INESEG
INTERNATIONAL ENGINEERING, SCIENCE AND EDUCATION GROUP

Middle East Journal of Science

https://dergipark.org.tr/mejs

e-ISSN:2618-6136 DOI: 10.23884/mejs.2019.5.2.02

MEJS

Research Article

EFFECT OF DIETARY SUPPLEMENTATION OF DIFFERENT MULTI-ENZYMES ON PRODUCTION PERFORMANCE AND EGG QUALITY CHARACTERISTICS IN LAYING HENS

***Özgür POLAT*[1], **Muzaffer DENLİ*[1]**

[1]Dicle University, Faculty of Agriculture, Department of Animal Science, 21280, Diyarbakir, Turkey

* Corresponding author; muzaffer.denli@gmail.com

Abstract: *The purpose of this study was to determine the effects of dietary addition of multi-enzymes in a different source of grains based diets on production performance and egg quality characteristics of laying hens. A total of 225, 24 weeks of old laying hens (Atak-S) were divided into 3 treatments with 5 replicates and 15 hens per replicate for 9 weeks. The control group was fed basal diet (without any supplementation) and treatment groups were fed basal diet supplemented 0.1% Enzyme-A (xylanase, ß-glucanase, cellulase, α-amilase, and protease) and 0.05 % Enzyme-B (xylanase, ß-glucanase). Productivity performance and egg quality parameters were checked weekly throughout the experiment. Dietary multi-enzymes supplementation significantly changed the shape index, yolk index and yolk color (L and a) at different weeks of trial (P<0.05). However, daily feed intake, egg production, average of egg weight and feed conversion rate were not affected by the addition of both multi-enzymes throughout the experiment. (P>0.05). Also, dietary addition two types of multi-enzymes did not affect the egg specific gravity, eggshell thickness, eggshell rate, albumen index, haugh units (P>0.05). As a result, the dietary addition of multi-enzyme did not affect the performance parameters but caused limited changes on some egg quality characteristics. of laying hens between 24-33 weeks of age.*

Keywords: *egg quality, laying hens, multi-enzymes, performance*

Received: November 21, 2019 Accepted: December 6, 2019

1. Introduction

Animal products play an important role in providing adequate and balanced nutrition that people need and maintaining the healthy lives of individuals. Therefore, one of the most consumed animal products is the egg. Eggs are the only food source that contains all the nutrients that human needs after breast milk. Especially the rich content of essential amino acids makes the egg an accepted source of quality animal protein [1]. As a matter of fact, the biological availability of egg protein is 100%; while this value is 85% in milk, 76% in fish and 74% in beef. With increasing interest in healthy and balanced nutrition in recent years, daily egg consumption per person has also increased. Therefore, with the increase in egg consumption and consumer awareness, the concept of egg quality has started to gain importance.

In determining the quality characteristics of the egg, many criteria related to external and internal quality are taken into consideration. Egg quality which is extremely important for the consumer, as well

as the producer, is determined by; egg weight, shell thickness, egg shape, shell pore and shell color [2]. In addition to this, the shape index, specific gravity, fracture strength, surface area, and fracture-crack egg ratio also play a decisive role in external quality. Although egg weight and size vary depending on genotype, it is also influenced by feeding and environmental factors [3]. Poor eggshell quality is an important problem that negatively affects egg production, reduces hatchability and increases embryo mortality. On the other hand, egg quality with low shell strength and high risk of infiltration of pathogenic bacteria is not preferred by consumers. Therefore, egg production with high shell defects breaks consumer perception of trust and leads to economic losses. In the studies, it has been reported that the use of feed supplementation added to the mixed feed is beneficial in improving egg quality and gives positive results [4]. After commonly used enzyme studies, such as xylanase, amylase, cellulase, and phytase, which affect a single substrate in poultry nutrition, it has been reported that the use of multienzyme acting on more than one substrate may further improve feed conversion rate and performance [5]. In a study, it was reported that the addition of a multienzyme has positive effects on the rate of feed conversion in laying hens, ether extract digestibility of non-starch polysaccharides (NSP), eggshell quality and n-3 fatty acid accumulation in the egg [6]. Lima et al. [7] reported that the addition of enzyme complex to mixed feed improves intestinal health and performance in laying hens and improves internal and external egg quality. The aim of this study is to determine the effects of adding different multienzyme to laying hens mixed feed on performance (feed intake, feed conversion rate, egg production, average egg weight), egg external quality (shape index, shell rate, shell thickness, specific gravity) and egg internal quality (yolk index, albumen index, Haugh unit and yolk color and blood and flesh stain) characteristics.

