

## Effects of dietary inclusion of different levels of alfalfa leaf meal on laying hens' performance and egg quality

Mutahar A. Al-shami<sup>1</sup>, Mohamed E. Salih<sup>2</sup> and Talha E. Abbas<sup>3</sup>

<sup>1-2</sup>Department of Animal Production, Faculty of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Shambat, Sudan; <sup>3</sup>Department of Animal Production, Faculty of Agricultural Technology and Fish Science, University of Alneelain, Khartoum, Sudan.

### Abstract

The experiment was designed to evaluate the effect of dietary supplementation of three levels (2%, 5% and 7%) of sun-dried alfalfa leaf meal on performance of layer hens, egg quality and cholesterol levels of egg yolk. A total of 48 hens (White Hisex) at point of lay (about 20 weeks old) were offered four experimental diets for 8 weeks. Iso-caloric and iso-nitrogenous experimental diets (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>) containing 0.0, 2, 5 and 7% alfalfa leaf meal, respectively were formulated. The results indicated that hens fed D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> laid eggs with significantly darker yellow colour comparing with those fed the control diet. Hens fed D<sub>4</sub> diet showed significantly lower yolk cholesterol, egg weight, hen-day egg production percentage, and poorer feed conversion ratio when compared to those fed the other diets. It was concluded 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.

**Key words:** Alfalfa Leaf Meal, Cholesterol, Laying Hens, Yolk Colour

### Introduction

Due to increase in population, poultry producers face an increase demand for poultry products. In Sudan, the cost of the poultry products is very high due to the increase of cost of feed ingredients. So researchers need to look for alternatives to the traditional energy and protein sources. Alfalfa (*Medicago sativa* L.) is a forage legume which is considered as a source of protein for ruminant because it contains plenty amount of protein and amino acids (Markovic et al., 2007). Baraniak (1995) and Markovic et al. (2007) mentioned that the fresh green leafy plants are better source of protein, energy and vitamins for animals, but their higher content of fiber limits their use for monogastric animals. Fletcher and Papa (1985) noticed that alfalfa has the colouring ability to egg yolk of laying hens because it contains high amount of xanthophylls. In addition, it was reported that alfalfa leaf meal reduced serum cholesterol of laying hens (Wen-jun et al., 2007), lowered serum and egg yolk cholesterol and increased eggshell thickness without any deleterious effects on laying hens performance (Güçlü et al., 2004). The study

aimed to evaluate effect of supplementation of alfalfa leaf meal on laying hens' performance, egg quality characteristics and cholesterol level in egg yolk.

### Materials and Methods

Fresh Alfalfa leaves were bought from Alhalfayah Market at Khartoum suburb. Both fresh leaves and stems were dried by oven for 24 hours. The collected dried Alfalfa Leaves were grinded into homogenous mixture using hammer mill. Then packaged in polythene bags and weighed to be mixed with other dietary ingredients. Samples of Alfalfa Leaf Meal (ALM) were chemically analyzed to determine their contents of dry matter, crude protein, crude fiber, ether extract and ash according to AOAC (1990).

Forty eight White Hisex laying hens (about 20 weeks old) bought from Coral Hatcheries and Feed Production Farms. All required practices (vaccination for Gumboro, Newcastle and Fowl Pox and de-beaking) were carried out during the brooding and rearing periods according to the recommended schedule. After arrival to the experimental house, all

**Corresponding author:** Talha Abbas, Department of Animal Production, Faculty of Agricultural Technology and Fish Science, Alneelain University, Sudan; P.O. Box 12702, Postal code 11121

hens were fed on the commercial diet. Birds were randomly distributed into 16 cages of 2 batteries. Batteries were placed in an open sided house. Three birds were allotted in each small cage and each cage represented one replicate for each treatment. The batteries were provided with longitudinal feeders and automatic drinkers. Light was provided for 15-17 hours in form of natural day light supplemented with artificial light in the evening by bulb lamps of 60 watt above the batteries. All possible steps were taken to avoid animal suffering at each stage of the experiment.

