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Additive Effect of NSP Enzymes and Phytase on Performance, Egg Quality, Nutrient Retention and Gut Health of Laying Hens Fed Corn-Soybean Meal Based Low Energy Diets*

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Abstract: *A trial was conducted to evaluate pure NSP enzyme combination derived from in vitro studies and commercially available phytase to corn-soybean meal based low energy diets singly and combination of both. The experiment was conducted by using completely randomized design on one hundred and fifty layer birds (40 weeks) of uniform body weight and production with five treatments, six replicates and five hens in each replicate for three laying periods with twenty eight days in each laying period. The performance was measured in terms of egg production, feed intake, weight changes, feed efficiency, egg quality, nutrient retention, and gut health. Egg production improved (P<0.05) with supplementation of phytase alone or in combination of phytase and NSP enzymes. No effect of supplementing NSP enzymes, phytase alone or in combination was observed on feed intake, FCR, egg quality traits and retentions of DM, OM and NFE. Significantly (P<0.05) higher retentions of CP, CF, EE, GE and phosphorus was observed with supplementation of NSP enzymes and similar trend was observed with both NSP and phytase to BD except for phosphorus indicating no associative effect of phytase and NSP enzymes on above nutrient retentions. Intestinal pH, viscosity and E. coli count significantly (P<0.05) reduced with supplementation of NSP enzymes and no further improvement was observed on these variables with supplementation of phytase with NSP enzymes. Gut histology revealed broad and disrupted villi with little goblet cell activity. No significant (P<0.05) effect on feed cost due to addition of phytase and/or NSP enzymes to BD was observed. The cost of feed to produce dozen eggs was comparable among SD, BD and BD supplemented with NSP enzymes and phytase.*
Key words: *Egg quality, Gut conditions, NSP enzymes, Nutrient retention, Phytase, Viscosity.*

I. Introduction:

In recent years there has been a concerted effort to improve the nutritive value of feedstuffs by using exogenous enzymes. On the basis of many publications it may be concluded that the nutritional and economic value of corn, soybean meal (SBM) and other ingredients commonly used in poultry diets in India can be improved by the addition of suitable combinations of NSP enzymes and phytase. An increase in the productive value with enzyme supplementation can be nutrient encapsulating effect of the cell walls and therefore improved energy and amino acid achieved by: (1) release of available phosphorus from phytate hydrolysis, (2) elimination of carbohydrate-protein linkages and therefore improved availability of amino acids, and (3) solubilization of cell wall, non-starch polysaccharides (NSP) for more effective hindgut fermentation and improved overall energy utilization, (4) hydrolysis of certain types of anti-nutritive properties of certain dietary components, including NSP, by their enzymatic hydrolysis to the prebiotic type components which, in turn, may facilitate gut development and health in young chickens. The use of phytase in poultry diets has become widespread because it reduces the P content of manure and can replace inorganic P in feed, thereby reducing P excretion and feed cost. Research on the effect of phytase in broiler diets has been extensive [1]; [2]; [3] where as research on the effect of phytase in layer diets has been less [4]; [5]; [6]; [7]. [8] Reported that commercial phytase increases the release of phytate P from various feedstuffs fed to layers and broilers from between 23 and 34.9% to between 46.8 and 72.4%, respectively. Interactions have been found between xylanase and phytase used in broiler diets [9]; [10]; [11], with phytase affecting the intestinal viscosity and xylanase affecting P availability. However, information on the effect of combining phytase and NSP enzymes on performance and nutrient digestibility in poultry has not been traced. An attempt has been made to explore the additive effect of NSP enzymes and phytase singly or in combination to low energy layer diets.

II. Materials and Methods

2.1. Experimental birds and diets

The study was conducted at All India Co-ordinated Research Project (AICRP) on Poultry breeding Hyderabad on one hundred and fifty layers (40 weeks) of uniform body weight and production to asses the effect of supplementing NSP enzymes and phytase to low energy corn-soybean meal based diets on egg production, egg quality and nutrient utilization by randomly allotting them to five dietary groups with six replicates per group and five birds per replicate. The dietary treatments were standard layer diet (2600 kcal ME/kg) (SD) and one low energy diet (2300 kcal ME/kg) supplemented with NSP enzyme complex (xylanase, 60000, cellulase, 400 and β -D glucanase 3200 IU/kg feed) and phytase at dose rates of 675 IU / kg. The details of the experimental diets are presented in TABLE 1. The ingredient and nutrient combination of experimental diets is given in TABLE 2. Feed was offered *ad libitum* to replicate groups of birds in cages for three periods of 28 days each and reared under standard management conditions.

