

## **EGG YOLK CHOLESTEROL AND PRODUCTIVE PERFORMANCE OF LAYING HENS INFLUENCED BY DIETARY CRUDE FIBER LEVELS UNDER DRINKING NATURAL SALINE WATER**

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### **ABSTRACT**

This study was conducted to investigate the influenced of four levels of dietary crude fibers (CF) on egg yolk cholesterol, productive and physiological performance of laying hens under drinking natural saline water (2000 ppm total dissolved solids). A total number of 120 White Leghorn layers from 44 to 56 weeks of age randomly divided into four equal groups (30 hens of each). Four levels of CF (3, 4, 5 and 6 %) as substitution of alfalfa meal. The treatments were tested for 12 weeks. The results showed that under condition of drinking natural saline water (2000 ppm total dissolved solids), feeding laying hens control diet which contained 3 % crude fiber had ( $P<0.05$ ) highest egg number, egg mass and feed consumption (g/day) (17.97, 30.99 g and 67.80 g, respectively) compared with other three groups which recorded no significant differences among each other's. Hens fed the highest-fiber diet (6 %) laid eggs with greatest shell, albumin and yolk weights % (4.53, 58.00 and 36.19, respectively) compared with other experimental groups. Feeding hens diet contained higher crude fiber level above 3 % had higher ( $P<0.05$ ) values of albumen height (mm) and shape index values with no significant differences in yolk index (%) and Haugh units. There were a gradual ( $P<0.05$ ) decrease in digestion coefficients of DM, OM, CP, ash, CF, NFE and EE with increasing crude fiber levels in hens diets; while, there were no significant differences in digestibility of fiber fractions (NDF, ADF and hemicelluloses). Water intake (ml/ bird/day) and water/feed intake increased ( $P<0.05$ ) with increasing crude fiber levels. So, hens fed 6 % CF consume more water being 236.00 ml/bird/day and 3.45 ml/g feed intake. Inclusion of graded levels of CF in laying hen diets tended to decrease ( $P<0.05$ ) concentrations of total lipids, triglycerides, phospholipids, cholesterol, total cholesterol, and low-density lipoproteins (LDL) in egg yolk. Hens fed diet contained 6 % CF decreased the egg yolk concentrations of the same respective parameters by 25.10, 22.22, 26.61, 22.38, 19.51 and 23.33 %, respectively compared to the control group. There were a significant decrease in serum total lipids (g/dl), cholesterols (mg/dl), triglyceride (mg/dl) and HDL values with increasing CF levels; however, AST and ALT activity was within a normal physiological range. Gizzard (%), edible giblets (%), digestive tract weight and digestive tract length had significantly increased with increasing dietary CF level; while, there were non-significant increases in carcass, liver and heart (%). The best value for economic efficiency and relative economic efficiency (117 %) had been recorded by hens fed diet contained 6 % CF as compared with the control (3 % CF).

It was concluded that, we can used crude fiber by up to 6 % in laying hens diets (44 to 56 weeks of age) reared under drinking natural saline to achieve acceptable productive and physiological performance and reduce the egg yolk cholesterol.

**Keywords:** Crude fiber, laying hens, productive performance, egg yolk cholesterol, blood metabolites, natural saline water.

## INTRODUCTION

Although eggs possess protein of significant biological value and are an excellent source of vitamins and minerals, many people limit their consumption of eggs because they associated high cholesterol content (1 large egg, 50 g, contains 213 mg of cholesterol) with cardiovascular disease (Zeidler, 2002). Higher cholesterol contents are an indication of higher fat deposition (Tewe and Bokanga 2001) which is not desirable in layers. Moreover, Butarbutar (2004) mentioned that elevated serum cholesterol in human has been strongly correlated with consuming greater amounts of cholesterol than normal, reducing the amount of high-cholesterol foods such as eggs will help reduce blood cholesterol levels (Weggemans *et al.*, 2001). Attention has recently focused on identifying ingredients or production methods that can facilitate a reduction in egg cholesterol; therefore, research centered mostly on diet composition (Jacob and Miles 2000).

Feeding of high fiber diets, however, is used as a strategy to control growth in some types of poultry such as chicken pullets to prevent excessive growth (Hester and Stevens, 1990); dietary fiber has been implicated in recent years as causing a reduction in serum and body cholesterol which referred to a natural hypocholesteremic agent. James and McNaughton (2012) found that non digestible component of animal's diets have major influence on both plasma cholesterol concentrations and turnover, as well as fecal excretion. Increasing dietary fiber has been shown to significantly decrease serum cholesterol and/or artery deposition of plaque in laying hens (Burr *et al.* 1985). Alfalfa, when added to a corn-soy laying hen diet, was the most effective of the fiber sources tested for decreasing egg cholesterol with the least loss of egg size, feed efficiency, and egg production (Zhang *et al.*, 2005). On the other hand; Menge *et al.* (2012) found that increasing the dietary fiber level from 4.1 to 17.7 % caused a reduction in serum cholesterol and an increase in egg yolk cholesterol. Story and Kritchevsky (1976) found that cellulose bound an average of 1.4 % of all the bile acids tested, whereas alfalfa bound 15.9 %; thus, alfalfa was most successful in reducing bile acids. During digestion in the intestine, cholesterol is the main component of bile acids secreted. The fiber coats the bile acids in the intestine and is excreted in the body, subsequently causing the body to draw cholesterol from the blood to form bile acids, and thus lowering blood cholesterol level (Zhang *et al.*, 2005). Results of this study attest to the hypocholesterolemic properties of crude fiber. Therefore, experiments were conducted to determine the effect of crude fiber level on yolk and body cholesterol and productive performance of laying hens using natural fiber sources (Alfalfa meal) that might be added to practical laying hen diets under drinking natural saline water.

