Global Veterinaria 6 (4): 409-416, 2011
ISSN 1992-6197

Egg Quality Characteristics and Productive Performance of Laying Hens Fed Olive Pulp Included Diets Supplemented with Enzyme

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Abstract: This study was conducted to investigate effects of dietary inclusion of olive pulp (OP), the remainder of olive cake after the removal of the seed fractions, with or without enzyme on performance of laying hens. A total number of 144 Lohmann LSL-Lite hens was randomly divided in 24 cages (n=6). Hens in 4 cages (replicates) were assigned to feed on one of the 6 experimental diets. Based on a 3×2 factorial arrangement of treatments including I-corn-soybean meal-based control-1 diet, II-corn-soybean meal-based control-2 diet included fish meal and soybean oil and III-control-2 diet included 174 g/kg olive pulp, with and without enzyme (0.0 and 0.08 g/kg); 6 iso-energetic and iso-nitrogenous experimental diets (ME =2720 Kcal/Kg and CP=150 g/Kg) were formulated. Egg quality characteristics were measured twice on weeks 3 and 7 of the experiment. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) as well as egg quality traits during 7-week trial period were analyzed based on completely randomized design using the GLM procedure of SAS. Dietary treatment did not have significant effect on EP and EM. Dietary inclusion of olive pulp increased FI and FCR as comparing to control diets. Enzyme supplementation did not significantly affect laying hens' performance and egg quality traits. Egg index, Haugh unit, egg gravity and egg abnormality were not significantly affected by dietary treatment. In the second egg sampling (wk 7), dietary inclusion of OP caused decreased egg index, Haugh unit, yolk color, egg shell weight and thickness as comparing to control diets ($P \leq 0.05$). In conclusion, OP can be included in laying hens diets up to 17% with no adverse effect on EP and EM; however, in terms of feed efficiency, egg shell weight and thickness, lower percentage of OP should be inserted into diets. Enzyme did not have beneficial effect on laying hens' performance and egg quality traits.

Key words: Olive pulp · Enzyme · Laying hens · Performance · Egg quality

INTRODUCTION

Corn has been used consistently as a major ingredient for poultry rations because of its high energy content and low cost. However, as corn markets tighten and corn supplies go to nonagricultural uses such as ethanol production, there appears to be a need for alternative grain sources for poultry rations [1]. To improve food resources to meet food requirements for the expected growth in poultry production, the search for non-conventional foodstuffs continues [2].

Olive pulp (OP) is the remainder of olive cake (the raw material resulting from extraction of olive oil) after the removal of the seed fractions. About 0.3 of cell wall fraction will be removed by sieving [3]. Due to low nutritive value (low in energy, digestible proteins and minerals and high in lignin), OP is seldom integrated into poultry feeding. The olive oil industry generates large amounts of byproducts that are harmful to the environment. According to the Food and Agriculture Organization of the United Nations [4], 2.7 millions of tones of olive oil are produced annually worldwide, 76% of which are produced in Europe, with Spain (35.2%), Italy (23.1%) and Greece (16.1%) being the highest olive oil producers. Other olive oil producers are Africa (12.5%), Asia (10.5%) and America (0.9%).

Non starch polysaccharides (NSP) are considered as anti-nutritives for poultry if they are present in diets at high concentrations. Soluble NSP increases the viscosity of the small intestinal chime, generally

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hampering the digestion process, whereas insoluble NSP impedes the access of endogenous enzymes to their substrates by physical entrapping [5-6]. A xyloglucan, one of the nonstarch polysaccharides (NSP) which has anti-nutritive effects on monogastrics such as poultry and pigs, from OP has been reported by Gil-Serrano and Tejero-Mateo [7]. Coimbra *et al.* [8] also showed the occurrence of the xylan-xyloglucan complexes in the OP cell walls. In addition, Rosa´rio and Domingues [9] extracted glucuronoxylans with a xylose/glucose ratio of 7: 1 from OP. Enzymes are used mainly to achieve consistency in performance and to reduce formulation costs by easing constraints on the inclusion level of some ingredients, such as soybean meal. Enzyme has been reported to alleviate the negative effects of NSP present in cell-wall constituent, by lowering gut viscosity and increasing nutrient digestibility [10-12] and on animal performance [13].

