Alfalfa Meal Supplementation Producing Vitamin E and Minerals Enriched Table Eggs

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Abstract. Eggs are inexpensive nutrition source and consist of fat soluble vitamins. Vitamin E, selenium and zinc are found in a small amount in eggs. Alfalfa is an inexpensive natural source of vitamin E, selenium and zinc. Therefore, a study was conducted to evaluate the effects of dried alfalfa in a ratio of 0%, 2%, 4%, 6%, 8% and 10% in diet additive on the increase ratio of vitamin E, selenium and zinc in eggs. In this experiment, a total of 180 birds (aged 27 weeks) were ranged in 6 treatments over three replicates including 10 birds of ISA-BROWN layer in each treatment. During the 17 weeks’ experiment period (two weeks of preliminary period), the experimental results showed a significant increase in vitamin E, selenium and zinc contents. A proportional increase in the ration of vitamin E, selenium and zinc were recorded with the increase of the ration of the dried alfalfa additive in the diet. The highest ratio of the dried alfalfa (10%) showed the highest increase in the vitamin E, selenium and zinc. Moreover, no significant differences were recorded between the treatments in term of egg production performance.

Key words: Alfalfa Meal, ISA-Brown, eggs performance, Vitamin E, Selenium and Zinc

1. INTRODUCTION

Poultry eggs are a good source of important nutrients. The egg represents a complete food with high-quality proteins, with a 2:1 ratio of unsaturated fats to saturated fat. It also represents a good source of iron, phosphorus, and other minerals as well as vitamins [1-6]. Eggs are also rich in phospholipids that can impact the absorption of intestinal cholesterol [1]. However, it is the best source of all the vital nutrients, its high cholesterol content [7] and saturated fatty acids (SFA) the main restrictions for egg customers [8, 9]. Egg quality is one of the main criteria of poultry husbandry and is affected by many factors, including nutrition. Alfalfa (Medicago sativa L.) a valuable source of n-3 fatty acids, vitamins, carotenoids, and minerals, which is reflected in the meat and eggs of poultry [10]. Alfalfa meal is rich in beta-carotene, xanthophyll and flavonoids, antioxidants, and other unknown growth and reproductive factors, and plays a role in the colour of the egg yolk [4], and is mostly added at low levels to the diets of laying hen [5]. In addition to its high xanthophyll content, alfalfa meal contains a high level of crude protein, depending on the variety and harvesting time [11-13]. Additionally, it is high in essential amino acids, poly- and mono-unsaturated fatty acids, vitamins (especially vitamin E), minerals, and organic acids [14, 15]. However, alfalfa meal includes anti-nutritional factors, such as cellulose, saponins, β-glucans, and xylans, which limit its usage due to digestion process interference [12, 16, 17]. Although anti-nutritional factors limited the use of alfalfa meal in layer diets, there are some advantages. The high saponin content (2–3%) and crude cellulose content (20–25%) reduce the cholesterol content in eggs [9, 12, 18-20], which can be healthier to human consumers [21]. It has been well known that the nutritive composition of eggs is
changed by the nutritional composition of the diet. In general, the transfer efficiency of dietary fat-soluble vitamins to the egg is lower than that of water-soluble vitamins [22, 23]. Limited studies investigated the effects of dried alfalfa meal (DAM) in diets for laying hens but the results have been inconsistent. The influence of dried alfalfa meal in laying hens is also not clear. Therefore, in the present experiment effects of 0, 2, 4, 6, 8, 10% dried alfalfa meal was added to the diet of laying hens on performance, egg production, and egg quality in term to enhance of vitamins E and some mineral in eggs were investigated [6, 24].

2. MATERIALS AND METHODS

2.1 Experimental design

The experiment was conducted at (Al-Shemal holding) integrated poultry project in (Qushtapa area, Erbil city). A part of the commercial layer farm was designated for the study. One hundred Eighty ISA-Brown penned in floor-litter system hens in lay, 27 weeks of age, were distributed into 6 treatments of 30 hens per treatment based on additives levels of dried alfalfa meal (DAM), as follows: Control diet (T1) - regular diet with no DAM, (T 2) - regular diet with 2% DAM, (T3) - regular diet with 4% DAM, (T4) - regular diet with 6% DAM, (T5) - regular diet with 8% DAM, (T6) - regular diet with 10% DAM. Each treatment was comprised of 3 indoor pens of 10 hens each that served as replicates. The observations per protocol were made over 17 weeks following a 2-week acclimation.

