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Effect of Enzyme Addition to Diets Containing Different Levels of Alfalfa Meal on Performance and Egg Quality Parameters of Laying Hens

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ABSTRACT

This study was conducted to investigate the effect of enzyme addition to diets containing different levels of alfalfa meal on performance, egg quality and egg yolk color in laying hens. Twenty-four weeks-old, 144 Lohmann-LSL laying hens were allocated to 8 experimental groups. The experiment, 4 different levels of alfalfa meal (0, 4, 8 and 12 %) and 2 different levels (0 and 1000 mg/kg) enzyme containing 8 different experimental diets were carried out with 6 replications according to 4x2 factorial design.

The results of study indicated that there were no differences in egg production, feed intake, feed conversion ratio, egg weight, egg mass and eggshell breaking strength among the treatment groups (P> 0.05). The eggshell thickness had no significantly affected by the dietary alfalfa meal levels and interaction groups, but eggshell thickness was significantly higher in group fed with containing enzyme than the group of without enzyme (P< 0.01). In the egg yolk color parameters, the L* value was significantly affected by dietary alfalfa meal levels (P<0.01), and the groups fed with alfalfa meal containing diets at 8 and 12% levels were significantly lower than the others (0 and 4 %). The a* and roche values were significantly and similarly affected by the interactions (P<0.05), and the groups fed with alfalfa meal (with or without enzyme) diets at 8 and 12% levels were significantly lower than the other groups.

In conclusion, the study results were observed that the addition of alfalfa and enzyme to laying hens diets did not cause a significant change in performance and egg quality parameters. However, it can be said that the addition of alfalfa meal at least 8% without adding enzyme to the diet causes an increase in egg yolk color.

1. Introduction

Alfalfa meal is rich in protein but has high cellulose concentrations. Alfalfa is well balanced in amino acids and is a rich source of vitamins as well as minerals. Dehydrated alfalfa meal is often used at very low levels in poultry diets, due to its high crude cellulose and low metabolic energy content, but it is a rich source of vitamins and carotenoids. Enzymes are added to improve the ability of birds to digest fibers, increase energy use, and overcome the negative effects of fibers on intestinal lumen activity and fecal consistency (Leeson and Summers, 2008). In particular, the use of enzymes to eliminate the negative effects of cellulose, which restricts the use of high amounts of alfalfa meal, is believed to contribute positively. In addition, beta-glucanase and arabinoxylanase can be added to alfalfa-containing diets to increase the performance and energy use of poultry (Mourao et al. 2006). When exogenous enzymes are supplemented, it may be possible to use alfalfa meal at moderate levels in poultry diets. Anhydrous alfalfa meal is high in xanthophylls and is generally used at very low levels in poultry feeding to increase the degree of pigmentation of egg yolk (Fetcher and Papa, 1985).

Recent studies (Güçlü et al., 2004) reported that the addition of alfalfa meal up to 9% had no negative effect on body weight, egg production, feed intake and feed efficiency in quail. Mourao et al. (2006) reported that alfalfa meal supplementation in diet reduces the intake, egg weight, egg production and egg mass in laying hens. Laudadio et al. (2014) reported that the partial substitution of soybean meal with low-fiber alfalfa meal had no adverse effect on the growth performance of laying hens. In addition, none of the egg production and egg quality characteristics examined were affected by dietary treatment except egg yolk color. Egg yolk color was higher in chickens fed with low-fiber alfalfa meal diet. Heywang (1950) reported that the addition of alfalfa meal to egg diets at 5, 10, 15 and 20% levels did not affect feed intake, but that more than 5% of the drugs reduced egg production.

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The aim of the study was to evaluate the effect of the addition of alfalfa meal and enzyme on performance and egg quality characteristics of laying hens.

2. Materials and Methods

Twenty-four weeks-old, 144 Lohmann-LSL laying hens were randomly allocated to 8 experimental groups with 18 hens each, 6 replicates per group. Hens were fed on a basal diet, containing 16.5 % crude protein and 2750 ME Kcal/kg (Table 1). Basal diet was formulated to meet or exceed nutrient requirements of laying hens as recommended by the NRC (1994). The diets were consisted of 4 different levels of alfalfa meal (0, 4, 8 and 12 %) and 2 different levels (with/without) enzyme (Farmazyme 3000 PROENX). A total of 8 experimental diets consisting of 4 different dietary alfalfa and 2 different enzyme levels were tested in 2x4 factorial design for 12 weeks. The hens were housed in an environmentally controlled room equipped with 48 metal battery cages. Hens were kept in cages (50 cm length, 50 cm width, 45 cm height) with 3 hens per cage. Feed and water were offered ad-libitum throughout the experiment. The lighting program was provided 16h lighting: 8h darkness in a day throughout the experimental period.

