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Use of Exogenous Enzymes on Laying Hens Feeding During the Second Production Cycle

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Abstract: The aim of this research was to evaluate the effect of exogenous enzymes on performance and egg quality of second production cycle laying hens. One hundred and sixty laying hens, 72 week-old, were used during five periods of 28 days each. Birds were distributed in a completely randomized design, with four treatments and five replicates, using eight birds per experimental unit. Treatments consisted in two diets enzyme-free (positive control (PC) and negative control (NC)) and two diets with exogenous enzymes addition (reformulated diet (RD) and "on top" diet (OT)). Feed consumption, egg weight and internal and external egg quality were not affected by treatments. Egg production (EP), egg mass (EM) and feed conversion by egg mass and egg dozen were worse when using NC. Exogenous enzymes addition (RD and OT) resulted in a performance similar or superior to PC diet for those variables and birds fed OT diet presented better results for EP and EM. There was no treatment effect on excreta humidity and tibial ashes and phosphorus. However, there was an effect due to enzymes supplementation on plasmatic calcium and phosphorus, with RD resulting in similar levels to PC and OT diet presenting higher levels, which indicate that the enzyme was efficient in turn those minerals available. Calcium tenor in tibia and bone resistance were altered by treatments, with lower values obtained in birds that received "on top" enzymatic supplementation. Supplementation with Allzyme® SSF and Allzyme® Vegpro™ was efficient in increasing egg production in second production cycle laying hens.

Key words: Additive, bone resistance, digestibility, economic viability, vegetable ingredients

Introduction

Vegetable ingredients used in poultry diets frequently have anti-nutritive factors, which reduce ingredient digestibility as well as nutrient availability of the entire diet.

Among anti-nutritive factors, the most common are the non-starch polysaccharides (NSPs), which increase intestinal viscosity, impairing endogenous enzymes action and absorption and phytate, which turn minerals unavailable, mainly bivalent metals. In order to act on NSPs, there are several enzyme complexes with effects on arabinoxylans digestion, composed of pentosans and B-glucans, others yet, have effects on α-galactosides and oligomannans. Specific for phytates, the enzyme phytase has been very efficient on releasing P from the ring-shaped structure of phytate, as well as from the above mentioned minerals as calcium, zinc, iron, manganese and others. According to Schang and Azcona (2003), the presence of pentosans in wheat, oligosaccharides in soybean and phytates in every vegetable ingredient limit energy, protein and phosphorus digestibility of diets. Using specific enzymes allows the improvement of these compounds utilization and nutrient digestibility, by and large, which contributes for animal's performance improvement (Dale, 2000; Vieira, 2003; Fernandes and Malaguido, 2004). Rezaei *et al.* (2007) indicate the use of phytase as a way to reduce phosphorus emission to the environment.

In general, enzymes are used in animal feeding aiming two well-defined purposes: to complement the enzymes insufficiently produced by the animal (amylases and proteases) and to provide animal those enzymes not synthesized by them (cellulases) (Fischer *et al.*, 2002). Besides, researches performed by Choct (2004) and Ferket (2004) have demonstrated beneficial changes on microbial intestinal population by supplementing exogenous enzymes in the diets. Such benefits occur due to a higher starch, protein and fat digestion rate in the small intestine, therefore limiting substrate for pathogenic flora that eventually exists.

Commercial preparations involving enzymes as amylase, xylanase, protease, α-galactosidase, pectinase, cellulose and lipase have been used successfully on poultry performance improvement (Garcia *et al.*, 2000). Soluble β-glucans and pentosans (xylose + arabinose) are observed in several cereals and are capable of forming gels, when in touch with water, creating viscous solutions that delay nutrient absorption. It is postulated that pentosans form complexes bonds with the albumen fraction of proteins. Pentosans, yet, raise the diet volume by water retention in the gastrointestinal tract, causing a decrease in feed intake. Pourreza *et al.* (2007) report that the improvement on nutrient availability attained by exogenous enzymes supplementation occurs due to NSPs hydrolysis and consequently, the negative effects reduction of those carbohydrates, resulting in a reduction of digesta viscosity.

According to Filer *et al*. (1999), the source and production process of an enzyme commercial supplement might have a positive effect on its properties, once enzyme production by solid state fermentation may generate additional activities not observed in enzymes produced by liquid immersion. The activity of phytase produced by genetically modified organism by liquid immersion fermentation in comparison *in vitro* to the activity of this enzyme produced by non genetically modified organism by solid state fermentation (Allzyme® SSF) in substrates as wheat, rice, soybean meal and initial ration for broiler chickens, the authors observed higher orthophosphate, amino nitrogen and reducing sugars release when using the second enzyme.