Iso-caloric and iso-nitrogenous four experimental diets, (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> of 0.0, 2, 5 and 7% ALM, respectively), were formulated depending on the chemical analysis of ALM. The experimental diets were formulated to meet the laying hen requirements according to NRC (1994) (Table2). Table (3) shows the

**Table 1: Chemical composition of alfalfa leaf meal (on dry matter basis)**

Item (%)	
Dry Matter	96.1
Crude Protein	22.75
Ether Extract	1.14
Crude Fiber	13.26
Ash	5.81
Nitrogen Free Extract	53.14
Calculated Metabolizable Energy (Kcal/Kg)	2300

**Table 2: Ingredients percentages of the experimental laying hen diets (on fresh basis)**

Ingredients (%)	Alfalfa Leaf Meal (%)			
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>
ALM	0.0	2.0	5.0	7.0
Sorghum. Grain.	70.00	70.00	69.94	68.47
Ground. Nut Meal	11.60	11.30	10.63	10.00
Wheat Bran	3.97	2.27	0.00	0.00
Limestone	8.10	8.10	8.10	8.10
Layer Concentrate*	5.00	5.00	5.00	5.00
Di-Cal. Phosphate	0.10	0.10	0.10	0.10
Salt (NaCl)	0.20	0.20	0.20	0.20
Choline	0.05	0.05	0.05	0.05
Antitoxin	0.20	0.20	0.20	0.20
Organic Acid	0.20	0.20	0.20	0.20
Lysine	0.31	0.31	0.31	0.31
Methionine	0.17	0.17	0.17	0.17
Vegetable Oil	0.10	0.10	0.10	0.20
Total	100	100	100	100

Hendrix \*Crude protein 40%, Crude fat 3%, Crude fiber 2%, Calcium 6%, Phosphorous (available) 5.8%, Lysine 6%, Methionine 2.8%, Methionine + Cystine 3.3%, Sodium 1.6%, ME (Kcal/kg) 2000, Vitamins: Vit. A 240,000 I.U/kg, Vit. D3 60,000 I.U/kg, Vit. E 800 mg/kg, Vit. K3 40 mg/kg, Vit. B1 30 mg/kg, Vit. B2 100 mg/kg, Vit B6 50 mg/kg, Vit. B12 400 mg/kg, D-pantothenic acid 130 mg/kg, Niacine 700 mg/kg, Antioxidant (BHT) 900 mg/kg, Choline chloride 8000 mg/kg, Folic acid 10 mg/kg, Manganese 1810 mg/kg, Zinc 1080 mg/kg, Iron 1000 mg/kg, Copper 151 mg/kg, Iodine 20 mg/kg, Selenium 5 mg/kg and Cobalt 20 mg/kg, Xanthophyll added, phytase added.

calculated chemical analysis of the layer experimental diets. Each experimental diet was allotted randomly to four replicates.

This experiment was conducted in the premises of Poultry Unit of the Department of Animal Production, Faculty of Agricultural Studies, Sudan University of Science and Technology, Shambat. The experimental birds were randomly distributed in the cages. The birds had approximately equal weights. The birds were subjected to adaptation period of one week to the batteries and fed on commercial diets before data collection. Clean fresh water and feed were offered *ad libitum*. During the experimental period the produced eggs per replicate were weighed daily and recorded to be computed for Hen-day egg production percentage and egg weight. Added and remained diet was weighed weekly to compute feed intake and feed conversion ratio. Mortality was recorded daily. At the end of every two weeks, eight eggs (2 eggs from each replicate) for each treatment were randomly selected, marked and individually weighed to be ready for studying egg quality characters (egg weight, egg length, egg diameter, egg shell thickness, yolk diameter, yolk height, albumin height and egg yolk colour). The studied eggs' dimensions were measured in millimetres (mm) using Digital Vernier Calliper. Egg yolk colour was measured using Yolk Colour Fan. During the last week of the experiment two eggs from each replicate (8 eggs per treatment) were randomly selected and marked to be used to determine the content of egg yolk cholesterol.

### Statistical Analysis

The experimental design used was a complete randomized design. Analysis of variance (ANOVA) (Steel and Torrie, 1980) was performed on all data using the General Linear Models procedure of SAS (1990). Differences between dietary treatments were tested using Duncan (1955) Multiple Range Tests.

