https://doi.org/10.22319/rmcp.v11i2.4843

Article

Effect of a multienzyme complex and a probiotic in laying hens fed sorghum-soybean-canola diets

Pedro Juárez Morales [a]

Arturo Cortes Cuevas [a*]

José Arce Menocal [b]

Juan Carlos Del Río García [c]

Gabriela Gómez Verduzco [a]

Ernesto Avila González [a]

[a] Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Centro de Enseñanza, Investigación y Extensión en Producción Avícola. Manuel M. López s/n Col. Zapotitlán, 13209, Tláhuac, Ciudad de México, México.

[b] Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Medicina Veterinaria y Zootecnia. Morelia, Michoacán, México.

[c] Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Cuautitlán. Estado de México, México.

*Corresponding author: cortesca68@gmail.com

Abstract:

This study aimed to evaluate the productive parameters and the concentrations of serum intestinal secretory IgA, cholesterol, LDL, and HDL of laying hens fed sorghum + soybean + canola diets with lower content of nutrients and added with a multienzyme complex (proteases, amylases, and xylanases) and a probiotic (*Bacillus subtilis*). A total of 180 Bovans White laying hens, between 42 and 54 wk of age, were randomly assigned to three treatments: 1) control diet; 2) low metabolizable energy diet (50 kcal/kg and 2 % of protein

and methionine and lysine amino acids) + enzymes; 3) diet number two + probiotic. Productive performance results showed a significant difference (P<0.05) in egg weight between both treatments; treatment 3, added with the enzymes and probiotic, increased egg weight, compared to treatment 2. Humoral immunity, cholesterol, LDL, and HDL variables showed no statistical differences (P> 0.05) between treatments. This study demonstrates that low-nutrient (ME, protein, and lysine and methionine amino acids) diets added with a multienzyme complex and probiotic allow similar results in productive parameters compared to the control diet, and without changing intestinal immunity and levels of cholesterol, high- and low-density lipoproteins in Bovans White hens.

Key words: Enzymes, *Bacillus subtilis*, Laying hen, Immunity, Cholesterol.

Received: 05/04/2018

Accepted: 26/06/2019

Introduction

In the last decade, the use of exogenous enzymes in poultry diets has increased in order to enhance energy and protein digestibility[1,2]. Non-starch polysaccharides (NSP) are the major components of fiber in traditional ingredients; previous studies indicate that sorghum contains around 6.5 % and soybean meal 3.3 % of these polysaccharides[3]. Cereal grains contain arabinoxylans and glucans, while soybean and canola contain arabinans, arabinogalactans, galactans, mannans, and pectins[4,5]. The NSP of cell walls, such as soluble and insoluble arabinoxylans, are degraded by xylanases, releasing nutrients encapsulated within the cell wall and improving access to endogenous enzymes[6,7].

In the food industry, xylanases and β-glucanases[8] are widely used to degrade NSP and reduce the loss of endogenous amino acids[9].

Products with multienzyme activity have shown that the combination of xylanases, amylases, and proteases enhances digestibility in poultry diets[10,11,12].

Amerah *et al*[9] reported that corn-soybean meal diets supplemented with proteases, amylases, and xylanases improved the ileal digestibility of protein, energy, as well as nitrogen retention in broiler chickens, which translated into higher productive performances. The addition of beneficial bacteria, such as those of the *Bacillus* genus, to poultry diets, is an alternative to the use of antibiotics for growth promotion[13,14,15].

Recent studies of a commercial product based on three *Bacillus* strains have shown positive effect in corn-soybean diets[16]. Since commercial poultry diets already include exogenous enzymes and probiotics, there is little information about how they interact with each other; enzymes are associated with an intestinal probiotic effect in chickens[12,17]. They also modulate the immune response and suppress the inflammatory immune reactions in the intestinal walls[18].

Moreover, a previous study reported that, in laying hens, the addition of probiotics improves feed conversion and egg quality (decrease in yolk cholesterol level, increase in shell thickness and egg weight), and decreases blood cholesterol levels[19].

This study aimed to evaluate, in Bovans White hens, the effect of the addition of a multienzyme complex (xylanases, proteases, and amylases) alone or combined with a probiotic (*Bacillus subtilis*) in sorghum-soybean-canola diets with low energy and protein content, on production parameters, production of intestinal secretory IgA, and serum levels of cholesterol, LDL, HDL.

Material and methods

The experiment was carried out in the facilities of the Center for Teaching, Research and Extension in Poultry Production (CEIEPA) of the Faculty of Veterinary Medicine and Animal Husbandry of the Universidad Nacional Autónoma de México (UNAM).

A total of 180 Bovans White 42-wk-old laying hens were randomly assigned to three treatments with five replicates of 12 hens each, which were distributed as follows: 1) control diet; 2) low metabolizable energy and protein diet (50 kcal/kg) with limiting methionine and lysine amino acids (2 % of control diet) + enzymes; 3) diet number two + probiotic.

