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Egg yolk cholesterol, egg quality, and performance in response to copper–methionine chelates and alfalfa powder supplementation in Nick chick laying hen diets

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ABSTRACT

This experiment was designed to investigate the effects of different levels of copper–methionine chelates and alfalfa powder on egg yolk cholesterol content, egg quality, and performance in laying hens. In this experiment, a total of 100 laying hens (Nick Chick strain, from 109 to 117 weeks of age) were arranged into five experimental treatments, consisting of 2 different dietary alfalfa powder and 2 different copper chelate levels in a (2x2) factorial completely randomized design (CRD). The treatments groups included: T1 Control (Corn and soybean meal), treatments T2 (3% alfalfa powder+50 mg/kg copper-methionine chelate), T3 (3% alfalfa powder+100 mg/kg copper-methionine chelate), T4 (6% alfalfa powder+50 mg/kg copper-methionine chelate) and T5 (6% alfalfa powder+100 mg/kg copper-methionine chelate). The results of this study have shown that, different levels of alfalfa powder and copper-methionine chelate in laying hens diet except feed conversion ratio and shell-less eggs, other performance parameters were not substantially affected by the level of 3% alfalfa powder throughout of experimental period (P>0.05). Moreover, different levels of alfalfa powder and copper-methionine chelate except egg yolk color had no significant effect on internal and external egg quality traits during the whole experimental period (P>0.05). As well as, the egg yolk color index was significantly increased by increasing the level of alfalfa powder in the laying hens' diet (P<0.001). The highest egg yolk color index was observed in treatment 5 which (containing 6% alfalfa powder+ 100 mg / kg copper-methionine chelate in per kg of diet). Meanwhile, the treatment group that received (6% alfalfa powder+100mg/kg copper- methionine chelate) the egg yolk cholesterol concentration was significantly decreased compared to the control group (P<0.001). Therefore, addition of alfalfa powder and copper methionine chelate to the diet of laying hens reduced egg yolk cholesterol and increased egg yolk color.

Keywords: *Alfalfa powder, Copper–methionine chelate, Egg yolk cholesterol, Laying hen*

INTRODUCTION

Copper has been added to poultry diets as an antimicrobial and growth promoter for many years. Supplementation of Cu in the form of chelates, complexes, or proteinates has been considered as an organic alternative in animal diets to alleviate these problems by lowering the effective usage level compared with in- organic Cu (Kim *et al.,* 2011). These metal-amino acid chelates or complexes furnish trace elements that are more efficiently absorbed from the gut than those provided by inorganic salts (Kim *et al.,* 2011; Ev *et al.,* 2014). These are heterocyclic compounds in which the metal ion is bound to two or more atoms of spatially oriented functional groups on the same ligand, building hales (Ev *et al.,* 2014).

Copper, in the form of chelates, complexes or proteinates are its organic forms and like other organic trace minerals, are usually considered for use in animal diet as alternatives to inorganic source (Chowdhury *et al.,* 2004). If organic Cu in the form of Cu–methionine (Cu–Met) chelate is better absorbed, this may save copper resources, decrease excretion of Cu and reduce environmental concern (Chowdhury *et al.,* 2004). It is well known; that the economic advantage of egg production could be affected highly by egg quality. Therefore, most researchers try to find out the best procedure in laying hens feed with different supplemented proteins and minerals to produce good quality eggs and meats for the market Adu *et al.* (2017).

Otherwise, layer nutrition with copper supplementation may play a significant role in affecting both internal and external egg qualities such as egg size, length, shell strength, yolk, and albumen status (Saldanha *et al.,* 2009). Some researchers have indicated that, dietary supplementation of various Cu sources, such as Cu Sulphate, Cu citrate, Cu chelate improve poultry performance, egg quality and reduce egg and blood cholesterol (Lim and Paik, 2006; Adu *et al.,* 2017). Today, chelate-mineral proteinates are used as feed additives, compounds and minerals, and amino acids or oligopeptides that are associated with better utilisation of minerals for animals compared to inorganic sources (Ev *et al.,* 2014). Reduced egg yolk and blood cholesterol concentration by the addition of 150 mg Cu-P/kg in the diet of laying hens (Jegede *et al.,* 2015).

