603.9
Soybean meal	393.0	353.8	314.8
Oil (veg)	27.0	36.8	46.5
Salt	4.5	4.5	4.5
DL-methionine	2.2	2.1	1.9
Di-Calcium Phosphate	19.0	18.1	16.5
Shell grit	7.1	6.7	6.70
Trace mineral mixture[1]	1.2	1.2	1.2
Vitamin premix[2]	0.40	0.40	0.40
Choline Chloride, 50%	0.6	0.60	0.60
Toxin Binder	2	2.0	2.0
Antibiotic	0.5	0.50	0.50
Coccidiostat	0.5	0.50	0.50
Total	1000	1000	1000
Nutrient Composition (Calculated)			
ME(kcal/kg)	2950.0	3050.0	3150.0
Protein (%)	22.5	21.0	19.50
Calcium (%)	0.90	0.85	0.800
Available phosphorus (%)	0.45	0.43	0.400
Lysine (%)	1.24	1.14	1.04
Methionine (%)	0.55	0.52	0.48
Crude fibre (%)	3.69	3.52	3.34

1. Trace mineral provided per kg diet: Manganese 120mg; Zinc 80mg; Iron 25mg; Copper 10mg; Iodine 1mg and Selenium 0.1mg.
2. Vitamin premix provided per kg diet: Vitamin A 20000IU; Vitamin D3 3000IU; Vitamin E 10mg; Vitamin K 2mg; Riboflavin 25mg; VitaminB1 1mg; Vitamin B6 2mg; Vitamin B12 40mcg and Niacin 15mg.

peroxides, and stimulate the immune system. The combined application of prebiotics and probiotics is called synbiotics [2]. Feed additives if incorporated in poultry feeds, can create favourable conditions in the intestine for the efficient digestion of feed [3, 4]. Many feed additives viz., NSP enzymes, prebiotics, probiotics, acidifier more or less help in maintaining gut directly or indirectly. In most of the experiments these additives have been used singly.If two or more such additives are used in combination, possibly their effects may complement and may have synergistic effect.

Keeping these objectives in mind the present study was conducted to exploit the synergistic effect of NSP enzymes, prebiotics, probiotics (synbiotics) and phytase, on performance, nutrient retention, gut health and histology.

Materials and Methods

Ethical approval: This research work was carried out after approval of Institutional Animal Ethics Committee.

Experimental design and sample collection: One hundred and fifty (150) day-old Cobb commercial broiler chicks were weighed, wing banded and randomly distributed into five experimental groups, six replicates per group and five birds per replicate. The NSP enzymes combination (xylanase 7500 IU/kg, cellulase 100 IU/kg and β- D- glucanase 100 IU/kg), prebiotic (MOS, 0.5g/kg), probiotic (*Saccharomyces boullardii*, 10^8 CFU/kg) and phytase (675 IU/kg) was tested at sub-optimal energy concentration (225 kcal/kg less ME than standard diet) [5]. The details of experimental diets are given in Table 1, 2 and 3. All replicate groups of chicks were offered the respective diets *ad libitum* for a period of 42 days. Weekly body weights and feed intake were recorded. At the end of experiment, a metabolic trial of 4 day duration was conducted to determine the nutrient utilization and balance of nutrients. The samples of each feed, feed

Table-4. Nutrient composition (% dry matter basis) of broiler finisher standard and basal diets (analyzed)

Diet	DM	OM	CP	EE	CF	NFE	Total ash	GE(kcal/g)	TP
Standard diet (SD)	92.15	93.93	19.85	6.22	3.34	64.52	6.07	3.85	0.46
Basal Diet (BD)	89.61	93.41	19.52	3.25	3.39	67.25	6.59	3.43	0.46
BD+ NSP enzyme	92.05	93.80	19.62	3.24	3.38	67.56	6.20	3.44	0.46
BD+ Synbiotics +Phytase	89.78	93.39	19.41	3.32	3.35	67.31	6.61	3.36	0.45
BD+NSP enzyme + Synbiotics +Phytase	90.58	93.49	19.46	3.35	3.38	67.24	6.57	3.34	0.46

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, GE: Gross energy, TP: Total phosphorus. Each value is average of duplicate analysis

Table-5. Effect of feeding low calorie diet supplemented with NSP enzymes, synbiotics and phytase on performance and cost economics of broiler chicken

