

## Associative Effect of Non-Starch Polysaccharide Enzymes and Probiotics on Performance, Nutrient Utilization and Gut Health of Broilers Fed Sub-Optimal Energy Diets.

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**Abstract:** The aim of this work was to investigate the associative effect of Non-starch Polysaccharide enzymes and probiotics on performance in broiler. One hundred and fifty day old Cobb broilers were randomly allocated in five experimental groups, six replicates per group and five birds per replicate. The experimental birds received corn-soy bean based standard diet (SD) and low calorie basal diet (BD) (225kcal/kg ME less) supplemented with NSP enzymes and/ or probiotics. Chicks fed BD supplemented with NSP enzymes and probiotics recorded significantly ( $P < 0.05$ ) higher body weight gain compared to BD and no effect on total feed intake was observed. The BD supplemented with probiotics alone or along with NSP enzymes improved ( $P < 0.01$ ) the FCR compared to BD group. The DM, CF, EE, NFE and phosphorus retentions were not influenced by supplementation of NSP enzymes and probiotics to BD. The OM, CP and GE retention was higher ( $P < 0.05$ ) in chicks supplemented with probiotics alone or in combination with NSP enzymes. Supplementation of NSP enzyme increased ( $P < 0.05$ ) the tibia ash content but no synergistic effect of probiotics and NSP enzymes was observed. The slaughter attributes in terms of dressing yield, breast yield, abdominal fat and weight of visceral organs were not affected by supplementation of NSP enzymes and probiotics alone or in combination to BD. Significant reduction ( $P < 0.05$ ) in intestinal pH, viscosity and *E. coli* count was observed with supplementation of both NSP enzymes and no synergistic effect of NSP enzymes and probiotics was observed on these variables. There was no effect of supplementing NSP enzymes along with probiotics to BD was observed on intestinal histology. Supplementation of NSP enzymes and probiotics singly or in combination significantly ( $P < 0.01$ ) reduced the feeding cost ( $P < 0.05$ ) and the cost per kg live weight gain.

**Key Words:** Non-starch Polysaccharide enzymes, probiotics, basal diet, gut health, live weight gain.

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### I. INTRODUCTION

Feed additives have two main groups. The nutrient feed additives (NFA) and non nutrient feed additives (NNFA). The NFA are added in the feed to correct quantity of the deficient nutrients in the rations, such as vitamin mix, mineral mix and single or the mixture of amino acids etc. [1]. While the NNFA such as color and taste enhancers, appetizers, enzymes, yeast, growth promoters and probiotics are added in the feed to improve or to accelerate the rate of feed or nutrient utilization, [2], [3]. Probiotics defined as live micro organisms, that when included in foods could influence the composition and activity of the gut microbiota, modulate the inflammatory response, improve non specific intestinal barrier and reinforce or modulate mucosal and systemic immune responses [4]. These live organisms after residing in intestinal tract, their metabolites acts as immune modulatory agent by activating specific and non specific host immune response in birds. Thus helping in prevention and control of various infectious diseases [5], [6]. It is extremely important for the highly intensive broiler production sector of the poultry industry to achieve performance optimization and minimization of economic losses while ensuring the safety of broiler meat via the control and elimination of foodborne pathogens [7]. In view of the above, present study was aimed to evaluate the effect of feeding NSP degrading enzymes either alone or along with probiotics in broilers fed corn soybean based sub-optimal energy diets.

### II. MATERIALS AND METHODS

#### 2.1. Experimental Design and Sample Collection

One hundred and fifty (150) day old Cobb commercial broiler chicks were weighed, wing banded and randomly distributed in to 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  $\beta$ - D- glucanase 100 IU/kg) and with probiotic (*Saccharomyces boulardii*,  $10^8$  CFU/kg) was tested at sub optimal energy concentration (225 kcal/kg) less ME than standard diet [8]. The details of experimental diets and ingredient composition 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 residue and feces pooled during 4 days period were ground and analyzed for proximate principles as per the method of [9]. After metabolic trial, 30 birds (of 6 birds from each diet by selecting one at random from each replicate) were slaughtered to assess the carcass characteristics.

## 2.2. Gut health

To study the effect of various dietary energy concentrations, supplementary effect of NSP enzymes with or without probiotic on gut health, the digesta was collected from distal portion of small intestine during slaughter. Approximately two g of digesta was taken in sterile eppendorf tubes for enumeration of *Escherichia coli*. Another 2 g of digesta was collected and centrifuged at 5000 rpm for 10 minutes at 20<sup>o</sup>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.

## 2.3. Histology of intestines

Representative pieces of deodenum of intestine were collected in 10% formal saline and preserved for histological studies. After proper fixation the intestine 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 [10]. The paraffin blocks were cut in to 5 $\mu$  thick sections and stained with routine H and E stain [11] and used for microscopic examination.

## 2.4. Statistical Analysis

The data were subjected to appropriate statistical analysis using Statistical Package for Social Sciences (SPSS) 16<sup>th</sup> version and comparison of means was tested using Duncan's multiple range tests [12]

# III. RESULTS AND DISCUSSION

## 3.1. Nutrient composition of experimental ration

Nutrient composition (% Dry matter basis) of broiler finisher standard and basal diets is presented in Table 4.