In addition, Kaya *et al.* (2018) have shown that egg yolk cholesterol considerably decreased by dietary copper supplementation in laying hens. No effect on egg production, egg weight, egg mass, FCR, and production of broken eggs was observed by dietary copper chelate supplementation in the form of copper-methionine (Fouad *et al.,* 2016). No beneficial effects on egg mass, egg production, egg quality, feed conversion ratio, and cholesterol level in egg yolk were found by the organic form of copper (Copper lysine) and inorganic (copper sulphate) in high inclusion levels in laying hens diet (Pekel and Alp, 2011).

Alfalfa (*Medicago sativa L.*) meal is a commercially available feedstuff moderately rich in protein but with high concentrations of fiber (Laudadio *et al.,* 2014), such powder is a commercially available feedstuff that is rich in protein (17.5%) with a well-balanced profile of amino acids, but with a high fibre content (24.1%) and a relatively low (Englmaierová *et al.,* 2019). Recent studies showed alfalfa could reduce the concentrations of cholesterol in the meat and egg yolk (Dong *et al.,* 2007; Krauze and Grela 2010). Alfalfa, as a natural source of carotenoids, increases concentrations of lutein, zeaxanthin, and β-carotene in egg yolks (Englmaierová *et al.*, 2019).

Alfalfa also contains high levels (2 to 3% dry matter) of saponins, which have showed hypocholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant activity(Olgun and Önder, 2015). 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 (Cufadar and Najm, 2020). Mutahar *et al.,* 2011) suggested that supplementation of alfalfa leaf meal always improved egg yolk colour and eggshell thickness but its inclusion in the diet above 5% adversely affect performance parameters.

Research has shown that alfalfa powder is rich in protein (17.5%), beta-carotene, xanthophyll, flavonoids, antioxidants, and other unidentified growth and reproductive factors, and plays

Table 1. Feed ingredients and chemical compositions of diets (%)

Ingredients (g Kg^{-1})	T_1[a]	T_2	T_3	T_4	T_5
Corn, Grain	66.287	62.987	62.987	59.688	59.688
Soybean Meal-44	16.107	15.521	15.521	14.935	14.935
Corn Gluten Meal	3.000	3.000	3.000	3.000	3.000
Alfalfa Powder	0.000	3.000	3.000	6.000	6.000
Wheat bran	2.000	2.000	2.000	2.000	2.000
Soybean Oil	0.245	1.232	1.232	2.220	2.220
Calcium CO_3	9.358	9.258	9.258	9.158	9.158
Dicalcium phosphate	1.384	1.370	1.370	1.356	1.356
Common Salt	0.189	0.167	0.167	0.145	0.145
Sodium bicarbonate	0.211	0.235	0.235	0.260	0.260
Mineral Premix[b]	0.250	0.250	0.250	0.250	0.250
Vitamin Premix[c]	0.250	0.250	0.250	0.250	0.250
DL-Methionine	0.144	0.150	0.150	0.155	0.155
L-Lysine- HCl	0.072	0.076	0.076	0.079	0.079
Zeolite	0.500	0.450	0.400	0.450	0.400
Cu- Methionine	0.000	0.050	0.100	0.050	0.100
Total	100.000	100.000	100.000	100.000	100.000
Nutrient Composition of experimental diets	The amount based on dry matter				
M (Kcal/Kg)	2750	2750	2750	2750	2750
Crude Protein (%)	15.19	15.19	15.19	15.19	15.19
Calcium (%)	3.91	3.91	3.91	3.91	3.91
Available Phosphorus (%)	0.34	0.34	0.34	0.34	0.34
Sodium (%)	0.15	0.15	0.15	0.15	0.15
Lysine (%)	0.71	0.71	0.71	0.71	0.71
Meth+ Cyst (%)	0.65	0.65	0.65	0.65	0.65
Threonine (%)	0.49	0.49	0.49	0.49	0.49

[a.]T: treatment 1 (control) contained (corn and soybean meal), treatments 2 ,3, 4 and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed. b. Composition Mineral Premix supplied per kg of diet: 99.2 mg; manganese (As manganese oxide form), 84.7 mg; zinc (As zinc oxide form), 50mg; iron, 10mg; copper (as a copper sulphate),50mg; iodine,250mg; choline chloride and 25mg; selenium. C. Composition Vitamin Premix supplied per kg of diet: vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 18,000 IU; vitamin K 3.5 mg; vitamin B1,800 mg; vitamin B6 (Pyridoxine), 150 mg; vitamin B12(Cobalamin), 3,000 mg; folic acid, 100mg; biotin, 100 mg; niacin (vitamin b3).

a vital role in pigmentation in the egg yolk (Yıldız *et al.*, 2020; Tkáčová *et al.*, 2015). However, Dong *et al.* (2007); Krauze and Grela (2010); Zheng *et al.* (2019) have demonstrated that reducing the concentrations of egg yolk cholesterol, egg production, feed intake, and eggshell quality by adding dry alfalfa to the diet of laying hens.