Diet	Body weight (g)		Feed intake (g)		Feed conversion ration		Feed cost per kg live weight gain (Rs)	
	0-3 wks.	0-6 wks.	0-3 wks.	0-6 wks.	0-3 wks.	0-6 wks.	0-3 wks	0-6 wks.
Standard diet (SD)	463.2	1494	726.0^{c}	3013^{a}	1.57^{b}	2.02^{ab}	31.55^{b}	40.50^{a}
Basal Diet (BD)	448.8	1436	789.7^{a}	2953^{ab}	1.77^{a}	2.06^{a}	34.08^{a}	39.57^{ab}
BD+ NSP enzyme	471.3	1495	774.5^{ab}	2895^{b}	1.64^{b}	1.94^{abc}	31.74^{b}	37.22^{bc}
BD + Synbiotics+ Phytase	479.6	1511	768.5^{b}	2886^{b}	1.61^{b}	1.91^{bc}	31.77^{b}	37.49^{bc}
BD + NSP enzymes+ Synbiotics + Phytase	486.4	1543	764.8^{b}	2874^{b}	1.57^{b}	1.86^{c}	35.92^{a}	36.57^{c}
SEM	4.67	13.94	4.82	16.14	0.02	0.02	0.41	0.44
P value	0.084	0.179	0.001	0.017	0.001	0.016	0.001	0.009

a-c: Means with different superscripts in a column differ significantly (P<0.05)

residue and feces pooled during 4 days period were ground and analyzed for proximate principles as per method described previously [6]. After metabolic trial, 30 birds comprising of 6 birds from each diet by selecting one at random from each replicate) were slaughtered to assess the carcass characteristics.

Gut health: To study the effect of dietary energy concentration, supplementary effect of NSP enzymes, synbiotics and phytase on gut health, the digesta was collected from distal portion of small intestine during slaughter. Approximately two grams of digesta was taken in sterile eppendorf tubes for enumeration of *E. coli*. Another 2 g of digesta was collected and centrifuged at 5000 rpm for 10 minutes at 20 °C. An aliquot of supernatant (0.5 to 1 ml) was collected and stored in capped vials for viscosity determination. The digesta collected in centrifuge tubes was utilized for measuring the pH.

Histology of intestines: Representative pieces of duodenum of intestine were collected in 10% formal saline and preserved for histological studies. After proper fixation the intestinal tissue was trimmed and subjected to over night washing, dehydration in various percentages of alcohol, cleaning in xylol, embedding in paraffin wax for preparation of blocks [7]. The paraffin blocks were cut into 5μ thick sections and stained with routine H & E stain [8] and used for microscopic examination.

Statistical analysis: The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) 16th version and comparison of means was tested using Duncan's multiple range tests [9].

Results

Nutrient composition of experimental ration: Nutrient composition (% dry matter basis) of broiler finisher standard and basal diets is presented in Table-4.

Body weight gain (g): The body weight gain in broiler chicks fed with BD supplemented with NSP enzymes, synbiotics and phytase is presented in Table-5. Significantly higher (P<0.01) weight gains were observed during 1st week in broiler chicks fed with basal diet (BD) supplemented with synbiotics and phytase (80.83g) or BD supplemented with NSP enzymes, synbiotics and phytase (81.47g) compared to BD (63.50g) and SD (68.57g). Starter phase, finisher phase and total period (1- 42 d) no difference in weight gain was observed among the chicks fed with SD, BD, BD supplemented with NSP enzymes, BD supplemented with synbiotics and phytase or in combination of synbiotics, phytase and NSP enzymes.

Feed intake (g/bird/day): The feed intake by chicks fed SD during starter phase was higher (P<0.01) than BD, BD supplemented with NSP enzymes, BD supplemented with synbiotics and phytase or BD in combination of all feed additives fed chicks (Table-5). Supplementation of NSP enzymes, synbiotics and phytase alone or in combination had significant effect on feed intake. Supplementation of NSP enzymes or synbiotics and phytase alone or in combination of all above feed additives to BD reduced (P<0.01) the feed intake. The starter phase feed intake was higher (P<0.01) in BD fed chicks than SD and comparable to BD supplemented with NSP enzymes. The addition of NSP enzymes, synbiotics and phytase to BD reduced (P<0.001) the feed intake in chicks but was higher than those fed SD. During finisher phase the feed intake by BD fed chicks was lower (P<0.001) than SD fed chicks and addition of feed additives alone or in combination had no effect on feed intake. While overall feed intake was higher (P<0.001) in SD but comparable to BD and