2.2 Adaptation of test laying hens

To eliminate the impact of the new ingredient and its differential additives levels, the hens under study were subjected to a period of adaptation for 2 weeks when the respective treatments were fed with the study diets allowing for adaptation of feed consumption and gut environment. Observations and data from the period of adaptation were not considered for this study.

2.3 Environment and the management

The standardized climate and administration is subjected to all the hens under analysis as follows. The hens are manually fed once a day 120 gm/ hens per day consumption as per breed standard were designed across all treatments. Continuous water, provided through a magnetic funnel, the dipolar magnetic field with powers 2000G made locally in the Ministry of Science and Technology-Iraq. Throughout treatments, similar climate and management is universally offered. Environmental conditions were maintained at 22-23 Celsius e temperature, 40-60% humidity, 30 Lux lighting for 16 h of lighting per day.

2.4 Diet formulations

The nutrients requirement was offered as recommended in the commercial ISA Brown layer’s guide, 2014*. Table (1) show the ratio composition and Table (2) shows calculated chemical analysis of feeding in a production period (2) according to the ISA Brown feed program guide in the year 2014.

Birtox: a mixture of active clinoptilolite, yeast and sepiolite and it’s a broad spectrum mycotoxin binder for feeds and feed ingredients. It is a synergistic blend of components that have many beneficial effects within the animal. It deactivates toxins both mechanically and physiologically. Therefore, the load on the liver decreases. Animals possess a naturally efficient detoxification system, mainly in liver.
Table (1): Ingredient composition calculated analysis of the basal diet (Kg/ Ton).

| Raw Material      | Quantity |
|-------------------|----------|
| 1 Corn            | 535.5    |
| 2 Soya bean       | 285.0    |
| 3 Oil             | 18.0     |
| 4 Wheat Bran      | 35.0     |
| 5 limestone       | 93.0     |
| 6 Salt            | 0.5      |
| 7 BIRMIX Y201     | 25.0     |
| 8 Birtox          | 1.0      |
| 9 METHIONINE      | 1.0      |
| 10 Di- Calcium- Phosphate | 6.0 |

BRIMIX 201: *vit. A=4,500,000 IU, vit. D=1,660,000 IU, vit. E=20,000 mg.kg-1, K3=1, mg.kg-1, vit.B1=1,800 mg.kg-1, vit. B2=2,500 mg. vit. B6=1,600 mg.kg-1, vit. B12=8.75 mg.kg-1, folic acid=600 mg.kg-calcium pentonite=5,500 mg.kg-1, niacinamid=18,000 mg.kg-1, biotin=60 mg cholin clorid=30,000 mg.kg-1, betain=65,000 mg.kg-1, cobalt=150 mg.kg-1, Iodine=380 mg.kg-1, Mn=45,800 mg.kg-1, cupper=6,500 mg.kg-1, Si=110 mg.kg-1, Zn=28,300 mg.kg-1, Fe=27,200mg.kg-1, Mo=350 mg.kg-1

2.5 Determination of external and internal egg quality:

2.5.1 Egg production (H.D %): Egg production was recorded daily per replicate of hens in all treatment groups and was calculated as follow:

\[ \text{Egg Production Hen Day (H.D) \%} = \frac{X}{Z} \times 100 \]

\( X = \) the number of eggs produced during a certain period.
\( Z = \) number of chickens at the same period.

2.5.2 Egg weight (g per egg): Egg was weighted individually at the end of each week; using a sensitive balance type Navigator TM (OHAUS R) and the average egg weight were taken weekly.

