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ORIGINAL ARTICLE

Dietary inclusion of raw faba bean instead of soybean meal and enzyme supplementation in laying hens: Effect on performance and egg quality

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Abstract An experiment was conducted with 160 Hisex Brown laying hens to evaluate the effect of different inclusion levels of faba bean (FB) and enzyme supplementation on productive performance and egg quality parameters. The experimental diets consisted of five levels of FB: 0% (control), 25%, 50%, 75% and 100%, substituting soybean meal (SBM), and two levels of enzyme supplementation (0 or 250 mg/kg). Each dietary treatment was assigned to four replicate groups and the experiment lasted 22 weeks. A positive relationship ($P < 0.05$) was found between FB inclusion and body weight (BW) change of hens when compared to those of the control treatment. Enzyme supplementation significantly affected the final hens' BW. Feed consumption (FC) of hens was statistically increased with increasing FB level up to 50%. Supplementing dietary enzyme mixture at 250 mg/kg led to improvement in FC at all studied ages ($P < 0.05$). Inclusion of 25% or 50% FB in diets had no adverse effects on feed conversion ratio (FCR) compared to the higher FB inclusion levels (75% or 100%). Egg weight (EW), egg number (EN) and egg mass (EM) were significantly ($P < 0.05$) influenced by FB inclusion in diet during the entire experimental periods, except for EN and EM at 20–24 weeks of age. Egg productive parameters were not influenced by enzyme mixture supplementation ($P > 0.05$). The main effect of FB levels replacing for SBM affected ($P < 0.05$) yolk and shell percentages, yolk index, yolk to albumen ratio, shell thickness and egg shape index. It can be concluded that FB and enzyme supplementation could be included

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in hens diet at less than 50% instead of SBM to support egg productive performance, however higher raw FB levels negatively affected egg production indices and quality.

1. Introduction

It is well known that prices of corn and soybean, which are mainly used in poultry diet, hit an all-time record high. Thus, there is an urgent need for affordable and nutritious feeds. Soybean meal (SBM) is the most commonly used protein source in poultry feeding and it is usually known for its high quality. Because SBM is the major by-product of oil extraction from soybean, costs and availability of SBM are strongly correlated with the price of agricultural commodities on the world market (Vicente et al., 2009; Jezierny et al., 2010). World market prices are influenced by variations in economic growth and changes in consumer product preferences and on weather conditions (Trostle, 2008; Jezierny et al., 2010; Cazzato et al., 2014). Therefore, price and availability SBM on global markets may change rapidly, thereby stimulating interest in maximizing the use of locally produced feed ingredients including grain legumes (Ravindran and Blair, 1992; Laudadio and Tufarelli, 2011).

Fava beans (*Vicia faba* L.) are widely produced in several countries, such as the Mediterranean area, and because of their good nutritional value, they can be used as an alternative protein source in place of soybean meal (Castanon and Perez-Lanza, 1990; Nalle et al., 2010). However, the presence

Table 1 Ingredients and experimental diets of laying hens.

	Dietary treatments				
	0	25	50	75	100
Ingredients, %					
Corn	60.58	59.76	58.81	58.24	57.00
Soybean meal (44%)	22.00	16.50	11.00	5.50	0.00
Faba beans (30%)[1]	00.00	5.50	11.00	16.50	22.00
Gluten meal (62%)	5.01	6.20	7.40	8.73	10.07
Limestone	8.17	8.20	8.30	8.32	8.35
Di-calcium	1.82	1.82	1.75	1.70	1.63
Layer premix[2]	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine HCL	0.01	0.08	0.11	0.15	0.18
Dl-methionine	0.09	0.14	0.15	0.16	0.17
Vegetable oil	1.72	1.20	0.78	0.10	0.00
Nutrient composition, %[3]					
Crude protein	18.00	18.01	18.00	18.00	18.00
ME, kcal/kg	2851	2851	2854	2849	2872
Ca	3.63	3.64	3.65	3.63	3.61
Non-phytate P	0.46	0.45	0.45	0.45	0.45
Lysine	0.85	0.85	0.85	0.85	0.85
Met + Cys	0.75	0.75	0.75	0.75	0.75
Crude fiber	3.08	3.06	3.19	3.31	3.43
Threonine	0.65	0.76	0.88	0.99	1.10
Linoleic acid	2.28	1.40	1.38	1.36	1.34
Ether extract	2.60	2.70	2.80	2.92	3.01
Nutrient composition, %[4]					
DM	89.16	89.00	88.79	88.70	88.68
OM	79.52	95.10	95.54	95.05	95.41
CP	17.97	18.08	17.71	17.78	18.01
EE	2.25	4.54	4.40	3.84	3.96
CF	2.79	3.12	3.09	2.96	2.90
Ash	2.48	4.90	4.46	4.45	4.59
Cost per ton[5]	2793	2803	2802	2781	2807

