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

M.E. Abd El-Hack a, M. Alagawany a, V. Laudadio b, R. Demauro b, V. Tufarelli b,*

a Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
b Department of Emergency and Organ Transplantation (DETO), Section of Veterinary Science and Animal Production, University of Bari ‘Aldo Moro’, Valenzano 70010, Bari, Italy

Received 8 February 2015; revised 14 April 2015; accepted 6 May 2015
Available online 14 May 2015

KEYWORDS
Faba bean; Enzyme; Performance; Egg quality; Layer

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...
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, %     | 60.58 | 59.76 | 58.81 | 58.24 | 57.00 |
| Corn               | 22.00 | 16.50 | 11.00 | 5.50 | 0.00 |
| Soybean meal (44%) | 0.00 | 5.50 | 11.00 | 16.50 | 22.00 |
| Faba beans (30%)   | 5.01 | 6.20 | 7.40 | 8.73 | 10.07 |
| Gluten meal (62%)  | 8.17 | 8.20 | 8.30 | 8.32 | 8.35 |
| Limestone          | 1.82 | 1.82 | 1.75 | 1.70 | 1.63 |
| Di-calcium         | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Salt               | 0.01 | 0.08 | 0.11 | 0.15 | 0.18 |
| L-Lysine HCL       | 0.09 | 0.14 | 0.15 | 0.16 | 0.17 |
| Vegetable oil      | 1.72 | 1.20 | 0.78 | 0.10 | 0.00 |

Nutrient composition, %

| Item                | 0  | 25 | 50 | 75 | 100 |
|---------------------|----|----|----|----|-----|
| 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, %

| Item                | 0  | 25 | 50 | 75 | 100 |
|---------------------|----|----|----|----|-----|
| DM                  | 89.16 | 89.00 | 88.79 | 88.70 | 88.68 |
| OM                  | 79.52 | 95.10 | 95.05 | 95.45 | 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        | 2793 | 2803 | 2802 | 2781 | 2807 |

1 Chemical composition of faba bean (%): dry matter 87; crude protein 30; crude fat 2.7; crude fiber 3.9; ash 3.70; Ca 0.10; P 0.46.
2 Contained per kg: vit. A, 8000 IU; vit. D3, 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 (100%)    | 1662            | 1978    | 316   |
| Enzyme (mg/kg diet) | 1672        | 2031    | 361   |
| FB × enzyme  |                 | 1667    | 2080  | 412    |