The body weight of hens was determined by weighing the hens individually at the beginning and end of the experiment. Egg production (EP) was recorded daily. Feed intake (FI) was calculated as the mean for the subgroup for the 12-week trial period (FI= given total feed - remaining feed in manger). Egg mass (EM) was calculated from the EP and egg weight (EW) data using the formula: EM = (EP % × EW)/Period (days). The feed conversion ratio (FCR) was calculated using the formula; FCR = FI/EM.

Table 1
Composition of experimental diets

Ingredients, %	Dietary alfalfa meal levels, %			
	0	4	8	12
Corn	52.0	50.0	46.5	45.6
Barley	10.0	6.0	4.35	0.0
Soybean meal (43.8 % crude protein)	26.05	26.40	26.10	26.30
Alfalfa meal[2]	0	4	8	12
Vegetable oil	0.40	2.15	3.80	5.40
Limestone	9.10	9.00	8.80	8.70
Di-calcium phosphate	1.70	1.70	1.70	1.65
Salt	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20
TOTAL	1000	1000	1000	1000
Calculated nutrient (% dry matter, DM)				
Metabolizable energy, Kcal/kg	2750	2753	2753	2750
Crude protein	16.48	16.55	16.51	16.53
Calcium	3.91	3.93	3.92	3.93
Available phosphorus	0.41	0.42	0.42	0.42
L-Lysine	0.88	0.88	0.88	0.89
DL-Methionine	0.44	0.44	0.44	0.44
Methionine+cystine	0.77	0.78	0.79	0.80

[1] Premix provided the following per kg of diet: retinly acetate, 4.0 mg; cholecalciferol, 0.055 mg; DL- α-tocopheryl acetate, 11 mg; nicotinic acid, 44 mg; calcium-D-pantothenate, 8.8 mg; riboflavin sodium phosphate 5.8 mg; thiamine hydrochloride 2.8 mg; cyanocobalamin, 0.66 mg; folic acid, 1mg; biotin, 0.11 mg; choline, 220 mg; Zn, 60 mg; Mn, 60 mg; Fe, 30 mg; Cu, 5 mg; I, 1.1 mg; Se, 0.1 mg.
[2] Alfalfa meal contains 13.2 % crude protein, 28.1 % crude cellulose.

The eggs were examined to determine the EW and eggshell quality characteristics (shell breaking strength, shell weight, and shell thickness) for collected eggs produced end of each period (28 days) for consecutive 2 days and sampled and analyses were done. Eggshell breaking strength was measured using a cantilever system by applying increasing pressure to - the broad pole of the shell using an Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). The eggs were then broken, and eggshell, albumen, and yolk were separated and weighed. The egg yolk colour was determined using the Egg Analyzer (ORKA Food Technology Ltd, Ramat Hasharon, Israel) based on Roche Yolk Colour Fan and Minolta CR-400 colorimeter (Konica Minolta, Japan).

The L*, a*, b* parameters correspond to the lightness (0 = black, 100 = white), redness (–100 = green, 100 = red), and yellowness (–100 = blue, 100 = yellow), respectively. Eggshells were weighed using a 0.01 g precision scale. Eggshell weight was calculated using the formula: eggshell weight (%) = [(eggshell weight (g)/EW (g))/100]. Eggshell thickness (including the membrane) was determined at three points on the eggs (one point on the air cell and two randomised points on the equator) using a micrometer (Mitutoyo Inc., Kawasaki, Japan).

Data were subjected to ANOVA using General Linear Model (GLM) procedure in Minitab (2000). Tukey's multiple range tests were applied to separate means. Statements of statistical significance were based on probability of $P<0.01$ and $P<0.05$.

3. Results and Discussion

The performance parameters are presented in Table 2. Dietary alfalfa and enzyme levels as a main factor, and their interactions had no significant effects on egg production, feed intake, feed conversion ratio, egg weight and egg mass among the treatment groups (P>0.05).