Diets are shown in Table 1. The positive control diet complies with the recommendations for the productive stage of the Bovans White strain and another control diet with low metabolizable energy (ME), protein, and essential amino acids. The reduction of these nutrients considerably reduced the amount of soybean meal and vegetable oil in the diet. Both were commercial diets and included phytase.

The low-nutrient diets were supplemented with two commercial products: a multienzyme complex (Axtra XAP^{R} 101 TPT, Dupont, Animal Nutrition) at a rate of 250 g/t of feed

containing xylanases (20,000 U/g derived from *Trichoderma longibrachiatum*), proteases (40,000 U/g derived from *Bacillus subtilis*), and amylases (2,000 U/g derived from *Bacillus licheniformis*); and a probiotic containing three strains of *B. subtilis* (Enviva PRO 201 GT^{R}, Dupont, Animal Nutrition 3E+08 CFU/g) at a rate of 250 g/t of feed.

Table 1: Composition and calculated analysis of basal sorghum-soybean meal-canola meal diets added with a multienzyme complex and a probiotic in 42- to 54-wk-old laying hens

Ingredients	**Control + (kg)**	**Control - (kg)**
Sorghum	653.780	675.850
Soybean meal	148.520	136.950
Canola	58.160	58.160
Vegetable oil	13.720	3.000
Calcium orthophosphate	9.190	9.200
Calcium carbonate	105.530	105.560
Salt	4.320	4.320
DL-Methionine 99 %	1.340	1.310
L-Lysine HCl 78 %	0.470	0.670
Vitamins and minerals *	2.400	2.400
Bacitracin MD 10 %	0.500	0.500
Choline chloride 60 %	0.500	0.500
Capsicum red pigment **	0.800	0.800
Larvicide	0.500	0.500
BHT Antioxidant	0.150	0.150
Apo-ester 10 %	0.050	0.050
Phytase	0.045	0.045
Total	1000	1000
	Calculated analysis	
Metabolizable energy, kcal/kg	2800	2750
Crude protein, %	15.79	15.41***
Total lysine. %	0.73	0.71***
Total Met+Cyst, %	0.67	0.66***
Total calcium, %	4.25	4.25
Available phosphorus, %	0.46	0.46
Sodium, %	0.18	0.18

* Vit A, 3000 000 IU; Vit D3, 750 000 IU; Vit E, 6 000 IU; Vit K3, 1.0 g; niacin, 25 g; biotin, 0.063 g; choline chloride, 250 g; selenium, 0.2 g; cobalt, 0.1 g; iodine, 0.3 g; copper, 10 g; zinc, 50 g; iron, 100 g; manganese, 100 g; excipient qs 1.000.00 g.

**Capsicum red pigment (Avired powder) plant-based colorant, 5 g/kg

***2 % reduction compared to the control diet.

Birds were housed in a naturally ventilated broiler house, in cages with three chickens, for 12 wk. Artificial and natural light were provided for a total of 16 h daily. The experimental laying hens were fed sorghum-soybean-canola diets in the form of flour, at a rate of 105 g/bird/d; with free access to water.

Weekly, during the 12 wk of the study, productive data were recorded and summarized; egg weight, feed consumption per bird per day, and conversion ratio. At the end of the study, 20 eggs per treatment were used to determine shell thickness manually using a micrometer without considering internal membranes; Haugh units and yolk color were determined using the QCM+ automated system from Technical Services and Supplies INC (TSS).

The intestinal antibody response was evaluated in five hens per treatment; animals were selected and processed in the slaughterhouse following the Official Mexican Standard NOM-033-ZOO-1995 for Humane slaughter of domestic and wild animals. The intestinal lumen of 10 cm ileal samples were washed three times with 10 ml of cold and sterile isotonic saline solution (ISS), the washing solution was collected and frozen at -20 °C until its subsequent evaluation with the ELISA test following the procedure previously described by Gómez[20].

At 54 wk of age, blood samples were collected from 30 hens (10 hens per treatment), each sample was centrifuged at 3,000 rpm/10 min to obtain the serum and determine the levels of cholesterol, LDL, and HDL in the Pathology Department of the Faculty of Veterinary Medicine and Animal Husbandry, UNAM. Results were transformed from mmol/dL to mg/dl using the conversion factor 0.0259.

Productive variables, egg yolk cholesterol, serum cholesterol, LDL, and HDL data were subjected to an analysis of variance based on a completely randomized design, and the differences between treatments were compared by Tukey test using the statistical software IBM SPSS Statistics v. 19[21].

Results

Table 2 shows the data obtained in 84 days of experimentation. Laying percentage, feed intake, and feed conversion results were similar ($P>0.05$) between treatments. However, egg weight was higher ($P<0.05$) in hens fed with the control diet (T1) and the reduced diet with enzymes + probiotic (T3).