Therefore, this research was developed aiming to evaluate the effects of exogenous enzymes supplementation on second production cycle laying hens (72 to 92 weeks) performance.

Materials and Methods

The trial was carried out at the Poultry Sector of Animal Science Department, belonging Federal University of Paraiba, Areia, Brazil.

One hundred and sixty semi-heavy Bovans Goldline laying hens, 72 week-old, were used, during five periods of 28 days each. Birds were distributed in a completely randomized design, with four treatments, five replicates and eight birds per experimental unit. Treatments consisted in two enzyme-free diets (positive control - PC and negative control - NC) and two diets with exogenous enzymes addition (reformulated diet - RD and "on top" - OT) (Table 1).

PC diet was formulated to attend laying hens requirements, containing 2,800 kcal of metabolizable energy (ME) per kg, 17% of crude protein (CP), 0.780% of digestible lysine (Lis_{dig}) and 0.670% of digestible methionine+cystine ($M+C_{dig}$), while NC diet was formulated with 5% reduction on ME, CP, Lis_{dig} and $M+C_{dig}$, providing 2,660 kcal of ME per kg, 16.15% of CP, 0.741% of Lis_{dig} and 0.636% of $M+C_{dig}$.

RD was reformulated with the addition of 0.015% of Allzyme™ SSF and 0.040% of Vegpro in order to provide similar nutritional levels to the NC. For RD calculation, it was considered the valorization of the main protein ingredient (soybean meal) in 7% for ME, CP and amino acids, as well as the enzyme (Allzyme™ SSF) nutritional matrix, which nutritionally contributed with 75 kcal of ME per kg, 0.2% of CP, 0.029% of lysine, 0.020% of methionine+cystine, 0.1% of Ca and 0.1% of available P, considering the dose used here.

OT diet corresponded to NC diet plus "on top" enzyme addition, meaning, no soybean meal valorization or enzyme nutritional matrix on ration calculation. Enzymes were added in the same amount as in RD.

Table 1: Composition of diets

Ingredient	Positive Control	Negative Control	Reformulated Diet	On Top
Corn	596.28	612.57	545.13	612.58
Soybean meal	243.56	206.58	164.08*	206.58
Wheat meal	-	42.06	161.72	42.06
Bone and meat meal	20.00	20.00	13.82	20.00
Vegetable oil	21.67	-	-	-
Calcitic limestone	101.50	101.89	106.72	101.89
Bicalcium phosphate	9.03	8.68	0.00	8.68
Choline chloride	0.70	0.70	0.70	0.70
Salt	3.68	3.69	3.99	3.69
DL - Methionine	1.92	1.78	1.97	1.78
L - Lysine Hcl	0.19	0.58	0.40	0.58
Caulim[1]	0.55	0.55	0.00	0.00
Etoxiquim[2]	0.10	0.10	0.10	0.10
Enradin[3]	0.05	0.05	0.05	0.05
Colistin[3]	0.02	0.02	0.02	0.02
Mineral premix[4]	0.50	0.50	0.50	0.50
Vitamin premix[5]	0.25	0.25	0.25	0.25
Vegpro	-	-	0.40	0.40
Allzyme SSF 75	-	-	0.15	0.15
Total	1000.00	1000.00	1000.00	1000.00
Nutritional composition				
ME, $kcal \cdot kg^{-1}$	2800	2660	2660	2660
Protein, %	17.000	16.150	16.150	16.150
Calcium, %	4.200	4.200	4.200	4.200
Available phosphorus, %	0.375	0.375	0.375	0.375
Digestible Lysine, %	0.780	0.741	0.741	0.741
Digestible Met+Cys, %	0.670	0.636	0.645	0.636
Digestible Methionine, %	0.435	0.408	0.408	0.408

[1]Inert; [2]Antioxidant; [3]Antimicrobian; [4]Mineral supplement (by product kg): Mn, 60,000 mg; Fe, 50,000 mg; Zn, 50,000 mg; Cu, 10,000 mg; Co, 2,000 mg; I, 1,000 mg;, [5]Vitamin supplement (by product kg): Vit. A - 15,000,000 UI, Vit. D_3 - 1,500,000 UI, Vit. E - 15,000 UI, Vit.B_1 - 2.0 g, Vit.B_2 - 4.0 g, VitB_6 - 3.0 g, Vit.B_{12} - 0.015 g, Nicotinic acid - 25 g, Panthotenic acid - 10 g, Biotin - 10 g, Vit.K_3 - 3,0 g, Folic acid - 1.0 g, Zinc bacitracin - 10 g, Se - 250 mg. *Soybean meal valorized in 7% of ME, CP and amino acids.

