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Use of Enzymes to Supplement Diets Containing Date Waste Meal for Lohmann White Layers

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Abstract: The current research objective was to determine the effect of enriched layers diets containing date waste meal (DWM) with a commercially prepared enzymes mixture on White Lohmann laying hens performance. Lohmann LSL-Classic White layers of (24-weeks old; n = 192) were randomly assigned to 8 groups of 24 birds each. Four formulated diets of 18% crude protein which contained 0, 5, 10 and/ or 15% DWM as a replacement for yellow corn, each diet was supplemented with of kemzyme (EZ) at 1 gm/kg diet until 44 weeks of age. Results showed that body weights and body weight changes due to 15% DWM inclusion and EZ supplementation were improved. Egg production, egg weight and feed conversion ratio were improved by EZ supplementation to diets containing 15% DWM. Enzyme and DWM supplementation to experimental diets had a positive effect on salmonella and *E. Coli* as well as fungi counts in the gastrointestinal tract. Also, EZ supplementation had improved nutrients digestibility coefficient of diets that contained higher DWM levels. Plasma total lipids were significantly ($P \leq 0.05$) decreased with higher DWM levels, while total lipids of fresh eggs were higher than that of stored eggs. On the other hand, EZ supplementation had no significant difference ($P \geq 0.05$) in this respect. Inclusion of 15% DWM significantly ($P \leq 0.05$) improved shell thickness and Haugh units with higher DWM levels. No significant differences were recorded for yolk color or index due to either of the dietary treatments. It is concluded that DWM can be included in laying diets as an alternative dietary ingredient up to 10% with no adverse effects on production, and showed an improvement on performance when compared to that of the control diet supplemented with enzymes mixture.

Key words: Laying hens, date waste meal, enzyme mixture, poultry diets

Introduction

Shortage of conventional feed ingredients is considered a major obstacle that is facing poultry industry development in many tropical and sub-tropical countries, prompting the search for other suitable raw materials "ingredients" to provide required nutrients for the bird. Incorporation of such products in poultry feed would help alleviating the problem of the scarcity of feed supply and may also reduce pollution problems.

Currently, date waste meal (DWM) is considered to be such a good ingredient that could partially overcome feed shortage. This product could be incorporated in poultry diets as a cheap untraditional feedstuff to reduce feeding costs and alleviates pollution problems (Al-Harthi, 2006). Dates are produced in abundance in many parts of the Middle East and Arab world, whereas over than 70% of the total world production of dates is produced in this area. Saudi Arabia with its varied agro-climatic conditions is one of few countries producing numerous varieties of dates. According to the published statistics of the Ministry of Agriculture and Water (2000), a considerable amount (20%) of produced dates is inedible and is not beneficial for human consumption due to its poor quality.

Substitution of grains in laying hen diets with date wastes has been reported by different investigators (Jumah *et al.*, 1973; Yeong *et al.*, 1981; Sawaya *et al.*, 1984; El-Boushy and Vanderpoel *et al.*, 1994), but the higher crude fiber content might be considered a limiting factor for the inclusion level of DWM. Fractionation of crude fiber of DWM indicated the presence of 39.7, 50.6, 11.9, 26.9 and 7.8% of acid detergent fiber, neutral detergent fiber, hemi-cellulose, cellulose and lignin, respectively (Ahmed, 1997). Moreover, there are accumulative evidences indicating that cell wall non-starch polysaccharides had an anti-nutritional activity in many mono-gastric animals (Choct, 2004). Fortunately, based on the recent biotechnology advances in feed additive, an extensive work has been done to improve the utilization of unconventional feedstuffs as a tool for reducing the economics in feed industry.

Benefits of supplementing non-starch polysaccharides rich poultry diets with exogenous enzymes are well documented (Bedford and Classen, 1992; Almirall *et al.*, 1995; Yu *et al.*, 1997; Wang *et al.*, 2005). Although nutrients in corn are generally considered highly available, a 2 to 3% improvement in feed conversion with enzyme supplementation has been reported (Cowan,