2. Materials and Methods

A total of 225, 24 weeks of old laying hens (Atak-S) were divided into 3 treatments with 5 replicates and 15 hens per replicate for 9 weeks. The control group was fed basal diet (without any supplementation) and treatment groups were fed basal diet supplemented 0.1% Enzyme-A (xylanase, ß-glucanase, cellulase, α-amilase, and protease) and 0.05 % Enzyme-B (xylanase, ß-glucanase). Performance and egg quality parameters were checked weekly throughout the experiment. Each kg of an enzyme A used in the assay contains 250.000 U + 790.000 EPU of xylanase, 1.000.000 U + 8.700 EPU of Beta-Glucanase, 350.000 U + 18.000 EPU of Cellulase, 350.000 U + 21.000 EPU of Alpha-Amylase and 7.500.000 U + 8.000 EPU of Protease. Each kilogram of enzyme B contains 200 U of xylanase and 138 U of Beta-Glucanase. The feed raw materials to be used in the study were obtained from a commercial feed factory and the mixed feeds of the experiment were prepared at the feed production facility of the Department of Animal Science, Agricultural Faculty of Dicle University. Enzyme A and B feed additives were added to the main mixed feed after pre-mixing.

The nutrient contents of the compound feed to be used in the experiments were prepared in accordance with the nutrient requirements of laying hens reported in NRC, 1994 [8]. The composition (%) and nutrient contents of the mixed feeds used in the study are shown in Table 1. The study was carried out in the Enriched Cage System in the laying hens experimental unit in the Poultry Research and Application Facility, Department of Animal Science, Agricultural Faculty of Dicle University. The enriched cage system has 3 floors and 5 cage sections on each floor. The animals in each cage compartment were group fed, and the feed and water were presented as *ad libitum.* Illumination of the experimental room was provided by fluorescence and 8 hours of dark and 16 hours of light program was applied daily. Determination of nutrient contents of feeds used in the experiment (except crude cellulose)

was performed according to the Weende analysis method [9] and the determination of crude cellulose according to the Lepper method. In the calculation of metabolic energy content of feeds, regression equation no. 9610 proposed by TSE was used [10].

Table 1. Ingredients and chemical composition of experimental diets (as-fed basis)

Ingredients	(%)
Corn	37.0
Soybean Meal (44% CP)	9.50
Full Fat Soybean	17.0
Sunflower Meal (32% CP)	13.0
Wheat	17.0
Dicalcium Phosphate (DCP)[a]	1.85
Calcium Carbonate	8.60
NaCI	0.30
Vitamin+ Mineral Premix[b]	0.25
Chemical Analysis	***(%)***
Dry Matter	90.70
Crude Ash	10.46
Crude Protein	17.00
Ether Extract	4.10
Calculated values	
ME (kcal/kg)	2744
Calcium (%)	3.81
Available Phosphor (%)	0.40
Na (%)	0.17
L-lysine (%)	0.78
Methionine+Cysteine (%)	0.59
Treonin (%)	0.61
Tryptophane (%)	0.21
Linoleik asit (%)	2.90

[a] Premix supplied per 1 kg; Calcium 24,5%, Phosphor; 18%.

[b] Premix supplied per 1 kg: vitamin A; 12.000.000 IU; vitamin D3; 2.500.000, vitamin E; 30.000 mg, vitamin K3; 4.000 mg; vitamin B1; 3.000 mg, vitamin B2; 7.000 mg, vitamin B12; 5.000 mg, vitamin B6; 5.000 mg, vitamin C; 50.000 mg, Niacin; 30.000 mg, Cal-D-Pantothenate; 10.000 mg, Biotin; 45 mg, Folic acid; 1.000 mg, Choline Chloride; 200.000 mg, Xanthate;1.500 mg, Manganese; 80.000 mg, Iron; 60.000 mg, Zinc; 60.000 mg, Co; 5.000 mg, Iodine; 1.000 mg, Cobalt; 200 mg, Selenium;150 mg.