During 20 days before the experiment, egg production was recorded and laying rate for this period was calculated to standardize the experimental units. Mean egg production by treatment was 66.3, 66.1, 66.5 and 66.4%, for PC, NC, RD and OT, respectively, with a general mean of 66.3 ± 1.1%. During birds distribution they were also weighed by pen, to calculate initial mean weight, which was further used as a covariant in statistical analysis.

Laying hens were housed in wire cages with 25 cm x 40 cm x 40 cm. Light program adopted was natural lighting and water was offered "ad libitum". Rations were supplied at large and offered twice a day, in order to avoid any waste.

Variable analyzed in each period were: Feed intake (g/bird/day), egg production (%), egg weight (g) and egg mass (g/bird/day), feed conversion by egg ass (kg/kg) and by egg dozen (kg/dz), yolk, albumen and shell weight (g) and percentage (%) and specific gravity.

Egg collection was performed twice a day (10:00 and 16:00 h) and intact and defected eggs and mortality were recorded in laying frequency sheet. Egg production in percentage was calculated through the division of total egg amount per experimental unit by number of birds. Eggs from the last three days of each experimental period were individually weighed in order to obtain eggs mean weight. Calculation of egg mass was performed through the product of egg production by mean egg weight, by experimental unit. Feed conversion by egg mass was calculates through the ratio between feed intake and egg mass produced. Feed conversion by egg dozen was calculated through the ratio between feed intake and egg production and the obtained value was multiplied by twelve.

Out of the total amount of eggs collected by replicate, six unites were selected for weight and percentage determination of yolk, albumen and shell, specific gravity and yolk pigmentation. After manual separation of these components, shells were placed in 105°C stove for four hours. Percentage was obtained by dividing those variables weights by mean egg weights and next, multiplying them by 100. Yolk pigmentation was determined by colorimetric fan (Roche®), which after visual comparison, it was attributed the numeric value from the fan to the most alike color of the yolk.

Specific gravity was determined by saline fluctuation method, as described by Hamiltom (1982). Every experimental period end, representative samples (two eggs) of each experimental unit were selected; further, eggs were immersed in different saline solutions with the correspondents adjustments for a volume of 25 liters of water, with densities varying from 1.060 to 1.100, with 0.0025 intervals.

A blood sample was collected from one bird per experimental unity at the 28° day from the fifth period, in order to analyze plasmatic calcium and phosphorus. Samples were centrifuges at 4,000 rpm during 15 minutes to obtain the plasma for P and Ca analyses, performed using a commercial kit from Labtest and a colorimetric spectrophotometer.

Bird's tibiae were collected; the left ones were used for break resistance and the right ones, after fat extraction in "Soxhlet" extractor, were placed in a 600°C oven for four hours (Silva, 1991) for ashes (TA), calcium (TCa) and phosphorus (TP) determination in tibia. Bones were placed in a horizontal position onto two holders and the pressure was applied in the center of these. The maximum amount of strength applied to the bone before its break was considered as break resistance.

Results were submitted to covariance analysis using the program SAS (2004). Birds initial weights were used as a covariant and in the event of significant differences, means were adjusted by the covariant and compared by Tukey's test. For yolk pigmentation, analysis was performed by chi-square test.

Results and Discussion

Results of feed intake, egg production, weight and mass and feed conversion by egg mass and egg dozen are presented in Table 2.

Feed intake and egg weight were not influenced by treatments ($P>0.05$). Similar results were found by Vukic and Wenk (1993), Pan *et al.* (1998) and Scheideler *et al.* (2005) that do not verify any influence of exogenous enzymes on feed intake by laying hens.

Treatments influenced ($P<0.01$) egg production, egg mass and feed conversion by egg mass and egg dozen. Egg production was reduced when birds received NC diet, demonstrating that providing 5% less ME, CP, Lis_{dig} and $M+C_{dig}$ was not enough to withstand egg production levels of PC. However, when RD was provided to the birds, laying rate increased in comparison to that of NC, demonstrating that enzyme utilization led to a nutrient digestibility increase, since diets were formulated to reach the same nutritional requirements. Concerning PC diet, egg production of laying hens fed RD was similar to it and this evidences once more, the better use of feed supplemented with enzymes, since nutritional levels calculated for the reformulation of this diet were 5% lower that those of PC diet. On the other hand, when OT diet was used, egg production overcame the production of birds receiving PC diet. This effect demonstrated that when OT diet is used, nutrient supply is increased even more, not only due to a higher digestibility of feed nutrients, but also due to the enzyme's nutritional matrix contribution.