2.5.3 Feed consumption: (One hundred twenty-gram feed/ per hen) were offered daily according to the feed requirement in commercial ISA -Brown guide, 2014, and all were consumed by hens.
2.5.4 Feed conversion ratio (FCR gr feed/ egg): Feed conversion ratio of laying hens was calculated weekly and recorded as a gram of feed consumed eggs produced for each week calculated as below equation:

\[
\text{FCR} (\text{gr. feed/ egg}) = \frac{\text{Feed intake during the period (gr)}}{\text{Egg mass produced during the same period (gr)}}
\]

2.5.5 Egg shape index (egg index)%: Recorded the measurements of length and width of the egg by Gans Gehartet vernier and egg shape index was calculated as the following equation: Egg shape index (%) = Egg width (cm) / Egg length (cm) *100

2.5.6 Egg Shell strength: Eggshell strength was measured using eggshell testing equipment (Egg Force Reader, OC-SPA, France Technical Services, and Supplies) and expressed as a unit of compression force that was exposed to the unit eggshell surface area (gr. /cm²).

2.5.7 Haugh Unit: Haugh unit was calculated from the values obtained from albumen height and egg weight employing the following equation:

\[
\text{Haugh Unit} = 100 \log (H + 7.57 - 1.7 W^{0.37}) \text{ As:}
\]

\[
H = \text{albumin height (mm)} \quad W = \text{egg weight (gr)}
\]

2.5.8 Yolk selenium & zinc content: Sample preparation: To prepare the sample for the analysis of the determination, a 0.5 g egg yolk was weighted sample of mineralized using 10 mL of ultra-pure nitric acid (HNO₃) (Merck, Germany) using a (Buchi) infrared block heating device (Buckinghamshire Germany). This process was carried out in three stages, at a temperature of 110C. Based on the content of selenium, and zinc determined in the egg yolk.

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**Table (2)**: Calculated chemical analysis of the basal diet.

| ANALYSIS | 1 | CRUDPTEIN % | 18.58 |
|----------|---|-------------|-------|
| 2        | ASH %        | 12.34       |
| 3        | CRUDFIBER %   | 3.35        |
| 4        | OIL          | 4.55        |
| 5        | METHIONINE %  | 0.48        |
| 6        | MET+CYCTINE%  | 0.73        |
| 7        | LYSINE %      | 1.10        |
| 8        | THERIONINE    | 0.68        |
| 9        | CALICUM       | 4.13        |
| 10       | AV.PHSPHOR    | 0.41        |
| 11       | T.PHOSPHOR.   | 0.47        |
| 12       | Na           | 0.16        |
| 13       | Cl           | 0.23        |
| 14       | K             | 0.76        |
| 15       | LINOLIC acid  | 2.16        |
| 16       | Ca/P          | 9.98        |
| 17       | ME.(Kcal/Kg)  | 2751.12     |
| 18       | ME/CP         | 148.09      |

*Shemal Company /Erbil/Iraq.
The content of elements was determined by atomic absorption spectrometry (AAS) using an (AAS9000) spectrometer (Skyray instrument). The sample in the form of a solution was introduced into the air-acetylene flame. The content of the element in the sample was determined based standard curves of elements.

2.5.9 Yolk Vitamin E-Content:

2.5.9.1 Sample extraction: weighted (0.3 g) of homogenized egg yolk sample were in an Eppendorf tube (Deltalab, Rubí, Spain), mixed with 1 mL of an acetonitrile: water 80:20 (v/v) solution by stirring in a Vortex (Stuart, Stone, United Kingdom) for 30 s, and then, centrifuged for 10 min at 14,000 rpm and 4 °C. The supernatant extract was then filtered through 0.22 µm filter) and injection into HPLC system analysis [25].

2.5.9.2 Instrumentation: The HPLC system were consisted of HPLC High Performance Liquid Chromatography (Hitachti Merck 7200 UK) and UV/Vis Detector at a flow rate of 1 mL min⁻¹ and column temperature of 25°C; The injection volume for the samples was set at 20 µL, and wave length 280 nm. Luna Omega C18 HPLC column (250 mm x 4.6 mm, 5 µm) from Phenomenex (California, USA) chromatographic column was used to separate the analytes. Chromatographic separation was carried out using the mobile phase which was consisted of 70 % acetonitrile and 30 % water.