Table 1: Details of diets of layer Experiment

Diet No	Diet
1	Standard control diet (SD)
2	Basal diet (BD)
3	BD + NSP enzymes
4	BD + Phytase
5	BD + NSP enzymes + Phytase

Table 2: Ingredient composition of experimental diets of layer Experiment

Ingredient (g/kg)	Standard Diet	Basal Diet
Maize	572.3	464.4
Soybean meal	258. 6	112.0
De oiled rice bran	50.0	50.0
Sunflower Cake	4.8	258.2
Salt	4.5	4.5
DL-Methionine	1.0	0.49
Di-Calcium phosphate	12.8	12.5
Shell grit	93.7	95.3
Trace mineral mixture[1]	1.2	1.2
Vitamin premix[2]	0.25	0.25
Choline Chloride (50%)	0.50	0.50
Liver Tonic	0.50	0.50
Total	1000	1000
Nutrient Composition (Calculated)		
ME(kcal/kg)	2600.0	2300.0
Protein (%)	17.0	17.0
Calcium (%)	3.60	3.60
Available phosphorus (%)	0.33	0.33
Lysine (%)	0.83	0.78
Methionine (%)	0.35	0.35
Crude Fiber (%)	3.50	6.83

[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 D_3, 3000IU; Vitamin K,2mg; Riboflavin, 25mg; VitaminB_1,1mg; Vitamin B_6, 2mg; Vitamin B_{12}, 40mcg and Niacin, 15 mg.

2.2. Criteria of response

Data on daily egg production was recorded for each period consisting of twenty-eight days. Percent hen day (HD) egg production was calculated for each treatment. Weekly feed intake was recorded and efficiency of feed utilization was expressed as feed intake / dozen eggs. The eggs laid during the last four consecutive days of last laying period were collected to assess egg quality parameters. Body weight of each bird was recorded at the beginning and end of laying period.

2.3. Nutrient retention studies

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 four days period were ground and analyzed for proximate principles as per the method of [12].

2.4. Gut health

To study the effect of NSP enzymes with or without phytase 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 20^0c. 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.

2.5. 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 [13]. The paraffin blocks were cut in to 5μ thick sections and stained with routine H and E stain [14] and used for microscopic examination.

2.6. Statistical Analysis

The data were subjected to appropriate statistical analysis using Statistical Package for Social Sciences (SPSS) 16th version and comparison of means was tested using Duncan's multiple range tests [15].

III. Results and Discussion

3.1. Nutrient composition of experimental ration

Nutrient composition (% Dry matter basis) of standard layer and basal diets is presented in TABLE 3

Table 3: Nutrient composition (% Dry matter basis) of layer diets (Analyzed)

Diet	Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	Nitrogen free extract	Total ash	Gross energy (kcal/g)	Total phosphorus
Standard diet (SD)	86.00	81.51	17.44	4.89	3.70	55.49	18.49	3.54	0.68
Basal Diet (BD)	84.31	81.39	17.02	4.31	6.84	53.21	18.61	3.15	0.62
BD + NSP enzyme	86.36	81.42	17.43	4.32	6.89	52.78	18.58	3.14	0.64
BD + Phytase	85.92	81.49	17.48	4.31	6.83	52.87	18.51	3.16	0.64
BD + NSP enzyme + Phytase	85.07	81.68	17.49	4.36	6.89	52.94	18.32	3.14	0.64

Each value is average of duplicate analysis

3.2. Hen day production (%)

The effect of supplementing NSP enzymes and phytase was observed on egg production. The overall average egg production/hen day production (%) though statistically insignificant, however, was numerically higher by 11% in NSP enzymes supplemented group (TABLE 4). In phytase and NSP enzymes plus phytase supplemented groups, the overall average egg production was 7.9% higher than Basal Diet (BD). [16] Reported no change in egg production with supplementation of xylanase and phytase individually or in combination to wheat based laying hen diets with low levels of phosphorus. Similarly [17] observed no significant difference in egg production on enzyme supplementation of phytase to corn soya based layer diets.