### Results

The effects of feeding laying hens diets of the different levels of ALM on feed intake were shown in Table (4). Birds fed diet of 7% ALM (P<0.05) consumed more feed than the other treatments. Table 5 showed that hens fed the diet of the highest level of ALM (7%) significantly (P<0.05) decreased egg weight on the 3<sup>rd</sup> to 8<sup>th</sup> weeks compared to those fed diet supplemented with lower percentages of ALM. Also, the higher level of ALM in the laying hens diet significantly (P<0.05) reduced hen-day egg production% as compared to the free or low ALM diets during 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks (Table 6). Hens offered diet supplemented with 7% ALM exhibited

**Table 3: Calculated chemical composition of the laying hens' diets (on dry matter basis)**

	Constituents							
	ME (Kcal/Kg)	CP%	Lys %	Meth %	Ca %	P %	Na %	CF %
D <sub>1</sub>	2806	17.48	0.86	0.44	3.37	0.41	0.17	3.42
D <sub>2</sub>	2811	17.50	0.85	0.43	3.37	0.40	0.17	3.17
D <sub>3</sub>	2815	17.46	0.83	0.42	3.37	0.40	0.83	2.81
D <sub>4</sub>	2804	17.40	0.82	0.42	3.37	0.39	0.17	2.72

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM

**Table 4: Feed intake (g/bird/day) of the layers during the experimental period**

	Weeks							
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	89±13.7 <sup>ab</sup>	94±10.4 <sup>a</sup>	92±4.7 <sup>a</sup>	101±4.2 <sup>a</sup>	103±3.2 <sup>a</sup>	109±5.5 <sup>a</sup>	113±4.4 <sup>a</sup>	111±6.5 <sup>a</sup>
D <sub>2</sub>	81±6.5 <sup>b</sup>	97±4.5 <sup>a</sup>	92±3.4 <sup>a</sup>	102±2.5 <sup>a</sup>	99±5.3 <sup>a</sup>	107±2.4 <sup>a</sup>	111±5.3 <sup>a</sup>	109±7.1 <sup>a</sup>
D <sub>3</sub>	81±10.6 <sup>b</sup>	90±16.9 <sup>a</sup>	94±9.0 <sup>a</sup>	101±20.5 <sup>a</sup>	99±24.9 <sup>a</sup>	96±14.4 <sup>a</sup>	109±18.7 <sup>a</sup>	120±28.2 <sup>a</sup>
D <sub>4</sub>	98±5.5 <sup>a</sup>	96±6.8 <sup>a</sup>	90±9.9 <sup>a</sup>	92±15.0 <sup>a</sup>	96±17.2 <sup>a</sup>	103±10.5 <sup>a</sup>	116±1.6 <sup>a</sup>	121±1.1 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 5: Egg weight (g) of layers fed ALM supplemented diets during the experimental period**

	Weeks							
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	44.5±1.32 <sup>a</sup>	47.2±0.63 <sup>a</sup>	50.5±1.57 <sup>a</sup>	52.1±1.16 <sup>a</sup>	53.2±1.58 <sup>a</sup>	55.6±1.38 <sup>a</sup>	56.6±1.58 <sup>a</sup>	57.2±1.53 <sup>a</sup>
D <sub>2</sub>	44.0±2.36 <sup>a</sup>	46.6±2.63 <sup>a</sup>	49.7±1.20 <sup>ab</sup>	52.4±0.85 <sup>a</sup>	53.2±0.93 <sup>a</sup>	55.6±1.15 <sup>a</sup>	56.0±0.92 <sup>a</sup>	56.0±1.27 <sup>ab</sup>
D <sub>3</sub>	44.5±2.73 <sup>a</sup>	48.2±3.77 <sup>a</sup>	50.9±3.13 <sup>a</sup>	51.7±2.73 <sup>a</sup>	50.4±1.82 <sup>b</sup>	53.6±3.24 <sup>a</sup>	54.4±3.16 <sup>a</sup>	54.7±2.91 <sup>ab</sup>
D <sub>4</sub>	43.9±2.34 <sup>a</sup>	46.0±2.86 <sup>a</sup>	47.0±1.16 <sup>b</sup>	47.4±0.93 <sup>b</sup>	48.5±2.24 <sup>b</sup>	49.9±0.97 <sup>b</sup>	51.2±1.26 <sup>b</sup>	53.6±0.72 <sup>b</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 6: Hen-day egg production (%) during the experimental periods**