Table 1: Composition and nutrients contents of the experimental diets

	Experimental diets			
DWM[1], %	0	5	10	15
Ingredients, %				
Corn yellow	61.300	58.230	55.170	52.100
Soybean meal (48% CP)	27.500	27.500	27.500	27.500
Di-Calcium Phosphate	1.821	1.745	1.670	1.594
Limestone	8.455	8.450	8.446	8.441
Salt (NaCl)	0.500	0.500	0.500	0.500
Vegetable oil	0.002	0.002	0.002	0.002
DL-Methionine, 99%	0.120	0.125	0.130	0.135
Vitamin+ Mineral premix[2]	0.250	0.250	0.250	0.250
DWM	-	3.065	6.130	9.195
Sand (filler)	0.052	0.128	0.203	0.278
Total	100.00	100.00	100.000	100.000
Nutrient (Calculated, %)				
Crude Protein	18.41	18.26	18.12	17.97
ME (kcal/kg)	2,850.00	2,850.00	2,850.00	2,850.00
Lysine	0.90	0.90	0.90	0.89
Methionine	0.40	0.40	0.40	0.40
Calcium	3.70	3.70	3.70	3.70
Available Phosphorus	0.45	0.45	0.45	0.45
Total Phosphorus	0.68	0.68	0.67	0.66

[1]DWM = Date waste meal. [2]Vitamins and Trace minerals premix supplied per kilogram of diet: vitamin A (retinly acetate); 12,000IU; vitamin E (all rac-α-tocopheryl acetate), 10IU; k_3 (menadione dimethypyrimidinol bisulfite), 3mg; vitamin D_3, 2,200ICU; riboflavin, 10mg; pantothenic acid (D-calcium pantothenate), 10mg; niacin, 20mg; Choline chloride, 500mg; vitamin B_{12} (cyanocobalamin), 10µg; vitamin B6, 105mg; thiamine (thiamine mononitrate), 2.2mg; folic acid, 1mg; D-biotin, 50µg; manganese (MnO), 55 mg; zinc (ZnO), 50 mg; iron ($FeSO_4 \cdot H_2O$), 30 mg; copper ($CuSO_4 \cdot 5H_2O$), 10 mg; selenium, 1mg and Ethoxyquin 3mg.

1993). The poultry feed industry has greatly increased its use of commercially available exogenous enzymes during the past 15 years. Supplementing exogenous enzymes can improve digestion of nutrients from feedstuffs, thereby decreasing feed costs, improving bird performance and decreasing environmental impact of land applied manure (Bedford and Schulze, 1998; Jaroni *et al.*, 1999; Silversides *et al.*, 2006).

Therefore, the main purpose of this study was to investigate the effect of replacing yellow corn with 0, 5, 10 and/or 15% DWM in layers diets that are supplemented with commercial enzyme cocktail mixture on performance of Lohmann LSL-Classic White hens.

Materials and Methods

The current experimental work was carried out at the Agriculture Research Center at Hada El-Sham, Faculty of Meteorology Environmental and Arid Land Agriculture, King Abdulaziz University. Lots of DWM used in this study were collected from varies suppliers across Saudi Arabia local markets. Date waste meal used consisted of low quality date, discarded date of the culling process, and old date of the previous year production. This date's mixture was processed by removing sand, herbage and

Table 2: Proximate analysis of date waste meal in comparison to yellow corn

Nutrient, %	DWM[1]	YC[2]
Moisture	10.05	11.00
Crude protein	3.70	8.50
Crude fiber	5.70	2.20
Ether extract	1.72	3.80
Ash	2.90	1.15
Nitrogen free extract	75.93	73.35
ME3 (kcal/kg)	3,549.00	4,225.76
Calcium	0.62	0.02
Total Phosphorus	0.54	0.28
Methionine	0.07	0.18
Lysine	0.21	0.26

[1]DWM = Date Waste Meal. [2]YC = Yellow Corn. [3]Metabolizable energy (ME) was calculated on the basis of its chemical composition according to Carpenter and Clegg (1956).

gravel. Then, it was sun-dried with 72 hours and ground in a heavy-duty high rotation hammer mill to pass through 1 mm. mesh sieve, producing a fine powder suitable for chemical analysis according (A.O.A.C., 1996). Experimental diets included 4 dietary levels of DWM of 0, 5, 10 and/ or 15%, to replace yellow corn with or without kemzyme[1] supplementation.

Enzyme description: The enzyme used in this study is kemzyme (EZ) which is a dry stabilized preparation manufactured by Chemix Company, Egypt. It is a multi-enzyme preparation (α - amylase, ß - glucanase, xylanase, protease, lipase and cellulase). The enzymes mixture was added at the level of 1g/kg diet. Each 1g of the enzyme contains 100 units cellulase, 250 units ß - glucanase, 400 units xylanase and 1000 units α-amylase.

Animals and diets: Diets calculated chemical analyses are presented in Table 1. Each experimental diet was formulated to meet nutrients recommendation of Lohmann LSL-Classic White management guide which met or exceeded the NRC (1994) recommendations. A total of one hundred and ninety two Lohmann LSL-Classic White[2] commercial laying hens, aging 24-weeks were randomly assigned to 8 experimental groups, each group was represented by hens in three replicates pens of 8 birds each and were kept under similar management conditions. Hens were housed in an environmentally controlled facility with a daily target temperature of 22°C. Feed and water were provided for *ad libitum* consumption. The proximate analysis of DWM in comparison to yellow corn is presented in Table 2.