Table-6. Nutrient retention, intestinal pH, viscosity, *E. coli* and tibia ash content of broilers fed basal diet supplemented with NSP enzymes, synbiotics and phytase

Diet	Nutrient retention (%)								Gut condition			Tibia ash(%)
	DM	OM	CP	CF	EE	NFE	GE	P	pH	V(%)	*E. coli* (cfu/ml)	
Standard diet (SD)	66.12	73.30^{a}	62.69^{a}	32.10	76.81	75.46^{b}	66.74^{cd}	31.14^{c}	6.17	8.12^{a}	4.34^{b}	46.39^{a}
Basal diet (BD)	67.20	68.87^{b}	54.75^{b}	29.06	71.29	77.08^{ab}	63.81^{d}	31.59^{c}	6.16	7.01^{b}	6.68^{a}	42.74^{b}
BD + NSP enzymes	69.89	72.12^{a}	60.72^{a}	32.18	74.56	79.51^{ab}	70.28^{bc}	32.70^{bc}	5.60	4.25^{c}	5.28^{b}	46.39^{a}
BD + synbiotics +phytase	68.57	72.22^{a}	61.89^{a}	32.15	74.56	82.12^{a}	72.27^{ab}	35.21^{ab}	5.92	4.71^{c}	1.32^{c}	47.74^{a}
BD + NSP enzymes + synbiotics + phytase	71.57	73.45^{a}	63.80^{a}	31.36	76.07	81.73^{a}	76.65^{a}	36.19^{a}	5.74	3.81^{c}	1.97^{c}	47.89^{a}
SEM	0.81	0.55	1.03	0.68	0.75	0.89	1.21	0.63	0.08	0.41	0.49	0.57
P value	0.222	0.038	0.026	0.586	0.165	0.056	0.001	0.017	0.074	0.001	0.001	0.007

P: Phosphorus, V: Viscosity, a-c: Means with different superscripts in a column differ significantly (P<0.05)

Table-7. Effect on slaughter characteristics of broilers fed low calorie diet supplemented with NSP enzymes, synbiotics and phytase

Diet	Dressing yield (%)	Breast yield (%)	Abdominal fat (%)	Visceral organs (% of body weight)		
				Liver	Heart	Gizzard
Standard diet (SD)	63.67	18.89	1.04	2.18	0.60^{b}	2.66
Basal diet(BD)	64.61	19.62	0.89	2.10	0.69^{a}	2.55
BD + NSP enzymes	64.78	18.39	1.07	2.25	0.62^{ab}	2.47
BD + Synbiotics + Phytase	64.57	19.05	0.64	2.09	0.67^{a}	2.29
BD + NSP enzymes + Synbiotics + Phytase	66.67	19.44	0.79	2.07	0.68^{a}	2.59
SEM	0.35	0.18	0.06	0.04	0.01	0.05
P value	0.066	0.236	0.156	0.710	0.046	0.135

Means with different superscripts in a column differ significantly (P<0.05)

addition of synbiotics, phytase and NSP enzymes reduced the feed intake compared to SD.

Feed conversion ratio: Supplementation of NSP enzymes or synbiotics with phytase or combination of all feed additives improved (P<0.01) the FCR compared to SD and BD (Table-5). Supplementation of synbiotics and phytase or NSP enzymes alongwith synbiotics and phytase to BD improved the FCR (P<0.01) in comparison to BD and SD. No significant effect of supplementing NSP enzymes, synbiotics and phytase was observed on FCR during finisher phase. The FCR of BD fed chicks during starter phase was higher (P<0.001) than those fed SD. Supplementing BD with NSP enzymes or synbiotics and phytase or combination of all feed additives improved the FCR in starter phase and comparable to SD. During finisher phase and overall period, the FCR was comparable between SD and BD. Synergistic effect of NSP enzymes, synbiotics and phytase was observed for FCR during finisher phase (1.99) and overall period (1.86) which improved (P<0.05) compared to BD (2.20) and SD (2.06).