	Weeks							
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	61±22.8 <sup>a</sup>	86±12.9 <sup>a</sup>	96±2.38 <sup>a</sup>	98±4.76 <sup>a</sup>	95±6.74 <sup>a</sup>	96±4.56 <sup>a</sup>	95±3.89 <sup>a</sup>	98±2.75 <sup>a</sup>
D <sub>2</sub>	68±12.5 <sup>a</sup>	93±6.15 <sup>a</sup>	89±5.99 <sup>a</sup>	90±9.52 <sup>ab</sup>	94±7.14 <sup>ab</sup>	99±2.38 <sup>a</sup>	98±2.75 <sup>a</sup>	94±9.02 <sup>a</sup>
D <sub>3</sub>	87±10.6 <sup>a</sup>	86±25.5 <sup>a</sup>	92±7.15 <sup>a</sup>	98±2.75 <sup>a</sup>	101±4.91 <sup>a</sup>	94±5.99 <sup>a</sup>	99±2.38 <sup>a</sup>	98±2.75 <sup>a</sup>
D <sub>4</sub>	76±12.6 <sup>a</sup>	92±2.75 <sup>a</sup>	95±4.76 <sup>a</sup>	86±4.77 <sup>b</sup>	73±7.27 <sup>b</sup>	81±9.53 <sup>b</sup>	95±8.25 <sup>a</sup>	100±0.00 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 7: Layers' feed conversion ratio (g feed/g egg) during the experimental period**

	Weeks							
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	3.79±1.79 <sup>a</sup>	2.35±0.32 <sup>a</sup>	1.89±0.13 <sup>a</sup>	2.00±0.15 <sup>a</sup>	2.04±0.19 <sup>b</sup>	2.04±0.17 <sup>b</sup>	2.10±0.11 <sup>b</sup>	1.99±0.15 <sup>a</sup>
D <sub>2</sub>	2.77±0.48 <sup>a</sup>	2.26±0.16 <sup>a</sup>	2.09±0.22 <sup>a</sup>	2.18±0.30 <sup>a</sup>	1.98±0.14 <sup>b</sup>	1.95±0.09 <sup>b</sup>	2.04±0.14 <sup>b</sup>	2.09±0.22 <sup>a</sup>
D <sub>3</sub>	2.13±0.37 <sup>a</sup>	2.27±0.31 <sup>a</sup>	2.02±0.22 <sup>a</sup>	2.01±0.42 <sup>a</sup>	1.96±0.51 <sup>b</sup>	1.92±0.34 <sup>b</sup>	2.02±0.26 <sup>b</sup>	2.25±0.45 <sup>a</sup>
D <sub>4</sub>	3.02±0.69 <sup>a</sup>	2.28±0.33 <sup>a</sup>	2.01±0.28 <sup>a</sup>	2.27±0.33 <sup>a</sup>	2.71±0.53 <sup>a</sup>	2.55±0.20 <sup>a</sup>	2.40±0.13 <sup>a</sup>	2.27±0.05 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 8: Egg shape index of layers fed ALM supplemented diets during the experimental.**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	77.3±1.76 <sup>a</sup>	77.0±2.20 <sup>a</sup>	77.4±2.99 <sup>a</sup>	76.5±3.20 <sup>a</sup>
D <sub>2</sub>	74.7±1.65 <sup>b</sup>	76.8±3.61 <sup>a</sup>	77.2±1.71 <sup>a</sup>	76.0±1.92 <sup>a</sup>
D <sub>3</sub>	77.7±2.06 <sup>a</sup>	77.6±2.19 <sup>a</sup>	77.8±6.92 <sup>a</sup>	77.3±1.87 <sup>a</sup>
D <sub>4</sub>	76.9±1.57 <sup>a</sup>	77.6±1.95 <sup>a</sup>	76.5±1.57 <sup>a</sup>	75.3±1.38 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a,b</sup>Values in the same column with different superscripts are significantly different (P<0.05)