3.4. Feed intake

Feed intake (g) was not influenced by supplementation of NSP enzymes and phytase alone or combination of both to BD (TABLE 4). Corroborating with the findings of [16] in layers fed wheat based diets supplemented with xylanase and phytase. [18] Observed no effect on feed intake with supplementation of phytase and zinc to maize and soybean meal based diets. Contrary to these findings, [17] observed significantly ($P<0.06$) more feed intake in phytase supplemented diets compared to basal diet.

3.5. Weight change

During 1st period weight loss was observed in all the treatment groups irrespective of supplementation of NSP enzymes and phytase to BD due to high environmental temperature. No effect of NSP enzymes and phytase was observed on body weight changes in subsequent cycles (TABLE 4).

3.6. Feed conversion ratio

The comparable feed intake, egg production and egg weight, resulted in no effect on feed conversion ratio (feed intake g/g of egg) with supplementation of NSP enzymes or phytase or combination of both to BD (TABLE 4). The results concurred with [18] where zinc and phytase supplementation to maize, soybean based diets had no effect on feed conversion efficiency and with [19] in birds fed diets containing xylanase and flavophospholipol individually or in combination.

Table 4 : percent hen day production, feed intake, FCR, cost per dozen eggs and weight gain of layers fed diets supplemented with NSP enzymes and phytase

Diet	Hen day production (%)	Feed intake(g/bird/ d)	Feed conversion ratio (g intake/g egg)	Cost of feeding (Rs.)	Cost per dozen eggs (Rs.)	Weight gain (g) (Total)	Intestinal weight (% of body weight)	Intestinal length (cm/100 g)
Standard Diet (SD)	66.27	107.6	2.99	58.38	37.82	108.5	2.27	9.24
Basal Diet (BD)	60.20	107.0	3.35	56.29	40.15	47.81	2.35	9.12
BD + NSP enzymes	64.35	109.2	3.31	58.74	38.35	57.42	2.38	9.49
BD + Probiotics	68.14	113.8	3.20	56.49	37.41	68.81	2.70	10.56
BD + NSP enzymes+ Probiotics	68.06	112.8	3.14	65.69	36.96	52.40	2.27	10.62
SEM	**1.13**	**2.37**	**0.07**	**1.45**	**0.78**	**13.97**	**0.08**	**0.28**
***P* Value**	**0.135**	**0.873**	**0.609**	**0.144**	**0.763**	**0.075**	**0.259**	**0.369**

Each value is average of 3 cycles of 28 days
Means with different superscripts in a column differ significantly (P>0.05)

3.7. Egg quality traits

Egg weight (g) and egg mass (g) were not affected by addition of NSP enzymes and phytase individually or in combination to BD (TABLE 5). [17] Also observed no effect on egg weight with supplementing phytase to corn soybean meal diets, but egg mass was significantly ($P<0.01$) affected by enzyme supplementation. [18] reported that supplementation of zinc and phytase to maize and soybean meal based diets had no effect on egg mass and egg weight. The Haugh unit score was comparable between BD and SD and was not affected by supplementation of NSP enzymes and phytase to BD (TABLE 5). [18] Also reported no effect of zinc and phytase supplementation to maize, soybean based diets on haugh unit score. [17] Reported insignificant effect of supplementation of phytase to corn soybean meal layer diets on haugh unit score. Albumen and yolk index were not influenced by supplementation of NSP enzymes and phytase individually or combination of both to BD (TABLE 5). [20] Found no effect of supplementation of manganese and phytase on yolk and albumen index in layers fed corn soya based diets. Shell weight (g) and shell thickness (mm) were not affected by NSP enzymes and phytase supplementation to BD (TABLE 5). [21] Reported no effect of phytase supplementation to low inorganic phosphorus, energy and protein diets on shell weight and shell thickness. Specific gravity was not affected by addition of BD with NSP enzymes and phytase individually or in combination (TABLE 5). Similar results have been reported by [17] with supplementation of two different sources of phytase to corn soybean meal diets.