Egg mass and feed conversion by egg mass and by egg dozen did not differ statistically between PC and NC diets, although numerically NC have presented the worst results. However, when the enzyme was added in RD those variables improved significantly in comparison to NC diet and was in the level with the values obtained wit PC diet. As for OT diet, enzyme supplementation promoted a higher egg mass production in comparison to PC diet and for feed conversion by egg mass and by egg dozen, OT diet resulted in similar values to those obtained wit PC and RD.

According to Yu and Chung (2004), the improvement in digestibility and diet's nutrient utilization through ME reduction in the diet supplemented with exogenous enzymes might be attributed to the decrease on energy losses due to caloric increment and to volatile fatty acids in excreta (as a result of inefficient microbial fermentation product). A great amount of starch can reach large intestine and can be degraded by microbial fermentation, resulting in a poor metabolic use in comparison to enzyme degradation with glucose absorption in the small intestine (Scheideler *et al.*, 2005). Thus, when enzymes degrading non-starch polysaccharides are used, cell wall is going to be broken, exposing increased starch amounts to digestive enzymes and more energy will be absorbed and will become available to production processes.

Likewise, Zanella *et al.* (1999) reported that supplementation with the enzymes α-amilase, protease

Table 2: Effect of exogenous enzymes on performance of second production cycle (72 to 92 weeks)

Treatment	Feed intake (g/bird/day)	Egg production (%)	Egg weight (g)	Egg mass (g/bird/day)	Feed conversion by egg mass (kg / kg)	Feed conversion by egg dozen (kg / dz)
PC	125.9000	55.800^{b}	71.7000	40.0000^{bc}	3.1600^{ab}	2.7400^{ab}
NC	126.6000	49.900^{c}	73.4000	36.6000^{c}	3.4600^{b}	3.0300^{b}
RD	130.3000	57.800^{ab}	71.3000	41.2000^{ab}	3.1700^{ab}	2.6900^{a}
OT	126.9000	61.000^{a}	73.7000	44.9000^{a}	2.8300^{a}	2.5000^{a}
Mean	127.4000	56.100	72.5000	40.7000	3.1500	2.7400
CV (%)	2.3000	3.700	3.8000	5.5000	6.6000	6.4000
Pr	0.1312	0.0001	0.4797	0.0003	0.0026	0.0022

PC = Positive Control; NC = Negative Control; RD = Reformulated Diet; OT = On Top; CV = Coefficient of variation; Pr = Probability. ^{a,b,c} Means followed by different letters in the same row differ statistically among each other, by Tukey's test, at 5% of probability.

and xylanase in reduced energy diet for broiler chickens resulted in better nutrient use and totally compensate the energetic reduction.

Von Bassenheim (2006), using a reformulated diet with Allzyme^{TM}SSF which nutritionally contributed with 120 kcal ME·kg^{-1}; 0.2% of CP; 0.029% of lysine; 0.011% of methionine; 0.1% of available P and 0.1% of Ca, found results in feed intake and egg production similar to those obtained for control diet. Using values of ME intake of birds fed control diet in comparison to the feed intake observed in laying hens fed the reformulated diet, the author concludes that the real ME income reached by Allzyme^{TM} SSF was of 75 kcal·kg^{-1} and that enzyme addition on reformulated diet was enough to sustain the same production level as control diet, making possible to reduce feed supplying costs.

Similarly, Fuente *et al.* (2006) did not find significant differences between second production cycle performance with control diet or reformulated diet with Allzyme^{TM} SSF providing50 kcal ME·kg^{-1}. On the other hand, Gregorio *et al.* (2006) found an egg production 1.8% higher in laying hens fed the reformulated diets (Allzyme^{TM} SSF providing 75 kcal ME) than control production.

This study results corroborate the technical viability of using exogenous enzymes on laying hens feeding, clearly demonstrating the recovery, or even the overcoming of performance levels on diets with reduced energy, protein amino acids and enzyme supplemented.