**Table 9: Egg shell thickness (mm) of layers fed ALM supplemented diets during the experimental period**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	0.38±0.018 <sup>b</sup>	0.36±0.009 <sup>ab</sup>	0.35±0.029 <sup>a</sup>	0.39±0.011 <sup>c</sup>
D <sub>2</sub>	0.36±0.021 <sup>b</sup>	0.34±0.021 <sup>b</sup>	0.37±0.010 <sup>a</sup>	0.38±0.016 <sup>c</sup>
D <sub>3</sub>	0.41±0.022 <sup>a</sup>	0.38±0.017 <sup>a</sup>	0.36±0.048 <sup>a</sup>	0.40±0.014 <sup>b</sup>
D <sub>4</sub>	0.37±0.029 <sup>b</sup>	0.36±0.027 <sup>ab</sup>	0.37±0.013 <sup>a</sup>	0.42±0.004 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a,b</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 10: Egg albumen height (mm) of layers fed ALM supplemented diets during the experimental period**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	7.28±0.53 <sup>b</sup>	7.24±0.96 <sup>a</sup>	9.17±0.66 <sup>a</sup>	8.68±1.30 <sup>a</sup>
D <sub>2</sub>	7.93±0.66 <sup>a</sup>	7.36±0.70	8.89±0.85 <sup>a</sup>	7.77±0.52 <sup>ab</sup>
D <sub>3</sub>	6.74±0.30 <sup>c</sup>	7.01±0.70 <sup>a</sup>	7.84±0.96 <sup>b</sup>	7.40±0.95 <sup>b</sup>
D <sub>4</sub>	7.18±0.40 <sup>bc</sup>	7.38±0.75 <sup>a</sup>	6.06±1.13 <sup>c</sup>	8.04±1.06 <sup>ab</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 11: Egg Haugh Unit of layers fed ALM supplemented diets during the experimental period**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	22.27±1.15 <sup>b</sup>	22.33±1.82 <sup>a</sup>	25.71±1.31 <sup>a</sup>	24.82±2.54 <sup>a</sup>
D <sub>2</sub>	23.65±1.32 <sup>a</sup>	22.55±1.35 <sup>a</sup>	25.13±1.66 <sup>a</sup>	22.96±1.10 <sup>ab</sup>
D <sub>3</sub>	21.22±0.50 <sup>b</sup>	21.90±1.50 <sup>a</sup>	23.20±1.82 <sup>b</sup>	22.44±1.86 <sup>b</sup>
D <sub>4</sub>	22.18±0.72 <sup>b</sup>	22.73±1.48 <sup>a</sup>	19.68±2.23 <sup>c</sup>	23.62±2.15 <sup>ab</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviations

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 12: Egg yolk index of layers fed ALM supplemented diets during the experimental period.**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	42.88±2.29 <sup>a</sup>	46.01±2.31 <sup>a</sup>	44.92±1.40 <sup>b</sup>	45.57±1.86 <sup>a</sup>
D <sub>2</sub>	43.29±1.91 <sup>a</sup>	46.49±2.26 <sup>a</sup>	47.14±1.74 <sup>a</sup>	45.52±3.02 <sup>a</sup>
D <sub>3</sub>	42.13±2.87 <sup>a</sup>	45.31±1.64 <sup>a</sup>	45.11±2.00 <sup>b</sup>	45.57±1.59 <sup>a</sup>
D <sub>4</sub>	41.77±2.18 <sup>a</sup>	46.76±2.61 <sup>a</sup>	44.61±2.48 <sup>b</sup>	44.19±2.41 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviation;

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 13: Scores of egg yolk colour of layers fed ALM supplemented diets during the experimental period.**

	Weeks			
	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>
D <sub>1</sub>	3.63±0.52 <sup>d</sup>	3.75±0.46 <sup>d</sup>	3.75±0.46 <sup>c</sup>	7.38±0.92 <sup>c</sup>
D <sub>2</sub>	5.38±0.52 <sup>c</sup>	5.38±0.52 <sup>c</sup>	8.50±0.76 <sup>b</sup>	8.88±0.64 <sup>b</sup>
D <sub>3</sub>	8.50±0.53 <sup>a</sup>	8.50±0.53 <sup>a</sup>	8.88±0.64 <sup>b</sup>	9.38±0.92 <sup>ab</sup>
D <sub>4</sub>	8.00±0.00 <sup>b</sup>	8.00±0.00 <sup>b</sup>	9.88±1.13 <sup>a</sup>	10.00±0.53 <sup>a</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM; ALM= Alfalfa leaf meal; Values are means ± standard deviation;

<sup>a-c</sup>Values in the same column with different superscripts are significantly different (P<0.05)**Table 14: Egg yolk cholesterol (mg/g) of layers fed the experimental diets**

	Overall Mean ± SE
D <sub>1</sub>	11.72±1.44 <sup>a</sup>
D <sub>2</sub>	10.62±0.88 <sup>ab</sup>
D <sub>3</sub>	10.55±1.33 <sup>ab</sup>
D <sub>4</sub>	10.08±0.88 <sup>b</sup>

D1: 0% ALM; D2: 2% ALM; D3: 5% ALM; D4: 7% ALM;

ALM= Alfalfa leaf meal; Values are means ± standard deviation; <sup>a,b</sup>Values in the same column with different superscripts are significantly different (P<0.05)

significantly (P<0.05) poorer feed conversion ratio when compared to those offered diet supplemented with zero, 2 or 5% ALM during the 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> weeks (Table 7).