Although many researchers have demonstrated the effect of alfalfa meal and chelate minerals on poultry and animal production, consequently there is no study investigating on effects of alfalfa powder and copper methionine chelate on performance, and egg quality in laying hens. Therefore, the objective of this study was to evaluate the effects of different levels of alfalfa powder and supplementation of copper–methionine chelates or its combination on egg yolk cholesterol content, egg quality, and performance in Nick Chick laying hens diets.

MATERIALS AND METHODS

The trials were approved (Ethics Number BASU-2020-12) by the Abbas Abad Research Ethics Committee (AEC) of the Bu-Ali- Sina University of Hamadan (BASU). This study has been performed in 2021, at the Abbas Abad research farm of the agriculture faculty at Bu-Ali-Sina University of Hamadan. To prepare alfalfa powder, first, dried alfalfas were purchased from

Table 2. Effects of different treatments on layer hens performance (whole experimental period).

Parameter / effect	EP %	EW (g)	EM (g)	DFI (g)	FCR (g feed/g egg)
Alfalfa powder (% / kg)					
3	67.279	68.338	45.965	102.864	2.316[a]
6	73.554	68.608	50.521	103.396	2.064[b]
SEM	3.460	0.631	2.449	2.301	0.083
Copper methionine chelate (mg/kg)					
50	69.491	67.585	46.944	104.189	2.276
100	71.341	69.360	49.542	102.071	2.103
SEM	3.460	0.631	2.449	2.301	0.083
Experimental treatments					
T1	65.982	66.377	43.641	100.871	2.466
T2	67.232	67.209	45.045	104.668	2.435
T3	67.325	69.467	46.885	101.060	2.195
T4	71.751	67.962	48.843	103.710	2.116
T5	75.357	69.255	52.200	103.083	2.010
SEM	5.678	1.153	3.887	3.280	0.150
P-value					
AP	0.218	0.766	0.207	0.872	0.049
Cu- chelate	0.710	0.064	0.464	0.524	0.162
AP x Cu- chelate	0.724	0.597	0.829	0.653	0.576
Week x AP	0.241	0.070	0.381	0.949	0.302
Week x Cu- chelate	0.840	0.253	0.769	0.870	0.631
Week x AP x Cu-chelate	0.358	0.365	0.376	0.814	0.407
Week	0.002	0.271	0.002	0.458	0.001
Treatment	0.752	0.300	0.573	0.902	0.174
Treatment x week	0.490	0.294	0.447	0.976	0.389

T: treatment 1 (control) contained (corn and soybean meal), treatments 2 ,3, 4 and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed. [a-b]Similar letters in the same column indicate no significant different between the means in Duncan's test at the error level of 0.05%. SEM: standard error of mean. Abbreviations: EP, egg production; EW, egg weight; EM, egg mass; DFI, daily feed intake; FCR, feed conversion ratio; AP, alfalfa powder; Cu-chelate, copper–methionine chelate.

Hamadan Province, then grinded into a homogenous mixture of powder by a small grinder machine.

Birds, House, and Management

In this experiment, a total of 100 laying hens (Nick Chick Strain, from 109-117 weeks of age were arranged in to 5 experimental treatments, 5 replicates, and 4 laying hens in each replication in a (2x2) factorial completely randomized design(CRD). First two weeks in an adaptation of the experimental diets were managed. During the experiment, the house temperature was kept between 18 and 22 degrees Celsius, and the humidity of the house varied between 55 to 65 percent. The hens were exposed to 16 hours of artificial light and 8 hours of the dark cycle.

Experimental Diets

Experimental diets were formulated according to Nick Chick laying hen nutritional recommendation guide (2016) by UFFDA software and Dry matter(DM), ether extract(EE), and crude protein (CP) were determined according to the methods of AOAC (2017). The ingredients and chemical composition of experimental diets are shown in (Table1). The experimental treatments consisted of 5 different diets, as follows: T1 Control (Corn and soybean meal), treatments T2(3% alfalfa powder+50 mg/kg copper-methionine chelate), T3(3% alfalfa powder+100 mg/kg copper-methionine chelate), T4(6% alfalfa powder+50 mg/kg copper-methionine chelate) and T5(6% alfalfa powder+100 mg/kg copper-methionine chelate).