The findings of present study in terms of performance parameters were consistent with the results of Khajali et al. (2007) who reported that the inclusion of alfalfa meal had no significant effect on egg production, egg weight, egg mass, feed conversion ratio. Laudadio et al. (2004) reported that the group containing 15 % alfalfa meal to diet had no significant effect on feed intake and feed efficiency compared with the control diet. Yuxin et al. (2004) showed that supplementation of alfalfa meal to laying hens diet had no significant effect on feed intake, egg weight and feed conversion ratio compared to untreated meals. As a result, they reported that diets containing 5% alfalfa meal are most suitable according to production performance in laying hens. Al-Shami et al. (2011), the addition of rations alfalfa and enzyme in laying hens, feed consumption, feed conversion rate, egg weight and egg production did not cause a significant difference. Olgun and Yıldız (2015) reported that the different dietary levels of alfalfa meal had no significant effect on body weight change, egg production, egg weight, egg mass, feed conversion ratio in quails. However, Maurao et al. (2006) demonstrated that egg production, egg mass and feed intake were significantly reduced by inclusion of alfalfa to laying hen diets at level of 15% and addition of beta-glucanase and xylanase could not overcome the situation. Halaj et al. (1998) showed that the addition of alfalfa meal to diet of laying hens rations had a positive effect on egg weight and egg mass. Feed consumption was higher in experimental groups, but had no effect on egg production.

Table 2
Effect of enzyme addition to diets containing different levels of alfalfa meal on performance of laying hens

Treatments	Egg production,%	Feed intake, g/d/hen	Feed conversion ratio, g feed/g egg	Egg weight, g	Egg mass, g/d/hen
Alfalfa meal (%)					
ALM-0	97.49	106.4	1.84	59.50	58.02
ALM-4	96.64	105.0	1.82	59.85	57.85
ALM-8	96.92	105.3	1.83	59.51	57.68
ALM-12	96.99	105.9	1.82	60.17	58.38
Pooled SEM	*0.536*	*0.666*	*0.025*	*0.674*	*0.775*
Enzyme (g/kg)					
0	96.70	105.6	1.85	59.36	57.41
1000	97.32	105.7	1.79	61.04	58.55
Pooled SEM	*0.379*	*0.471*	*0.018*	*0.476*	*0.548*
*Alfalfa*Enzyme*					
ALM-0*0	97.49	107.1	1.84	59.87	58.38
ALM-0*1000	97.49	105.6	1.84	59.13	57.66
ALM-4*0	95.32	104.4	1.87	58.95	56.19
ALM-4*1000	97.95	105.7	1.78	60.74	59.51
ALM-8*0	96.89	104.6	1.82	59.31	57.48
ALM-8*1000	96.96	106.0	1.84	59.72	57.88
ALM-12*0	97.09	106.2	1.85	59.31	57.60
ALM-12*1000	96.89	105.6	1.79	61.04	59.16
Pooled SEM	*0.758*	*0.942*	*0.035*	*0.952*	*1.100*

The eggshell quality parameters are presented in Table 3. Dietary alfalfa levels and enzyme addition, and their interactions had no significant effects on eggshell weight and eggshell breaking strength (P> 0.05). Eggshell thickness was significantly affected by the dietary enzyme addition (P<0.05), but it was not affected by dietary alfalfa and interactions of groups.

Similar results have been reported in previous studies. Khajali et al. (2007) found that the inclusion of alfalfa in the laying hen diet had no significant effects on eggs shell thickness and shell breaking strength. In another study, the use of alfalfa meal in laying hens diets have determined that there is a significant level effect on the relative eggshell weight. However, eggshell thickness was found to be significantly higher in alfalfa meal groups than in the control group (Yuxin et al., 2004). Laudadio et al. (2004), reported that the use of alfalfa meal to laying hens diets does not adversely affect any feature related to egg shell quality. It was stated that they have similar average values between the groups using alfalfa meal and control group for eggshell thickness and eggshell breaking strength parameters. These findings agree with those reported by Al-Shami et al. (2011), who observed that eggshell thickness increased due the addition of enzyme to the diets containing 5 or 7% alfalfa meal compared to control and 2% alfalfa meal diet. Olgun and Yıldız (2015) reported that the different dietary levels of alfal-

fa meal had no significant effect on egg shell breaking strength in quails. Some research results, which are partly inconsistent with the current results, that Mourao et al. (2006) and Khajali et al. (2007) who reported that addition of alfalfa meal and enzyme to laying hens diets had no effect on eggshell thickness.