## MATERIALS AND METHODS

The present work was carried out at South Sinai Experimental Research Station (Ras-Suder City) which belongs to the Desert Research Center. An experiment were conducted using 120 White Leghorn layers from 44 to 56 week of age to evaluate the effect of dietary crude fiber levels on

egg yolk cholesterol and layer performance. Laying hens were randomly taken and distributed in four experimental groups. Each group contained 30 birds. Birds in all treatments were reared under similar hygienic and managerial conditions and randomly divided into three equal replicates (10 hens each).

Experimental diets (Table 1), the basal diet was modified by substitution of alfalfa meal for diets control (3 %), 4, 5 and 6 % crude fiber, adjustments were made in the amount of corn and soybean meal in order to keep the diets at approximately equal protein (17 %) and energy content (2800 kcal ME /kg) which formulated to meet Hassan *et al.* (2012) recommendations under drinking natural saline water 2000 ppm (Table 2). Feed and water were offered *ad-libitum*. All hens were kept under the same managerial and environmental conditions and artificial lighting (16 hours of light per day) through the experimental periods. Body weights were recorded at the beginning and at the end of the experiment (40 and 52 weeks of age, respectively). Egg weight and egg number were recorded daily to calculate the egg production percentage and egg mass (g/hen/day). Feed consumption (g/hen/day) and feed conversion values (g feed /g eggs) were recorded biweekly.

**Table (1): Chemical composition (%) of experimental diets**

Ingredients (%)	Experimental diets (Crude fiber levels)			
	Control (3 %)	4 %	5 %	6 %
Yellow corn	58.70	56.90	54.50	53.00
Soybean meal (44 % CP)	25.00	21.00	18.50	16.30
Corn gluten meal (60 %)	1.50	4.00	5.00	5.70
Wheat bran	0.00	0.50	0.00	0.00
Vegetable oil	2.70	3.00	3.50	3.50
Alfaalfa meal*	0.00	4.00	8.00	11.50
Limestone	8.50	7.00	7.00	6.50
Dicalcium phosphate	2.60	2.60	2.50	2.50
NaCl	0.30	0.30	0.30	0.30
L-Lysine-HCl	0.10	0.10	0.10	0.10
DL- Methionine	0.10	0.10	0.10	0.10
Vit& Min premix**	0.30	0.30	0.30	0.30
Choline chloride	0.20	0.20	0.20	0.20
Total	100	100	100	100
<b>Calculated analysis</b>				
ME, K cal/kg	2796	2805	2776	2715
Crude protein (%)	17	17	16.90	16.63
C/P ratio	164	164	164	164
Calcium (%)	4.20	4.20	4.20	4.20
T. Phosphorus (%)	0.75	0.75	0.75	0.75
Lysine (%)	0.90	0.90	0.90	0.90
Methionine & Cystine (%)	0.70	0.70	0.70	0.70
Crude fiber (%)	3.00	4.00	5.00	6.00

\*Alfaalfa, meal: 90.0 % DM, 12%CP, 2.1%EE, 30 %CF, 40% ADF, 56% NDF, 82% Lig., 32.5%Calcium, 0.25% T. Phosphorus, 0.54 % Lys., 0.33% Met.+Cys, 900 ME(Kcl/Kg).

\*\* Each 3 Kg of Vit and Min. premix contains: 10000000 IU Vit. A; 2000000 IU.vitD3; 10000mg Vit.E; 1000mg Vit.K3; 1000mg Vit. B1;5000mgVit. B2; 10 mg Vit. B12; 1500mg Vit . B6;30000mg Niacin; 10000mg Pantothenic acid; 1000mg Folic acid ;50mg Biotin; 300000 mg Choline; 4000 mg Copper; 300mg Iodine; 30000 mg Iron; 50000 mg Zinc; 60000 mg Manganese; 100mg Selenium; 100mg Cobalt and CaCO3 as carrier to 3000g.

**Table (2): Chemical analysis of Well water**

Parameters	Well water
Total dissolved solids (mg/l)	2000
Electric conductivity (ds/m)	3.68
pH	7.50
Sodium (Na <sup>+</sup> , mg/l)	30.27
Potassium (K <sup>+</sup> , mg/l)	0.22
Calcium (Ca <sup>++</sup> , mg/l)	6.17
Magnesium (Mg <sup>++</sup> , mg/l)	7.83
Hardness (mg/l)	14.00
Carbonate (CO <sub>3</sub> <sup>-</sup> , mg/l)	0.33
Bicarbonate (HCO <sub>3</sub> <sup>-</sup> , mg/l)	2.73
Chloride (Cl <sup>-</sup> , mg/l)	22.09

At the end of the experiment, egg quality parameters were measured using 80 eggs (20 eggs / each treatment group). These measurements involved yolk, albumen and shell weight percentage. Egg shell thickness was measured in  $\mu\text{m}$  using a micrometer; Egg surface area (ESA) was calculated according to Paganelli *et al.* (1974) as  $ESA = (4.835 \times W^{0.662} \text{ Cm}^2)$  where  $W$ =Egg mass in grams. Shell weight per unit surface area (SWUSA) was calculated by dividing shell weight (including the adhering membranes) in mg by egg surface area (ESA) and specific gravity according to Harms *et al.* (1990). Egg shape index was computed as the ratio of egg width to the length (Awosanya *et al.*, 1998). Yolk index was calculated according to Funk *et al.* (1958), as yolk height divided by yolk diameter. Haugh unit was calculated according to Eisen *et al.* (1962) using the calculation chart for rapid conversion of egg weight and albumen height.