[1] Chemical composition of faba bean (%): dry matter 87; crude protein 30; crude fat 2.7; crude fiber 9.3; ash 3.70; Ca 0.10; P 0.46.
[2] Contained per kg: vit. A, 8000 IU; vit. D_3, 1300 ICU, vit. E 5 mg; vit. K, 2 mg; vit. B1, 0.7 mg; vit. B2, 3 mg; vit. B6, 1.5 mg, vit. B12, 7 mg; Biotin 0.1 mg; Pantothenic acid, 6 g; Niacin, 20 g; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Copper, 6 mg; Iodine, 1 mg; Selenium, 0.5 mg; Cobalt, 1 mg.
[3] Calculated according to NRC (1994).
[4] Chemical analysis according to AOAC (2006).
[5] Calculated according to the price of feed ingredients when the experiment was started.

Table 2 Live body weight and body weight change of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Body weight (g)		
		Initial	Final	Change
FB[1] (%)				
	0	1662	1864[b]	201[c]
	25	1668	1992[ab]	323[ab]
	50	1670	1978[ab]	306[b]
	75	1672	2031[ab]	361[ab]
	100	1668	2080[a]	412[a]
Enzyme (mg/kg diet)				
	0	1667	1967[b]	300[b]
	250	1670	2012[a]	342[a]
FB[1] × enzyme				
0	0	1661	1843	181[c]
	250	1662	1884	222[c]
25	0	1668	1912	244[c]
	250	1669	2073	403[a]
50	0	1668	2024	355[b]
	250	1672	2038	366[b]
75	0	1668	1997	329[bc]
	250	1675	1959	283[c]
100	0	1667	2057	389[b]
	250	1670	2104	434[a]
SEM[2]		1.99	70.05	69.37
Two-way ANOVA		*P*-value[3]		
FB[1]		0.051	< 0.001	0.041
Enzyme		0.148	0.028	0.048
FB[1] × enzyme		0.572	0.504	0.043

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment *P*-value.

of anti-nutritional factors (ANFs), such as vicine and convicine, precludes the incorporation of FB into poultry diets (Perella et al., 2009; Laudadio et al., 2011). Moreover, some concerns were observed for high faba inclusion into broiler diets, which might result in increased feces viscosity due to the high non-starch polysaccharide (NSP) contents. The NSP are not degraded by monogastric digestive enzymes impairing the gastro intestinal tract functions. The NSP contents vary accordingly in species and cultivars (Kocher et al., 2000), and the attempt to reduce the negative effects of NSP involves enzymes' addition to diets (Moschini et al., 2005).

The effects of faba bean (FB) inclusion in feeds for laying hens have been studied for many years, but with conflicting results. Perez-Maldonado et al. (1999) showed that the optimum level of inclusion of FB in laying hen diets was about 250 g/kg. It has also been shown that yolk color, albumen quality and Haugh unit scores are not affected by concentrations as high as 300 g/kg of FB in ration (Guillaume and Bellec, 1977). Laudadio and Tufarelli (2010) reported that most of the ANFs found in the beans are located in the dehulled cotyledon, which led them to recommend dehulling and micronizing the FB for optimum efficiency of seeds.

Therefore, the objective of this study was to explore in hens' diet an appropriate inclusion level of raw FB as an alternative protein source instead of SBM with or without enzyme mixture supplementation on the productive performance and egg quality criteria of laying hens during the early stage of production.

2. Materials and methods

This study was conducted at the Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt. All experimental procedures were carried out according to the Local Experimental Animal Care Committee, and approved by the ethics of the institutional committee. Birds were cared for using husbandry guidelines derived from Zagazig University standard operating procedures.

2.1. Birds, experimental design and diets

One hundred sixty Hisex Brown laying hens were used in the experiment. A completely randomized design was used, with four replications of four hens each; four birds were housed per (50 × 50 × 45 cm) wire cage. The cages were equipped with a nipple drinker and trough feeders. The bird