The objectives of the present experiment were to investigate the effects of endo-xylanase (Nutrase Xyla 500®) on performance of laying hens fed maize/soybean diet including OP.

MATERIALS AND METHODS

All procedures used in this seven-week experiment were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research". A total number of 144 Lohmann LSL-Lite hens 80-week-old Lohmann LSL-Lite hens with an average egg production rate of 90.6 ±4.8% (late laying phase) and 1460±24 g live body weight, were obtained from a commercial supplier. After a week of adaptation, the hens were randomly allocated to one of four experimental diets. Hens were semi-randomly distributed between 24 cages (n=6). The hens were housed in laying cages made from galvanized metal wire which provided approximately 430 cm2/hen. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple waterer. Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a weekly basis.

Based on a 3×2 factorial arrangement of treatments, 6 iso-energetic and iso-nitrogenous (ME =2720 Kcal/Kg and CP=150 g/Kg) experimental diets were formulated. Hens in 4 cages (replicates) were assigned to feed on one of the 6 experimental diets including: I-corn-soybean meal-based control-1 diet, II-corn-soybean meal-based control-2 diet included fish meal and soybean oil and III-control-2 diet included 174 g/kg OP; diets IV to VI were similar to diets I to III supplemented with enzyme (0.0 and 0.08 g/kg of Nutrase Xyla ®), respectively.

Nutrase® Xyla is a bacterial endo-1,4-beta-xylanase, designed for use in pig and poultry rations with a high content of arabinoxylans. Nutrase® Xyla is produced by *Bacillus subtilis*, which makes it today the only EU registered bacterial endo-xylanase preparation available on the market. The approximate analysis of the OP used in this study is: dry matter (DM=93%), crude protein (CP= 6.06%), ether extract (EE%= 7.6%), crude fiber (CF%= 48.2), Ash (7.4%), Calciume (Ca%=0.6) and total phosphorous (P%= 0.07). Fatty acid composition (%) of oils from OP used in this study is: total saturated fatty acids (ΣSFA= 15.01%), total monounsaturated fatty acids (ΣMUFA= 71.38%) and total polyunsaturated fatty acids (ΣPUFA= 9.10%).

Egg quality characteristics were measured twice on weeks 3 and 7 of experiment and each time all eggs during three frequent days were used. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) as well as egg quality traits during 7-week trial period was analyzed based on completely randomized design using the GLM procedure of SAS. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Effects of dietary OP inclusion and enzyme supplementation on EP, EM, FI and FCR are presented in Tables 2-5. There was no significant interaction between dietary OP inclusion and enzyme supplementation on the measured productive performance parameters and egg characteristics throughout the experimental period, except for yolk color in the second egg sampling on week 7. Including OP in diet did not have significant effects on EP and EM, except for weeks 4-5 of trail. Hens fed OP-included diets had decreased EP compared to hens fed the control-I diet during weeks 4-5. Dietary inclusion of OP increased FI and FCR in hens compared to hens fed the control diets. Studies concerning feeding OP to monogastrics are limited [14]. Several research studies were conducted to investigate the feasibility of utilizing OP in broiler rations. The proportion of OP in its rations is variable. There seems to be a limit between 50 and 100g/ kg [3, 15]. Diets with different levels of fiber