Laying Performance

During along experimental period, to determine egg production(EP), egg weight(EW), egg mass(EM), daily feed intake(DFI), feed conversion ratio(FCR), broken egg(BE) and Shell-less egg(SLE) data were recorded daily. In addition, calculated body weight changes at the beginning and at the end of experimental period by individual weighed birds.

Internal and External Egg Quality Traits

Internal and external egg quality parameters were measured at the end of each week. For these objects, two eggs from each replicate were randomly selected, numbered, and transferred to the laboratory. First, the eggs were weighed with a digital scale of 0.01 g sensitive and then the desired external and internal egg quality traits were measured by (Laudadio *et al.,* 2014) method.

Egg Yolk Cholesterol and Triglyceride Content

The egg yolk cholesterol and triglyceride concentration were determined at the end of experimental period. For these goals two eggs in a replicate (10 eggs per treatment) were randomly collected, weighted, and transferred to the poultry laboratory, then egg yolk cholesterol and triglyceride concentrations were measured by (Zlatkis, Zak, and Boyle 1953; Washburn and Nix 1974) methods.

Statistical Analysis

At the end of 8 weeks' research, the collected data were subjected to analysis using the General Linear Model (GLM) procedure in Statistical Analysis System (SAS) (version 9.4). Significant differences among the treatment of means were tested at ($p<0.05$) by using Duncan's multiple range test.

Table 3. Effects of different treatments on the average percentage of broken eggs, soft eggs and body weight changes in layer hens at the end of the experimental

Parameter / effect	BE (%)	SLE (%)	BWC (g)
Alfalfa powder % / kg			
3	2.267	4.611[a]	31.900
6	2.740	2.224[b]	23.525
SEM	0.523	0.749	28.330
Copper methionine chelate mg/kg			
50	2.359	3.616	40.050
100	2.646	3.219	15.375
SEM	0.523	0.749	28.330
Experimental treatments			
T1	2.206	2.831	25.560
T2	1.850	5.074	38.800
T3	2.683	4.148	25.000
T4	2.869	2.157	41.300
T5	2.610	2.290	5.750
SEM	0.812	0.950	35.976
P-value			
AP	0.532	0.039	0.837
Cu- chelate	0.704	0.713	0.547
AP x Cu- chelate	0.472	0.624	0.790
Treatment	0.902	0.173	0.959

T: treatment 1 (control) contained (corn and soybean meal), treatments 2 ,3, 4 and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed. [a-b] Similar letters in the same column indicate no significant different between the means in Duncan's test at the error level of 0.05%. SEM: standard error of mean. Abbreviations: BE, broken egg; SLE, shell-less egg; BWC, body weight change; AP, alfalfa powder; Cu- chelate, copper–methionine chelate.

RESULTS AND DISCUSSION

The results of this study have shown that no significant effect on performance parameters was found by different levels of alfalfa powder and copper–methionine chelate during the whole experimental period except feed conversion ratio (P >0.05) (Table2). FCR was considerably better by a 6% level of alfalfa powder among other levels (P<0.05). affected such level may be due to the age of layer hens and combination of alfalfa powder with copper-methionine supplementation. However, in experimental treatments section, no experimental treatments had a significant effect on performance parameters during the whole experimental period (P>0.05). Our result has shown that time (week) was significant on egg production (EP), egg mass (EM) and feed conversion ratio (FCR) (P<0.05) (Table 2). In addition, their interactions between alfalfa powder and copper-methionine chelate on performance parameters were also not significant (P>0.05).

The findings of present study in terms of performance parameters some researcher's results have shown their agreement to these findings. Yıldız A.Ö *et al.* (2020) demonstrated that alfalfa meal can negatively affect the perfor-

Table 4. Effects of different treatments in layer hens on internal egg quality traits at the whole experimental period.