Table 3 Feed consumption of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Feed intake (g/d)					
		20–24 weeks	24–28 weeks	28–32 weeks	32–36 weeks	36–42 weeks	20–42 weeks
FB[1] (%)							
	0	103.08[a]	110.42[a]	105.01[ab]	106.36[ab]	107.71[ab]	106.52[a]
	25	100.30[ab]	110.38[a]	108.44[ab]	107.46[ab]	107.95[ab]	106.90[a]
	50	95.58[b]	109.13[a]	111.65[a]	110.96[a]	111.30[a]	107.72[a]
	75	88.43[c]	96.45[b]	102.83[b]	99.96[b]	101.39[b]	97.81[b]
	100	86.64[c]	86.91[c]	89.98[c]	81.35[c]	85.66[c]	86.11[c]
Enzyme (mg/kg diet)							
	0	91.98[b]	102.04	103.31	99.47	101.86	99.73
	250	97.63[a]	103.28	103.85	102.96	103.74	102.29
FB[1] × enzyme							
0	0	106.81[a]	115.93[a]	109.56	111.15	112.74	111.24[a]
	250	99.34[b]	104.91[bc]	100.46	101.57	102.68	101.79[ab]
25	0	97.85[b]	107.51[b]	109.55	105.95	107.75	105.72[ab]
	250	102.74[ab]	113.26[ab]	107.32	108.97	108.15	108.09[a]
50	0	89.78[c]	108.39[b]	108.81	112.32	110.56	105.97[ab]
	250	101.39[ab]	109.87[b]	114.49	109.60	112.04	109.47[a]
75	0	85.27[d]	93.59[d]	99.75	93.33	96.54	93.70[c]
	250	91.60[c]	99.31[c]	105.90	106.58	106.24	101.93[b]
100	0	80.20[d]	84.77[f]	88.87	74.60	81.73	82.14[d]
	250	93.08[c]	89.05[e]	91.10	88.09	89.60	90.19[c]
SEM [2]		3.27	2.23	3.63	4.56	3.85	2.94
Two-way ANOVA		*P*-value[3]					
FB[1]		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Enzyme		0.011	0.387	0.814	0.236	0.447	0.179
FB[1] × enzyme		0.033	0.003	0.220	0.069	0.115	0.034

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$ or 0.01).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment *P*-value.

house was provided with programmable lighting and adequate ventilation. The lighting program at the start of the trial was 14 h of light and was increased by 15 min each week to 17 h of light. The diets and water were provided *ad libitum* throughout the experiment. Each experimental diet was formulated to meet nutrient recommendation of Hi-sex Brown management guide which met or exceed the NRC (1994) recommendations. Experimental diets were isocaloric (2800 kcal of ME/kg) and isonitrogenous (18% CP). The duration of the experiment was 22 weeks (between 20 and 42 weeks of age). The diets were arranged in a 2 × 5 factorial design, with the variable being broken faba bean substituted for soybean meal at five levels (0%, 25%, 50%, 75% and 100%, respectively) and enzyme (Galozyme) supplementation at two levels (0 and 250 mg/kg, respectively). The diets were fed as a mash. Ingredients and diet analysis, according to the Association of Official Analytical Chemists (AOAC, 2006) procedures, are reported in Table 1. The exogenous cellulolytic enzyme was purchased from a feed additives company (MultiVita Animal Nutrition® Co., Egypt) and added at 250 mg/kg diet being supplied with beta-glucanase 2300 U/g, xylanase 20,000 U/g, cellulase-complex 3000 U/g, alpha-amylase 400 U/g, protease 200 U/g.

2.2. Data collection and egg parameters

Laying hens' body weight (BW) was registered at the start and end of the feeding trial. The BW changes (BWC) were calculated accordingly. Feed consumption (FC) was recorded weekly and adjusted for mortalities, while feed conversion ratio (FCR) was calculated as the egg mass (EM) value divided by the amount of feed consumed. Eggs were daily collected and egg production was calculated on a hen-day basis. Egg weight (EW) and egg number were recorded daily to calculate the egg mass (egg number × egg weight).

2.3. Egg quality criteria

Eggs were examined for interior and exterior quality. Egg components were monthly measured using three eggs from each treatment replicate. Eggs were weighed, then egg length and width were determined before breaking. The egg was carefully broken on a glass plate (35 × 25 cm) to measure both internal and external egg quality characteristics. Yolks were separated from albumen. Egg shells were cleaned of any adhering albumen. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight. Egg shape index was computed as the ratio of egg width to the length