Table 1: Ingredients and composition of experimental diets

Live pulp	-	-	-	-	17.40	17.40
Nutrase 500	-	0.08	-	0.08	-	0.08
Feed ingredients	g / 100 g diet					
Corn	67.11	67.11	57.28	57.28	48.37	48.37
Fish meal	0.00	0.00	4.90	4.90	4.90	4.90
Soybean meal	20.97	20.97	15.76	15.76	15.02	15.02
Olive pulp	-	-	-	-	17.40	17.40
Soybean oil	0.00	0.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	1.17	1.17	1.29	1.29	1.34	1.34
Lime stone	8.78	8.78	8.28	8.28	7.98	7.98
Common salt	0.29	0.29	0.22	0.22	0.23	0.23
Nutrase 500	-	0.08	-	0.08	-	0.08
Vit. & Min. Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Sand	1.06	0.98	7.68	7.60	0.12	0.04
DL-Methionine	0.12	0.12	0.09	0.09	0.13	0.13
Calculated analyses						
ME (Kcal/kg)	2720.00	2720.00	2720.00	2720.00	2720.00	2720.00
Crude protein (%)	15.00	15.00	15.00	15.00	15.00	15.00
Calcium (%)	3.67	3.67	3.67	3.67	3.67	3.67
Available P (%)	0.33	0.33	0.33	0.33	0.33	0.33
Lys (%)	0.73	0.73	0.82	0.82	0.77	0.77
Met (%)	0.36	0.36	0.38	0.38	0.40	0.40
Met & Cys (%)	0.62	0.62	0.62	0.62	0.62	0.62

[1]Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg. 'Vitamins mix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K; 4 mg; vitamin B\2, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg

Table 2: Egg production (%) of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase)

Treatments	Production (%)				
Week	1	2-3	4-5	6-7	1-7
Olive pulp (OP)					
0 (g/100g diet)	86.31±4.89	94.64±3.37	94.46±5.77^{a}	94.05±7.31	93.23±4.31
0 (g/100g diet)	83.33±9.26	93.15±5.35	91.81±4.78^{ab}	93.45±4.76	91.45±3.96
17.4 (g/100g diet)	81.55±8.70	89.14±6.47	88.24±4.65^{b}	90.77±6.93	88.26±5.65
Nutrase Xyla (N)					
0.00 (g/100g diet)	84.72±4.81	92.56±6.11	90.38±5.57	91.47±5.96	90.50±4.83
0.08 (g/100g diet)	82.74±10.06	92.06±5.13	92.64±5.46	94.05±6.66	91.46±5.24
SEM	1.587	1.128	1.126	1.290	1.011
CV	9.81	5.91	5.05	6.51	4.97
	P values				
Olive pulp (OP)	0.52	0.14	0.05	0.52	0.11
Nutrase Xyla (N)	0.56	0.83	0.24	0.31	0.61
OP × N	0.65	0.56	0.07	0.12	0.14

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$), SEM= Standard error of means

Table 3: Egg mass (g egg/hen/day) of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase)

Treatments	Egg mass (g/hen/day)				
Week	1	2-3	4-5	6-7	1-7
Olive pulp (OP)					
0 (g/100g diet)	52.02±2.78	58.53±1.59	59.06±4.21	58.47±4.81	57.73±2.70
0 (g/100g diet)	50.62±5.59	58.03±3.75	56.61±3.73	57.98±3.70	56.55±3.02
17.4 (g/100g diet)	49.61±5.42	55.30±3.53	60.07±15.57	55.58±5.07	55.93±5.83
Nutrase Xyla (N)					
0.00 (g/100g diet)	51.24±3.54	57.18±4.17	59.49±12.79	56.60±4.53	56.83±4.95
0.08 (g/100g diet)	50.27±5.71	57.39±2.34	57.67±3.69	58.09±4.65	56.65±2.97
SEM	0.954	0.675	1.889	0.929	0.815
CV	9.91	5.85	16.07	7.79	7.16
	P values				
Olive pulp (OP)	0.64	0.15	0.75	0.40	0.67
Nutrase Xyla (N)	0.64	0.88	0.64	0.42	0.92
OP × N	0.72	0.97	0.21	0.22	0.22

a-b Means within a column (within main effects) with no common superscript differ significantly (p <0.05), SEM= Standard error of means

Table 4: Feed intake (g/hen/day) of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase)