At the beginning of the experiment, all hens were weighed and were left in the experimental group cages as their live weight and egg yield levels same. During the experiment, the egg production, feed intake and egg weight of the animals were measured weekly and feed conversion rate was calculated using the data obtained. Feed Conversion Rate (FCR) = Feed Consumption (g)/Total Egg Weight (g). Internal and external quality analyses were performed on 15 eggs collected weekly from each group on the same day. Egg weight was determined by weighing with precision balance (0.01g) every other day. Egg Shape Index (ESI): The width and length of the egg were measured by digital caliper and calculated

using the formula ESI = (Width of egg / Length of the egg) x 100. Egg Specific Gravity was measured with a density analyzer consisting of precision balance, beaker, and apparatus. For this purpose, the weight of the eggs which were kept at room temperature for 24 hours was first weighed in the air and then the weight in the water has an average temperature of 20-22 ^{o}C was calculated to determine the specific gravity of the egg.

The shells taken from the middle parts of the broken eggshell under laboratory conditions were measured by digital micrometer after drying and separating the membranes. The shelling rate was determined by the ratio of the value of the eggshells obtained with the precision balance after the membrane was removed and dried to the egg weight. Egg yolk color was determined by digital colorimeter (Minolta CR-300) in *L**, *a** and *b**. Albumen Index (AI): For this purpose, the eggs were broken on a clean glass so that they could not spread and then the albumen breadth and albumen lengths were measured using a digital caliper. The height of white was measured with digital foot micrometer and calculated with the formula AI = [albumen height (mm) / ((albumen length (mm) + albumen width (mm)) / 2)] x100. Yolk Index (YI): The diameter of the egg yolk was measured by digital caliper and the height was measured by digital foot micrometer and it was determined by the formula; YI = [(Yolk height / Yolk diameter) x 100]. Haugh Unit was calculated by using the egg weight and albumen height and by using formula; Haugh Unit = 100 Log (H + 7.57-1.7G 0.37). H: Albumen height (mm), G: Egg weight (g). Statistical analysis of the data obtained at the end of the experiment was performed using the SPSS 18.0 package program [11]. The analysis of variance of the averages was performed with the General Linear Model (GLM) ANOVA. Tukey's multiple comparison test was used to compare the differences between means.

3. Results and Discussion

No difference was found between the groups in terms of productivity performance (average feed intake, feed conversion ratio, egg production, and egg weight) in 24-33 week periods (P> 0.05) (Table 2). The findings obtained from this study were found to be consistent with some of the previous literature and it was observed that the addition of a single enzyme or multi-enzyme did not change feed intake in laying hens [12]. However, the addition of multi enzymes [13] and, phytase [14] increased feed intake. On the contrary, Torki et al. [15] reported that the addition of β-glucanase and xylanase or β-mannose-containing enzymes reduced feed intake. In another study, it was reported that the addition of protease to protein-restricted diets did not affect treatments in terms of feed intake [15]. Since the enzymes do not have an aromatic taste and are not used for appetizing purposes, it can be said that their addition to diets is not expected to have any effect on feed intake. However, it is thought that the discrepancy between the literature is affected by factors that change the efficiency of the enzyme such as laying hens, dietary raw material types, environmental and climatic conditions used in the studies, and changes in the feed intake.

Table 2. Effects of multi-enzyme supplementation in laying hens diet on performance parameters (age of 24-33 weeks)

Parameters	Groups			SEM	P-value
	Control	Enzyme A	Enzyme B		
Feed consumption, g/day	117.7	117.1	117.5	0.182	0.310
Feed conservation ratio	2.4	2.4	2.4	0.020	0.779
Egg production, %	86.4	86.4	87.1	0.771	0.211
Egg weight, g	55.4	55.8	55.2	0.174	0.335

The differences between means in the same row with different letters are significant ($P < 0.05$). SEM: Standard Error of Mean