Table 4 Feed conversion ratio of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Feed conversion ratio (g feed/g egg)					
		20–24 weeks	24–28 weeks	28–32 weeks	32–36 weeks	36–42 weeks	20–42 weeks
FB[1] (%)							
	0	1.97	1.73	1.58[c]	1.58[b]	1.64[b]	1.70[b]
	25	2.08	1.64	1.61[c]	1.58[b]	1.60[b]	1.70[b]
	50	1.87	1.65	1.71[bc]	1.67[b]	1.69[b]	1.72[b]
	75	2.02	1.88	1.87[b]	1.86[a]	1.89[a]	1.90[a]
	100	1.88	1.81	2.11[a]	1.90[a]	1.91[a]	1.92[a]
Enzyme (mg/kg diet)							
	0	1.94	1.76	1.75	1.67	1.73	1.77
	250	1.99	1.73	1.80	1.77	1.76	1.81
FB[1] × enzyme							
0	0	1.99	1.73	1.63	1.63	1.68	1.73
	250	1.95	1.73	1.53	1.54	1.61	1.67
25	0	2.15	1.59	1.62	1.58	1.59	1.70
	250	2.01	1.70	1.59	1.58	1.60	1.70
50	0	1.75	1.67	1.68	1.71	1.70	1.71
	250	1.98	1.64	1.73	1.64	1.68	1.73
75	0	1.96	2.02	1.74	1.73	1.85	1.86
	250	2.09	1.73	2.01	1.98	1.92	1.95
100	0	1.86	1.79	2.07	1.69	1.81	1.84
	250	1.91	1.84	2.16	2.12	2.01	2.01
SEM [2]		0.11	0.09	0.08	0.08	0.07	0.06
Two-way ANOVA		*P*-value[3]					
FB[1]		0.276	0.105	< 0.001	0.001	< 0.001	0.001
Enzyme		0.503	0.623	0.325	0.080	0.402	0.328
Faba bean × enzyme		0.519	0.337	0.277	0.082	0.392	0.497

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$ or 0.01).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment *P*-value.

(Awosanya et al., 1998). Yolk index was calculated according to Funk et al. (1958), as average yolk height divided by yolk diameter (mm) following removal of the yolk from the albumen. The Haugh unit was calculated as: Haugh units (%) = 100 × log (H + 7.57 − 1.7 $W^{0.37}$, where H is the height of the albumen and W is the weight of the egg) according the formula proposed by Card and Nesheim (1972). The eggs were examined for shell quality by shell thickness (with shell membrane) of the eggs was measured by micrometer. Shell thickness was a mean value of measurements at 3 locations on the eggs (air cell, equator, and sharp end).

2.4. Statistical analysis

Data were analyzed using the general liner model procedure in SPSS (version, 21). A 5 × 2 factorial design was used to analyze data of performance as a response to five levels of faba bean and two concentrations of enzyme. Differences among groups means were detected using two way analysis of variance (ANOVA). Duncan's multiple range test was applied to separate means (Duncan, 1995). Statements of statistical significance were based on probability of (P < 0.05). The model used was: $Y_{ij} = \mu + D_i + A_j + DA_{ij} + e_{ij}$, where: Y_{ij} = an observation, μ = the overall mean, D_i = fixed effect of FB levels, A_j = fixed effect of enzyme concentrations, DA_{ij} = fixed effect of interaction between FB and enzyme levels and e_{ij} = random error associated to each observation.

3. Results and discussion

3.1. Chemical composition of faba bean

The chemical composition of FB used in the experiment is shown in Table 1. Results of proximate analysis for FB yielded values of 87% for dry matter (DM), 30% for crude protein (CP), 2.7 for ether extract (EE), 9.3% for crude fiber (CF), 3.70% for ash, 0.10% for Ca and 0.46% for total P (TP). The CP value 30% of FB was similar to the value recorded by Koivunen et al. (2014) and higher than that recorded 24% according to NRC (1994). This means that the protein content of SBM is 1.5 fold of that FB content. The DM, CP, EE, Ca and TP contents of SBM were clearly higher than FB (NRC, 1994), while the CF content of FB was higher than SBM. The EE content in FB obtained in the present study (2.7%) was approximately the same of those recorded by Koivunen et al. (2014) and higher than that reported by NRC (1994). Overall, reliable values of the nutrient content of feed constituents are vital to create more accurate diet