Parameter/effect	YC	YW (g)	HU	YI (%)	AI (%)	ALP
Alfalfa powder % / kg						
3	8.281[b]	19.908	75.800	38.943	7.252[b]	63.098
6	8.606[a]	20.187	77.933	39.425	7.669[a]	62.848
SEM	0.081	0.218	0.992	0.297	0.076	0.544
Copper methionine chelate mg/kg						
50	8.375	19.767	76.491	39.011	7.287	62.909
100	8.513	20.327	77.242	39.357	7.633	63.036
SEM	0.081	0.218	0.992	0.297	0.076	0.544
Experimental treatments						
T1	7.125[c]	20.196	75.483	39.697	7.369	61.772
T2	8.238[b]	19.840	75.502	38.698	7.026	62.879
T3	8.325[b]	19.975	76.097	39.188	7.478	63.316
T4	8.513[b]	19.695	77.480	39.324	7.549	62.939
T5	8.700[a]	20.678	78.386	39.526	7.788	62.756
SEM	0.098	0.309	1.131	0.508	0.297	0.524
p-value						
AP	0.045	0.124	0.897	0.369	0.011	0.951
Cu-chelate	0.230	0.096	0.613	0.515	0.155	0.486
AP x Cu- chelate	0.947	0.745	0.541	0.609	0.897	0.626
Week x AP	0.189	0.199	0.352	0.465	0.026	0.379
Week x Cu- chelate	0.020	0.004	0.903	0.698	0.883	0.284
Week x AP x Cu-chelate	0.564	0.325	0.089	0.745	0.900	0.160
Week	0.003	0.032	0.235	0.587	0.032	0.312
Treatment	0.0001	0.230	0.303	0.691	0.495	0.338
Treatment x week	0.004	0.000	0.144	0.009	0.003	0.024

T: treatment 1 (control) contained (corn and soybean meal), treatments 2,3, 4, and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed. [a-b] Similar letters in the same column indicate no significant different between the means in Duncan's test at the error level of 0.05%. SEM: standard error of the mean. Abbreviations: YC, yolk colour; YW, yolk weight; HU, haugh unit; YI, yolk index; AI, albumin index; ALP, albumin percent; AP, alfalfa powder; Cu-chelate, copper–methionine chelate.

Table 5. Effects of different treatments on external egg quality traits in layers' hen at the whole experimental period

Parameter / effect	ST (mm)	SW (gr)	ESI (%)	ESA (cm^2)	ESP %	ESG	(SWUSA) mg/cm^2
Alfalfa powder % / kg							
3	0.557	5.828	72.833	70.775	8.316	1.077	0.082
6	0.553	5.928	73.214	71.129	8.425	1.078	0.083
SEM	0.007	0.119	0.544	0.901	0.086	1.000	0.001
Copper methionine chelate mg/kg							
50	0.555	5.758	72.765	70.089	8.339	1.077	0.082
100	0.555	5.998	73.282	71.814	8.401	1.078	0.083
SEM	0.007	0.119	0.544	0.901	0.086	1.000	0.001
Experimental treatments							
T1	0.531	5.516	72.175	68.798	8.194	1.076	0.080
T2	0.553	5.639	72.496	69.931	8.182	1.076	0.080
T3	0.560	6.018	73.169	71.618	8.449	1.078	0.084
T4	0.557	5.877	73.033	70.248	8.496	1.078	0.084
T5	0.550	5.979	73.395	72.010	8.353	1.077	0.083
SEM	0.009	0.168	0.659	1.053	0.125	0.000	0.001
P-value							
AP	0.902	0.640	0.682	0.370	0.516	0.515	0.853
Cu-chelate	0.377	0.985	0.343	0.732	0.631	0.629	0.744
AP x Cu- chelate	0.483	0.839	0.636	0.569	0.552	0.555	0.763
Week x AP	0.860	0.988	0.433	0.779	0.864	0.859	0.914
Week x Cu- chelate	0.347	0.256	0.893	0.023	0.738	0.732	0.860
Week x AP x Cu-chelate	0.626	0.602	0.130	0.367	0.912	0.906	0.901
Week	0.930	0.579	0.424	0.779	0.665	0.657	0.921
Treatment	0.186	0.190	0.679	0.229	0.299	0.298	0.213
Treatment x week	0.324	0.730	0.045	0.087	0.826	0.822	0.887

T: treatment 1 (control) contained (corn and soybean meal), treatments 2 ,3, 4 and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed.