At the end of the experimental feeding period, digestion trial was conducted using 12 White Leghorn hens (three from each treatment) to determine the digestion coefficients of the experimental diets. Hens were individually housed in metabolic cages. The digestibility trials extended for 9 days; 5 days as a preliminary period followed by 4 days as collection period. During the main period, excreta were collected daily and weighed, dried at 60 °C, bulked, finally ground and stored for chemical analysis. The faecal nitrogen was determined according to Jakobsen *et al.* (1960). The digestion coefficients (%) of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) of the experimental diets were estimated chemical analysis of the experimental diets and faeces were assayed using methods of the Association Official Analytical Chemists (A.O.A.C., 1990). Four hens from each treatment were randomly chosen for slaughter test and carcass parts were weighed and calculated as a percentage of live body weight.

Blood samples were withdrawn (three times during experiment periods) from the wing vein into tube. Serum was collected to determine total protein, total lipid, glucose, triglycerides, cholesterol, alanine transaminase (ALT), aspartic transaminase (AST) and high density lipoprotein (HDL) calorimetrically by using commercial kits.

Eggs were collected for chemical analysis during the last 3 days of the experimental period. Twelve eggs per each treatment were taken at

random, and then were weighted, cracked and their yolks were separated. Then each 4 yolks were pooled and homogenized and considered one samples i.e. each treatment had 3 samples of these pooled egg yolks for chemical analysis.

These samples of the pooled yolks were freeze and stored at -20 °C until the chemical analysis was performed. Egg yolk samples were analyzed for total lipids, triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and phospholipids. Total lipid content of egg yolk was determined gravimetrically after extraction with chloroform: methanol (2:1) according to (Folch *et al.*, 1957). Triglycerides content of egg yolk was determined calorimetrically according to the method of (Lowell *et al.*, 1973). The developed color was read at 410 nm.

The economic efficiency of the experimental diet was calculated based upon the differences in both selling revenue and feeding cost, which was calculated according to the price of the experimental diets and egg production during the year of 2013. Data obtained were statistically analyzed using the general Linear Model Procedure (SAS, 1996), all the characteristics were performed in conformity by one way analysis model. Duncan's multiple range tests was used to test the significance of mean differences (Duncan, 1955).

## **RESULTS AND DISCUSSION**

### **1. Productive performance and feed utilization**

Dietary fiber levels had no significant effects on final body weights and egg weight (Table 3). These results are in agreement with the results of Amjad and Yasin (2008). Hens fed control diet which contained 3 % crude fiber had highest ( $P<0.05$ ) egg number, egg mass and feed consumption (17.97, 30.99 and 67.80, respectively) compared with other three groups which recorded no significant differences among each other's. On the other hand, increasing dietary fiber level had delayed ( $P<0.05$ ) the feed conversion values with any significant differences among the three levels of 4, 5 or 6 %. These results are in agreement with that of Menge *et al.* (2012).

### **2. Egg quality measurements**

Data of egg quality measurements for 56 weeks old Leghorn laying hens fed diets containing different CF levels are presented in Table (4). In our results, hens fed the highest-fiber diet (6 %) laid eggs with greatest shell, albumin and yolk weights (4.53, 58.00 and 36.19 %, respectively) compared with other experimental groups. These results are in agreement with Hassan (2011), whereas disagreed with those of Menge *et al.* (2012) who reported that Egg yolk weights numerically decreased as fiber was increased in laying hen diets.

**Table (3): Body weight, feed utilization and productive performance (Means  $\pm$ SE) of White leghorn hens as affected by dietary crude fiber (CF) levels**

Crude fiber levels	Parameters			
	Initial body weight (g)	Final body weight (g)	Egg production (%)	Egg Number
Control (3 %)	1355 $\pm$ 21.38	1367 $\pm$ 60.22	62.40 <sup>a</sup> $\pm$ 2.73	17.97 <sup>a</sup> $\pm$ 0.73
4 %	1334 $\pm$ 19.97	1350 $\pm$ 49.00	59.90 <sup>a</sup> $\pm$ 0.73	16.40 <sup>b</sup> $\pm$ 0.56
5 %	1340 $\pm$ 33.45	1353 $\pm$ 53.40	57.00 <sup>b</sup> $\pm$ 1.98	16.50 <sup>b</sup> $\pm$ 0.44
6 %	1350 $\pm$ 43.00	1365 $\pm$ 25.00	56.87 <sup>b</sup> $\pm$ 0.73	16.46 <sup>b</sup> $\pm$ 0.32
	Egg weight (g)	Egg mass (g/hen/day)	Feed consumption (g/day)	Feed conversion (g/feed/g egg)
Control (3 %)	48.28 $\pm$ 1.00	30.99 <sup>a</sup> $\pm$ 1.48	67.80 <sup>a</sup> $\pm$ 2.98	2.19 <sup>b</sup> $\pm$ 0.11
4 %	48.33 $\pm$ 1.80	28.31 <sup>b</sup> $\pm$ 1.00	64.00 <sup>b</sup> $\pm$ 2.09	2.26 <sup>a</sup> $\pm$ 0.10
5 %	48.40 $\pm$ 0.98	28.52 <sup>b</sup> $\pm$ 1.22	63.10 <sup>b</sup> $\pm$ 1.96	2.21 <sup>a</sup> $\pm$ 0.07
6 %	47.95 $\pm$ 1.19	28.19 <sup>b</sup> $\pm$ 1.09	62.45 <sup>b</sup> $\pm$ 3.08	2.22 <sup>a</sup> $\pm$ 0.03

a, b Means in the same column in each classification bearing different letters differ significantly ( $P \leq 0.05$ ).