Table 5: Egg quality traits of layers fed low calorie diet supplemented with NSP enzymes and phytase

Diet	Egg weight (g)	Egg Mass (g)	Haugh unit	Albumin Index	Yolk Index	Shell weight (g)	Shell Thickness (mm)	Specific gravity (unit)
Standard Diet (SD)	53.65	35.13	73.93	0.733	0.267	5.37	0.338	1.100
Basal Diet (BD)	52.98	35.92	67.97	0.633	0.267	5.50	0.343	1.149
BD + NSP enzymes	52.81	40.80	75.00	0.800	0.277	5.20	0.340	1.151
BD + Phytase	53.53	36.45	71.25	0.667	0.272	5.72	0.342	1.155
BD + NSP enzymes + Phytase	52.06	35.06	77.53	0.800	0.262	5.43	0.348	1.126
SEM	**0.42**	**1.06**	**1.34**	**0.03**	**0.002**	**0.07**	**0.003**	**0.009**
***P* value**	**0.793**	**0.419**	**0.202**	**0.219**	**0.371**	**0.273**	**0.899**	**0.368**

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

3.8. Nutrient retention

The retention of Dry Matter (DM), Organic Matter (OM) and Nitrogen Free Extract (NFE) were not affected by supplementation of NSP enzymes and phytase (TABLE 6). While Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), Gross Energy (GE) and phosphorus retentions significantly ($P<0.05$) improved in NSP enzymes and phytase supplemented groups. The CP retention was lower in BD than SD, supplementing NSP enzymes or phytase to BD increased CP retention and was comparable to BD but the combination of NSP enzymes and phytase added to BD had no effect on CP retention and was lower than SD. The EE and GE retentions were lower in phytase supplemented group compared to SD, while retentions of these nutrients with

NSP enzymes supplementation to BD improved and was comparable to SD. No associative effect of NSP enzymes and phytase was observed on nutrient retention. The results are in agreement with [19] who reported that supplementation of xylanase and flavophosphorus as well as their combination in laying hen diets had only slight influence on apparent nitrogen corrected ME and digestibility of OM, the greatest effects were observed on digestibility of CP and fat, the latter being additionally influenced by age. Per cent tibia ash content of BD supplemented with NSP enzymes and phytase was insignificantly (P>0.05) higher than BD by 3.06%. [22] Reported that the addition of 600 FTU/kg phytase improved the metabolism of nutrients in nutritionally deficient diets. [21] Reported increased tibia ash per cent on supplementation of phytase to low inorganic phosphorus, energy and protein diets. Phosphorus digestibility significantly improved on supplementing phytase to corn soybean meal based diets [17].

3.9. Gut conditions

Supplementation of NSP enzymes and phytase individually reduced pH of intestinal contents, where as combination had no effect on intestinal pH (TABLE 6). Intestinal viscosity was reduced (P<0.01) in BD supplemented with NSP enzymes and/ or phytase (TABLE 6). The *E. coli* count decreased (P<0.01) in supplemented groups compared to SD and BD (TABLE 6).The results are in line with [23] who studied the synergistic effect of prebiotics, probiotics and acidifier singly or in combination in broiler chickens and reported reduced total coli form count. [24] Studied the efficiency of a gut acidifier, a probiotics and antibacterial feed additive in female commercial broiler diets and recorded a reduction in the total coliform count in the crop and cecal contents. Viscosity values obtained are in agreements with [25] who studied the effect of different feed additives Allzyme, Avilamycin, Avimos, Biomos, yeast extract, Avizyme, xylanase, Gustar alone or in combination could reduce the gut viscosity. There was no influence of supplementation of NSP enzymes, phytase alone or combination of both to BD on, intestinal length (TABLE 6).

