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DIETARY INCLUSION OF A LIPASE ENZYME IN THE BIOPRODUCTIVE INDICATORS OF LAYING PULLETS

INCLUSIÓN DIETÉTICA DE UNA ENZIMA LIPASA EN LOS INDICADORES BIOPRODUCTIVOS DE POLLITAS PONEDORAS

S. N. ZÚÑIGA[1], J. A. VACA[1], Y. MARTÍNEZ[2*], R. RODRÍGUEZ[3]

[1]*Centro de Investigación y Enseñanza Avícola, Departamento de Ciencia y Producción Agropecuaria, Escuela Agrícola Panamericana. Zamorano, Honduras*

[2]*Facultad de Medicina Veterinaria, Universidad de Fondwa. Leogane, Haití*

[3]*Centro de Estudio de Producción Animal, Facultad de Ciencias Agropecuarias, Universidad de Granma. Granma, Cuba*

* *E-mail: ceoyordan@hotmail.com*

A total of 700 one-day-old Dekalb White® birds were randomized into two treatments and seven repetitions to evaluate the inclusion of 0.01 % of a lipase enzyme with an energy contribution of 0.42 MJ/kg during five productive stages: starter 1 (1- 3 weeks) starter 2 (4-6 weeks), grower (7-10 weeks), development (11-15) and pre-lay (16-17). The inclusion of lipase decreased the cost of the diets by 12.21 USD/t in relation to the control treatment. During weeks 1-3, 7-10 and 16-17 no notable changes (P>0.05) were recorded for body weight, feed intake, feed conversion ratio and viability. However, in weeks 4-6, the inclusion of lipase improved (P<0.05) body weight (408.96 vs 449.36 g) and feed conversion ratio (2.32 vs 1.95). However, this treatment (lipase) increased (P<0.05) feed intake and feed conversion ratio in weeks 11-15 (3.24 vs 4.11). In the global period (1-17 weeks), no productive indicator of the pullets changed (P>0.05) due to the effect of the experimental diets. It is recommended to include the lipase enzyme (Lipase AN6) in hypocaloric diets (-0.42 MJ/kg) to reduce its cost, without affecting the bioproductive indicators of laying pullets.

Keywords: *slow-growing birds, economical diet, exogenous enzyme, productivity*

Un total de 700 aves Dekalb White® de un día de edad se aleatorizaron en dos tratamientos y siete repeticiones para evaluar la inclusión de 0.01 % de una enzima lipasa con aporte energético de 0.42 MJ/kg durante cinco etapas productivas: inicio 1 (1-3 semanas) inicio 2 (4-6 semanas), crecimiento (7-10 semanas), desarrollo (11-15) y prepostura (16-17). La inclusión de la lipasa disminuyó el costo de las dietas en 12.21 USD/t en relación con el al tratamiento control. Durante las semanas de 1-3, 7-10 y 16-17 no se registraron cambios notables (P>0.05) para el peso vivo, consumo de alimento, conversión alimentaria y viabilidad. Sin embargo, en las semanas 4-6, la inclusión de lipasa mejoró (P<0.05) el peso vivo (408.96 vs 449.36 g) y la conversión alimentaria (2.32 vs 1.95). No obstante, este tratamiento (lipasa) incrementó (P<0.05) el consumo de alimento y la conversión alimentaria en las semanas 11-15 (3.24 vs 4.11). En el período global (1-17 semanas), ningún indicador productivo de las pollitas cambió (P>0.05) por efecto de las dietas experimentales. Se recomienda incluir la enzima lipasa (Lipase AN6) en dietas hipocalóricas (-0.42 MJ/kg) para reducir su costo, sin afectar los indicadores bioproductivos de pollitas ponedoras.

Palabras clave: *aves de crecimiento lento, dieta económica, enzima exógena, productividad*

Currently, exogenous enzymes are commonly used in poultry diets, especially to enhance growth performance, contribute to environmentally friendly production and increase economic feasibility (Aftab and Bedford 2018). Also, exogenous enzymes have been used individually or in combination with various natural alternatives to eliminate and/or restrict the use of antibiotic growth promoters in poultry (Cowieson and Kluenter 2019).