Table 2: Productive variables of 42- to 54-week-old Bovans hens fed sorghum-soybean-canola diets

Diets	Laying (%)	Egg weight (g)	Feed intake/bird/d (g)	Feed conversion (g/g)
1) Control diet	92.2±1.9	60.0±0.4a	104±0.5	1.88±0.04
2) Low-nutrient diet + enzymes	92.8±1.7	58.9±0.7b	104±0.8	1.90±0.03
3) Diet 2 + probiotic	91.5±1.7	59.2±0.4ab	104±0.8	1.91±0.03

Values ± standard error

[a,b] Values with different letters are statistically different ($P<0.05$).

Table 3 shows data obtained during the experiment on egg quality. The results of Haugh units, shell thickness, and DSM yolk color fan were similar between treatments ($P>0.05$).

Table 3: Quality variables of eggs laid by 42-wk-old hens fed sorghum-soybean meal-canola meal diets

Diets	Shell thickness (µm)	Haugh units	Yolk color (DSM fan)
1) Control diet	337±7.7	90.8±2.8	9.4±2.8
2) Low-nutrient diet + enzymes	352±7.2	92.2±2.5	9.2±0.4
3) Diet 2 + probiotic	347±9.2	91.2±1.8	9.4±0.5

Values ± standard error.

($P>0.05$).

Table 4 shows the average results of the analyses of serum cholesterol, LDL, HDL, and the production of intestinal secretory IgA. There were no statistical differences between treatments ($P>0.05$).

Table 4: Changes in serum cholesterol, LDH, HDL, and secretory IgA content in 42 to 54 wk-old Bovans hens fed sorghum-soybean meal-canola meal diets

Diets	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Secretory IgA (%)
1) Control diet	100.2±26.2	13.9±10.8	30.0±2.5	40.9±30.4
2) Low-nutrient diet + enzymes	109.7±15.7	17.3±2.3	37.0±5.2	68.7±34.0
3) Diet 2 + probiotic	122.9±21.1	16.2±0.9	35.7±5.0	63.0±27.9

Values ± standard error.

($P>0.05$).

Discussion

These results are similar to those reported by Wena *et al*[22], who evaluated an enzyme cocktail in corn-soybean diets with lower nutrient content, and found that the nutritional value of the feed was improved; for this reason, enzymes are used in the food industry to reduce formulation costs without affecting productive behavior[8]; similarly, in this study, nutrient reduced diets, based on the nutritional recommendations for the Bovans White strain, had a lower content of soybean meal and oil.

Sobczak and Kozlowski[23] evaluated the effect of adding *Bacillus subtilis* on egg production without significant changes in egg weight, laying percentage, feed intake, and feed conversion. Other studies also show that the use of probiotics in hen diets has no influence on productive performance[24].

Higher egg weights were observed in diets without nutrient reduction, which probably suggests that the reduction of 50 kcal of ME in sorghum-soybean-canola+enzymes diets did not provide a sufficient amount of ME for the multienzyme complex. However, the diets supplemented with the enzymes and probiotic increased egg weight, probably due to the promotion of a more favorable microbiome and enhanced intestinal health caused by *Bacillus subtilis*. Amerah *et al*[9] also demonstrated that amylases, xylanases, and proteases, alone or combined, increase non-starchy polysaccharides digestibility. However, a different study[16] evaluated the productive performance of broilers fed diets supplemented with phytase, alone or combined with xylanases, amylases, proteases, and *Bacillus amyloliqueciens* as a probiotic; there were no significant positive effects. Although the combination did increase the apparent ileal digestibility of sugars and fat, with an increase in ME; it also reduced the pathogenic bacteria populations.

A previous study reports that corn-soybean-canola meal diets supplemented with carbohydrases and proteases increase weight gain and feed conversion; it also enhances protein digestibility and metabolizable energy[25]; these results differ from findings of this study since these productive parameters were not improved. The cholesterol, HDL, and LDH data showed no significant difference in this study; however, Salma *et al*[26] used different concentrations of a *Rhodobacter capsulatus* probiotic in hens fed corn-soybean diets and reported that the diets with a higher concentration of the probiotic increased serum high-density lipoproteins (HDL), cholesterol, and the atherogenic index. Recently, a study reported[3] that the addition of 0, 250, 450, and 900 U/kg of xylanases derived from the fermentation of *Bacillus subtilis* to corn-wheat-soybean meal diets fed to Hy-Line Brown hens, did not improve the productive parameters; however, there was an effect on

shell thickness and Haugh units; which does not agree with the results of the present study.

Conclusions and implications

The use of a multienzyme complex of amylases, proteases, and xylanases plus a *Bacillus subtilis* spore-based probiotic in sorghum-soybean-canola diets with the nutritional recommendations for the Bovans White strain, allows reducing the ME in 50 kcal/kg, as well as the protein and essential amino acids, lysine and methionine, in 2 %, with no detrimental effect on the productive performance of 42- to 54 wk-old Bovans White hens. The enzyme complex and *Bacillus subtilis* probiotic did not affect the intestinal secretory IgA or serum cholesterol, HDL, and LDH values.

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