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 con-
vicine, precludes the incorporation of FB into poultry diets
(Perella et al., 2009; Laudadio et al., 2011). Moreover, some
corcerns 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 con-
tents 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 opti-
mum 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 concentra-
tions 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 mix-
ture 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 (%)                     |                   |             |             |             |             |             |             |
| 0 25 50 75 100             |                   |             |             |             |             |             |             |
| 0 103.08a 110.42a 105.01ab 106.36b 107.71ab 106.52a |             |             |             |             |             |             |             |
| 25 100.30ab 110.38a 108.44b 107.46b 107.95ab 106.90a |             |             |             |             |             |             |             |
| 50 95.58b 109.13a 111.65c 110.96a 111.30c 107.72a |             |             |             |             |             |             |             |
| 75 88.43c 96.45b 102.83b 99.96b 101.39b 97.81b |             |             |             |             |             |             |             |
| 100 86.64d 86.91e 89.98c 81.35c 85.66e 86.11e |             |             |             |             |             |             |             |
| Enzyme (mg/kg diet)        |                   |             |             |             |             |             |             |
| 0 91.98b 102.04 103.31 99.47 101.86 99.73 |             |             |             |             |             |             |             |
| 250 97.63 103.28 103.85 102.96 103.74 102.29 |             |             |             |             |             |             |             |
| FB (%) × enzyme            |                   |             |             |             |             |             |             |
| 0 0 250                    |                   |             |             |             |             |             |             |
| 0 106.81a 115.93a 109.56 111.15 112.74 111.24a |             |             |             |             |             |             |             |
| 250 99.34a 104.91bc 100.46 101.57 102.68 101.79ab |             |             |             |             |             |             |             |
| 0 97.85b 107.51b 109.55 105.95 107.75 105.72ab |             |             |             |             |             |             |             |
| 250 102.74ab 113.26b 107.32 108.97 108.15 108.09ab |             |             |             |             |             |             |             |
| 50 0 100.39b 108.18 112.32 110.36 105.97ab |             |             |             |             |             |             |             |
| 250 108.39b 109.87b 114.49 109.60 112.04 109.47b |             |             |             |             |             |             |             |
| 75 0 8.52 93.33 65.54 93.70c |             |             |             |             |             |             |             |
| 250 91.60c 97.93a 105.90 106.58 106.24 101.93c |             |             |             |             |             |             |             |
| 100 0 80.20d 39.77f 88.87 74.60 81.73 82.14d |             |             |             |             |             |             |             |
| 250 68.49e 89.05e 91.10 88.09 89.60 90.19e |             |             |             |             |             |             |             |
| SEM 2                      |                   |             |             |             |             |             |             |
| 2 3.27 2.23 3.63 4.56 3.85 2.94 |             |             |             |             |             |             |             |
| Two-way ANOVA             |                   |             |             |             |             |             |             |
| P-value                   |                   |             |             |             |             |             |             |
| FB (%)                    | <0.001            | <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 (%) × 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 recommendations 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** (%)     |            |            |            |            |            |            |
| 0              | 1.97       | 1.73       | 1.58<sup>c</sup> | 1.58<sup>b</sup> | 1.64<sup>b</sup> | 1.70<sup>b</sup> |
| 25             | 2.08       | 1.64       | 1.61<sup>c</sup> | 1.58<sup>b</sup> | 1.60<sup>b</sup> | 1.70<sup>b</sup> |
| 50             | 1.87       | 1.65       | 1.71<sup>bc</sup> | 1.67<sup>b</sup> | 1.69<sup>b</sup> | 1.72<sup>b</sup> |
| 75             | 2.02       | 1.88       | 1.87<sup>b</sup> | 1.86<sup>a</sup> | 1.89<sup>a</sup> | 1.90<sup>a</sup> |
| 100            | 1.88       | 1.81       | 2.11<sup>a</sup> | 1.90<sup>a</sup> | 1.91<sup>a</sup> | 1.92<sup>a</sup> |
| **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** (%) × enzyme |            |            |            |            |            |            |
| 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             | 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             | 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             | 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            | 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** <sup>2</sup> | 0.11       | 0.09       | 0.08       | 0.08       | 0.07       | 0.06       |
| **Two-way ANOVA** | **P-value**<sup>3</sup> |            |            |            |            |            |
| FB <sup>1</sup> | 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).

<sup>1</sup> FB: faba bean.
<sup>2</sup> SEM: standard error of the means.
<sup>3</sup> Overall treatment P-value.
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: Yij = l + Di + Aj + DAij + ei, where: Yij = an observation, l = the overall mean, Di = fixed effect of FB levels, Aj = fixed effect of enzyme concentrations, DAij = fixed effect of interaction between FB and enzyme levels and ei = 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