Measurements: The following criteria were measured and / or calculated: Body weight, mortality rate (Data not

[1]Kemzyme: enzyme cocktail preparation manufactured by Chemix Company, Egypt. supplemented at 1g/kg diet as recommended by the producer (Finn Feeds international, LTD, UK).

[2]Lohmann Tierzucht GmbH. Abschnede 64, 27472 Cuxhaven, P.O.Box 460, 27454 Cuxhaven, Germany

Table 3: Effect of different dietary date waste meal levels and enzyme mixture supplementation on egg numbers of Lohmann White layers from 24 to 44 weeks of age

		Egg number (egg/hen/4-wk)[2]					
Treatments		4-27	28-31	32-35	36-39	40-44	Overall[2]
DWM[1],%	0	24.62^{a}	26.62^{a}	25.32^{a}	23.80^{a}	23.75^{a}	24.82^{a}
	5	23.33^{b}	24.96^{b}	24.85^{ab}	22.40^{b}	24.19^{a}	23.95^{b}
	10	21.96^{c}	24.15^{bc}	24.26^{bc}	22.38^{b}	23.03^{b}	23.16^{b}
	15	21.60^{c}	24.02^{c}	23.69^{c}	22.06^{b}	22.83^{b}	22.84^{b}
EZ[3]	-	21.83^{b}	24.79	24.44	22.39	23.15^{b}	23.32
	+	23.91^{a}	25.09	24.62	22.92	23.75^{a}	24.06
DWM x EZ	0	25.44^{a}	26.16^{ab}	25.20^{a}	23.33^{ab}	24.97^{a}	25.02^{a}
	5	23.80^{cd}	25.65^{b}	25.19^{a}	23.33^{ab}	23.03^{bc}	24.20^{c}
-	10	19.16^{e}	24.02^{cd}	24.26^{ab}	21.92^{cd}	22.71^{c}	22.41^{d}
	15	18.93^{e}	23.32^{d}	23.11^{b}	21.00^{d}	21.90^{d}	21.65^{d}
	0	24.98^{ab}	27.08^{a}	25.45^{a}	24.26^{a}	24.79^{a}	25.31^{ab}
+	5	24.03^{bc}	24.98^{bc}	24.49^{a}	23.12^{abc}	23.41^{bc}	24.01^{ab}
	10	23.79^{cd}	24.27^{cd}	24.26^{ab}	22.84^{bc}	23.03^{bc}	23.64^{b}
	15	22.85^{d}	24.02^{cd}	24.26^{ab}	21.46^{d}	23.76^{b}	23.27^{c}
SEM		1.62	1.88	1.81	1.91	1.14	1.78
Probabilities							
DWM		***[4]	***	***	***	***	***
EZ		***	NS[5]	NS	NS	**	NS
DWM x EZ		***	**	*	***	***	***

[a-d] Means within a column with no common superscripts differ significantly (P = 0.05). [1]DWM: Date waste meal. [2]Overall: 24 to 44 weeks of age. [3]EZ: kemzyme enzyme mixture. [4]***: ($P \leq 0.001$); **: ($P \leq 0.01$); *: ($P \leq 0.05$). [5]NS: not significant ($P > 0.05$).

shown), egg number, egg weights were measured and feed conversion ratio was calculated starting 24 wks of age. At the end of the experiment, a freshly collected sample of five eggs and another five eggs stored for 15 days under normal room temperature (22°C) were collected from each treatment for egg quality measurements, which included shell thickness, Haugh unit (Stadleman, 1977), yolk color using yolk color fan score and yolk index (Well, 1968). Also, a similar egg sample size as described above were collected from each treatment for yolk lipids analysis as described by Fisher and Leveille (1957) using commercial kits produced by Diamond Diagnostic[3]. Estimated slaughter yield were also carried out by randomly using three hens around the average of body weight from each group. Then, were deprived of feed for 12 hours, weighed and slaughtered to complete bleeding, followed by plucking feathers then weighted. Carcass weight, dressing, abdominal fat, intestine weight, oviduct and ovary weight percentage were recorded and intestine length was also determined.