Table 5 Egg weight of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Egg weight (g)					
		20–24 weeks	24–28 weeks	28–32 weeks	32–36 weeks	36–42 weeks	20–42 weeks
FB[1] *(%)*							
	0	64.08[a]	70.15[a]	69.96[b]	71.40[a]	70.51[ab]	69.22[a]
	25	64.04[a]	70.63[a]	71.48[a]	71.77[a]	71.29[a]	69.84[a]
	50	64.21[a]	69.88[a]	69.69[b]	70.43[a]	70.00[b]	68.84[a]
	75	61.32[b]	65.50[b]	66.79[c]	65.68[b]	65.99[c]	65.06[b]
	100	61.48[b]	65.73[b]	63.07[d]	62.02[c]	63.61[d]	63.18[c]
Enzyme (mg/kg diet)							
	0	63.19	67.86	68.02	67.88	67.92	66.97
	250	62.87	68.90	68.37	68.64	68.64	67.48
FB[1] *× enzyme*							
0	0	64.79	72.06[a]	70.07[a]	71.43[a]	71.18[a]	69.91[a]
	250	63.37	68.24[ab]	69.85[a]	71.38[a]	69.83[a]	68.54[a]
25	0	64.30	69.74[a]	70.82[a]	69.85[ab]	70.14[a]	68.97[a]
	250	63.79	71.51[a]	72.15[a]	73.69[a]	72.45[a]	70.72[a]
50	0	64.08	68.13[ab]	69.28[a]	69.79[ab]	69.07[a]	68.07[a]
	250	64.34	71.64[a]	70.10[a]	71.06[a]	70.93[a]	69.61[a]
75	0	61.85	63.79[c]	67.06[b]	65.06[b]	65.30[b]	64.61[b]
	250	60.79	67.21[bc]	66.52[b]	66.31[bc]	66.68[b]	65.50[b]
100	0	60.93	65.58[c]	62.88[c]	63.27[c]	63.91[b]	63.32[b]
	250	62.03	65.89[c]	63.26[c]	60.78[c]	63.31[b]	63.05[b]
SEM [2]		0.83	0.81	0.64	0.84	0.58	0.54
Two-way ANOVA		P-value[3]					
FB[1]		0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Enzyme		0.544	0.052	0.397	0.166	0.061	0.151
FB[1] × enzyme		0.566	< 0.001	0.614	0.014	0.013	0.040

Means in the same column within each classification bearing different letters are significantly different (P < 0.05 or 0.01).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment P-value.

formulations however, several factors affect the physical and nutritional characteristics of FB causing variability.

3.2. Hens productive performance

The effects of different dietary FB levels and enzyme supplementation on final BW and changes are shown in Table 2. There was a positive relationship ($P < 0.05$) between FB inclusion and BW indices of Hisex hens when compared to the control treatment. These results are in line with findings of Brufau et al. (1998), Perez-Maldonado et al. (1999) who found that addition of FB into the diet significantly affected BW of chicks. In a previous study, Laudadio and Tufarelli (2010) did not observe improvements in BW change resulting from increased processed FB inclusion in the hens' diet. In the current study, enzyme mixture supplementation significantly ($P < 0.05$) increased the final BW and change. These results disagreed with data obtained by Ghazalah et al. (2011) who stated that enzyme addition to layer diet had no effect on BW gain. No significant interaction between FB levels and enzyme supplementation was noticed for BW traits.

Table 3 summarizes the effect of treatments on feed consumption (FC). The FC was influenced ($P < 0.05$) by FB throughout the experimental period. The FC of hens was increased with increasing FB level up to 50% as alternative protein source, while the high levels (75% or 100%) of FB recorded the worst value of FC during the periods 28–32, 32–36, 36–42 and 20–42 weeks of age, respectively. This observation was expected, because FB contains relatively high contents of ANF, which are a primary contributor of performance retardation (Nalle et al. 2010).

Previous data regarding the effects of dietary FB on FC of laying hens are consistent. In a study, Laudadio and Tufarelli (2010) showed that FB inclusion at 24% in the diets significantly affected feed intake. On the other hand, Fru-Nji et al. (2007) reported that dietary FB at different inclusion levels had no significant effect on intake of hens.

Supplemental dietary enzyme mixture (250 mg/kg diet) led to improvement in FC at all studied periods ($P < 0.05$). This improvement in FC with exogenous enzyme supplementation compared to control diet could be attributed to some stimulators and compounds enhancing ingestion, digestion and absorption of certain nutrients in diet. There were no differences in FC due to the interaction between FB inclusion and enzyme addition through 28–32, 32–36 and 36–42 weeks old ($P < 0.05$). While, during periods 20–24, 24–28 and 20–42 weeks of age, FC was influenced by the interaction-effect. The highest amount of FC was achieved by hens that consumed the control diet without enzyme supplementation, but