Nutrient retention: The supplementation of NSP enzymes, synbiotics and phytase to BD improved (P<0.05) utilization of OM, CP, NFE, GE and phosphorus compared to BD (Table-6). The OM retention was higher (P<0.05) for BD supplemented with NSP enzymes, synbiotics and phytase (73.45%) followed by SD (73.30%) and lowest DM retention was observed in BD (68.87%). Similar to OM the retention of CP was highest (P<0.05) for BD supplemented with NSP enzymes, synbiotics and phytase (63.80%) than BD (54.75%). Supplementing synbiotics and phytase to BD increased CP retention and it reached to the level of SD. The retention of NFE improved (P<0.05) with addition of synbiotics, phytase and NSP enzymes to BD and the NFE retention was comparable to SD and BD with NSP enzymes. The GE retention was higher (P<0.01) for BD supplemented with NSP enzymes, synbiotics and phytase (76.65%) followed by BD supplemented with synbiotics and phytase (72.27%) and lowest GE retention was observed in BD (63.81%). Addition of NSP enzymes in combination with synbiotics and phytase improved (P<0.005) phosphorus retention significantly compared to SD (31.14%), BD (31.59%), BD supplemented with NSP enzymes (32.70%). However the phosphorus retention was comparable between synbiotics group and BD supplemented with NSP enzymes, synbiotics and phytase. The tibia ash content was lower in BD (42.74%) compared to SD (46.39). Supplementing BD with NSP enzymes or with synbiotics and phytase or combination of synbiotics, phytase and NSP enzymes improved the tibia ash content and was comparable to SD (Table-6).

Carcass characteristics: The slaughter attributes in terms of dressing yield, breast yield, abdominal fat and visceral organs *viz*., liver, heart and gizzard is presented in Table-7.

No significant (P<0.05) effect was observed among broilers fed SD, BD, BD supplemented with NSP enzymes, synbiotics and phytase alone or in combination on dressing yield, breast yield, and abdominal fat and it varied from 63.67 to 66.67% 18.39 to 19.44% and 0.64 to 1.07%, respectively. Similarly no significant effect (P<0.05) of supplementation of BD with NSP enzymes, combination of synbiotics and phytase or combination of synbiotics, phytase and NSP enzymes was observed on liver, gizzard weights (Table-7) and the values ranged from 2.07 to 2.25% and 2.29 to 2.66%, respectively.

Gut conditions: No significant ($P<0.05$) effect of supplementation of various feed additives to BD was observed on intestinal pH and the values varied between 5.60 and 6.17. The intestinal viscosity varied from 3.81 to 8.12%. The supplementation of BD with NSP enzymes (4.25%) or synbiotics and phytase (4.71 %) or combination of all feed additives (3.81%) reduced ($P<0.01$) the intestinal viscosity compared to SD (8.12 %) and BD (7.01 %) (Table-6). The intestinal viscosity was higher in SD compared to BD. The *E. coli* count ($\log_{10}$) in intestinal contents was lowest ($P<0.001$) in SD (4.34) compared to BD (6.68) (Table 6). Supplementation of synbiotics with phytase (1.32) or combination of all feed additives (1.97) lowered the *E. coli* count in intestinal contents in comparison to BD and SD.

Gut histology: The supplementation of NSP enzymes along with synbiotics and phytase did not influence the intestinal histology, except few broad elongated and folded, congested villi with presence of few goblet cells was noted (Figure-1).

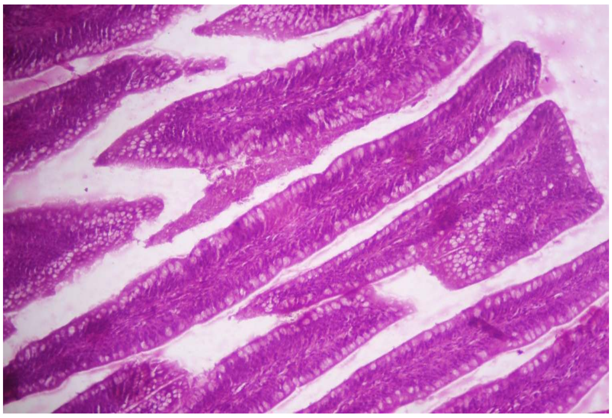

Figure-1. H & E section of duodenum showing broad villi at tip with distinct goblet cell activity