**Table (4): Egg components and egg quality parameters (Means  $\pm$ SE) of White leghorn hens as affected by dietary crude fiber levels**

Crude fiber levels	Egg components			
	Egg weight (g)	Shell weight (%)	Yolk weight (%)	Albumen weight (%)
Control (3 %)	45.68 $\pm$ 0.93	3.97 <sup>c</sup> $\pm$ 0.19	36.18 <sup>a</sup> $\pm$ 0.73	54.80 <sup>b</sup> $\pm$ 0.67
4 %	45.50 $\pm$ 0.77	4.14 <sup>b</sup> $\pm$ 0.12	35.10 <sup>c</sup> $\pm$ 0.18	55.97 <sup>a</sup> $\pm$ 0.70
5 %	45.70 $\pm$ 0.58	4.42 <sup>ab</sup> $\pm$ 0.23	33.37 <sup>b</sup> $\pm$ 0.88	57.78 <sup>a</sup> $\pm$ 0.23
6 %	45.40 $\pm$ 0.91	4.53 <sup>a</sup> $\pm$ 0.13	36.19 <sup>a</sup> $\pm$ 0.96	58.00 <sup>a</sup> $\pm$ 0.25
	Exterior quality			
	Egg specific gravity	Shell thickness (mm)	ESA (cm <sup>2</sup> )	SWUSA (mg/cm <sup>2</sup> )
Control (3 %)	1.0098 $\pm$ 0.03	0.334 $\pm$ 0.01	43.89 <sup>b</sup> $\pm$ 1.40	94.39 $\pm$ 3.41
4 %	1.0097 $\pm$ 0.03	0.341 $\pm$ 0.01	44.81 <sup>a</sup> $\pm$ 1.38	96.37 $\pm$ 2.00
5 %	1.0096 $\pm$ 0.02	0.339 $\pm$ 0.00	44.54 <sup>a</sup> $\pm$ 1.05	95.80 $\pm$ 2.15
6 %	1.0098 $\pm$ 0.04	0.340 $\pm$ 0.01	44.68 <sup>a</sup> $\pm$ 1.11	96.09 $\pm$ 1.66
	Interior quality			
	Albumen height (mm)	Haugh units	Shape index	Yolk index (%)
Control (3 %)	4.76 <sup>b</sup> $\pm$ 0.03	72.00 $\pm$ 0.65	73.18 <sup>b</sup> $\pm$ 0.97	42.17 $\pm$ 0.73
4 %	5.30 <sup>a</sup> $\pm$ 0.22	71.02 $\pm$ 0.54	75.00 <sup>a</sup> $\pm$ 0.47	42.00 $\pm$ 0.33
5 %	5.34 <sup>a</sup> $\pm$ 0.11	70.00 $\pm$ 0.43	75.43 <sup>a</sup> $\pm$ 0.88	41.90 $\pm$ 0.17
6 %	5.51 <sup>a</sup> $\pm$ 0.02	71.11 $\pm$ 0.87	75.98 <sup>a</sup> $\pm$ 0.69	42.08 $\pm$ 0.51

a,b,c, Means in the same column in each classification bearing different letters differ significantly ( $P \leq 0.05$ ).

### 2.1. Exterior quality

Hens fed diet contained 6 % crude fiber recorded the highest ( $P < 0.05$ ) values of egg surface area (ESA) by 1.79 % as compared with hens fed 3 % crude fiber, however, values of shell thickness, egg specific

gravity and SWUSA showed no significant differences among experimental groups. Results of a study on laying birds fed some agro-industrial by products (with different crude fiber contents) revealed no significant effect of dietary treatments on egg shell thickness which were all well above the value (0.3 mm) reported to be optimum for thickness of chicken egg shells which will not adversely result in breakages (Donkoh1 and Zanu, 2010).

**2.2. Interior quality**

Feeding hens diet contained higher crude fiber level above 3 % has higher ( $P < 0.05$ ) values of albumen height (mm) and shape index values with no significant differences on yolk index % and haugh units. This means that diets contained high level in crude fiber (such as by products or roughages) did not show any adverse effects on the haugh unit values of eggs (Donkoh1 and Zanu, 2010). On the other hand, El-Deek *et al.* (2009) reported that fed laying hens with different levels of crude fiber showed no significant effect on yolk index and significantly higher shell thickness than those fed the control diet.

**3. Digestion coefficients (%)**

Data presented in Table (5) indicated that there were a gradual decrease ( $P < 0.05$ ) in digestion coefficients of DM, OM, CP, ash, CF, NFE and EE with increasing crude fiber levels in hens diets; while, there were no significant differences on digestibility of fiber fractions (NDF, ADF and hemicelluloses). Nelson *et al.* (2007) reported a reduction in body weight for birds fed on agro-industrial by-products based diet which may due to addition of fiber to the diet can lead to a lower apparent digestibility coefficients.