Table 6 Egg number of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Egg number per month					
		20–24 weeks	24–28 weeks	28–32 weeks	32–36 weeks	36–42 weeks	20–42 weeks
FB[1] (%)							
	0	24.43	27.25[a]	28.39[a]	28.11[a]	27.82[a]	27.20[a]
	25	23.22	28.55[a]	28.26[a]	28.33[a]	28.41[a]	27.35[a]
	50	23.94	28.25[a]	28.08[a]	28.12[a]	28.16[a]	27.31[a]
	75	21.55	24.00[b]	24.78[b]	24.59[b]	24.39[b]	23.86[b]
	100	22.50	22.05[c]	20.50[c]	20.89[c]	21.28[c]	21.44[c]
Enzyme (mg/kg diet)							
	0	22.63	25.94	26.27	26.18	26.10	25.42
	250	23.62	26.10	25.74	25.83	25.92	25.44
FB[1] × enzyme							
0	0	24.84	27.86[ab]	28.68[a]	28.47[a]	28.27[a]	27.62[a]
	250	24.02	26.64[ab]	28.11[a]	27.74[a]	27.37[a]	26.78[a]
25	0	22.00	29.11[a]	28.56[a]	28.69[a]	28.83[a]	27.44[a]
	250	24.44	28.00[a]	27.97[a]	27.98[a]	27.98[a]	27.27[a]
50	0	24.00	28.50[a]	27.90[a]	28.05[a]	28.20[a]	27.33[a]
	250	23.89	28.00[a]	28.26[a]	28.20[a]	28.13[a]	27.30[a]
75	0	21.11	22.33[bc]	25.70[b]	24.86[b]	24.01[b]	23.60[b]
	250	22.00	25.67[b]	23.87[b]	24.32[b]	24.77[b]	24.12[b]
100	0	21.22	21.89[c]	20.51[c]	20.85[c]	21.19[c]	21.13[c]
	250	23.77	22.22[c]	20.50[c]	20.93[c]	21.36[c]	21.76[c]
SEM [2]		1.42	0.88	0.96	0.86	0.80	0.82
Two-way ANOVA		*P*-value[3]					
FB[1]		0.298	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Enzyme		0.281	0.766	0.398	0.523	0.728	0.967
FB[1] × enzyme		0.699	0.045	0.040	0.039	0.048	0.046

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$ or 0.01).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment *P*-value.

lower amount of FC was recorded when fed diet containing 100% FB with no enzyme supplementation.

Results from this study showed that inclusion of 25% or 50% FB instead of SBM in laying hen diets had no adverse effects on FCR. These results are in agreement with those reported by Fru-Nji et al. (2007) elucidating that FCR was significantly increased by FB inclusion. Indeed, Laudadio and Tufarelli (2010) stated that inclusion of the alternative protein source as FB at level of 24% in the laying hen diets significantly influenced feed intake without any negative impact on feed efficiency compared to a control diet.

There were no effects of enzyme supplementation or the interaction between FB inclusion and enzyme addition on FCR during the experiment (Table 4). Previous results regarding the effects of dietary enzyme on FCR are inconsistent. However, it could be stated that enzyme mixture supplementation to layer diets has been found to improve performance including FCR (Benabdeljelil and Arbaoui, 1994).

In Tables 5–7 are reported the effect of diets containing different levels of FB on egg production parameters in laying hens. In general, the EW, EN and EM were significantly ($P < 0.05$) influenced by FB inclusion in diets during all of experimental periods, except for EN and EM at 20–24 weeks of age. Compared to the control diet, the EW, EN and EM of birds receiving dietary FB up to 50% were not statistically influenced ($P > 0.05$). Hens consuming 25% FB diet had marginally higher EW, EN and EM than those fed with control or 50% FB diets. The 75% or 100% inclusion of FB significantly decreased EW, EN and EM ($P < 0.05$) compared to other treatments. The EW, EN and EM values in our study were in accordance with findings obtained by Olaboro et al. (1981) and more recently by Laudadio and Tufarelli (2010). According to Fru-Nji et al. (2007) the reduction in EN and EM production with increasing dietary FB may be attributed to ANF in FB content, and may be due to the deficient indispensable amino acid (such lysine, threonine and sulfur amino acids) content of the FB. In this study, lysine and methionine were supplied to achieve the balance and nutrient requirements of hens. The variation in chemical composition of the tested diets seemed not to be related to EW and EM losses. Moreover, we assumed that the reduction in EW, EN and EM values observed between the low levels (25% and 50%) and the high levels (75% and 100%) of FB was mainly due to ANF (vicine and convicine) and may be due to different amounts of dietary linoleic acid content. The depression in EN which is observed with 75% or 100% FB instead of soybean might be due to a reduction in the yolk fraction. On the other hand, increases in layer body weight were negatively correlated with egg production parameters (EN and EM). Stability of BW in layers receiving diets supplemented with FB could be considered a favorable factor in increasing productive performance as noticed by Aydin et al. (2008).