Table (8) showed that ALM had no significant (P>0.05) effect on the egg shape index during the whole experimental period. Higher levels of ALM in the laying hens' diet (5% and 7%) resulted in a significant (P<0.05) increase in eggshell thickness compared to zero or 2% levels of ALM during the 2<sup>nd</sup>,

4<sup>th</sup> and 8<sup>th</sup> weeks (Table 9). During 2<sup>nd</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks, hens fed the diet supplemented with 5% ALM achieved significantly ( $P<0.05$ ) lower egg albumin height compared to those fed the diets supplemented with zero or 2% ALM (Table 10). Higher levels of ALM in the laying hens' diets (5% and 7%) resulted in a significantly ( $P<0.05$ ) lower egg Haugh Unit compared to those of zero or 2% levels during the 2<sup>nd</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks (Table 11). Only during the 6<sup>th</sup> week of the experimental period, addition of 2% ALM to laying hen diets' resulted in a significantly ( $P<0.05$ ) higher egg yolk index than other treatments (Table 12). Egg yolk colour was significantly ( $P<0.05$ ) improved by addition of 5% ALM to diet during the 2<sup>nd</sup> and 4<sup>th</sup> weeks, and by addition of 7% ALM during the 6<sup>th</sup> and 8<sup>th</sup> weeks (Table 13). Table 14 exhibited that supplementation of 7% ALM to laying hen diets' significantly ( $P<0.05$ ) reduced egg yolk cholesterol when compared to the control diet.

## Discussion

The findings observed in Table (1) were in disagreement with the findings reported by Elfaki (2009) who found alfalfa meal to be consisted of 17.5% crude protein, 26.65% crude fibre, 1.65% crude fat (ether extract) and 8.91% ash. This disagreement may be due to the different stages of growth at the plant cutting (Aganga and Omphile, 2005). The decrease in egg weight (Table 5) and hen-day egg production % (Table 6) in addition to poor feed conversion ratio (Table 7) disagreed with the findings of Yu-xin et al. (2004), Güçlü et al. (2004) and Khajali et al., (2007) who reported that addition of alfalfa meal (10%, 7% and 9%, respectively) to laying hens diet had no significant effect on feed intake, egg production and feed conversion ratio. Disagreement of these findings may be due to differences in varieties or stage of growth at cutting of the supplemented alfalfa.

The insignificant ( $P>0.05$ ) effect of supplementation of ALM to laying hens' diet on egg shape index (Table 8) was also reported by Yu-xin et al. (2004) and Khajali et al. (2007). An increase in eggshell thickness occurred as a result of offering laying hens' diets containing 5 and 7% ALM compared to those given diets supplemented with zero or 2% ALM (Table 9). This result was similar to that noticed by Yu-xin et al. (2004) and Güçlü et al. (2004), but disagreed with the finding reported by Khajali et al. (2007) who found that supplementation of alfalfa meal in laying hens' diet had no significant ( $P>0.05$ ) effect on eggshell thickness. The adverse effect produced by addition of 5% ALM to laying hen diets' on egg albumin height (Table 10) was in contrast to the result obtained by Khajali et al. (2007) who reported that addition of alfalfa meal to laying hens' diet had no