| Items | Egg weight of laying hens as affected by faba bean levels and enzyme supplementation during the experimental periods. |
|-------|------------------------------------------------------------------------------------------------------------------|
|       | Egg weight (g)                                                                                                     | 20–24 weeks | 24–28 weeks | 28–32 weeks | 32–36 weeks | 36–42 weeks | 20–42 weeks |
| **FB** (%) |                                                                                                                   |             |             |             |             |             |             |
| 0     | 64.08<sup>a</sup>                                                                                                  | 70.15<sup>a</sup> | 69.96<sup>b</sup> | 71.40<sup>a</sup> | 70.51<sup>ab</sup> | 69.22<sup>a</sup> |
| 25    | 64.94<sup>a</sup>                                                                                                  | 70.63<sup>a</sup> | 71.48<sup>a</sup> | 71.77<sup>a</sup> | 71.29<sup>a</sup> | 69.84<sup>a</sup> |
| 50    | 64.21<sup>a</sup>                                                                                                  | 69.88<sup>a</sup> | 69.69<sup>b</sup> | 70.43<sup>a</sup> | 70.00<sup>b</sup> | 68.84<sup>a</sup> |
| 75    | 61.32<sup>b</sup>                                                                                                  | 65.50<sup>b</sup> | 66.79<sup>c</sup> | 65.68<sup>b</sup> | 65.99<sup>c</sup> | 65.06<sup>b</sup> |
| 100   | 61.48<sup>b</sup>                                                                                                  | 65.73<sup>b</sup> | 63.07<sup>d</sup> | 62.02<sup>d</sup> | 63.61<sup>d</sup> | 63.18<sup>d</sup> |
| **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** (%)× enzyme |                                                                                                                   |             |             |             |             |             |             |
| 0     | 64.79                                                                                                             | 72.06<sup>a</sup> | 70.07<sup>a</sup> | 71.43<sup>a</sup> | 71.18<sup>a</sup> | 69.91<sup>a</sup> |
| 250   | 63.37                                                                                                             | 68.24<sup>ab</sup> | 69.85<sup>a</sup> | 71.38<sup>a</sup> | 69.83<sup>a</sup> | 68.54<sup>a</sup> |
| 25    | 64.30                                                                                                             | 69.74<sup>a</sup> | 70.82<sup>a</sup> | 69.85<sup>ab</sup> | 70.14<sup>a</sup> | 68.97<sup>a</sup> |
| 50    | 63.79                                                                                                             | 71.51<sup>a</sup> | 72.15<sup>a</sup> | 73.69<sup>a</sup> | 72.45<sup>a</sup> | 70.72<sup>a</sup> |
| 250   | 64.08                                                                                                             | 68.13<sup>ab</sup> | 69.28<sup>a</sup> | 69.79<sup>ab</sup> | 69.07<sup>a</sup> | 68.07<sup>a</sup> |
| 75    | 63.48                                                                                                             | 71.64<sup>a</sup> | 70.10<sup>a</sup> | 71.06<sup>a</sup> | 70.92<sup>a</sup> | 69.61<sup>a</sup> |
| 250   | 61.85                                                                                                             | 63.79<sup>b</sup> | 67.06<sup>b</sup> | 65.06<sup>b</sup> | 65.30<sup>b</sup> | 64.61<sup>b</sup> |
| 100   | 60.79                                                                                                             | 67.21<sup>bc</sup> | 66.52<sup>b</sup> | 66.31<sup>bc</sup> | 66.68<sup>b</sup> | 65.50<sup>b</sup> |
| 250   | 60.93                                                                                                             | 65.58<sup>c</sup> | 62.88<sup>c</sup> | 63.27<sup>c</sup> | 63.91<sup>b</sup> | 63.32<sup>b</sup> |
|      | SEM<sup>2</sup>                                                                                                    | 0.83        | 0.81        | 0.64        | 0.84        | 0.58        | 0.54        |
|      | **Two-way ANOVA**                                                                                                  | **P-value3** |             |             |             |             |             |
|      | FB (%)                                                                                                             | 0.001       | <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 (%)× 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
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       | 1913b       | 1986a       | 2007a       | 1962a       | 1887a       |
| 25    | 1493       | 2016a       | 2020a       | 2033a       | 2025a       | 1917a       |
| 50    | 1538       | 1973a       | 1957a       | 1980a       | 1971a       | 1884a       |
| 75    | 1321       | 1578b       | 1654b       | 1617b       | 1611b       | 1556b       |
| 100   | 1385       | 1451b       | 1293a       | 1294a       | 1353c       | 1355c       |
|       |            |            |            |            |            |            |
| Enzyme (mg/kg diet) |          |            |            |            |            |            |
| 0     | 1432       | 1769       | 1795       | 1787       | 1782       | 1713       |
| 250   | 1488       | 1803       | 1769       | 1785       | 1787       | 1726       |
|       |            |            |            |            |            |            |
| FB (1) × enzyme |          |            |            |            |            |            |
| 0     | 1609       | 2007a       | 2009a       | 2034a       | 2012a       | 1934a       |
| 250   | 1522       | 1818b       | 1963a       | 1980a       | 1911a       | 1839a       |
| 25    | 1417       | 2030a       | 2022a       | 2005a       | 2022a       | 1899a       |
| 250   | 1569       | 2033a       | 2018a       | 2061a       | 2027a       | 1936a       |
| 50    | 1538       | 1942b       | 1933a       | 1957a       | 1947a       | 1863a       |
| 250   | 1537       | 2005c       | 1981a       | 2003a       | 1995a       | 1904b       |
| 75    | 1305       | 1431c       | 1725c       | 1622c       | 1573c       | 1531b       |
| 250   | 1336       | 1725c       | 1584b       | 1613b       | 1650b       | 1382b       |
| 100   | 1293       | 1436c       | 1289a       | 1320a       | 1335c       | 1338c       |
| 250   | 1477       | 1465c       | 1297a       | 1270a       | 1352c       | 1372c       |
|       |            |            |            |            |            |            |
| 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 during all experimental periods, Tables 5–7.