Table 7 Egg mass of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Items		Egg mass (g)					
		20–24 weeks	24–28 weeks	28–32 weeks	32–36 weeks	36–42 weeks	20–42 weeks
FB[1] (%)							
	0	1566	1913[a]	1986[a]	2007[a]	1962[a]	1887[a]
	25	1493	2016[a]	2020[a]	2033[a]	2025[a]	1917[a]
	50	1538	1973[a]	1957[a]	1980[a]	1971[a]	1884[a]
	75	1321	1578[b]	1654[b]	1617[b]	1611[b]	1556[b]
	100	1385	1451[b]	1293[c]	1294[c]	1353[c]	1355[c]
Enzyme (mg/kg diet)							
	0	1432	1769	1795	1787	1782	1713
	250	1488	1803	1769	1785	1787	1726
FB[1] × enzyme							
0	0	1609	2007[a]	2009[a]	2034[a]	2012[a]	1934[a]
	250	1522	1818[b]	1963[a]	1980[a]	1911[a]	1839[a]
25	0	1417	2030[a]	2022[a]	2005[a]	2022[a]	1899[a]
	250	1569	2003[a]	2018[a]	2061[a]	2027[a]	1936[a]
50	0	1538	1942[b]	1933[a]	1957[a]	1947[a]	1863[a]
	250	1537	2005[a]	1981[a]	2003[a]	1995[a]	1904[a]
75	0	1305	1431[d]	1725[b]	1622[b]	1573[b]	1531[b]
	250	1336	1725[c]	1584[b]	1613[b]	1650[b]	1582[b]
100	0	1293	1436[d]	1289[c]	1320[c]	1355[c]	1338[c]
	250	1477	1465[d]	1297[c]	1270[c]	1352[c]	1372[c]
SEM [2]		100	68.94	63.63	62.40	58.73	58.14
Two-way ANOVA		*P*-value[3]					
FB[1]		0.099	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Enzyme		0.383	0.444	0.511	0.956	0.889	0.721
FB[1] × enzyme		0.651	0.026	0.028	0.036	0.040	0.045

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$ or 0.01).

1 FB: faba bean.

2 SEM: standard error of the means.

3 Overall treatment *P*-value.

Magoda and Gous (2011) stated that egg production parameters were unaffected by the different levels of FB in the layer diets. As the FB inclusion level increased, egg weight decreased (Mateos and Puchal, 1982; Perez-Maldonado et al. 1999). The use of FB in poultry diets is restricted due to its content of ANF (Crepon et al. 2010). Heat treatments are recommended to allow a maximum inclusion FB level in laying hen diets (Marquardt and Campbell 1973). Crepon et al. (2010) observed that the use of raw FB with a high ANFs (mainly vicine and convicine) content cannot exceed 7% of the diet, but it is possible to include FB at 20% if the FB used has a low ANF content.

Irrespective of FB inclusion in the layer diets, egg production parameters were not influenced by enzyme mixture supplementation during all of experimental periods, Tables 5–7. Values of EM were similar ($P = 0.721$) for all bird treatment groups at 1713 g in birds fed on the control diet and 1726 g in birds fed on the diet supplemented with 250 mg enzyme/kg diet during the whole period. The same effect as EM was noticed on EW and EN. These results partially agree with Ghazalah et al. (2011) who reported that enzyme supplementation had no significant affect on egg weight. Conversely, enzyme addition significantly improved egg production by 2.4% and egg mass by 2.9%.

Egg production criteria were significantly ($P < 0.05$) influenced by the interaction between FB levels and enzyme supplementation during all experimental periods vs. 20–24 weeks of age. The highest values of EW, EN and EM were achieved by the hens that consumed diets containing 0%, 25% or 50% FB with or without enzyme addition; while, the birds fed diets containing 75% or 100% FB recorded the lowest values of egg production parameters.

3.3. Egg quality criteria

The effect of different dietary levels of FB and enzyme supplementation and their interaction on egg quality of laying hens are reported in Table 8. The main effect of FB levels replacing SBM statistically ($P < 0.05$) affected yolk%, shell%, yolk index, yolk:albumen ratio, shell thickness and egg shape index. The results of the current study are consistent with those of Laudadio and Tufarelli (2010) who found that dietary FB inclusion had no negative influence on Haugh unit scores as well as on albumen percent. Egg shape index was reduced when FB were incorporated over 75% instead of SBM into the layer diets (76.04 vs. 79.92, respectively; $P < 0.001$). The highest values of yolk and shell percentages as well as shell

Table 8 Egg quality criteria of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods.