Table 3
Effect of enzyme addition to diets containing different levels of alfalfa meal on egg quality parameters of laying hens

Treatments	Eggshell weight, g	Eggshell thickness, mm	Eggshell breaking strength, kg
Alfalfa meal (%)			
ALM-0	5.87	0.395	4.68
ALM-4	5.92	0.393	4.54
ALM-8	5.98	0.399	4.85
ALM-12	5.97	0.398	4.77
Pooled SEM	*0.077*	*0.0023*	*0.087*
Enzyme (g/kg)			
0	5.89	0.394[b]	4.69
1000	5.98	0.399[a]	4.79
Pooled SEM	*0.541*	*0.0017*	*0.062*
*Alfalfa*Enzyme*			
ALM-0*0	5.86	0.392	4.60
ALM-0*1000	5.88	0.398	4.76
ALM-4*0	5.75	0.386	4.44
ALM-4*1000	6.09	0.400	4.64
ALM-8*0	5.99	0.398	4.97
ALM-8*1000	5.97	0.401	4.72
ALM-12*0	5.94	0.399	4.75
ALM-12*1000	5.99	0.397	4.79
Pooled SEM	*0.108*	*0.0033*	*0.123*

[a, b, c:] Within a column, values not sharing a common superscript are statistically different; P<0.05

The parameters of egg yolk color are presented in Table 4. In the egg yolk color parameters, the L* value was significantly affected by dietary alfalfa meal levels (P<0.01), and the groups fed with alfalfa meal containing diets at 8 and 12% levels were significantly lower than the others (0 and 4 %). The a* and roche values were significantly and similarly affected by the interactions (P<0.05), and the groups fed with alfalfa meal (with or without enzyme) diets at 8 and 12% levels were significantly lower than the others. The b* value was not affected by any of the treatments. The effect of enzyme addition to diet on all egg yolk color parameters were insignificant.

According to the results of the present study, in general, an increase in egg yolk color density was observed with the use of alfalfa meal to the diet. Khajali et al. (2007) found that laying hens fed with diets containing alfalfa meal tended to produce eggs with higher score of yolk pigmentation assessed by egg yolk color fan. In this study, enzyme supplementation had no impact on egg yolk color. It is known that alfalfa meal causes an increase in egg yolk pigmentation due to its high xanthophyll content (Laudadio et al., 2004). Yuxin et al. (2004) reported that the alfalfa meal increased, egg yolk color increased and was significantly higher than those who did not add alfalfa flour. It recommends the addition of 5% alfalfa flour to be optimal. Halaj et al. (1998) found that egg yolk colour scores revealed significant differences among all treatments with the increase of yolk colour as the level of alfalfa increases. Al-Shami et al. (2011) reported similar results. In this study, it was observed that the egg yolk color improved by increasing the alfalfa meal level in the diets that the containing the enzyme of laying hens.

Table 4
Effect of enzyme addition to diets containing different levels of alfalfa meal on egg yolk color parameters of laying hens

Treatments	L*, Lightness	a*, Redness	b*, Yellowness	Roche Color Score
Alfalfa meal (%)				
ALM-0	62.69[A]	4.35	49.65	7.24
ALM-4	61.74[A]	4.90	48.84	7.93
ALM-8	59.99[B]	7.22	48.62	9.07
ALM-12	59.99[B]	7.32	48.44	9.14
Pooled SEM	*0.296*	*0.162*	*0.524*	*0.117*

Table 4(Continuation)
Effect of enzyme addition to diets containing different levels of alfalfa meal on egg yolk color parameters of laying hens

Enzyme (g/kg)				
0	61.37	5.69	49.32	8.27
1000	60.84	6.21	48.46	8.42
Pooled SEM	*0.210*	*0.114*	*0.371*	*0.083*
*Alfalfa*Enzyme*				
ALM-0*0	62.72	4.35^{c}	49.50	7.36^{c}
ALM-0*1000	62.66	4.36^{c}	49.81	7.11^{c}
ALM-4*0	62.02	4.23^{c}	49.08	7.56^{c}
ALM-4*1000	61.43	5.56^{b}	48.60	8.31^{b}
ALM-8*0	60.21	7.22^{a}	49.42	9.14^{a}
ALM-8*1000	59.78	7.23^{a}	47.81	9.00^{a}
ALM-12*0	60.49	6.97^{a}	49.26	9.03^{a}
ALM-12*1000	59.50	7.67^{a}	47.62	9.25^{a}
Pooled SEM	*0.419*	*0.228*	*0.741*	*0.166*

$^{A, B:}$ Within a column, values not sharing a common superscript are statistically different; P<0.01
$^{a, b, c:}$ Within a column, values not sharing a common superscript are statistically different; P<0.05

In conclusion, according to the results of this study, it was observed that the addition of alfalfa meal and enzyme to the laying hens diets did not cause a significant change in performance and egg quality parameters. Addition of alfalfa meal to the diet at 8% level caused an increase in egg yolk color without adding enzyme to the diet. However, the addition of enzyme to diets containing 4% alfalfa meal had an effect on egg yolk color increase.

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