[a-b] Similar letters in the same column indicate no significant different between the means in Duncan's test at the error level of 0.05%. SEM: standard error of the mean. Abbreviations: ST, shell thickness; SW, shell weight; ESI, egg shape index; ESA, egg surface area; ESP, egg shell percent; ESG, egg specific gravity; SWUSA, shell weight per unit surface area; AP, alfalfa powder; Cu-chelate, copper–methionine chelate.

mance of laying hens. Moreover, in accordance with our results, Balevi and Coskun (2004); Khajali *et al.* (2007[a]); Laudadio *et al.* (2014); Olgun and Önder (2015); Zafar and Fatima (2018) narrated that,addition of alfalfa powder and Cu supplement in laying hens diet had no significant results(P>0.05). Moreover, Manangi *et al.* (2015) described that, increasing Copper from organic or inorganic sources had no effect on egg production, egg weight, FCR and broken egg rate. Another study performed by Chowdhury *et al.* (2004), they noted that feed consumption and FCR did not improve by adding 100 mg cu-methionine compared with the control (contained 20.5 mg cu/kg diet). On the other hand, Heywang (1950) reported that addition of alfalfa powder to the layer diets at the levels of 50, 100, 150 and 200 g/kg had no effect on feed intake (FI), but an addition of more than 5% decreased egg production.

This negative effect in the performance parameters of laying hens may be due to anti-nutritional factors such as saponins, cellulose tannins, and high levels of fibre in alfalfa content (A. Yıldız, Şentürk, and Olgun 2020) and age of birds. In addition, Mourão *et al*. (2006); Chiou *et al*. (1997) have demonstrated that copper is a strong oxidant, and may damage the gizzard and

Table 6. The effect of treatments in layer hens on EYC and TG content in layers' hen

Parameter / effect	EYC mg/g	TG mg/g
Alfalfa powder % / kg		
3	13.4328	11.591[b]
6	11.3532	12.557[a]
SEM	0.094	0.090
Copper methionine chelate mg/kg		
50	12.870	11.959
100	11.916	12.189
SEM	0.094	0.090
Experimental treatments		
T1	14.480 [a]	10.610[c]
T2	13.564 [b]	11.552[b]
T3	13.302 [b]	11.630[b]
T4	12.177 [c]	12.366[a]
T5	10.530 [d]	12.748[a]
SEM	0.143	0.130
P-value		
AP	<.0001	<.0001
Cu- chelate	<.0001	0.093
AP x Cu- chelate	<.0001	0.255
Treatment	<.0001	<.0001

T: treatment 1 (control) contained (corn and soybean meal), treatments 2 ,3, 4 and 5 respectively contained 3 and 6% alfalfa powder and each with 50 and 100 mg/kg of copper–methionine chelate per kg of feed. [a-b] Similar letters in the same column indicate no significant different between the means in Duncan's test at the error level of 0.05%. SEM: standard error of the mean. Abbreviations: EYC, egg yolk cholesterol; TG, triglyceride; AP, alfalfa powder; Cu-chelate, copper–methionine chelate.

oral cavity, and leads to reduced feed intake. In terms of, the average percentage of broken eggs, and body weight changes at the end of the experimental period except for shell-less eggs were not significant changes by different levels of alfalfa powder and copper–methionine chelate (P>0.05) (Table3). These findings are supported by the results of (Manangi *et al.,* 2015) they have described that egg production, egg weight, FCR, and broken egg rate, were not affected by increasing Copper in the laying hen's diet. Laudadio *et al.* (2014) have reported that the number of broken eggs was not affected by the addition of, 15% of alfalfa powder in laying hens' diet. On the other hand, in the experimental treatments section, no experimental treatments had a significant effect on broken eggs, shell-less eggs and body weight changes at the end of the experimental period (P>0.05). Furthermore, the effects different levels of alfalfa powder and copper-methionine chelates on the internal and external egg quality traits of laying hens (109 to 117 weeks of age) at the whole experimental period were presented in (Table4) and (Table5). These tables, have shown that different levels of alfalfa powder except for egg yolk color and albumin index, no significant effect was found on the internal and external egg quality(P>0.05). Except egg yolk color index, other treatments were also not found significant in the internal and external parameters (P<0.05).

In treatment 5 which included (6% alfalfa powder+100 mg/kg copper-methionine chelate) in per kg of layer diet, the egg yolk color index was very significantly higher among the other treatment (P<0.0001). Researches shown that alfalfa powder is high in xanthophyll such as lutein and zeaxanthin, which increase egg yolk pigment (Laudadio *et al.,* 2014). The finding of the present study has shown that egg yolk weight, shell weight, and egg surface area indexes in the first, second, fourth, and seventh weeks of the experiment were significantly effected by 100 mg copper-methionine chelate per kg of laying diet (P<0.05).