Treatments	Feed intake (g/hen/day)				
Week	1	2-3	4-5	6-7	1-7
Olive pulp (OP)					
0 (g/100g diet)	117.29±5.39	114.17±5.62^{b}	112.16±3.09^{b}	115.77±1.83^{b}	114.50±3.20^{b}
0 (g/100g diet)	117.72±2.56	115.81±4.88^{ab}	112.92±5.44^{b}	115.99±115.99^{b}	115.31±3.78^{b}
17.4 (g/100g diet)	120.00±0.00	119.54±0.85^{a}	119.24±1.40^{a}	120.00±0.00^{a}	119.65±0.64^{a}
Nutrase Xyla (N)					
0.00 (g/100g diet)	118.90±1.84	117.30±3.13	114.41±4.18	117.06±2.64	116.63±2.59
0.08 (g/100g diet)	117.78±4.66	115.71±5.96	115.13±5.52	117.45±3.45	116.34±4.51
SEM	0.717	0.964	0.980	0.615	0.734
CV	3.01	3.82	3.37	2.02	2.56
	P values				
Olive pulp (OP)	0.29	0.07	0.00	0.00	0.01
Nutrase Xyla (N)	0.45	0.40	0.65	0.69	0.81
OP × N	0.60	0.62	0.60	0.28	0.49

a-b Means within a column (within main effects) with no common superscript differ significantly (p <0.05), SEM= Standard error of means

Table 5: Feed conversion ratio (g: g) of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase)

Treatments	Feed conversion ratio (g feed: g egg)				
Week	1	2-3	4-5	6-7	1-7
Olive pulp (OP)					
0 (g/100g diet)	2.26±0.17	1.95±0.11^{b}	1.91±0.15	1.99±0.18	2.00±0.12^{b}
0 (g/100g diet)	2.35±0.31	2.00±0.17^{b}	2.00±0.10	2.01±0.10	2.05±0.12^{ab}
17.4 (g/100g diet)	2.44±0.27	2.17±0.13^{a}	2.10±0.27	2.18±0.21	2.19±0.17^{a}
Nutrase Xyla (N)					
0.00 (g/100g diet)	2.33±0.19	2.06±0.18	2.00±0.23	2.08±0.18	2.09±0.16
0.08 (g/100g diet)	2.37±0.32	2.02±0.14	2.00±0.17	2.03±0.19	2.07±0.16
SEM	0.053	0.033	0.040	0.038	0.032
CV	11.44	7.18	9.10	8.02	6.51
	P values				
Olive pulp (OP)	0.40	0.02	0.14	0.07	0.03
Nutrase Xyla (N)	0.70	0.46	0.92	0.50	0.79
OP × N	0.59	0.81	0.13	0.15	0.20

a-b Means within a column (within main effects) with no common superscript differ significantly (p <0.05), SEM= Standard error of means

Table 6: Egg quality characteristics of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase) in first egg sampling (week 3)

Treatments	Egg quality characteristics (week 3)			
Parameter	Egg weight (g)	Egg index	Yolk index	Hugh unit
Olive pulp (OP)				
0 (g/100g diet)	58.76±7.44	71.18±9.01	36.02±6.66	72.15±6.12
0 (g/100g diet)	62.25±2.46	75.67±1.42	37.71±4.91	73.12±7.60
17.4 (g/100g diet)	64.44±4.62	74.96±2.34	38.39±1.07	72.08±3.05
Nutrase Xyla (N)				
0.00 (g/100g diet)	62.02±2.35	72.98±7.68	37.76±4.35	71.44±4.52
0.08 (g/100g diet)	61.60±7.68	74.89±2.00	36.99±5.21	73.46±6.66
SEM	1.134	1.138	0.962	1.156
CV	8.04	7.57	13.61	7.77
	P values			
Olive pulp (OP)	0.10	0.25	0.64	0.92
Nutrase Xyla (N)	0.84	0.41	0.72	0.39
OP × N	0.09	0.58	0.71	0.14

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$), SEM= Standard error of means

Table 7: Egg quality characteristics of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase) in first egg sampling (week 3-continue)