Item		Albumen%	Yolk%	Shell%	Haugh unit	Yolk index	Yolk:albumin ratio	Shell thickness (mm)	Egg shape index
FB[1] (%)									
	0	65.99	20.97[c]	13.03[a]	85.34	49.80[a]	0.319[c]	0.355[a]	79.92[a]
	25	65.06	22.29[bc]	11.44[b]	96.53	47.80[b]	0.343[bc]	0.352[a]	80.63[a]
	50	64.31	26.63[a]	12.73[a]	95.04	48.44[ab]	0.414[a]	0.358[a]	80.26[a]
	75	66.45	23.33[b]	12.35[ab]	95.56	47.40[b]	0.355[b]	0.337[b]	76.04[b]
	100	65.27	23.06[b]	11.65[b]	97.26	48.56[ab]	0.353[b]	0.333[b]	77.24[b]
Enzyme (mg/kg diet)									
	0	65.67	23.94[a]	12.71[a]	92.70[b]	49.01[a]	0.366[a]	0.343	79.62[a]
	250	65.17	22.57[b]	11.77[b]	95.19[a]	47.79[b]	0.348[b]	0.351	78.01[b]
FB[1] × enzyme									
0	0	63.82	22.27[c]	13.86[a]	85.34[d]	49.76	0.350[c]	0.355[b]	81.82[a]
	250	68.16	19.66[d]	12.17[ab]	85.35[d]	49.85	0.288[f]	0.355[b]	78.02[b]
25	0	65.16	23.73[c]	11.10[b]	94.39[a]	48.04	0.363[c]	0.338[c]	81.10[a]
	250	64.97	20.86[d]	11.78[b]	98.67[a]	47.57	0.323[d]	0.366[a]	80.17[a]
50	0	65.25	28.26[a]	13.89[a]	91.51[c]	49.15	0.433[a]	0.370[a]	80.25[a]
	250	63.37	25.01[b]	11.61[b]	98.56[a]	47.74	0.395[b]	0.346[bc]	80.27[a]
75	0	67.43	23.23[c]	13.63[a]	97.22[a]	48.87	0.353[c]	0.330[c]	76.85[bc]
	250	65.48	23.44[c]	11.07[b]	93.89[b]	45.93	0.358[c]	0.345[bc]	75.23[c]
100	0	66.68	22.23[c]	11.08[b]	95.04[b]	49.24	0.330[c]	0.323[c]	78.11[b]
	250	63.87	23.89[c]	12.23[ab]	99.48[a]	47.89	0.375[bc]	0.343[bc]	76.37[bc]
SEM[2]		2.29	0.66	0.442	0.70	0.77	0.01	0.007	0.51
Two-way ANOVA		*P*-value[3]							
FB[1]		0.899	< 0.001	0.004	< 0.001	0.044	< 0.001	0.002	< 0.001
Enzyme		0.743	0.003	0.002	< 0.001	0.019	0.023	0.066	< 0.001
FB[1] × enzyme		0.547	0.002	< 0.001	< 0.001	0.372	0.001	0.004	0.013

Means in the same column within each classification bearing different letters are significantly different ($P < 0.05$ or 0.01).
[1] FB: faba bean.
[2] SEM: standard error of the means.
[3] Overall treatment *P*-value.

thickness and yolk:albumen ratios were achieved by 50% of FB diet compared to the other diets.

Data presented in Table 8 reveal that egg quality criteria were affected by enzyme supplementation except for albumin and shell thickness ($P < 0.05$). The highest rates of yolk and shell percent, yolk and egg shape index were obtained by birds fed the control diet without enzyme supplementation. On the other hand, Haugh unit scores and yolk:albumen ratio were increased with enzyme addition. Similar results were obtained by Ghazalah et al. (2011). Conversely, Deniz et al. (2013) noted that there was no effect of enzyme cocktail supplementation on Haugh unit score of laying hens during experiment.

Exterior and interior egg quality criteria were statistically affected by the interaction between FB levels and enzyme supplementation effect except for albumen percent and yolk index (Table 8; $P < 0.05$). Yolk and shell percent as well as yolk to albumin ration and shell thickness were higher for hens fed 50% FB without enzyme supplementation compared with hens fed the other diets. However, the diet containing 100% FB with 250 mg enzyme per kg diet resulted in an increase in Haugh unit score (99.48) compared with eggs from hens fed other diets ($P < 0.05$). Our results coincided with Robblee et al. (1977) who found that Haugh unit increased as the amount of FB in laying hen diets increased. The highest value of egg shape index was achieved by hens consuming the corn-soya diet compared to the other diets.

In conclusion, in view of our findings, it could be concluded that FB and enzyme supplementations could be included in laying hen diets at level less than 50% instead of SBM in order to improve egg productive performance, since higher FB levels can negatively affect egg production indices and egg quality.

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