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:albumen ratio | Shell thickness (mm) | Egg shape index |
|------|-------------|----------|-----------|------------|-------------|--------------------|---------------------|----------------|
| **FB** (%) | | | | | | | | |
| 0 | 65.99 | 20.97<sup>a</sup> | 13.03<sup>b</sup> | 85.34 | 49.80<sup>a</sup> | 0.319<sup>c</sup> | 0.355<sup>a</sup> | 79.92<sup>a</sup> |
| 25 | 65.06 | 22.29<sup>bc</sup> | 11.44<sup>b</sup> | 96.53 | 47.80<sup>b</sup> | 0.343<sup>bc</sup> | 0.352<sup>a</sup> | 80.63<sup>a</sup> |
| 50 | 64.31 | 26.63<sup>a</sup> | 12.73<sup>a</sup> | 95.04 | 48.44<sup>ab</sup> | 0.414<sup>a</sup> | 0.358<sup>a</sup> | 80.26<sup>a</sup> |
| 75 | 66.45 | 23.33<sup>b</sup> | 12.35<sup>bc</sup> | 95.56 | 47.40<sup>b</sup> | 0.355<sup>b</sup> | 0.337<sup>b</sup> | 76.04<sup>b</sup> |
| 100 | 65.27 | 23.06<sup>b</sup> | 11.65<sup>b</sup> | 97.26 | 48.56<sup>ab</sup> | 0.353<sup>b</sup> | 0.333<sup>b</sup> | 77.24<sup>b</sup> |
| **Enzyme (mg/kg diet)** | | | | | | | | |
| 0 | 65.67 | 23.94<sup>a</sup> | 12.71<sup>a</sup> | 92.70<sup>b</sup> | 49.01<sup>a</sup> | 0.366<sup>a</sup> | 0.343 | 79.62<sup>a</sup> |
| 250 | 65.17 | 22.57<sup>b</sup> | 11.77<sup>b</sup> | 95.19<sup>a</sup> | 47.79<sup>b</sup> | 0.348<sup>b</sup> | 0.351 | 78.01<sup>b</sup> |
| **FB** (%) × enzyme | | | | | | | | |
| 0 | 65.82 | 22.27<sup>a</sup> | 13.86<sup>a</sup> | 85.34<sup>d</sup> | 49.76 | 0.350<sup>c</sup> | 0.355<sup>b</sup> | 81.82<sup>a</sup> |
| 25 | 65.16 | 19.66<sup>b</sup> | 12.17<sup>bc</sup> | 85.35<sup>b</sup> | 49.85 | 0.288<sup>c</sup> | 0.355<sup>b</sup> | 78.02<sup>b</sup> |
| 50 | 65.25 | 28.26<sup>a</sup> | 13.89<sup>a</sup> | 91.51<sup>c</sup> | 49.15 | 0.433<sup>a</sup> | 0.370<sup>a</sup> | 80.25<sup>a</sup> |
| 75 | 63.37 | 25.01<sup>b</sup> | 11.61<sup>b</sup> | 98.56<sup>b</sup> | 47.74 | 0.395<sup>b</sup> | 0.346<sup>bc</sup> | 80.27<sup>a</sup> |
| 100 | 67.43 | 23.23<sup>c</sup> | 13.63<sup>a</sup> | 97.22<sup>a</sup> | 48.87 | 0.353<sup>c</sup> | 0.330<sup>c</sup> | 76.85<sup>bc</sup> |
| 250 | 65.48 | 23.44<sup>c</sup> | 11.07<sup>b</sup> | 93.89<sup>b</sup> | 45.93 | 0.358<sup>c</sup> | 0.345<sup>bc</sup> | 75.23<sup>c</sup> |
| **SEM<sup>2</sup>** | 2.29 | 0.66 | 0.442 | 0.70 | 0.77 | 0.01 | 0.007 | 0.51 |
| **Two-way ANOVA P-value<sup>3</sup>** | | | | | | | | |
| FB<sup>1</sup> | 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<sup>1</sup> (%) × 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).