The findings of present study on internal and external egg quality parameters were congruent with the results of Kaya *et al.* (2017); Lim and Paik (2006); Pekel and Alp (2011) who found that inclusion of supplementation of copper chelate in laying hens diets had no significant effect on egg quality traits, except for shell strength and egg yolk colour (P>0.05). Meanwhile, increasing the level of alfalfa powder in the laying hen's diet, the egg yolk colour index was improved. These results are close to previous studies (Al-Nasry 2010; Khajali *et al.,* 2007; A. Ö. Yıldız, Şentürk, and Olgun 2020; Englmaierová, Skřivan, and Vít 2019b; Laudadio *et al.,* 2014; Khudhur, Najm, and Cufadar 2020; Hongxia and Xifeng 2004) who affirm that existing alfalfa powder and copper chelate in laying hens diets, significantly increased egg yolk colour than those who did not include alfalfa powder.

On the other hand, Grela *et al.* (2020) also reported that, adding 3% alfalfa protein concentrates in to the layer hen's diet had no significant effect on internal egg parameters such as: haugh unit, yolk weight, yolk index and albumin index (P> 0.05). Moreover, the effects different levels of alfalfa powder and copper- methionine chelates on egg yolk cholesterol and triglyceride content of laying hens (109 to 117 weeks of age) at the end of the experimental period were shown in (Table 6). This result indicated that, different levels of alfalfa powder had a significant effect on egg yolk triglyceride (P<0.05).

However, the experimental treatments on egg yolk cholesterol and triglyceride were significant (P<0.05). The current study's findings showed that, egg yolk cholesterol was affected by treatment 5 which included (6% alfalfa powder+ 100 mg/kg copper methionine chelate) and significant decreased was observed compared to the control group (P< 0.05). Moreover, treatments that hade alfalfa powder and copper-methionine chelate significantly reduced egg yolk cholesterol and also increased egg yolk triglyceride compared to the control group (P<0.05). Parallel to the findings of the present study, Olgun and Önder (2015) observed that egg yolk cholesterol content considerably decreased by adding 4% alfalfa powder to the laying hens' diets. Most of researchers have shown that, decreasing of egg yolk cholesterol may be the cause of saponins, they have the capacity to link cholesterol and steroids, impeding its absorption and consequently reducing egg yolk and plasma cholesterol concentrations (Kocaoglu Guclu *et al.,* 2004; Mourão *et al.,* 2006; Klita *et al.,* 1996; A. Ö. Yıldız, Şentürk, and Olgun 2020). On the other hand, previous researches (Bakalli *et al.,* 1995; Konjufca, Pesti, and Bakalli 1997; Balevi and Coskun 2004; Idowu *et al.,* 2006; Jegede *et al.,* 2015; Kaya *et al.,* 2017) have shown that, copper supplementation reduce egg yolk and plasma cholesterol, because copper decreased hepatic glutathione formation, which in turn reduces the activity of 3-hydroxy-3-methylglutaryl coenzyme A(HMGCoA) reductase. In this case, some researchers (Zheng *et al*., 2019; AL-Haweizy & AL-Sardary, 2007; Kocaoğlu Güçlü *et al*., 2004) have reported that egg yolk cholesterol decreased by alfalfa in laying hens' diet. Adding saponins to the diet (Khubeiz and Shirif, 2020) or increasing cellulose levels (Mourão *et al.,* 2006) are effective at reducing egg cholesterol content. Similarly, decreased egg yolk total cholesterol concentration have reported by additional copper supplementation in the diet of laying hens (Kaya *et al.,* 2018; Olgun, Yazgan and Cufadar 2013).

CONCLUSION

The feed conversion ratio and shell-less eggs were considerably increased by the level of 3% alfalfa powder (P<0.05). However, egg yolk colour and albumin index were considerably increased by increasing the level of alfalfa powder and copper–methionine chelates per kg of the diets. in addition, egg yolk cholesterol was remarkably decreased by increasing the level of alfalfa powder and copper–methionine chelates compared with the control treatment. Therefore, this study, recommended that inclusion of 3% alfalfa powder and 100 mg/kg copper- methionine supplementation in per kg of layer birds diets as a growth promoter, could be better for increasing egg yolk colour and considerably modified egg yolk cholesterol.

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