Another interesting effect of enzyme supplementation on second production cycle laying hens could be observed concerning recovery and maintenance of egg production rate. It is well known that layers in final cycle tend to reduce laying rate as their age increases (Oliveira *et al.*, 2002). This event became clear in birds receiving PC diet, which egg production slightly increased from 72 to 76 weeks and after that decreased gradually. When laying hens received NC diet, this egg production drop with the age increase was even more visible. However, when rations supplemented with exogenous enzymes were supplied, there was a recovery on laying rate in comparison to birds fed the NC diet from 80th week on. Using RD, there was a smoother reduction on laying rate until the 84th week than with NC diet and from this age on to the end of the trial (92 week-old) egg production was kept in levels superior to those of PC diet. On the other hand, when enzyme supplementation was made "on top", the recovery and maintenance of laying rate occurred earlier and from the 84th week, values were superior to those obtained with PC diet. Therefore, one can suppose that nutritional requirement for the maintenance of egg production rate of laying hens with the age increase are greater than the nutritional income provided by the PC diet, or yet, the feed use by these birds is decreased as the age increases. Thus, diet supplementation of these birds with exogenous enzymes was efficient in increase nutrient availability for egg production.

Internal and external egg quality were not affected by treatments (P>0.05). Since no difference were observed between the results obtained with PC diet in comparison to NC, one can assume that nutrient reduction, was not high enough to affect weight and proportion of egg components, although it has affected laying rate. The grater nutrient availability by enzyme addition was directed to the egg amount increase, not much changing egg quality traits.

Results related to excreta humidity, plasmatic Ca and P, tibial ashes, Ca and P and bone resistance are presented in Table 3.

There was no treatment effect on excreta humidity and tibial ashes and phosphorus (P>0.05).

According to Opalinski (2006), viscosity is directly proportional to excreta humidity, due to the greater water retention by non-starch polysaccharides (NSPs). A decrease in the excreta humidity level is noticed when especially glucanases are added to the diets (Choct, 2004). In this study, it could be expected an increase on excreta humidity for RD, since it contains higher amount of wheat meal and hence, higher levels of NSPs. However, this effect was not observed, probably due to enzymes action on those polysaccharides, resulting in excreta humidity, similar to the other treatments.

However, there was an effect of enzymatic supplementation on plasmatic calcium and phosphorus (P<0.01), because although every other diets have been formulated to provide the same available calcium and phosphorus amount, in RD formulation it was considered the income of 0.1% of available P and 0.1% of Ca due to the inclusion of 0.015% of Allzyme SSF®,

Table 3: Effect of exogenous enzymes on excreta humidity (EH), plasmatic Ca (PCa) and P (PP) levels, tibial ashes (TA), Ca (Tca), (TP) and tibial break resistance (TR) in second production cycle laying hens (72 to 92 week)

Treatment	EH (%)	PCa (mg/dL)	PP (mg/dL)	TA (%)	TCa (%)	TP (%)	TR(kgf/mm)
PC	76.5000	2.8600^{b}	1.5300^{b}	56.0000	26.5000^{a}	12.6000	14.0000^{a}
NC	76.9000	2.6900^{b}	1.1600^{c}	63.6000	22.5000^{b}	12.4000	14.4000^{a}
RD	76.4000	2.7200^{b}	1.2400^{bc}	68.3000	21.1000^{bc}	12.6000	18.1000^{a}
OT	77.5000	3.3900^{a}	1.8400^{a}	57.0000	19.9000^{c}	12.5000	6.0000^{b}
Mean	76.8000	2.9100	1.4400	61.2000	22.5000	12.5000	13.1000
CV (%)	1.6000	8.8500	10.7100	12.2800	3.6500	4.2700	20.3800
Pr	0.4977	0.0087	0.0001	0.1186	0.0001	0.8632	0.0030

[1]Chi-square test; PC = Positive Control; NC = Negative Control; RD = Reformulated Diet; OT = On Top; CV = Coefficient of variation; Pr = Probability

resulting similar to those of PC indicate that enzyme was in fact effective in turn these mineral available. The highest plasmatic levels of Ca and P of birds receiving OT diet corroborate with this statement, because enzymatic supplementation increased these mineral availability, resulting in plasmatic levels augmented in comparison to PC diet.

Tibial calcium level and bone resistance were influenced by treatments ($P<0.01$), and the lowest values were obtained with birds receiving enzymatic supplementation "on top". A possible explanation is that these birds also presented a higher egg production and hence, higher calcium demand for eggshell formation, which is constituted mainly of calcium carbonate. According to Brandao *et al.* (2007), during the eggshell formation process, part of the calcium used for it is mobilized from the bones, no matter what are the calcium levels on diet. Even if birds consume ideal quantities of calcium, around 30% of the shell calcium comes from the bones.

Conclusion: Either the nutritional requirements for production rate maintenance in second production cycle laying hens are greater than the nutritional levels provided by the positive control, or the efficient use of these feeds diminishes as bird age.

Supplementation with Allzyme® SSF and Allzyme® Vegpro™ was efficient in increasing egg production in second production cycle laying hens.

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