<sup>1</sup> FB: faba bean.
<sup>2</sup> SEM: standard error of the means.
<sup>3</sup> 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 albumin 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 cornsoya 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.

References

AOAC. 2006. Official Methods of Analysis Association. Off. Anal. Chem., 18th ed. Washington, DC.

Awosanya, B., Joseph, J.K., Oloosebiakan, O.D., 1998. Effect of age of birds on shell quality and component yield of egg. Nig. J. Anim. Prod. 25, 68–70.

Aydin, R., Karaman, M., Cicek, T., Yardibi, H., 2008. Black cumin (Nigella sativa L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality, and decreases egg cholesterol. Poult. Sci. 87, 2590–2595.

Benabeljelil, K., Arboou, M.I., 1994. Effects of enzyme supplementation of barley based diets on hen performance and egg quality. Anim. Feed Sci. Technol. 48, 325–334.

Brufau, J., Boros, D., Marquardt, R.R., 1998. Influence of growing season, tannin content and autoclave treatment on the nutritive value of near-isogenic lines of fava beans (Vicia faba L.) when fed to leghorn chicks. Br. Poult. Sci. 39, 97–105.

Card, L.E., Nesheim, M.C., 1972. Poultry Production, 11th ed. Lea and Fèbiger, Philadelphia, PA.

Castanon, J.I., Perez-Lanza, C.J., 1990. Substitution of fixed amounts of soybean meal for field beans (Vicia faba), sweet lupins (Lupinus albus), cull peas (Pisum sativum) and vetches (Vicia sativa) in diets for high performance laying Leghorn hens. Br. Poult. Sci. 31, 173–180.

Cazzato, E., Laudadio, V., Ceci, E., Tufarelli, V., 2014. Effect of sulphur fertilization on fatty acid composition of fava bean (Vicia faba L.), white lupin (Lupinus albus L.) and pea (Pisum sativum L.) grains. J. Food Agric. Environ. 12, 136–138.

Crepon, K., Marget, P., Peyronnet, C., Carroue, B., Arese, P., Duc, G., 2010. Nutritional value of fava bean (Vicia faba L.) seeds for feed and food. Field Crops Res. 115, 329–339.

Deniz, G., Gencoglu, H., Gezen, S.S., Turkmen, I.I., Orman, A., Kara, C., 2013. Effects of feeding corn distiller’s dried grains with solubles with and without enzyme cocktail supplementation to laying hens on performance, egg quality, selected manure parameters, and feed cost. Livest. Sci. 152, 174–181.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11, 1–42.

Fru-Nji, F., Niess, E., Pfeffer, E., 2007. Effect of graded replacement of soybean meal by fava beans (Vicia faba L.) or field peas (Pisum sativum L.) in rations for laying hens on egg production and quality. J. Poult. Sci. 44, 34–41.

Funk, E.M., Froning, G., Grottes, G., Forward, R., Kinder, J., 1958. Quality of eggs laid by caged layers. World Poult. Sci. J. 15, 207.

Ghazalah, A.A., Abd-Elsamee, M.O., Moustafa, E.S., 2011. Use of distillers dried grains with soluble (DDGS) as replacement for soybean meal in laying hen diets. Int. J. Poult. Sci. 10, 505–513.