**Table (5): Digestion coefficients (%) and water intake of nutrients as affected by dietary crude fiber (CF) levels**

Crude fiber levels	Digestion coefficients (%)			
	DM	OM	CP	ASH
Control (3 %)	81.22 <sup>a</sup> ±4.06	84.51 <sup>a</sup> ±3.42	78.21 <sup>a</sup> ±2.56	75.41 <sup>a</sup> ±5.46
4 %	80.00 <sup>ab</sup> ±1.06	79.39 <sup>b</sup> ±2.41	67.33 <sup>a</sup> ±2.25	61.10 <sup>b</sup> ±4.03
5 %	78.52 <sup>b</sup> ±5.01	75.51 <sup>b</sup> ±5.00	64.52 <sup>b</sup> ±2.34	54.02 <sup>c</sup> ±4.06
6 %	69.06 <sup>c</sup> ±2.13	62.42 <sup>c</sup> ±3.00	60.02 <sup>c</sup> ±2.76	46.11 <sup>c</sup> ±5.44
	CF	EE	NFE	NDF
Control (3 %)	18.45 <sup>a</sup> ±6.05	90.75 <sup>a</sup> ±7.06	91.31 <sup>a</sup> ±1.91	24.96±3.45
4 %	16.60 <sup>b</sup> ±5.12	74.53 <sup>b</sup> ±6.87	86.82 <sup>ab</sup> ±1.01	21.38±2.90
5 %	14.51 <sup>c</sup> ±4.54	71.80 <sup>b</sup> ±4.45	84.42 <sup>b</sup> ±1.00	22.89±3.91
6 %	12.92 <sup>c</sup> ±5.26	69.00 <sup>b</sup> ±4.36	74.59 <sup>c</sup> ±2.00	20.27±1.88
	ADF	Hemicelluloses	Water intake (ml/ bird/day)	Water ml/g feed intake
Control (3 %)	9.84±3.00	15.12±4.93	191.00 <sup>c</sup> ±24.48	2.82 <sup>b</sup> ±0.90
4 %	6.14±3.45	15.24±2.12	206.40 <sup>b</sup> ±45.00	3.04 <sup>a</sup> ±0.58
5 %	5.58±3.04	17.31±3.10	220.00 <sup>b</sup> ±60.00	3.23 <sup>a</sup> ±0.40
6 %	5.84±3.00	15.43±2.15	236.00 <sup>a</sup> ±47.00	3.45 <sup>a</sup> ±0.38

a,b,c Means in the same column in each classification bearing different letters differ significantly ( $P \leq 0.05$ ).

Feeds high in crude fiber, such as roughages, will require more water for ingestion than feeds low in crude fiber. Because water functions as a lubricant in the transport of feed and aids in the excretion of waste products from the body, the intake must equal the output lost through urine, feces and evaporation. As an example, during protein metabolism, uric acid and urea are produced and must be removed through the kidneys. Water is needed to dissolve the urea, uric acid, phosphates and other minerals for easy passage through the urinary tract (Hellee *et al.*, 2009). It was clear in our study that water intake (ml/bird/day) and water ml/g feed intake increased ( $P < 0.05$ ) with increasing crude fiber levels; So, hens fed 6 % CF consume more water being 236.00 ml/ bird/day and 3.45 ml/g feed intake. Aderemi *et al.* (2006) refer to the relation between feed intake, water intake and digestion mechanism; that the decrease in feed intake of some by products based diet could be due to high fiber ingestion which led to accumulate increase water intake that created a stomach fill sensation and a subsequent depression of appetite.

#### 4. Egg yolk composition

Inclusion of graded levels of CF in laying hen diets tended to decrease ( $P < 0.05$ ) concentrations of total lipids, triglycerides, phospholipids, cholesterol, total cholesterol, and low-density lipoproteins (LDL) in egg yolk (Table 6). Hens fed diet contained 6 % CF decreased the egg yolk concentrations of same respective parameters by 25.10, 22.22, 26.61, 22.38, 19.51 and 23.33 %, respectively compared to the control group (Table 6); such decreases could be attributed to high fiber content of Alfalfa meal (Table 1). Similar reports were obtained by Hashish and Abd El-Samee (2012) who demonstrated that yolk cholesterol of yolk and cholesterol per yolk decreased as hens were fed increasing dietary fiber levels.

**Table (6): Egg yolk composition (Means  $\pm$ SE) of White leghorn hens as affected by dietary crude fiber levels**

Crude fiber levels	Egg yolk composition			
	Yolk weight (g)	Total lipids (g/yolk)	Triglyceride (g/yolk)	Phospholipids (g/yolk)
Control (3 %)	16.53 $\pm$ 0.23	2.55 <sup>a</sup> $\pm$ 0.01	1.62 <sup>a</sup> $\pm$ 0.06	0.496 <sup>a</sup> $\pm$ 0.01
4 %	15.97 $\pm$ 0.13	2.10 <sup>b</sup> $\pm$ 0.11	1.47 <sup>b</sup> $\pm$ 0.04	0.423 <sup>b</sup> $\pm$ 0.01
5 %	16.25 $\pm$ 0.58	2.12 <sup>b</sup> $\pm$ 0.04	1.41 <sup>b</sup> $\pm$ 0.01	0.421 <sup>b</sup> $\pm$ 0.02
6 %	17.15 $\pm$ 0.93	1.91 <sup>c</sup> $\pm$ 0.03	1.26 <sup>c</sup> $\pm$ 0.09	0.364 <sup>c</sup> $\pm$ 0.01
	Cholesterol (mg/g yolk)	Total cholesterol (g/yolk)	LDL (mg/yolk)	HDL (mg/yolk)
Control (3 %)	223.16 <sup>a</sup> $\pm$ 0.13	3.69 <sup>a</sup> $\pm$ 0.01	30.00 <sup>a</sup> $\pm$ 0.01	6.45 $\pm$ 0.11
4 %	193.24 <sup>b</sup> $\pm$ 0.13	3.09 <sup>b</sup> $\pm$ 0.13	26.00 <sup>b</sup> $\pm$ 0.02	6.32 $\pm$ 0.01
5 %	193.38 <sup>b</sup> $\pm$ 0.58	3.14 <sup>b</sup> $\pm$ 0.13	25.00 <sup>b</sup> $\pm$ 0.04	6.14 $\pm$ 0.13
6 %	173.22 <sup>c</sup> $\pm$ 0.93	2.97 <sup>c</sup> $\pm$ 0.13	23.00 <sup>c</sup> $\pm$ 0.06	6.10 $\pm$ 0.01