To determine nutrients digestibility coefficients, samples of diets and dried excreta were taken from each treatment. Excreta were collected from each pen at the end the experiment (44 wks) for measuring nutrients digestibility and excreta nitrogen content. At 44 wks of age, all hens were euthanized by cervical dislocation, and ileal contents (from Meckel's diverticulum to the ileo-cecal junction) were collected and pooled per pen for analysis of chromium and nitrogen Chromic oxide was added to experimental diets at 3 gm/kg (0.3%) as an inert marker. Chromic oxide was analyzed according to Czarnocki *et al.* (1961). Excreta and ileal samples were frozen and stored at -20°C subsequent to collection until further analyzed. Frozen ileal and frozen excreta samples were thawed, transferred to aluminum pans and placed in a 65°C oven for 3 days to determine dry matter. After drying, all samples were ground through a 1-mm screen to prep for analysis. Values obtained were used to calculate nutrient retention and ileal digestibility using the following equation: % Nutrient digestibility = 100-((Diet Cr_2O_3 / Fecal Cr_2O_3 * Fecal nutrient / Diet nutrient) * 100). Fecal nitrogen was determined according to the procedure of Jakobsen *et al.* (1960).

Statistical analysis: All data was analyzed using the GLM procedures of SAS for a Complete Randomized Design (CRD). All dietary treatments (four DWM inclusion levels and two enzyme levels) were considered fixed effects. Significant treatment differences were established using the LSMEANS statement in SAS (SAS, 2003). A 2 x 4 factorial arrangement of treatments was implemented and the following model was used to determine differences between treatment groups: $Y_{ij} = \mu + a_i + b_j + (ab)_{ij} + e_{ij}$
Where Y_{ij} = variable measured; μ = overall mean; a_i = effect of the i^{th} level of A; b_j = effect of the j^{th} level of B; $(ab)_{ij}$ = interaction effect of the i^{th} level of A and the j^{th} level of B and e_{ij} = error component. Significance of difference was based on the probability of a type III error set at ($P \leq 0.05$). The differences among means were tested utilizing Duncan's multiple range test (Duncan, 1955).

[3]Diamond diagnostic: 333 Fiske Street. Holliston, Massachusetts 01746 USA

Table 4: Effect of different dietary date waste meal levels and enzyme mixture supplementation on egg weight of Lohmann White layers from 24 to 44 weeks of age

Treatments		Egg weight (gm) Experimental periods (wks)					
		24-27	28-31	32-35	36-39	40-44	Overall[2]
DWM[1],%	0	56.80^{a}	58.25^{a}	57.50^{ab}	58.65	56.85	57.61^{a}
	5	55.00^{ab}	56.60^{b}	58.45^{a}	58.55	57.10	57.14^{ab}
	10	55.70^{ab}	58.25^{a}	57.15^{ab}	58.75	57.50	57.47^{ab}
	15	54.20^{b}	58.20^{a}	56.75^{b}	57.90	57.30	56.87^{b}
EZ[3]	-	54.95	57.65	56.98^{b}	58.45	56.80	56.70
	+	55.90	58.00	57.95^{a}	58.48	57.58	57.58
DWM x EZ	0	56.90^{a}	58.10^{a}	58.70^{ab}	57.80^{bc}	56.60	57.62^{ab}
	5	55.40^{a}	58.40^{a}	58.60^{ab}	60.10^{a}	57.60	58.00^{bc}
-	10	52.20^{b}	55.90^{b}	57.10^{bc}	58.30^{abc}	57.70	57.54^{abc}
	15	55.30^{a}	58.20^{a}	57.40^{bc}	57.60$_{bc}$	57.30	56.40^{c}
	0	56.10^{a}	58.40^{a}	56.10^{c}	59.50^{ab}	57.00	57.42^{bc}
+	5	56.20^{a}	57.30^{ab}	59.80^{a}	58.20a^{bc}	56.60	57.62^{a}
	10	56.70^{a}	58.20^{a}	55.70^{c}	59.20^{ab}	56.70	57.30^{abc}
	15	54.60^{a}	58.10^{a}	56.30^{c}	57.00^{c}	58.00	56.80^{c}
SEM		4.62	2.81	3.04	2.84	2.33	2.54
Probabilities							
DWM		*[4]	*	**	NS[5]	NS	*
EZ		NS	NS	*	NS	NS	NS
DWM x EZ		*	*	***	**	NS	***

[a-c]Means within a column with no common superscripts differ significantly ($P \leq 0.05$). [1]DWM: Date waste meal. [2]Overall: 24 to 44 weeks of age. [3]EZ: kemzyme enzyme mixture. [4]***: ($P \leq 0.001$); **: ($P \leq 0.01$); *: ($P \leq 0.05$). [5]NS: not significant ($P > 0.05$).