Guillaume, J., Bellec, R., 1977. Use of field beans (Vicia faba L.) in diets for laying hens. Br. Poult. Sci. 18, 573–583.

Jezierny, D., Mosenthin, R., Bauer, E., 2010. The use of grain legumes as a protein source in pig nutrition: a review. Anim. Feed Sci. Technol. 157 (111–128), 2010.

Koivunen, E., Tuuaninen, P., Valkonen, E., Rossow, L., Valaja, J., 2014. Use of faba beans (Vicia faba L.) in diets of laying hens. Agric. Food Sci. 23, 165–172.

Kocher, A., Choct, M., Hughes, R.J., Broz, J., 2000. Effect of food enzymes on utilisation of lupin carbohydrates by broilers. Br. Poult. Sci. 41, 75–82.

Laudadio, V., Ceci, E., Tufarelli, V., 2011. Productive traits and meat fatty acid profile of broiler chickens fed diets containing micronized fava beans (Vicia faba L. var. minor) as the main protein source. J. Appl. Poult. Res. 20, 12–20.

Laudadio, V., Tufarelli, V., 2011. Dehulled-micronised lupin (Lupinus albus L. cv. Multitalia) as the main protein source for broilers: influence on growth performance, carcass traits and meat fatty acid composition. J. Sci. Food Agric. 91, 2081–2087.

Laudadio, V., Tufarelli, V., 2010. Treated fava bean (Vicia faba var. minor) as substitute for soybean meal in diet of early phase laying hens: egg-laying performance and egg quality. Poult. Sci. 89, 2299–2303.

Magoda, S.F., Gous, R.M., 2011. Evaluation of dehulled fava bean (Vicia faba cv. Fiord) as a protein source for laying hens. South Afr. J. Anim. Sci. 41, 87–93.

Marquardt, R.R., Campbell, L.D., 1973. Raw and autoclaved fava beans in chick diets. Can. J. Anim. Sci. 53, 741–746.

Mateos, G.G., Puchal, F., 1982. The nutritional value of broad beans for laying hens. Br. Poult. Sci. 23, 1–6.

Moschini, M., Masoero, F., Prandini, A., Fusconi, G., Morlacchini, M., Piva, G., 2005. Raw pea (Pisum sativum), raw fava bean (Vicia faba var. minor) and raw lupin (Lupinus albus var. multitalia) as alternative protein sources in broiler diets. Ital. J. Anim. Sci. 4, 59–69.

Nalle, C.L., Ravindran, V., Ravindran, G., 2010. Nutritional value of fava beans (Vicia faba L.) for broilers: apparent metabolizable energy, ileal amino acid digestibility and production performance. Anim. Feed Sci. Technol. 156, 104–111.

NRC. 1994. Nutrient Requirements of Poultry, ninth ed. Natl. Acad. Press, Washington, DC.

Olaboro, G., Campbell, L.D., Marquardt, R.R., 1981. Influence of fava bean fraction on egg weight among laying hens fed test diets for a short time period. Can. J. Anim. Sci. 61, 751–755.

Perella, F., Mugnai, C., Dal Bosco, A., Sirri, F., Cestola, E., Castellini, C., 2009. Faba bean (Vicia faba var. minor) as a protein source for...
organic chickens: performance and carcass characteristics. Ital. J. Anim. Sci. 8, 575–584.
Perez-Maldonado, R.A., Mannion, P.F., Farrell, D.J., 1999. Optimum inclusion of field peas, faba beans, chick peas and sweet lupins in poultry diets. I. Chemical composition and layer experiments. Br. Poult. Sci. 40, 667–673.
Ravindran, V., Blair, R., 1992. Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. World Poult. Sci. J. 48, 205–231.
Robblee, A.R., Clandinin, D.R., Hardin, R.T., Milne, G.R., Darlington, K., 1977. Studies on the use of faba beans in rations for laying hens. Can. J. Anim. Sci. 57, 421–425.
Trostle, R., 2008. Global agricultural supply and demand: factors contributing to the recent increase in food commodity prices. USDA Economic Research Service, Washington, DC. A Report from the Economic Research Service.