a,b,c, Means in the same column in each classification bearing different letters differ significantly ( $P \leq 0.05$ ).

Total cholesterol per yolk decreased ( $P < 0.05$ ) by 4.39, 10.38 and 13.29 % by feeding crude dietary fiber levels of 4.41, 6.68, and 8.79 %, respectively; to hens as compared to a corn-soybean meal basal diet containing 2.05 % crude fiber (James and McNaughton, 2012).

Based on the hypothesis formulated by Story *et al.* (1979) that increased excretion of bile acids and bile salts induced by the ingestion of fiber would lead to a reduction in the cholesterol pool, a reduction in yolk cholesterol concentration might have been expected as a result of the marked increase in dietary fiber intake by the hens. It affects cholesterol metabolism of laying hens by decreasing absorption of cholesterol, binding with bile salts in the intestinal tract (Hargis, 1988).

The major egg yolk phospholipids are phosphatidyl choline and phosphatidyl ethanolamines which are lower cholesterol reduced liquid egg yolk than control. This is probably due to their partial absorption and precipitation with O-cyclodextrin during the cholesterol reduction process (Awad *et al.*, 1997). On the other hand, some researchers refer to steroids compounds (saponin) that contained in some feeds materials like Alfalfa meal. The hyp-cholesterolemic action of saponins is thought to be mediated in part by their binding cholesterol and bile acids in the intestinal lumen, thus enhancing the excretion of these steroids in the faeces (Francis *et al.*, 2002). As a result, cholesterol metabolism is accelerated and its circulating levels are decreased. Also, McGonigle and McCracken (2002) fed laying hens 20 % source of steroid saponins (steroidal saponin) and observed no effect on yolk weight or cholesterol content. Kaya *et al.* (2003) considering that laying hens are normally fed little, if any dietary cholesterol, the lack of effect of dietary saponins on plasma and egg yolk cholesterol concentrations is not surprising given that the hypocholesterolemic action of these compounds may be primarily mediated via cholesterol binding in the intestine.

#### **5. Blood constituents**

Serum parameters of Leghorn laying hens, measured in the study were estimated to show the metabolic and health status of hens as affected by feeding different levels of CF (Table 7). There were a significant decrease in total lipids, cholesterols, triglyceride and high density of lipoprotein (HDL) values with increasing CF levels. However, serum glucose insignificantly decreased ( $P \geq 0.05$ ) with increasing CF levels. On the other hand, AST and ALT concentrations give an indication of liver function in animals. In the present study, AST and ALT activity was within a normal physiological range, indicating no damage to the liver as increases of their activities have been reported to be associated with hepatic necrosis and other disease conditions related to histopathological changes (Abdel Rahman *et al.*, 2000). Literatures in many ways had demonstrated the effects of some factors on blood parameters as followed; Oladunjoye, *et al.* (2010) refers to lower egg yolk cholesterol values observed in birds at 70 and 80 % inclusion level can be attributed to higher fiber content of the diets. This supports the hypothesis that increased dietary fiber often result in reduction in availability of cholesterol for incorporation into lipoproteins (Karadjole *et al.* (1999).

**Table (7): Some Blood constituents (Means  $\pm$ SE) of White leghorn hens as affected by dietary crude fiber levels**

Crude fiber levels	Blood constituents			
	Total protein (g/dl)	Total Lipids (g/dl)	AST (i. u./l)	ALT (i. u./l)
Control (3 %)	9.97 $\pm$ 0.31	49.17 <sup>a</sup> $\pm$ 1.00	24.20 $\pm$ 0.10	11.12 $\pm$ 0.15
4 %	10.02 $\pm$ 0.10	45.11 <sup>a</sup> $\pm$ 1.20	25.01 $\pm$ 0.30	11.35 $\pm$ 0.13
5 %	10.56 $\pm$ 0.50	41.03 <sup>b</sup> $\pm$ 1.31	25.23 $\pm$ 0.10	11.72 $\pm$ 0.10
6 %	11.01 $\pm$ 0.21	39.90 <sup>b</sup> $\pm$ 1.22	25.00 $\pm$ 0.10	11.53 $\pm$ 0.18
	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL
Control (3 %)	224.00 $\pm$ 14.00	128.24 <sup>a</sup> $\pm$ 11.13	760.00 <sup>a</sup> $\pm$ 12.43	68.02 <sup>a</sup> $\pm$ 4.10
4 %	213.00 $\pm$ 12.10	87.30 <sup>bc</sup> $\pm$ 5.58	550.27 <sup>b</sup> $\pm$ 21.63	24.30 <sup>b</sup> $\pm$ 2.48
5 %	209.00 $\pm$ 9.00	91.20 <sup>c</sup> $\pm$ 6.92	509.00 <sup>b</sup> $\pm$ 26.13	25.50 <sup>b</sup> $\pm$ 0.69
6 %	203.00 $\pm$ 4.13	99.04 <sup>c</sup> $\pm$ 13.03	386.24 <sup>c</sup> $\pm$ 34.10	21.05 <sup>c</sup> $\pm$ 3.00

a,b,c Means in the same column in each classification bearing different letters differ significantly (P $\leq$  0.05).