Lipases are enzymes with functional properties applied to animal production and in various fields: agrochemistry, medicine, pharmaceutical and food industry (Cavalcante *et al.* 2021). Lipases also participate in the hydrolysis of

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triglycerides into fatty acids and glycerol, which is important for controlling blood lipid levels (Olivecrona 2016). Specifically, in poultry, lipid assimilation occurs at the lipid-water interface because this biomolecule (lipids) is insoluble in aqueous medium, contrary to digestive enzymes (Gole *et al.* 2022). Upadhaya *et al*. (2019) reported that birds in the first days of life produce low concentrations of pancreatic lipase, which can provoke poor digestibility of lipids (mainly saturated fatty acids) and also affect feed efficiency.

Castro and Kim *et al*. (2021) reported positive results when they used a dietary lipase in the early life stages of broilers. However, Movagharnejad *et al*. (2020) did not find improvements in growth performance of chickens when they used a lipase enzyme combined with lysophospholipids in the diets. Few studies have focused on the use of lipase enzymes in low-calorie diets in slow-growing birds such as laying pullets, especially to reduce the use of energy corrector, reduce production costs, maintain and improve the feed efficiency of this poultry category, which affects the productivity of the future laying hen. The objective of the present study was to evaluate the effect of a lipase enzyme (Lipase AN6) on the bioproductive indicators of laying pullets.

Materials and methods

Experimental location

The experiment was developed at the Poultry Research and Teaching Center of the Pan-American Agricultural School, Zamorano, located 32 km between Tegucigalpa-Danlí, Honduras. The average annual temperature is 26 °C, with average precipitation of 1100 mm and altitude of 800 m a.s.l.

Animals, experimental design and treatments

A total of 700 Dekalb White® laying pullets, one day old and sexed, were placed for 17 weeks according to a completely randomized design with two treatments and seven repetitions per treatment. The treatments consisted of basal diets (T1), formulated with corn and soymeal, and the inclusion of 0.01 % lipase in hypocaloric diets (T2) (-0.42 MJ/kg). The lipase enzyme (Lipase AN6) was purchased from company "Enzimas y Productos Químicos S.A. (Enziquim)", Mexico. Also, the company's recommendation for the inclusion level and energy release (0.42 MJ /kg) of the enzyme was considered.

Five experimental periods were used: 1-3 weeks, 4-6 weeks, 7-10 weeks, 11-15 weeks and 16-17 weeks. The diets were prepared in the feed factory of the Poultry Research and Teaching Center, University of Zamorano. The requirements described in the manual of the genetic line used (Hendrix-Genetics 2020) were considered. Table 1 shows the ingredients and nutritional contributions of the experimental diets.

Experimental conditions

Each repetition corresponded to a pen with dimensions of 5.92 m^2 each (1.6 × 3.7 m), where 50 pullets/pen were located, at a rate of 10.13 pullets/m^2. Feed and water were offered *ad libitum* in hopper feeders and dual automatic drinkers, respectively. Temperature and ventilation in the house were controlled by gas brooders, curtain management, and fans, respectively. The house was disinfected with quaternary ammonium (5 %) 24 h before the arrival of the batch of pullets. No medications or therapeutic veterinary care were administered throughout the experimental stage.

Productive indicators

All bioproductive indicators were determined in periods 1-3 (starter 1), 4-6 (starter 2), 7-10 (grower), 11-15 (development) and 16-17 weeks (pre-lay). Viability was determined by the live animals among those existing at the beginning of the experiment. The initial and final weighing of each stage was carried out individually on a SARTORIUS model BL 1500 digital scale with precision ± 0.1 g. Cumulative feed intake was determined using the offer and rejection method. Feed conversion ratio was calculated as the amount of feed ingested for a gain of 1 g of body weight (BW). At week 17, uniformity was calculated according to the coefficient of variation (CV, %).

Statistical analysis

A completely randomized design was used. The Student's t test was performed for two independent samples using the SPSS 1/23/2014 program (SPSS Inc., Chicago, IL, USA). Values of $P<0.05$ were taken to indicate significant differences. Viability was determined by comparison of proportions using the COMPRAPRO 1.0® program (Font *et al.* 2007).

Results and discussion

Table 2 shows the effect of the inclusion of an exogenous enzyme (lipase) on growth performance of laying pullets fed with low-calorie diets. In the initial stage (starter 1, 1-3 weeks), the diet with the inclusion of lipase did not affect body weight, feed intake, feed conversion ratio and viability. However, in the second stage (starter 2, 4-6 weeks), lipase improved body weight and reduced feed conversion ratio, without modifying feed intake and viability.