Treatments	Egg quality characteristics (week 3-continue)			
Parameter	Yolk color	Gravity	Shell weight	Shell thickness
Olive pulp (OP)				
0 (g/100g diet)	6.25±0.95	1.04±0.13	5.65±0.56	34.75±3.61
0 (g/100g diet)	5.62±0.55	1.09±0.00	6.11±0.47	37.46±1.88
17.4 (g/100g diet)	6.00±0.47	1.09±0.00	5.95±0.39	36.17±2.12
Nutrase Xyla (N)				
0.00 (g/100g diet)	6.25±0.40	1.09±0.00	5.94±0.35	36.28±1.30
0.08 (g/100g diet)	5.67±0.84	1.06±0.10	5.87±0.62	35.97±3.78
SEM	0.145	0.015	0.102	0.566
CV	11.14	6.80	7.83	7.66
	P values			
Olive pulp (OP)	0.19	0.37	0.16	0.17
Nutrase Xyla (N)	0.06	0.32	0.70	0.79
OP × N	0.91	0.43	0.14	0.57

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$), SEM= Standard error of means

Table 8: Egg quality characteristics of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase) in second egg sampling (week 7)

Treatments	Egg quality characteristics (week 7)			
Parameter	Egg weight (g)	Egg index	Yolk index	Hugh unit
Olive pulp (OP)				
0 (g/100g diet)	65.13±3.72	75.45±0.95^{a}	41.41±5.10	69.69±3.71^{a}
0 (g/100g diet)	65.10±2.83	74.73±0.87^{ab}	42.54±0.91	69.13±2.87^{a}
17.4 (g/100g diet)	63.02±1.07	73.99±0.89^{b}	41.94±1.73	65.78±2.05^{b}
Nutrase Xyla (N)				
0.00 (g/100g diet)	63.67±2.63	74.82±0.93	42.36±1.11	68.01±3.39
0.08 (g/100g diet)	65.16±2.94	74.62±1.20	41.57±4.23	68.39±3.40
SEM	0.578	0.215	0.623	0.680
CV	3.86	1.27	8.01	4.27
	P values			
Olive pulp (OP)	0.18	0.02	0.80	0.03
Nutrase Xyla (N)	0.16	0.61	0.57	0.75
OP × N	0.08	0.66	0.92	0.20

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$), SEM= Standard error of means

Table 9: Egg quality characteristics of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme (Nutrase Xyla®: a commercial cocktail glycosidase-based enzyme with main activity of endo-xylanase) in second egg sampling (week 7-continue)

Treatments		Egg quality characteristics (week 7-continue)			
Parameter		Yolk color	Gravity	Shell weight	Shell thickness
Olive pulp (OP)					
0 (g/100g diet)		6.54±0.43	1.07±0.00	6.01±0.52[a]	37.58±2.44[a]
0 (g/100g diet)		6.42±0.43	1.07±0.00	5.81±0.34[a]	36.12±2.07[ab]
17.4 (g/100g diet)		5.92±0.39	1.47±1.13	5.33±0.30[b]	34.67±1.70[b]
Nutrase Xyla (N)					
0.00 (g/100g diet)		6.28±0.63	1.07±0.00	5.65±0.36	36.30±2.00
0.08 (g/100g diet)		6.30±0.30	1.34±0.92	5.79±0.58	35.94±2.71
SEM		0.099	0.133	0.098	0.478
CV		5.76	54.28	6.48	5.61
		P values			
Olive pulp (OP)		0.01	0.39	0.00	0.03
Nutrase Xyla (N)		0.87	0.33	0.35	0.67
OP × N		0.02	0.39	0.09	0.15
OP	N				
0	-	6.75±0.32[a]			
0	+	6.33±0.47[a]			
0	-	6.50±0.58[a]			
0	+	6.33±0.27[a]			
17.4	-	5.58±0.17[b]			
17.4	+	6.24±0.16[a]			
CV		5.76			
P values		0.01			

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$), SEM= Standard error of means

showed certain influence on gastrointestinal tract weight, length and content [16-17]. The different levels of olive cake had no effect on carcass cuts of broilers carcass when fed OP at levels up to 100g/kg [18]. Rabayaa *et al*. [19] who incorporated OP in four of the experimental groups at rates of 2.5, 5, 7.5 and 10% in both starter and finisher feeds to replace similar rates of yellow corn reported that weight gain of chicks was the same in chicks consuming up to 7.5% of OP. However, weight gain of chicks fed the level of 10% OP had the lowest significant weight gain. In addition, similar trends were observed in chicks for FI and feed conversion efficiency.