Table 6: Nutrient retention, intestinal pH, viscosity, *E. coli* and tibia ash content of layers fed low calorie diet supplemented with NSP enzymes and phytase

Diet	Nutrient retention (%)								Gut condition			Tibia ash (%)
	DM	OM	CP	CF	EE	NFE	GE	Phosphorus	pH	Viscosity (cP)	*E. coli* count (cfu/ml)	
Standard Diet(SD)	63.71	60.58	66.18^{a}	40.38^{b}	76.55^{a}	64.63	69.92_{a}	32.52^{bc}	6.17^{a}	6.29^{a}	2.54^{a}	39.07
Basal Diet (BD)	57.76	60.90	59.04^{b}	40.14^{b}	73.45^{c}	64.13	59.66^{b}	30.58^{c}	6.18^{a}	6.15^{ab}	2.32^{ab}	36.09
BD + NSP enzymes	60.86	64.65	64.35^{a}	43.77^{a}	76.65^{a}	66.70	63.24^{b}	33.58^{abc}	5.73^{b}	4.07^{c}	1.47^{bc}	39.22
BD + Phytase	57.31	60.88	62.89^{ab}	40.84^{b}	75.77^{ab}	64.05	65.36^{ab}	35.39^{ab}	5.84^{ab}	5.38^{b}	0.95^{c}	38.76
BD + NSP enzymes + Phytase	58.20	60.70	64.88^{a}	44.30^{a}	74.33^{bc}	64.48	63.65^{b}	36.55^{a}	5.97^{ab}	3.89^{c}	1.59^{bc}	39.15
SEM	**1.01**	**0.79**	**0.83**	**0.54**	**0.37**	**0.80**	**1.01**	**0.60**	**0.06**	**0.25**	**0.18**	**0.85**
***P* value**	**0.221**	**0.445**	**0.053**	**0.014**	**0.008**	**0.858**	**0.013**	**0.005**	**0.033**	**0.001**	**0.011**	**0.785**

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

3.10. Gut histology

The addition of NSP enzymes and phytase alone or combination of both to BD had not shown any positive influence on gut morphology and the intestinal sections revealed broad and disrupted villi with little goblet cell activity (Fig. 1). These results agree with the findings of the [26] who observed decreased number of goblet cells, goblet cell size in enzyme supplemented barley (60%) based broiler diet when compared to the un supplemented group.

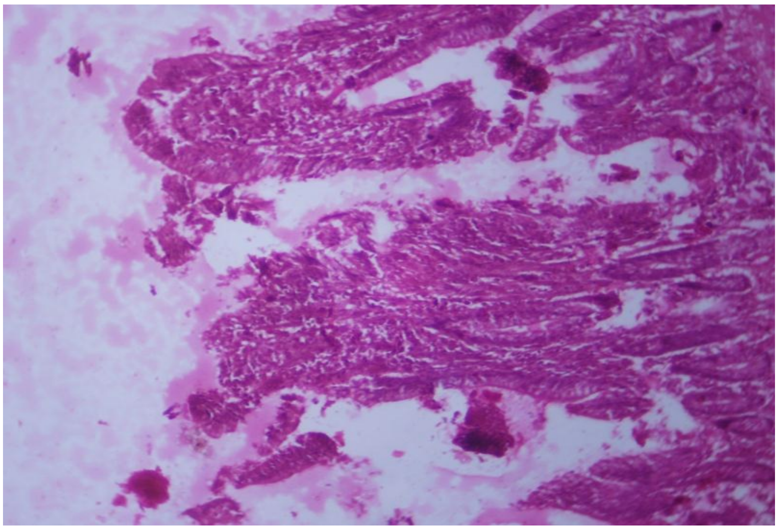

Figure.1: H & E section of deodenum showing broad and disrupted villi with little goblet cell activity (x200)

3.11. Cost of feeding

The overall average feed cost to produce dozen eggs was less by 6.87% in BD supplemented with NSP enzymes and phytase compared to BD (TABLE 4) indicating better utilization of low calorie diets (BD) when supplemented with these feed additives. The overall cost of feeding through out the experimental period between SD, BD and BD supplemented with phytase and/or NSP enzymes do not differ significantly (TABLE 4). The results are in agreement with [27] who reported supplementation of enzymes to diets varying in energy produced 0.45 cents more profit than hens fed low energy diets without enzyme. [28] Reported lower cost of egg production by 2 and 3 paise/egg on enzymes supplementation to standard ration as well as to low dense ration than their respective control.

IV. Conclusion

Overall, supplementing NSP enzymes selected from *in vitro* studies along with phytase improved egg production and decreased average feed cost to produce dozen eggs. Associative effect of NSP enzymes with phytase was observed for nutrient retentions, gut health in layers fed low energy diets at reduced cost of production.

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