significant ( $P>0.05$ ) effect on egg albumin height. Egg Haugh Unit significantly ( $P>0.05$ ) decreased in 5 and 7% fed ALM compared to those fed diet containing zero or 2% ALM (Table 11). This finding disagreed with that observed by Yu-xin et al. (2004) who reported that offering laying hens diet supplemented with ALM resulted in significantly higher egg Haugh Unit. Significant ( $P>0.05$ ) improvement in egg yolk colour as a result of supplementation of diets with 5 and 7% ALM (Table 13) was expected due to the presence of higher level of xanthophylls in alfalfa meal (Fletcher and Papa, 1985). This result was in agreement with the result observed by Yu-xin et al. (2004) and Khajali et al. (2007). A reduction in egg yolk cholesterol was observed due to supplementation of diet with 7% ALM compared to control diet (Table 14) may be a result of saponins content of alfalfa meal which has been reported to have hypocholesterolaemic effect (Sidhu and Oakenfull, 1986). These findings were also reported in other studies (Güçlü et al., 2004; Cheng-zhang et al., 2005; Khajali et al., 2007; Wen-jun et al., 2007). Disagreement of some of the current findings with previous reported results may be due to the use of different varieties of alfalfa with different chemical compositions, or due to the inclusion of different levels of alfalfa meal.

## Conclusion

It was concluded that supplementation of 5% ALM to laying hens diet improved egg yolk colour and eggshell thickness without adverse effect on performance parameters, but level above 5% adversely affected performance parameters.

## References

- Aganga, A.A. and Omphile, U.J. 2005. Chemical composition of *Medicago sativa* (ALFALFA) cut at different stages of growth at Gaborone Botswana. *Journal of Animal and Veterinary Advances*, 4 (1): 1-2.
- Association of Official Analytical Chemists. 1990. Official Method of Analysis, 15th ed., Arlington, Virginia, USA. Pp: 807-809.
- Baraniak, B. 1995. The chemical composition of protein concentrate obtained from alfalfa juice mixed with buckwheat juice. *Current Advances in Buckwheat Research*, 2:855-860.
- Cheng-zhang, W., Yu-xin, Y., Xi-feng, H. Hong-xia, L. and Chun-mei, Z. 2005. Study on effect of *Medicago sativa* meal on egg yolk cholesterol concentration of layers. *Acta Pratacultural Science*, 2: D (Abstract).
- Duncan, D.B. 1955. Multiple Range and Multiple F-tests. *Biometrics*, 11: 1-42.
- Elfaki, A.E. 2009. Effects of different levels of alfalfa

- meal on layer, broiler performance and cholesterol content. Ph.D. Thesis, Sudan University of Science and Technology, Sudan.
- Fletcher, D. and Papa, C.M. 1985. Utilization and yolk colouring capability of dietary xanthophylls from yellow corn, corn gluten meal, alfalfa and coastal Bermuda grass. *Poultry Science*, 64: 1458-1463.
- Güçlü, B.K., Işcan, K.M., Uyanik, F., Eren, M. and Ağca, A.C. 2004. Effect of Alfalfa meal in diets on laying quails on performance, egg quality and some serum parameters. *Archives of Animal Nutrition*, 58 (3): 255-263.
- Khajali, F., Eshraghi, M., Zamani, F. and Fathi, E. 2007. Supplementation of exogenous enzymes to laying hen diets containing alfalfa: Influence upon performance and egg yolk cholesterol and pigmentation. Proceeding In: 16<sup>th</sup> European Symposium on Poultry Nutrition, France.
- Markovic, J., Radovic, J., Lugic, Z. and Sokolovic, D. 2007. The effect of development stage on chemical composition of alfalfa leaf and stem. *Biotechnology in Animal Husbandry*, 23: 383-388.
- NRC, National Research Council. 1994. Nutrient Requirement of Poultry. 9<sup>th</sup> (ed.), National Academic Press, Washington, DC.
- SAS Institute. 1990. SAS/STAT User's Guide: Statistics. Release 8.2 SAS Institute Inc., Cary, NC.
- Sidhu, G.S. and Oakenfull, P.G. 1986. A mechanism for the hypocholesterolaemic activity of saponins. *British Journal of Nutrition*, 55: 643-649.
- Steel, R.G.D. and Torrie, J.H. 1980. Principles and procedures of statistics. A Biochemical Approach. 2<sup>nd</sup> (ed.), McGraw-Hill, New York, NY.
- Wen-jun, G., Kuan-hu, D. and Xian-jun, H. 2007. Effect of adding alfalfa leaf meal to hen diet on serum parameters in the late part of laying stage. *Livestock and Poultry Industry*, 10: 2 (Abstract).
- Yu-xin, Y., Cheng-zhang, W., Hong-xia, L., Chun-mei, Z. and Xi-feng, H. 2004. Effect of alfalfa meal on production performance, egg quality and egg yolk colour of layer. *Journal of Huazhong Agricultural*, 3: 8 (Abstract).