Results and Discussion

Results of egg number as affected by different inclusion levels of DWM and EZ supplementation throughout the entire experimental periods are presented in Table 3. Overall, DWM inclusion decreased ($P \leq 0.001$) egg number values as compared with that of the control group. This reduction was significant to the extent of 3.50, 6.69 and 7.98% for 5, 10 and 15% DWM substitution of yellow corn, respectively. However, egg number for birds fed 5% DWM substitution for YC was insignificant ($P > 0.05$) during (32 to 35 wks) and (40 to 44 wks) periods as compared to those fed the control diet. Similarly, Najib *et al.* (1995) found that a significant decline in egg production percent was noted for hens fed diets containing 28% DWM. Overall, EZ supplementation (24 to 44 wks of age) insignificantly improved egg number by 3.17% over that of the un-supplemented diet. However, the increment rate was only significant; during period (24 to 27 wks; $P \leq 0.001$) and (40 to 44 wks; $P \leq 0.01$), respectively. Enzymes mixture supplementation to layer feeds have been reported to improve layers performance including FCR (Benabdeljelil and Arbaoui 1994; Vukic Vranjes and Wenk 1995) energy utilization (Wyatt and Goodman 1993; Vukic Vranjes and Wenk 1995) and laying rate (Poultry International, 1996). This improvement appears to be a result of microbial fermentation of soluabilized NSPs (Vukic Vranjes and Wenk, 1995) and the subsequently higher absorption of volatile fatty acids (Choct *et al.*, 1995). Our results are in agreement with the results of Abd El-Ghany *et al.* (1997) and El-Full (2000) who reported that there was insignificant increase in egg production due to EZ supplementation. On the other hand, (Arafat, 2002; Shakmak, 2003; Yakout *et al.*, 2004) reported that EZ supplementation to laying hens diets significantly improved egg production. Overall, a significant ($P \leq 0.001$) DWM x EZ was noted, of which dietary EZ supplementation to experimental diets did not significantly improve egg number ($P > 0.05$). However, increasing dietary DWM inclusion levels reduced egg number ($P \leq 0.001$), asfeeding the 15% DWM level lowered egg number when hens were fed diets with no EZ or with EZ by 13.57 and 8.06%, respectively as compared to the control group.

Data presented in Table 4 indicated that overall there was no significant effect ($P > 0.05$) of DWM at 5 or 10% inclusion levels on egg weight as compared to that of the control. However, DWM inclusion level of 15% showed a significant reduction ($P \leq 0.05$) as compared to that of the control group by 1.28%. These results are in contrast with previously reported results by (Najib *et al.*, 1995; El-Bogdady 1995; Radwan *et al.*, 1997; Perez *et al.*, 2000; Mahmoud, 2005) who reported similar average egg weights among groups received different levels of date pits containing diets. In regard to EZ supplementation, overall egg weight was not influenced by EZ 0.1% addition expect that of period (32 to 35) weeks which had the highest ($P \leq 0.05$) value, which might be due to the improvement in nutrients utilization due to EZ addition (El - Deek *et al.*, 2003 and Choct,

Table 5: Effect of different dietary date waste meal levels and enzyme mixture supplementation on feed conversion ratio of Lohmann White layers from 24 to 44 weeks of age

		FCR[2] (gm feed/ gm egg mass)					
		Experimental periods (wks)					
		24-27	28-31	32-35	36-39	40-44	Overall[3]
DWM[1],%	0	2.02^{b}	1.99^{c}	2.33^{b}	2.43^{b}	2.62	2.28^{b}
	5	2.32^{a}	2.15^{b}	2.40^{ab}	2.62^{a}	2.72	2.44^{b}
	10	2.36^{a}	2.22^{ab}	2.44^{a}	2.57^{a}	2.71	2.46^{a}
	15	2.40^{a}	2.26^{a}	2.45a	2.64^{a}	2.72	2.49^{a}
EZ[4]	-	2.44^{a}	2.17	2.44^{a}	2.59	2.77^{a}	2.48^{a}
	+	2.11^{b}	2.13	2.37^{b}	2.54	2.62^{b}	2.35^{b}
DWM x EZ	0	1.96^{c}	2.04^{de}	2.27^{b}	2.53^{b}	2.50^{b}	2.26^{e}
	5	2.45^{ab}	2.08^{cd}	2.40^{ab}	2.52^{b}	2.86^{a}	2.46^{ab}
-	10	2.65^{a}	2.30^{d}	2.44^{a}	2.65^{ab}	2.81^{a}	2.57^{bc}
	15	2.69^{d}	2.27^{ab}	2.37^{ab}	2.68^{ab}	2.89^{a}	2.58^{a}
	0	2.03^{c}	1.95^{e}	2.40^{ab}	2.34^{c}	2.43^{b}	2.23^{de}
+	5	2.14^{c}	2.16^{bc}	2.39^{ab}	2.60^{ab}	2.94^{a}	2.45^{cd}
	10	2.09^{c}	2.21^{ab}	2.51^{a}	2.49^{bc}	2.55^{b}	2.37^{bc}
	15	2.19^{bc}	2.21^{ab}	2.47^{a}	2.72^{a}	2.55^{b}	2.43^{bc}
SEM		0.42	0.19	0.22	0.27	0.33	0.29
Probabilities							
DWM		***[5]	***	*	**	NS[6]	***
EZ		***	NS	*	NS	**	***
DWM x EZ		***	*	**	**	***	***