Some researchers [15,16,17] reported that the addition of multi-enzyme improved the feed conversion rate in laying hens. In contrast, some researchers found the opposite results [18, 19, 20]. As it is known, 'FCR' is the ratio of feed intake to egg weight in laying hens. There are similarities between these results with our results. It is seen that the literature on the effect of enzyme addition to laying hens diets on egg production is incompatible. In related studies [12, 13, 19, 20] reported that the enzyme addition did not change egg production. On the other hand, Khan et al. [21] reported that dietary enzymes increased egg production in laying hens. Differences between the results may be due to the level of difference of enzymes added to the feed. There was no difference between treatment groups with respect to the average egg weight ($P > 0.05$). These results are in agreement with the results of other researchers [12, 13, 22]. The effects of multi-enzyme supplementation in laying hen diet on external and internal egg quality characteristics are given in Table 3.

Table 3. Effects of multi-enzyme supplementation in laying hens diet on external and internal egg quality characteristics (age of 24-33 weeks)

Measurements	Groups			SEM	P-value
	Control	Enzyme A	Enzyme B		
Shell rate, %	12.0	11.8	12.1	0.090	0.299
Shell thickness, mm	0.37	0.37	0.37	0.002	0.923
Specific gravity, g/cm^3	1.08	1.07	1.08	0.003	0.085
Shape index	77.2^a	76.5^b	75.9^c	0.170	0.017
Yolk index	48.1^a	46.9^b	46.7^b	0.250	0.040
Albumen index	3.9	3.8	3.7	0.050	0.333
Haugh unit	74.6	73.4	73.4	0.520	0.569
L* value	52.4	51.0	49.6	1.068	0.177
a* value	19.4	17.3	17.1	0.643	0.275
b* value	31.2	30.1	29.4	0.504	0.342

The differences between means in the same row with different letters are significant ($P < 0.05$). SEM: Standard Error of Mean.

The results showed that the dietary addition of either enzyme A or enzyme B decreased shape index value when compared with the control group ($P<0.05$). However, the egg quality indices such as eggshell

thickness, shell rate, specific gravity, albumen index, yolk colors, and blood spots were not affected by the diets (P>0.05). Similar results have been reported by various researchers [13, 15, 21]. Contrary Yaghobfar [23] reported that the addition of ß-glucanase and xylanase to barley-based diets reduced the eggshell thickness in laying hens. These different results may be due to the content or levels of enzymes used in feed.

Conclusion

In conclusion, our results showed that the addition of different multi-enzyme sources to mixed feeds did not affect the production performance of hens aged between 24-33 weeks and no changed egg quality characteristics tested except egg shape and yolk index

Acknowledgments

This research was supported by the Scientific Research Project Fund of Dicle University (DÜBAP) under project number Ziraat.19.001.

References

[1] Açıkgöz, Z., Özkan, K., "Yumurta tüketiminin beslenme ve sağlık üzerine etkisi". *Hayvancılık' 96 Ulusal Kongresi.18-20 Eylül, Bornova-İzmir,* pp.305-312, 1996

[2] Durmuş, İ. "Effect of egg quality traits on hatching results." *Akademik Ziraat Dergisi*, 3, 95-97, 2014.

[3] Shanawany, M.M., "Hatching weight in relation to egg weight in domestic birds" *World's Poultry Science Journal*, *43*(2), 107-115, 1987.

[4] Świątkiewicz, S., Arczewska-Włosek, A., Krawczyk, J., Puchała, M., Józefiak D., "Dietary factors improving eggshell quality: an updated review with special emphasis on microelements and feed additives" World's Poultry Science Journal, 71, 83-94, 2015.

[5] Kutlu, H.R. and Şahin, A., "Kanatlı beslemede güncel çalışmalar ve gelecek için öneriler. *Hayvansal Üretim,* 58, (2), 66-79, 2017.

[6] Jia, W., Slominski, B.A., Guenter, W., Humphreys, A., Jones O., "The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed" *Poultry Science*, 87, 2005-2014, 2008

[7] Lima, M.R.D., Costa, F.G.P., Goulart, C.D.C., Pinheiro, S.G., Souza, R.B.D., Morais, S.A.D.N. and Lima, R.C., "Nutritional reduction of protein and usage of enzyme in the diet of light layers" *Revista Brasileira de Zootecnia*, *41*(9), 2055-2063, 2012.