Egg quality characteristics of Lohman LSL-lite hens fed diets including olive pulp supplemented with enzyme in the first (week 3) and the second egg sampling (week 7) are presented in Tables 6-9. Egg index, Haugh unit and egg gravity was not significantly affected by dietary treatment in first sampling (week 3). Dietary inclusion of OP decreased egg index, Haugh unit, shell weight and thickness in the second egg sampling (week 7) compared to hens fed the control diets.

Enzyme supplementation did not significantly affect on laying performance of hens and egg quality characteristics. Interaction between dietary OP inclusion and enzyme supplementation on yolk color was significant in second egg sampling on week 7. Hens fed OP-included diet with no enzyme did have the lowest egg yolk color compared to hens fed the other experimental diets.

Chickens do not produce some enzymes, such as galactosidases (xylanase/β-glucanase); thus, corn-soybean-based diets without supplemented enzymes such as xylanases and pectinases might result in gas accumulation in the gut and diarrhea [20, 21]. Xylanase has been used in combination with other enzyme(s) (xylanase-based cocktail enzyme) in layer diets [20, 22-29] to counteract the effects of NSP; however, the results obtained with different experimental conditions and diets have been inconclusive. The degree of improvement obtained by adding enzymes to the diet depends on many factors including the level of antinutritive factor in the diet, the spectrum and concentration of enzymes used; the type of animal and the age of the animal (young animals tend to respond better to enzymes than older animals); type of gut micro flora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the micro flora in their intestines, have a greater capacity to deal with negative viscosity effects [30]. Typically, enzymes added to layer feed appear to have little effect on egg mass but improve feed efficiency [31]. Pirgozliev *et al.* [32] who examined the effect of dietary xylanase on the availability of nutrients for laying hens when fed on wheat-rye-soy-based diets reported that the AME and nitrogen metabolisability coefficients of xylanase-supplemented diets were greater than the control diet. In addition, they reported supplementary xylanase significantly improved the coefficients of metabolisability of indispensable, dispensable and total amino acids. Their data suggested that use of a xylanase might improve the metabolisability of some nutrients, but that such effects might not always benefit production parameters. Feed intake and feed conversion ratio for egg production were not affected by xylanase. Their data suggested that use of a xylanase might improve the metabolisability of some nutrients, but that such effects might not always benefit production parameters [32]. Diet supplementation with an enzyme cocktail providing 7 U/g of α-1, 6-galactosidase and 22 U/g of β-1, 4-mannanase significantly improved feed conversion of Lohmann Brown-Lite laying hens [33].

Gunawardana *et al.* [34] who evaluated the effects of Rovabio (a natural mixture of enzymes produced by the organism *Penicillium funiculosum*), dietary energy and protein on performance, egg composition, egg solids and egg quality of commercial Leghorns in phase 2, reported dietary protein significantly increased feed consumption but decreased yolk color. As dietary energy increased, feed consumption decreased and yolk color increased. They also found a significant interaction among dietary protein, energy and Rovabio on egg production, BW, egg mass, feed conversion and yolk solids. Egg weight of hens fed the diets supplemented with enzyme was significantly greater than that of hens fed the diets without enzyme during wk 3 and 4. However, enzyme did not significantly influence average egg weight [34]. In the research study by Pirgozliev *et al.* [32] the yolk color of the birds receiving xylanase was darker than the yolk of the birds given the control diet. In addition, birds receiving xylanase had a significantly higher weight gain than those fed on the unsupplemented diet. Dirty and cracked eggs in their study were not affected by xylanase [32]. The improvement in feed conversion for Lohmann Brown-Lite laying hens fed the enzyme cocktail (providing 7 U/g of α-1, 6-galactosidase and 22 U/g of β-1, 4-mannanase) supplemented diet was accompanied by an increase in albumen weight and percentage and a decrease in the percentage of yolk and the ratio of yolk to albumin in the eggs [33].

In conclusion, OP can be included in laying hens diets up to 17% with no adverse effect on EP and EM; however, in terms of feed efficiency, egg shell weight and thickness and yolk color, lower percentage of OP should be inserted into diets. Enzyme did not have beneficial effect on laying hens' performance and egg quality traits.

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