[a-e]Means within a column with no common superscripts differ significantly (P≤0.05). [1]DWM: Date waste meal. [2]FCR: Feed conversion ratio. [3]Overall: 20 to 40 weeks of age. [4]EZ: kemzyme enzyme mixture. [5]***: (P≤0.001); **: (P≤0.01); *: (P≤0.05). [6]NS: not significant (P>0.05).

2004). A significant DWM x EZ interaction (P≤0.001) was noted overall, although there was no significant (P > 0.05) EZ main affect was noted, all the interaction significant effects were due to a significant (P≤0.05) DWM dietary inclusions. Feeding hens diets without EZ supplementation had numerically higher egg weights as compared to those produced from hens fed EZ supplemented diets. As for DWM fed hens, increasing DWM inclusion level from 0 to 10% resulted in a subsequent significant (P≤0.001) increase in egg weight. However, feeding the highest DWM level (15%) had lowered average egg weights by 1.59% as compared to the control group.

All dietary treatments including significant DWM and EZ main effects and interactions (P≤0.001) on feed conversion ratio are illustrated in Table 5. It is clear that DWM substitution for yellow corn impaired feed conversion ratio values through all studied periods and overall (P≤0.001). This impairment was to the extent of 6.56 (5% DWM), 7.31 (10% DWM) and 9.21% (15% DWM) for the overall period. On the other hand, overall EZ addition improved (P≤0.001) feed conversion ratio by about 5.24% over the EZ un-supplemented diets. These results agree with those found by (Hataba *et al.*, 1994; Mathlouti *et al.*, 2003), who reported a significant improvement in feed conversion ratio observed when hens where fed diets supplemented with enzymes mixture. A significant DWM x EZ (P≤0.001) was also noted, of which feeding hens the control diet (0.0 DWM) with EZ supplementation improved feed conversion ratio better than those fed no EZ supplemented diets.

Table 6: Effect of different dietary date waste meal levels and enzyme mixture supplementation on egg quality of Lohmann White layers fresh and stored eggs at 44 weeks of age

Treatments			Shell thickness (mm.)	Haugh unit	Yolk color	Yolk index (%)
DWM[1],%	EZ[2]	C[3]				
0	-	F[4]	0.33	97.60	3.10	52.18
5			0.35	105.20	3.10	52.20
10			0.34	100.90	3.30	52.70
15			0.34	104.00	3.30	52.20
0	+		0.34	96.30	3.20	56.70
5			0.33	99.40	3.40	56.60
10			0.35	99.60	3.60	56.60
15			0.35	100.10	3.20	57.20
0	-	S[5]	0.32	82.00	3.60	47.60
5			0.34	82.40	3.80	48.80
10			0.34	82.60	3.70	48.20
15			0.34	83.70	3.60	49.40
0	+		0.33	82.80	3.50	47.60
5			0.33	83.40	3.50	49.40
10			0.33	81.60	3.60	48.20
15			0.33	84.60	3.70	48.20
SEM			0.02	3.50	0.49	2.50
Probabilities						
DWMxEZ			*[6]	***	NS	**
EZxC			NS[7]	***	NS	**
DWMxC			NS	***	NS	**
DWMxEZxC			NS	**	NS	*

[1]DWM: Date waste meal. [2]EZ: kemzyme enzyme mixture. [3]C: Egg condition fresh vs. stored. [4]F: Fresh. [5]S: Stored. [6]***: (P ≤ 0.001); **: (P≤0.01); *: (P≤0.05). [7]NS: not significant (P>0.05).