[8] NRC, "Nutrient Requirements of Poultry" *8th Rev. Edn., National Academy Press, Washington, DC.* 1984.

[9] Naumann, C., Bassler, R., "Methodenbuch, Band III, Die Chemische Untersuchung von Futtermitteln" *VDLUFA-Verlag, Frankfurt, Germany*, 1993.

[10] TSE, "TS 9610 Animal Feeds- Determination of Metabolizable Energy (Chemical Method)", 1994.

[11] SPSS INC. PASW Statistics for Windows, version 18.0. Chicago, 2009.

[12] Lee, K.W., Choi, Y.I., Moon, E.J., Oh, S.T., Lee, H.H., Kang, C.W. and An, B.K., "Evaluation of dietary multiple enzyme preparation (Natuzyme) in laying hens" *Asian-Australasian journal of animal sciences*, *27*(12), 1749, 2014.

[13] Banghban-Kanani, P., Hosseintabar-Ghasemabad, B., Azimi-Youvalari, S., Seidavi, A., Ayaşan, T., Laudadio, V., Tufarelli, V., "Effect of different levels of sunflower meal and multi-enzyme complex on performance, biochemical parameters and antioxidant status of laying hens" *South African Journal of Animal Science,* 48 (2): 390-399, 2018.

[14] Orhan, Z.Ç., The Effects of Phytase Supplementatıon on The Performance and Egg Quality Of Free-Range Layers, MSc. Thesis, Namık Kemal University, Tekirdag, 2016.

[15] Torki, M., Mirzaee, M., Habibian, M., "Effects of barley cultivar and dietary supplemental enzyme on performance, egg quality traits, and selected blood parameters of laying hens" *Poultry Science Journal*, 4 (1), 1-12, 2014.

[16] Joshua, A.O., " Effect of protease supplementation on the performance of laying chickens fed marginally deficient protein diets" *Journal for Studies in Management and Planning*, *2* (11), 20-29, 2016.

[17] Attia, Y.A., El-Tahawy, W.S., Abd El-Hamid, A.E.A., Nizza, A., Al-Harthi, M.A., El-Kelway, M.I., Bovera, F., "Effect of feed form, pellet diameter and enzymes supplementation on carcass characteristics, meat quality, blood plasma constituents and stress indicators of broilers" *Archiv Tierzucht,* 57 (30), 1-14, 2014.

[18] Yalçin, S., "Yumurta tavuğu rasyonlarında enzim, probiyotik ve antibiyotik kullanılması" *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *49*(2), 135-141, 2002.

[19] Scheideler, S.E., Beck, M.M., Abudabos, A. and Wyatt, C.L., "Multiple-enzyme (Avizyme) supplementation of corn-soy-based layer diets" *Journal of applied poultry research*, *14*(1), 77-86, 2005.

[20] Vieira, B.S., Pedrozo, S.A., Barbosa, V., Tavares, J.M.N., Beloli, I.G.C., Melo Silva, G.M., Neto, H.R.L., Júnior, J.G.C., Corrêa G.S.S. 2016. "Phytase and protease supplementation for laying hens in peak egg production" *Ciências Agrárias, Londrina,* 37 (6): 4285-4294, 2016.

[21] Khan, S.H., Atif, M., Mukhtar, N., Rehman, A. and Fareed, G., " Effects of supplementation of multi-enzyme and multi-species probiotic on production performance, egg quality, cholesterol level and immune system in laying hens" *Journal of Applied Animal Research, 39*(4), 386-398, 2011.

[22] Benabdejelil K., Arbaoui M.I., "Effect of enzyme supplementation of barley-based diets on hen performance and egg quality" *Animal Feed Science and Technology*, 48 : 325- 334, 1994.

[23] Yaghobfar, A., Boldaji, F., Shrifi, S.D., "Effects of enzyme supplement on nutrient digestibility, metabolizable energy, egg production, egg quality and intestinal morphology of the broiler chicks and layer hens fed hull-less barley based diets" *Pakistan Journal of Biological Sciences,* 10 (14), 2257-2266, 2007.