Cholesterol concentration was significantly lower when laying hens were fed diets containing alfalfa meal, wood shavings, oats, or rice mill feed when compared to laying hens fed a basal diet. The mechanism of egg cholesterol reduction is thought to be through the lowering of plasma concentrations of LDL (James and McNaughton, 2012). During digestion in the intestine, cholesterol is the main component of bile acids secreted. The fiber coats the bile acids in the intestine and is excreted in the body, subsequently causing the body to draw cholesterol from the blood to form bile acids, and thus lowering blood cholesterol level (Yukio and Tatsuro, 2011). Demonstrated mechanisms responsible for this cholesterol-lowering effect include viscosity effects in the gut, bile acid binding capacity and, possibly, the capability to inhibit hepatic cholesterol synthesis via short-chain fatty acids generated by colonic fermentation of viscous fibers (Wu *et al.*, 2003).

Generally, feeding fiber to laying hens also dilutes the available energy content of a diet and, as a result, may limit energy intake and potentially reduce hepatic cholesterol production, especially if prior energy intake had been excessive (Naber, 1990). This may partially explain the observations of Weiss and Scott (1979), who fed laying hens diets containing 50% wheat bran, oat hulls, or alfalfa meal substituted iso-nitrogenously for part of the corn and soybean meal in the control diet and reported that yolk cholesterol contents were decreased by 19.8% (wheat bran), 16.2 % (oat hulls), and 7.8% (alfalfa). James and McNaughton (2012) found no significant differences in cholesterol level were found due to dietary fiber level. However, plasma triglycerides decreased as hens were fed diets with increasing dietary fiber levels. Triglycerides level decreased as dietary fiber increased. Menge, *et al.* (2012) reported that increasing the dietary fiber level from 4.1 to 17.7 % by the addition of cellulose reduced serum cholesterol but increased the cholesterol concentration in the yolk and differences in yolk triglyceride levels were not significant.

### 6. Carcass traits

Data in the present study showed that gizzard (%), edible giblets (%), digestive tract weight (g) and digestive tract length (cm) had significantly increased with increasing dietary CF level, while there were non-significant increases in carcass, liver and heart % (Table 8). There were reversed opinions; Yukio and Tatsuro (2011) cellulose feeding did not result in any significant effect on absolute weight of the above organs. However; Zhang *et al.* (2005) reported that high fiber consumption on by-product diets was increased gizzard weights in both, probably related to volume of feed, increased time spent grinding the feed and increased frequency of mechanical contraction of the gizzard muscle for further feed digestion in the intestine. Results of Graham and Aman (1991) summarized that gizzard weight a percentage of body weight was significantly influenced by dietary energy and fiber contents of the diets.

**Table (8): Some Carcass traits (Means ±SE) of White leghorn hens as affected by dietary crude fiber levels**

Crude fiber levels	Carcass traits			
	Pre-slaughter weight (g)	Carcass (%)	Liver (%)	Gizzard (%)
Control (3 %)	1573.06±14.22	64.71±1.47	1.37±0.02	1.91 <sup>b</sup> ±0.09
4 %	1578.00±0.01	64.62±1.23	1.25±0.01	2.36 <sup>ab</sup> ±0.03
5 %	1564.11±12.00	65.56±1.00	1.25±0.01	2.03 <sup>b</sup> ±0.01
6 %	1644.23±11.02	74.29±1.08	1.28±0.01	2.61 <sup>a</sup> ±0.01
	Heart (%)	Edible giblets* (%)	Digestive tract weight (g)	Digestive tract length (cm)
Control (3 %)	0.61± 0.001	3.89± 0.43	2.00 <sup>b</sup> ±0.10	97.11 <sup>d</sup> ±0.33
4 %	0.59±0.001	4.20± 0.12	2.10 <sup>b</sup> ±0.11	117.20 <sup>c</sup> ±0.15
5 %	0.56±0.001	3.64± 0.33	2.55 <sup>a</sup> ±0.10	127.09 <sup>b</sup> ±0.44
6 %	0.61±0.001	4.50± 0.21	2.96 <sup>a</sup> ±0.09	143.00 <sup>a</sup> ±0.35

a,b,c,d Means in the same column in each classification bearing different letters differ significantly (P ≤ 0.05).

\*Edible giblets (%) = liver + gizzard + heart.

### 7. Economical efficiency

Results of economical efficiency (EE) and relative economical efficiency (REE) estimated for different treatments during experiment are shown in Table (9). According to the input-output, economical efficiency and relative economical efficiency were ranged between 1.15-1.34 and 100-117 % for the control and experimental treatments. The best value for EE and REE had been recorded by hens fed diet contained 6 % CF as compared with the control group (3 % CF). This may be due to the prices of the test ingredients and also had good egg mass and good revenue. On the other hand, Donkohand Zanu (2010) reported that the results of the cost-benefit analysis derived from feeding various dietary fibrous agro-industrial by-products indicated that the inclusion of rice bran, maize bran, brewers, spent grains, Alfalfa hay and cocoa pod husk in laying hen diets resulted in economic gains.