Furthermore, the control group had better feed conversion ratio when compared to the highest DWM

Table 7: Effect of different dietary date waste meal levels and enzyme mixture supplementation on some slaughter traits[1] of Lohmann White layers at 44 weeks of age

Treatments		Abdominal fat (%)	Intestine wt.(%)	Intestine length (cm.)	Oviduct wt.(%)	Ovary wt.(%)	Liver wt.(%)
DWM[2], %	0	2.38[a]	5.23[a]	143.50[c]	3.78[a]	2.92	2.17[a]
	5	2.41[a]	4.36[b]	147.00[b]	3.78[a]	3.24	2.21[a]
	10	1.99[b]	5.37[a]	146.83[b]	3.19[b]	2.96	2.14[a]
	15	1.03[b]	4.92[ab]	154.83[a]	3.36[b]	2.71	1.98[b]
EZ[3]	-	1.62[b]	4.95	146.58[b]	3.31[b]	2.81	2.18
	+	2.12[a]	4.98	149.50[a]	3.70[a]	3.10	2.07
DWM x EZ							
-	0	2.31[a]	5.54[a]	140.00[b]	3.88[a]	2.86	1.95
	5	2.35[a]	4.29[b]	139.33[b]	3.71[ab]	3.37	2.19
	10	2.23[a]	5.37[a]	151.00[ab]	3.38[b]	3.23	2.20
	15	1.23[b]	4.76[ab]	156.00[a]	3.85[a]	2.94	1.91
+							
	0	2.38[a]	5.37[a]	142.67[c]	3.66[a]	2.97	2.06
	5	2.43a	4.43[b]	147.67[b]	3.70[a]	3.11	2.04
	10	2.03a	4.93[ab]	154.00[a]	2.99[b]	2.68	2.22
	15	1.38[b]	5.09[a]	153.67[ab]	2.87[c]	2.49	2.39
SEM		0.48	0.78	3.58	0.48	0.53	0.22
Probabilities							
DWM		*[4]	**	***	*	NS[5]	*
EZ		***	NS	**	**	NS	NS
DWM x EZ		**	***	**	*	NS	NS

[a-d] Means within a column with no common superscripts differ significantly (P $\leq$ 0.05). [1]%; percent of body weight. [2]DWM: Date waste meal. [3]EZ: kemzyme enzyme mixture. [4]***: (P $\leq$ 0.001); **: (P $\leq$ 0.01); *: (P $\leq$ 0.05). 5NS: not significant (P > 0.05)

level (15%), with EZ supplemented diets (8.23%), or without EZ supplementation (12.40%), respectively.

Selected egg quality traits are shown in Table 6. Both DWM levels replacing yellow corn and EZ supplementation insignificantly (P$\leq$0.05) affected yolk color and shell thickness for fresh and stored eggs, respectively. The opposite was true for Haugh unit and yolk index which were significantly improved (P$\leq$0.01, P$\leq$0.05) by DWM and EZ supplementation, for fresh and stored egg, respectively. This improvement might be attributed to anti-microbial enhancing effects due to EZ supplementation and digestive improvement effects due to the DWM inclusion levels (Cowan, 1993; Bedford; Schulze, 1998; Jaroni *et al.*, 1999; Silversides *et al.*, 2006). Haugh units showed a significant DWM x EZ x C interaction (P$\leq$0.01), of which in general fresh eggs had higher Haugh units over stored eggs. Looking at the fresh eggs, feeding 5% DWM with no EZ supplementation had the highest Haugh units (105.2) over all other DWM supplemented diets. Also, increasing DWM inclusion levels up to 15% increased Haugh units for the EZ supplemented diets fed hens as compared to the control group (96.3 *vs.* 100.1), respectively. Moreover, feeding the highest DWM (15% with no EZ) resulted in higher Haugh units as compared to feeding 15% DWM with EZ supplementation. As for the stored eggs, feeding 15% DWM with EZ supplementation had the highest Haugh units (84.6) over all other DWM supplemented diets. Also, increasing DWM inclusion levels up to 15% increased Haugh units for the EZ non-supplemented diets fed hens as compared to the control group (82 vs. 83.7), respectively. Moreover, feeding the highest DWM (15% with EZ) resulted in higher Haugh units as compared to feeding 15% DWM with no EZ supplementation. Another significant DWM x EZ x C interaction was noted (P < 0.01), of which in general fresh eggs had higher yolk index over stored eggs. Looking at the fresh eggs, there was a numerical increase in yolk index with increasing DWM inclusion levels for the no EZ supplemented diets. Also, increasing DWM inclusion levels up to 15% increased yolk index for the EZ supplemented diets fed hens as compared to the control group (57.2 *vs.* 56.7), respectively. Moreover, feeding the highest DWM (15% with EZ) resulted in higher yolk index as compared to feeding 15% DWM without EZ supplementation. As for the stored eggs, feeding 15% DWM with no EZ supplementation had the highest Haugh units (49.4) over all other DWM supplemented diets. Also, increasing DWM inclusion levels up to 15% increased yolk index for the EZ non-supplemented diets fed hens as compared to the control group (49.4 *vs.* 47.6), respectively. Moreover, feeding the highest DWM (15% without EZ) resulted in higher yolk index as compared to feeding 15% DWM with EZ supplementation 49.4 *vs.* 48.2, respectively.