## 3.2. Body weight gain

Highest weight gains were recorded in BD supplemented with NSP enzymes and probiotics (Table 5). The total gain (0-42 d) in broilers was lowest ( $P < 0.05$ ) when fed BD and insignificant ( $P > 0.05$ ) increase in body weight gain was observed with addition of either NSP enzymes or probiotics, while adding both the above feed additives to BD significantly increased ( $P < 0.05$ ) the total gain. Body weight gains improved with supplementation of *saccharomyces cerevisiae* to broiler diet at 1, 1.5 and 2 % level. [13][19] also found increase in weight gain of broilers fed diets supplemented with probiotics at 0.01% level. Contrary to the present findings [14] reported no effect of supplementing probiotics (protexin 0.1%,  $6 \times 10^7$  CFU/kg) to broiler rations.

## 3.3. Feed intake

Supplementation of BD either with NSP enzymes or probiotics or their combination had no effect on feed intake during starter and finisher phases (Table 5). The results are in agreement with [14], [15] and [7] where no effect on feed intake was observed with probiotics in broilers. On the other hand [13] observed higher feed consumption in broilers fed diets supplemented with *saccharomyces cerevisiae* at 1, 1.5 or 2% level.

## 3.4. Feed conversion ratio

The Feed conversion ratio (FCR) in the BD was either poor or comparable to SD. During starter phase, the NSP enzymes or probiotics improved FCR and during finisher and overall period synergistic effect of NSP enzymes and probiotics was observed on FCR (Table 5). The results are in line with [13],[19], [15] and [1] who reported improved feed: gain ratio with inclusion of probiotics. [14] Observed no effect on FCR when broiler diet was supplemented with probiotics (protexin 0.1%,  $6 \times 10^7$  CFU/kg) alone or with antibiotic growth promoter (Flavomycin, 0.1%) and organic acid mixture to corn soy diets.

## 3.5. Nutrient retention

Nutrient retention was positively influenced by supplementation of NSP enzymes and probiotics to BD (Table 6). The OM, CP and GE retentions were higher in supplemented groups compared to SD and BD without feed additives. The DM, CF, NFE and phosphorus retention were unaffected. The tibia ash content was lower in BD and improved with NSP enzymes and probiotics addition to BD. The results are in agreement with Mountzouriset *al.*, (2010) [7] who reported improved total tract digestibility of DM, OM, ash, CP, EE and AMEn with supplementation of probiotics and avilangem at different inclusion levels. Similarly [16] reported

that addition of probiotics, livol and antibiotics to broiler diets improved digestibility of DM and other nutrients as well as the retention of nitrogen, calcium and phosphorus in all the treatment groups compared to control.

### 3.6. Carcass characteristics

Probiotic or NSP enzymes supplementation to BD had no effect on slaughter attributes (Table 5). The dressing per cent and breast yield was 2.34 and 6.3% higher than SD which might be due to higher weight gains and nutrient retentions recorded in this group. Supplementation of NSP enzymes and probiotics had no effect on abdominal fat, visceral organs viz., liver, heart and gizzard. These results are in agreement with [17] and [18] who reported supplementation of enzymes to broilers diets had no effect on various carcass characteristics.

### 3.7. Gut conditions

Viscosity, *E. coli* counts and pH in intestines decreased with supplementation of NSP enzymes or probiotics compared to BD and SD (Table 6). Similarly [14] observed significant ( $P<0.05$ ) decrease in caecal and ileal gram negative bacteria counts at 21<sup>st</sup> and 42 days with dietary addition of antibiotic growth promoter flavomycin, probiotics and organic acid mixture.

### 3.8. Gut histology

Dietary supplementation of NSP enzymes and probiotics had no effect on villus length and globular activity. Similarly [14] studied effects of supplementing anti-biotic growth promoter (Flavomycin; 0.1%), probiotics (Protexin; 0.1%,  $6 \times 10^7$  CFU/g) and organic acid mixture (Genex; 0.2 %) to corn soy based diet in broilers in combination or alone and found that, jejunum and ileal crypt depth, the ratio of villus height to crypt depth and villus width at 21<sup>st</sup> and 42<sup>nd</sup> days of age were not affected by treatments ( $P<0.05$ ). However, probiotic treatment increased ( $P<0.05$ ) jejunum and ileum villi height at 21<sup>st</sup> and 42<sup>nd</sup> days of age compared to non supplemented basal diet.

### 3.9. Cost economics

The overall feed cost of production (Rs. /kg gain) was statistically comparable between SD and BD (Table 5). Supplementation of probiotics or NSP enzymes reduced ( $P<0.01$ ) feed cost of production compared to SD and further reduction was observed with supplementation of both these feed additives to BD. Thus clearly indicating that feeding of low calorie diet with addition of NSP enzymes and probiotics could be formulated to reduce the cost of production considerably for better returns. Similar findings were observed by [19] with supplementation of various growth promoters Biosafe plus (*lactic acid bacillus* 30 billion CFU/g, live yeast culture, 800 billion CFU/g), Albac (Zinc bacitracin 10%) and combination of both at 0.01% and 0.02% on performance of broilers.

## IV. CONCLUSION

Supplementation of NSP enzymes along with probiotics to BD (-225 kcal lower ME than SD) significantly ( $P<0.05$ ) improved total weight gain, feed conversion ratio, digestibility of most of the nutrients and reduced ( $P<0.05$ ) intestinal pH, viscosity and *E. coli* count in broilers. There was no effect on slaughter characteristics with reduction in energy and supplementation of NSP enzymes and probiotics. The feed cost per kg live weight gain significantly ( $P<0.05$ ) reduced with supplementation of NSP enzymes with prebiotics.

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