**Table (9): Input and output analysis and economical efficiency of White Leghorn hens as affected by dietary crude fiber levels**

Crude fiber levels	Parameters			
	Price/kg feed (L.E.)	Total feed intake /hen (kg)	Total feed coast/hen (L.E.)	Total number of egg/hen
Control (3 %)	2.52	5.965	15.03	53.91
4 %	2.52	5.376	13.55	49.20
5 %	2.47	5.300	13.09	49.50
6 %	2.41	5.246	12.64	49.38
	Total price of eggs/hen (L.E.) <sup>1</sup>	Net revenue/hen (L.E.) <sup>2</sup>	Economical efficiency (E.E.)	Relative EE (%) <sup>3</sup>
Control (3 %)	32.35	17.32	1.15	100
4 %	29.52	15.97	1.18	103
5 %	29.70	16.61	1.27	110
6 %	29.63	16.99	1.34	117

1-Price of an egg at the time of experimental period = 0.60 L.E.

2-Net revenue per unit of total feed coast.

3- Relative economical efficiency % of the control, assuming that relative EE of the control (L1) =100.

It was concluded that, we can used crude fiber by up to 6 % in laying hens diets (44 to 56 weeks of age) reared under drinking natural saline to achieve acceptable productive and physiological performance and reduce the egg yolk cholesterol.

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## كوليستيرول البيضة والأداء الإنتاجي للدجاج البياض المتأثر بمستوى الألياف الخام تحت ظروف شرب الماء المالح الطبيعي

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أجريت هذه الدراسة لبحث تأثير استخدام أربعة مستويات من الألياف الخام على كوليستيرول البيضة والأداء الإنتاجي والفسيولوجي للدجاج البياض تحت ظروف شرب الماء المالح الطبيعي (٢٠٠٠ جزء في المليون أملاح كلية ذائبة). استخدم في هذه الدراسة عدد ١٢٠ دجاجة بياضة من عمر ٤٤-٥٦ أسبوع قسمت عشوائياً إلى أربعة مجاميع متساوية (٣٠ دجاجة لكل معاملة). استخدم أربعة مستويات من الألياف الخام واختبرت العلائق على الدجاجات لمدة ١٢ أسبوعاً.

أوضحت النتائج ما يلي:

- أدت تغذية الدجاج البياض على مستوى ٣ % ألياف خام إلى تحقيق أعلى عدد للبيض وكتلة البيض وأعلى كمية إستهلاك يومي للغذاء مع عدم وجود اختلافات معنوية بين الثلاث مستويات الأخرى.
  - سجل الدجاج البياض المغذى على أعلى مستوى معنوي من الألياف الخام أعلى نسبة لوزن القشرة والبيض و الصفار مقارنة بالمستويات التجريبية الأخرى.
  - أدت تغذية الدجاج البياض على عليقة تحتوى على مستوى أعلى من ٣ % ألياف خام إلى تحقيق أعلى قيم معنوية لارتفاع بياض البيضة ودليل شكل البيضة مع عدم وجود أى تأثيرات معنوية على دليل الصفار أو وحدات هاو.
  - أدت زيادة مستوى الألياف الخام في علائق الدجاج البياض إلى انخفاض معنوي في معاملات الهضم للمادة الجافة والمادة العضوية والبروتين الخام والرماد الخام ومستخلص الإثير والمستخلص الخالي من النيتروجين بينما لم تظهر أى تأثيرات معنوية على معاملات هضم مكونات الألياف.
  - أدت زيادة مستوى الألياف الخام في علائق الدجاج البياض إلى زيادة معنوية لكمية ماء الشرب حيث سجلت الدجاجات المغذاه على عليقة بها ٦ % ألياف خام أعلى كمية استهلاك لماء الشرب (حوالي ٢٣٦ مل/طنان/اليوم و ٣,٤٥ مل/جم غذاء مأكول).
  - أدت تغذية الدجاج البياض على عليقة تحتوى على مستوى أعلى من ٣ % ألياف خام إلى انخفاض معنوي في قيم الدهون الكلية والجليسريدات الثلاثية والفسفوليبيدات والكوليستيرول في صفار البيض.
  - أدت تغذية الدجاج البياض على عليقة تحتوى على مستوى أعلى من ٣ % ألياف خام إلى انخفاض معنوي في تركيزات الدهون الكلية والكوليستيرول والجليسريدات الثلاثية لسيرم الدم.
  - أدت زيادة مستوى الألياف الخام إلى زيادة معنوية في وزن القونصة والأحشاء المأكولة ووزن القناة الهضمية وطول القناة الهضمية مع عدم وجود أى تأثيرات معنوية على وزن الكبد أو القلب.
  - أظهر الدجاج المغذى على العليقة المحتوية على ٦ % ألياف خام أفضل قيم للكفاءة الاقتصادية والكفاءة الاقتصادية النسبية (١١٧%) مقارنة بباقي المجاميع التجريبية.
- من نتائج هذه الدراسة يتضح أنه يمكن استخدام مستوى ألياف خام بنسبة تصل إلى ٦ % في علائق الدجاج البياض (٤٤ إلى ٥٦ أسبوعاً) والمربى تحت ظروف شرب الماء المالح الطبيعي (٢٠٠٠ جزء في المليون أملاح كلية ذائبة) وذلك لتحقيق أفضل أداء إنتاجي وفسيولوجي وتقليل كوليستيرول البيضة.

قام بتحكيم البحث

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