Cost comparison: The cost of production per kg live weight gain during starter and finisher phases of broiler production is given in Table-5. The cost of production per kg live weight gain during starter phase was higher ($P<0.01$) for BD (Rs. 34.08) compared to SD (Rs. 31.55). Supplementing BD with NSP enzymes (Rs. 31.74) or synbiotics alongwith phytase (Rs. 31.77) reduced the cost of production per kg weight gain compared to BD, but was similar to SD. Addition of all the feed additives to BD increased the feed cost (Rs. 35.92) compared to all other groups during starter phase. During finisher phase, cost of production was highest ($P<0.01$) for SD (Rs. 40.50) but comparable with BD (Rs. 39.57). Supplementation of NSP enzymes, synbiotics along with phytase or all feed additives reduced ($P<0.01$) the cost of production compared to SD during finisher phase. Similarly the overall cost of production was similar between SD and BD. Feeding of BD containing either NSP enzymes (Rs. 37.22) or synbiotics with phytase (Rs. 37.49) reduced the cost of feed to SD (Rs. 40.50), but comparable to that of BD (Rs. 39.57). Supplementing all the above feed additives to BD significantly ($P<0.01$) reduced the cost of production (Rs. 36.57) compared to BD and SD.

Discussion

Body weight gain: Effect of supplementing different feed additives, NSP enzymes, synbiotics and phytase to BD individually or combination of all had significantly ($P<0.005$) improved body weight gain during 1st week of experiment (Table-5). The starter, finisher and total weight gains recorded was though comparable among various groups, higher weight gain of 7.48% was recorded with supplementation of all feed additives to BD. The results are in agreement with [10,11] who reported improvement in weight gains with supplementation of various feed additives (avilamycin, allzyme, avimos, biomos, yeast extract, xylanase, avizyme and gustor) individually or in combination. Improvement in live weight gain was also reported [12] when broiler diets supplemented with direct fed microbials (DFM), antibiotic growth promoters (AGP) and Biomos compared to control. However, contrary to present findings, addition of prebiotics (galacto oligosacharides) and probiotics (*Bifiodobacteriam lactic* 300x10^9 cells/g) individually or in combination had no effect on body weight gain [13].

Feed intake: During starter period feed intake (g/d) was higher ($P<0.05$) in BD compared to SD and BD supplemented with feed additives (Table-5). However, significantly higher ($P<0.01$) feed intake was recorded in SD fed birds during finisher period compared to BD and BD supplemented with various feed additives. Feed intake was lowest in BD supplemented with various feed additives.

The results are in line with a previous report [10] who studied that effect of various feed additives (Avilamycin, Allzyme, Avimos, Biomos, Yeast extract, xylanase, Avizyme and Gustor) individually and combination had no effect on DM intake over the entire 1-28 day experimental period. Mean feed intake for the whole period was numerically greatest for the birds fed on positive control. The average daily feed intake (ADFI) of birds fed with direct fed microbials (DFM), antibiotic growth promoters (AGP) and Biomos was insignificant compared to control [12]. It was found no effect of feed additives (xylanase, protease and phytase) when supplemented to nutritionally marginal (2870 kcal of ME/kg, 0.85% Ca and 0.24% available P) corn soy bean based diets on feed intake [14]. However, a study [11] reported that diets supplemented with performance enhancers (prebiotics, probiotics and organic acids either alone or in combination) had significantly influenced feed intake for 21 day ($P<0.05$) period but not at 0 to 42 day period.

Feed conversion ratio: Birds fed with BD had poorer

(P<0.05) feed conversion efficiency compared with those fed with SD. Supplementation of NSP enzymes, synbiotics and phytase and combination of all improved feed conversion efficiency linearly indicating synergistic effect of addition of two or more feed additives to low calorie diets (Table-5). Earlier it was reported that significant improvement in FCR with addition of various feed additives (avilamycin, allzyme, avimos, biomass, yeast extract, avizyme and gustor), combination of prebiotics and probiotics compared to control and xylanase, protease, amylase and phytase to nutritionally marginal (2870 kcal of ME/kg, 0.85% Ca and 0.24% available P) corn soy bean based diets [10, 11, 14]. However another study [15] observed that addition of xylanase, amylase, protease and phytase alone to low density diets had no effect on feed conversion ratio, but combination had significantly (P<0.05) improved feed efficiency compared to the negative control.