STATISTICAL ANALYSIS:
The experiments were executed as a complete randomized design (CRD), performance data , factorial for vitamins and mineral data analyzed using the SAS (2005). Duncan’s multiple range tests were used to compare differences among treatment means [26]

3. Result and Discussion

3.1 Egg production performance

Table (3) showed a non-significant difference (P≤0.05) in egg production (H.D %) level, F.C.R, egg weight, eggshell strength measuring of Hough unit and egg shape index among all treatments in the diets of different concentrations of Alfalfa in comparison with the group of the regular diet. There was a dose-dependent decrease in egg performance parameters in groups of DAM diet. Those results may be related to the high fiber content and a relatively low energy amount in the alfalfa diet [15]. Another explanation for the negative effect of DAM on the production performance in the hen is the richness of alfalfa with tannins. Tannins are considered as regulators and stabilizers of microflora populations in the intestine, [27], tannins can negatively affect the protein digestibility in monogastric animals [10], and may cause negative changes in the small intestine histology, affect the microbial status of jejunum content, and a finally may affect performance parameters in chickens [10]. Negative effects of alfalfa on egg performance parameters may be explained by saponins' high content of alfalfa, which may reduce the palatability of the diet [28]. Our results agreed with the previous studies, [17] concluded that the inclusion of alfalfa in diets of laying hens reduced bird performance, body weight, and egg mass. [10], showed that egg production and feed conversion ratio were negatively affected by the addition of 4% alfalfa meal to the diets of laying hens at 39–51 weeks of age. In contrast, [29] reported that the addition of 5%, 8%, or 10% alfalfa meal to diets did not impact egg weight or eggshell quality from laying hens at 20 weeks of age. Similar results were observed by [19] when a 10% alfalfa meal was added to the diet of laying hens. [18] demonstrated that the eggshell quality was not affected by the addition of alfalfa meal at 3% inclusion; however, it was positively affected by the addition of 6% and 9% alfalfa meal to the diet of quail at 10 weeks of age. Another study, which used quail breeders [20], reported that dietary supplementation with 1–8% alfalfa meal did not affect eggshell strength. Similarly, [13] reported that the addition of 5% alfalfa meal to wheat-based diets did not affect the eggshell thickness or weight, specific
gravity, or the egg shape index from laying hens aged 40 weeks. Moreover, [12] stated that the addition of a 15% alfalfa meal containing a high level of cellulose (26.7% dry matter basis) did not adversely affect the eggshell quality of hens aged 18 weeks. In contrast, [10] reported that the supplementation of 4% alfalfa meal to diet decreased the eggshell thickness and the eggshell strength of laying hens at 39 weeks of age. Most studies showed that the addition of alfalfa meal to the diets either did not affect or tended to improve the eggshell quality. The addition of alfalfa meal to the diet reduced the populations of pathogenic microorganisms in the intestine and increased beneficial microorganisms, such as Lactobacillus spp. [29], and there was a linear relationship between the beneficial microorganisms in the intestine and the eggshell quality [30].

Table (3) The effect of alfalfa levels on production parameters

| Attribute            | T1 (Control) | T2 %2 | T3 %4 | T4 %6 | T5 %8 | T6 %10 |
|----------------------|--------------|-------|-------|-------|-------|--------|
| Hen Day Production % | 80.82 ± 1.09 a | 82.7 ± 5.86 a | 84.61 ± 2.14 a | 84.13 ± 2.68 a | 79.71 ± 1.25a | 82.73 ± 3.63a |
| FCR / 1 Egg Production | 148.53 ± 1.99 a | 146.67 ± 11.1 a | 142.01 ± 3.51 a | 142.93 ± 4.48 a | 150.62 ± 2.38 a | 145.59 ± 6.21 a |
| Egg Weight (g)      | 61.71 ± 0.57 a | 61.15 ± 0.56 a | 61.32 ± 0.63 a | 60.5 ± 0.29 a | 60.69 ± 0.19 a | 60.32 ± 0.4 a |
| Shell Strength      | 1390.6 ± 15.85 a | 1346.6 ± 140.3 a | 1414.47 ± 36.46 a | 1300.42 ± 7.95 a | 1365.9 ± 123.19 a | 1380.32 ± 95.19 a |
| Hough Unit          | 94.1 ± 1.04 a | 92.38 ± 0.45 a | 93.26 ± 0.75 a | 92.57 ± 0.23 a | 93.66 ± 0.58 a | 94.6 ± 0.93 a |
| Egg shape Index %   | 81.27 ± 0.4 a | 80.49 ± 0.21 a | 81.32 ± 0.6 a | 81.36 ± 0.45 a | 80.82 ± 0.38 a | 80.84 ± 0.46 a |