In the grower (7-10 weeks) and pre-lay (16-17) stages, no productive indicator changed due to the effect of the experimental groups. However, in the development stage (11-15 weeks), the hypocaloric diet with lipase increased

Table 1. Ingredients and nutritional contributions of Dekalb White® pullet diets (1-17 weeks)

Ingredients, %	1-3 weeks		4-6 weeks		7-10 weeks		11-15 weeks		16-17 weeks	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Cornmeal	55.745	58.255	60.64	61.28	55.82	58.22	53.95	56.42	54.71	57.13
Soymeal	34.11	33.74	29.92	29.37	26.90	26.62	21.86	21.56	26.37	26.07
Premix[1]	0.50	0.50	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Common salt	0.28	0.28	0.23	0.23	0.23	0.23	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.23	0.23	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Palm oil	3.82	1.68	1.60	0.00	4.71	2.6	4.98	2.84	6.08	3.95
Wheat bran	2.00	2.00	3.00	4.50	8.00	8.00	15.00	15.00	4.00	4.00
Choline	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.20	0.20	0.20	0.20	0.18	0.17	0.13	0.12	0.19	0.19
L-Threonine	0.00	0.00	0.04	0.04	0.03	0.02	0.0	0.00	0.00	0.00
L-Lysine	0.09	0.09	0.11	0.11	0.09	0.09	0.06	0.06	0.02	0.02
Calcium carbonate	1.75	1.75	1.68	1.68	1.61	1.61	1.61	1.61	5.95	5.95
Monocalcium phosphate	1.03	1.02	1.60	1.60	1.45	1.45	1.18	1.15	1.45	1.45
Mycotoxin sequestrant	0.075	0.075	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Exogenous enzymes[2]	0.07	0.07	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipase enzyme	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
Costs (USD/t)	548.64	536.55	537.04	524.83	537.98	525.57	511.80	499.33	538.6	526.74
Contributions (%)										
Metabolizable energy (MJ/kg)	12.34	11.92	11.92	11.51	12.34	11.92	12.13	11.72	12.34	11.92
Crude protein	20	20	18.5	18.5	17.5	17.5	16.00	16.00	16.50	16.50
Ca	1.05	1.05	1.00	1.00	0.95	0.95	0.90	0.90	2.50	2.50
Available P	0.45	0.45	0.47	0.47	0.45	0.45	0.40	0.40	0.43	0.43
Lysine	1.10	1.10	0.96	0.96	0.88	0.88	0.76	0.76	0.78	0.78
Methionine+Cystine	0.80	0.80	0.72	0.72	0.67	0.67	0.59	0.59	0.66	0.66
Threonine	0.70	0.70	0.65	0.65	0.60	0.60	0.52	0.52	0.55	0.55
Na	0.18	0.18	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18
Cl	0.20	0.20	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18

[1]Each kg contains: vit. A 10 x 10^6 I.U., D3 1.5x 10^6 I.U., K3 2100 mg, E 10000 mg, thiamine, 800 mg, riboflavin 2500 mg, pantothenic acid 10000 mg, pyridoxine 2500 mg, folic acid 250 mg, biotin 100 mg, vit. B12 15 mg, manganese 60000 mg, copper 8000 mg, iron 60000 mg, zinc 50000 mg, selenium 200 mg, iodine 800 mg, cobalt 500 mg, antioxidant 125000 mg.

[2]Lumis Lbzyme X50® multienzyme complex is composed by xylanase (25000 U/g), mannanase (250 IU/g), beta-glucanase (2500 IU/g), cellulase (400000 U/g), pectinase (80 IU/g), galactosidase (100 U/g), protease (2500 Hut/g), amylase (60000 U/g) and phytases (15000 FTU/g).

feed intake and feed conversion ratio; also, uniformity was similar between treatments, according to the coefficient of variation.

One of the aims of the experiment was to verify whether the lipase enzyme, which provides 0.42 MJ/kg of metabolizable energy (according to information from the company Enziquim), reduces the diet cost (table 1), without modifying the growth performance of laying pullets from birth to pre-lay (1-17 weeks), considering that young birds have low enzymatic activity (Temairaev *et al*. 2020). This is the case of endogenous lipase, which is produced in the pancreas and participates in lipid metabolism to catabolize the hydrolysis of triglycerides to glycerol and free fatty acids (Wickramasuriya *et al*. 2020).