Nutrient retention: The supplementation of NSP enzymes, synbiotics and phytase alone or in combination of all improved the retention of OM, CP, NFE, GE and phosphorus (Table 6). Whereas DM, CF and EE retentions were not influenced by dietary treatments compared to BD and SD. BD with synbiotics and phytase influenced the retention of OM, CP, CF and NFE. On the whole, the addition of NSP enzymes, synbiotics and phytase to BD improved (P<0.05) retention of these nutrients significantly. The results are in agreement with earlier report on improvement in AME and phosphorous retention by supplementation of phytase alone or in combination with NSP degrading enzymes and citric acid [16].

Supplementation of exogenous enzymes to the broiler diet improved starch digestibility and consequently DM, OM, CP and energy digestibilities. Improvement in ileal apparent digestibility co-efficient (ADC) of CP and EE was reported [17] with addition of avilamycin (2.5 g/kg of diet) and further improvement was observed when fortified with probiotics (10^8 CFU/ kg) on total tract ADC for DM, ash, EE and ME values. The apparent digestibility of DM, OM, CP, EE, starch and energy were increased (P<0.05) with supplementation of enzymes [18]. However, a previous study [15] observed no improvement in apparent total tract retention of DM and energy as phytase or xylanase, amylase, protease could influence the retention including phosphorus.

Tibia ash content was significantly (P<0.001) higher in supplemented groups and SD compared to BD (Table 6). This might be due to synergistic effect of feed additives. The results are in agreement with [19] who reported the benefits of NSP enzymes and phytase supplementation to broiler diets which improved bone mineralization and reached to the level of positive control diet.

Carcass characteristics: The birds receiving different feed additives, either singly or in combination recorded in significantly (P>0.05) higher dressing per cent and breast yield per cent than SD and BD (Table 7). Similarly, dietary treatments had no effect on abdominal fat pad weight, liver and gizzard.

There was no effect of biomas, protexin and acidifier individually or combination of all on edible carcass yield, liver weight and gizzard weight [20]. However, on the other hand another study [21] reported improved dressing yield per cent (P<0.05) and breast yield per cent and other meat yield traits when supplemented with or without growth promoters (flavomycin, avilamycin, genex and avila m/z) and were comparable (P>0.05) among the groups.

Gut conditions: Supplementation of NSP enzymes, synbiotics and phytase alone and combination had no effect on intestinal pH values recorded among the treatment groups (Table-6). Whereas viscosity and *E. coli* count significantly (P<0.01) reduced in feed additives supplemented groups compared to SD and BD. The intestinal pH recorded were in agreement with earlier findings [20] that observed supplementing prebiotics, probiotics and acidifier singly or in combination had no effect on pH. Viscosity recorded was in agreement with the earlier report [22] which indicated that the exogenous enzyme supplementation significantly (P<0.05) reduced the digesta viscosity. Previously significant effect on lactobacilli and coliform counts in ileum of the diets supplemented with different feed additives alone or in combination were reported [10].

Gut histology: The supplementation of NSP enzymes with synbiotics and phytase to BD resulted in broad villi at tip with distinct goblet cell activity (Figure 1). The effect of synbiotics (BIOMIN IMBO) increased (P < 0.001) villus height/crypt depth ratio and villus height in ileum [23].

Cost comparison: Feed cost per kg live weight gain was significantly (P<0.01) reduced by Rs. 3.93 and Rs. 3.00 in all feed additive group compared to SD and BD, respectively (Table-5). Similarly, reduced cost of production was observed in earlier studies [21] with supplementation of different growth promoters such (flavomycin, avilamycin, genex and avila m/z) in broiler diets. Feeding low calorie diet fortified with feed additives like NSP enzymes, synbiotics and phytase resulted in low cost of production and better returns.

Conclusion

From this study, it can be concluded that supplementing NSP enzyme, synbiotics and phytase in combination has exerted synergistic effect on body weight gain feed conversion efficiency, improvement in nutrient retentions and gut health at reduced cost of production in broilers fed corn-soybean meal based low energy diets.

Authors' contributions

JN and DN designed the experiment, implemented the design, analyzed data and prepared the manuscript; YRR and STVR revised manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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