*Mean values within the same row were insignificantly different (P≥0.05)
3.2 Effect of alfalfa levels on Vitamin E (IU / g) Contents in egg yolk

Figure (1) observed that the role of additive alfalfa with different levels as shown in among treatment was significant (P≤0.05) the best value was observed in T6 on months 3, 1, and 2 respectively. For an overview as average for three months was (3.64) as shown in figure 1. The increased amounts of β-carotene may explain this result in alfalfa which is considered a valuable source of it.

Those results came inconsistent with other researches which concluded that Alfalfa had a positive effect on the physical parameters of yolk and albumen quality, [10, 24] also found that alfalfa meal in the diet significantly increased yolk colour which is related to the amount of β-carotene.

The positive effect of DAM on the yolk characteristics seen in this analysis, relative to the dosage, can be correlated with a high content of β-carotene and this pigment is associated with a high amount of β-carotene is responsible for the dark color of egg yolk [12] demonstrated that laying hens receiving low-fiber alfalfa meals in the diet was characterized by a higher concentration of β-carotene in both blood serum and egg yolk concluded that alfalfa contains a high level of lutein as well as β-carotene [31]. Those results may also be explained by increased antioxidant component; retinol which the most abundant in egg yolk. the higher amount of β-carotene in the two groups fed sprouts of the metabolic product of β-carotene is retinol. In alfalfa and flax eggs, even tocopherols and tocotrienols were higher, particularly Alpha-T, alpha-T3, and γ-T3. Lutein and zeaxanthin were also more concentrated in the eggs of sprout groups; in particular, lutein was greater in the T6. This result confirmed by [32], serum AA and retinol concentrations increased with increasing amounts of AA in the diet. AA protected retinol against oxidation and increased serum retinol levels in parallel with increased serum AA level it can be attributed as explained by [33] in their reports of eggs enriched with lutein, selenium, vitamin E, and DHA [32].

Figure 1. The effect of alfalfa levels on Vitamin E (IU / g) Contents in egg yolk
3.3 Effect of alfalfa levels on Selenium & zin (mg / g) Contents in egg yolk.

Figure 2. significant (P≤0.05) changes among all treatments with different levels of alfalfa and differences of three months for both selenium and zinc. Selenium and zinc concentrations were increased with increasing DAM concentration in the diet. A higher concentration of selenium and zinc respectively was noticed in the group of T6. This may be related to the chemical content of alfalfa. Selenium (Se) is a component of several important seleno-proteins and enzymes required for functions including antioxidant defense, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility, and reproduction [33]. Selenium has a positive effect on egg production, some egg quality parameters, and selenium deposition in egg yolk [34]. High contents of crude proteins, minerals, and oil were measured in alfalfa leaves [35]. Selenium, as a component of several glutathione peroxidase enzymes, actively participates in lipid peroxide removal from cells; compassionate the use of vitamin E for this purpose [33]. This could be beneficial in producing a healthy product for human consumption and prevent oxidation of products during storage. It has been demonstrated in chicks that Se preserves the integrity of the pancreas, which allows normal fat digestion and vitamin E absorption [35].

![Graph showing selenium and zinc levels](image)

Figure 2. The effect of alfalfa levels on Selenium & zinc (mg /g) Contents in egg yolk.

**Conclusion & Recommendation**

This study demonstrated the relevant transfer of bioactive compounds from alfalfa meal to egg yolk. An increase proportion was found between increasing the level of the dried alfalfa additive and the level of the studied parameters. Thus, an increase in the dried alfalfa additive from 0% to 10% had increased vitamin E from 2.06 IU to 4.5 IU, selenium from 0.64 mg/g to 0.73 mg/g and zinc from 1.42 mg/g to 1.59 mg/g respectively. Moreover, it will be worth conducting further studies to investigate the effects of higher levels dried alfalfa (compared to the levels used in this study) on the above studied parameters and egg production performance.

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