Feed intake of pullets in weeks 1-3 (starter 1) remained unchanged despite the energy reduction (0.42 MJ/kg) in the diet when the lipase enzyme was included (table 2). According to Lamot *et al*. (2017) and Barzegar *et al*. (2020), the energy concentration in the diet has a direct influence on feed intake in birds, which demonstrates that the diets had a similar contribution of metabolizable energy (table 1). Bakare *et al*. (2021) reported growing interest in using exogenous enzymes in poultry diets to improve nutrient utilization and decrease diet costs. Apparently, the use of lipase indicated higher availability of nutrients (lipids, fatty acids and glycerol), important to maximize enzymatic activity in young animals (Valentini *et al*. 2020). Furthermore, oral administration of lipase reduced the diet cost (1-3 weeks) by 12.09 USD/t (table 1).

Table 2. Effect of the inclusion of a lipase enzyme on the bioproductive indicators of Dekalb White® laying pullets

Items	Experimental treatments		SEM ±	P value
	T1	T2		
1-3 weeks				
Initial body weight, g	35.71	35.15	0.430	0.3741
Body weight, g	189.50	187.62	1.953	0.5108
Feed intake, g	396.00	394.86	1.063	0.4624
Feed conversion ratio	2.58	2.59	0.028	0.7492
Viability, %	98.00	97.71	0.841	0.8147
4-6 weeks				
Body weight, g	408.96	449.36	5.413	0.0010
Feed intake, g	507.23	497.12	8.748	0.4292
Feed conversion ratio	2.32	1.95	0.057	0.0010
Viability, %	99.14	99.12	0.411	0.9764
7-10 weeks				
Body weight, g	684.24	687.95	5.388	0.6351
Feed intake, g	892.05	914.56	18.060	0.3954
Feed conversion ratio	3.24	3.73	0.093	0.0524
11-15 weeks				
Body weight, g	1064.60	1088.55	9.823	0.1105
Feed intake, g	1561.39	1645.73	33.967	0.0154
Feed conversion ratio	3.24	4.11	0.054	0.0010
Viability, %	99.41	99.71	0.336	0.5367
16-17 weeks				
Body weight, g	1190.39	1199.19	8.099	0.4573
Feed intake, g	685.39	652.91	19.455	0.2615
Feed conversion ratio	5.52	5.95	0.288	0.3124
1-17 weeks				
Body weight, g	4042.06	4105.17	63.759	0.4974
Feed intake, g	3.50	3.52	0.037	0.6238
Uniformity	2.35	2.20		

T1 control diet, T2 inclusion of 0.01 % of a lipase enzyme in hypocaloric diets (-0.42 MJ/kg).

The use of the lipase enzyme in diets without the energy corrector (African palm oil) decreased the cost of the diets by 12.21 USD/t (table 1) and promoted body weight from weeks 4-6 (table 2). Zhu *et al*. (2014) reported that the use of a lipase enzyme in hypocaloric diets improved the activity of pancreatic enzymes and pepsin, and also increased the height and surface area of the villi of the jejunum and ileum in poultry. This confirms that, in young birds, the use of lipase favors the digestion and absorption of lipids and directly affects the feeding efficiency of these slow-growing animals. Likewise, Siqueira *et al*. (2021) recommended the use of exogenous lipases in diets, because birds do not produce lingual and gastric lipase. It is in the gizzard and small intestine where lipid emulsification occurs. However, young birds have an immature digestive system, which causes low digestibility of lipids and less use of the metabolizable energy of the diet.

The experimental group with lipase caused better feeding efficiency of the birds in the initial phase 2 (table 2). Although there are still contradictions about the metabolizable energy requirements in the early stages of life in pullets, Savoldi *et al*. (2012) stated that diets with high levels of metabolizable energy during the initial phase favor weight gain and feed conversion ratio. Furthermore, Noy and Sklan (1995) found that poultry have fat digestibility of 85 % in the first days after hatching, and only growth factor improves the digestibility of this biomolecule. Thus, the use of lipase enzymes could enhance the absorption of lipids in the early stages of life of birds. Upadhaya *et al*. (2019) reported in broilers that lipase supplementation improved pellet durability, endogenous enzyme production, and absorption of lipids and fat-soluble vitamins.

During the growth stage (7-10 weeks), productive indicators were similar in birds fed with the inclusion of 0.01 % lipase in hypocaloric diets (11.92 MJ/kg) and reduction of African palm oil (2.11 %) and of the cost of the diet at 12.41 USD/t (table 1). The results demonstrate that the dietary inclusion of exogenous lipase has a greater growth-promoting effect in the weeks prior (4-6 weeks) to the growth phase (7-10 weeks). de Oliveira *et al.* (2019) found that the use of a dietary lipase changed the feed consumption of birds, with greater effectiveness in the initial stages. Studies with lipase enzymes in fast-growing birds (chickens) are insufficient (Munir and Maqsood 2013). Al-Marzooqi and Leeson (2000) reported that increasing levels of a lipase enzyme did not change the growth performance and relative weight of internal organs during 42 d of age. Apparently, in slow-growing birds (such as pullets), the effect is more evident. However, other studies with this lipase enzyme are necessary to verify this hypothesis.

During the development phase (11-15 weeks), the pullets are fed with maintenance diets, low in metabolizable energy and crude protein and rich in fibrous compounds, the body weight of the experimental groups was similar to that reported by the manual of the genetic line (Hendrix-Genetic 2022). The diets cost with lipase decreased by 12.47 USD/t (table 1, 11-15 weeks). However, this experimental treatment increased feed intake and, therefore, feed conversion ratio (table 2). Nogueira *et al.* (2013) found that soy lecithin supplementation, alone or in combination with a lipase enzyme, decreased production efficiency due to increased feed conversion ratio of broilers. Other studies reported that dietary inclusion with 0.075 % lipase improved lipid absorption. However, feed intake and body weight gain in poultry decreased, due to the high concentration of the exogenous enzyme (lipase) and contamination with cholecystokinin (Al-Marzooqi and Leeson 1999). Apparently, the stimulation of voluntary consumption in this productive stage (development) is related to the fact that lipase has less energy release than in initial stages, thus birds consume more feed due to the energy deficit for organic functions. An opposite effect was reported by Wu *et al.* (2005), who report that diets with high energy concentration decrease the voluntary consumption of poultry in all productive stages.

In pre-laying (16-17 weeks), the energy requirement is directly related to body weight, ambient temperature, feathering and future egg production (Hadinia *et al*. 2018). The inclusion of lipase reduced the use of African palm oil by 2.14 % and the cost of diets by 11.86 USD/t (table 1). Apparently, the enzyme supplied the missing energy in the hypocaloric diet (-0.42 MJ/kg), since the productive performance was similar for both treatments. Although no studies were found on the effect of lipase enzymes in the pre-laying stage, Castro and Kim (2021) reported that the inclusion of a lipase enzyme in finishing diets (29-42 days) increased body weight gain and decreased the negative effects of diets with low nutrient concentrations in fast-growing birds.

Overall (1-17 weeks), the use of the lipase enzyme (Lipase AN6) maintained the bioproductive indicators of laying pullets fed with low-calorie diets and reduced the use of the energy corrector (African palm oil) between 0 to 2.14 % in the diet. Similar results were noted by Suresh *et al*. (2014), who found no changes for feed intake and feed conversion ratio when they used treatments that included lipid emulsifying agents (0.2 g of lipase and 0.2 g/kg of soy lecithin). Furthermore, Lichovníková *et al*. (2002) reported that the addition of lipase to hypocaloric diets kept productive indicators unchanged in 72-week-old laying hens. For their part, Meng *et al*. (2004) noted that the productivity and nutrient digestibility of broilers was not affected by the dietary inclusion of exogenous enzymes (including lipase).

Uniformity is one of the most important indicators in the production of pullets, since it evaluates the homogeneity of the batch, which has a direct impact on the future layer (Asensio *et al*. 2020). According to Delgado *et al*. (2020), a CV of 8 % corresponds to very uniform batches. These authors found that the CV increases with the age of the pullets. This demonstrates that both experimental groups had a uniform flock at week 17 of age (table 2) and that lipase did not modify this productive indicator. Sweeney *et al*. (2022) reported that the growth of pullets with a similar body weight in the flock influences the age at first egg, laying persistence and egg weight uniformity of laying hens. In this study, regrouping was not necessary to homogenize the flock, which is a common practice in raising pullets and replacing breeders (García *et al*. 2019).

Conclusions

Dietary inclusion with 0.01 % of Lipase AN6 with an energy contribution of 0.42 MJ/kg decreased the level of inclusion of the African palm oil and the cost of diets in all productive stages. Likewise, using this lipase enzyme did not affect the productivity and uniformity of Dekalb White® laying pullets (1-17 weeks)

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