Slaughter traits are presented in Table 7, of which ovary weight percent of hens fed dietary DWM (0, 5, 10 or 15%) had statistically similarvalues to those fed the control diet (P > 0.05), whereas, means values of abdominal fat, intestine, oviduct and liver weight percentages were decreased (P$\leq$0.01) as compared with those of the

Table 8: Effect of different dietary date waste meal levels and enzyme mixture supplementation on total protein and lipids concentration of plasma and lipid content of fresh and stored egg yolk of Lohmann White layers at 44 weeks of age

Treatments		Plasma		Yolk Total Lipid	
EZ[1]	DWM[2], %	Total Protein g/100 ml	Total Lipid g/100 ml	Fresh	Stored
-	0%	5.84	3.00	269[a]	185.04[b]
	5%	5.83	3.65	256.23[cb]	209.52[a]
	10%	5.32	4.62	230.12[c]	163.06[c]
	15%	5.23	3.80	222.22[c]	147.42[d]
+	0%	4.56	3.03	214.38[c]	155.71[ad]
	5%	5.34	2.86	247.04[b]	202.75[a]
	10%	4.84	4.35	261.15[cb]	194.42[cb]
	15%	5.54	2.34	272.77[a]	164.90[c]
EZ	-	5.56	3.77[a]	244.48	176.2[6]
	+	5.07	3.14[b]	248.84	179.46
DWM, %	0	5.20	3.01[b]	241.87	170.37[cb]
	5	5.59	3.25[b]	251.64	206.13[a]
	10	5.08	4.49[a]	245.64	178.74[b]
	15	5.38	3.07[b]	247.50	156.16[c]
SEM	0.76	0.58	8.71	12.58	
Probabilities					
EZ	NS[3]	*[4]	NS	NS	
DWM	NS	**	NS	**	
EZ x DWM	NS	NS	**	**	

[a-c]Means within a column with no common superscripts differ significantly (P ≤ 0.05). EZ: kemzyme enzyme mixture. [2]DWM: Date waste meal. [3]NS: not significant (P > 0.05). [4]**: (P ≤ 0.01); *: (P ≤ 0.05).

control group. The opposite was true with the intestine length which increased (P≤0.001) with increasing the DWM dietary inclusion 154.83 cm for the 15% DWM. These results are in line with those reported by (Kamel *et al.*, 1981; Onwudikee, 1986; Soliman, 1996; Radwan *et al.*, 1997) on grower chicks and laying hens, respectively. Also, the significant decrease (P≤0.05) in abdominal fat percentage of 15% DWM diet is similar to the findings of (Onwudikee, 1986; Hammad, 1996; Abd El - Rahman *et al.*, 1999). This might be due to estrogen (estrone) hormone effect in date meal. El-Borai and El-Borai (1988) reported that estrone acts as a stimulator of fat deposition within muscle fibers followed by decrease in abdominal fat. Diets supplementation with EZ, irrespective of dietary DWM, increased (P≤0.001) abdominal fat values, oviduct percent, and intestine length. This trend was also seen with ovary and intestine weight percent but without significance (P > 0.05). Similarly, increasing abdominal fat by EZ addition has been reported in many previous studies of (Pisarski and Wozciks, 1994; Jamroz *et al.*, 1996; Alam *et al.*, 2003). The opposite was true with liver percentage which was decreased with increasing DWM levels. Garcia *et al.* (2003) found that liver weights as a percentage of body weight decreased with α -amylase supplementation to diets based on corn-soybean meal. A significant DWM x EZ interaction (P≤0.01) was noted regarding intestine length, of which feeding diets with 15% DWM without EZ supplementation had the longest intestine as compared to all other dietary treatments including the control one. As shown in the Table 8, plasma total protein and lipids concentration had followed a similar trend as a result of feeding dietary DWM treatments during the experimental period. Statistically, the reduction rate in plasma total protein was not significant (P > 0.05); however, it increased (P≤0.01) plasma total lipids. These results are in agreement with those reported by Cherry and Jones (1982) who indicated that a fraction of fiber such as pectin and lignin conjugates with bile acids resulting in an increase in bile acid excretion associated with the absorption of lipids. Similar trend was also seen with yolk total lipids of fresh and stored egg which were decreased (P ≤ 0.01) with increasing the inclusion level of DWM. It is very important for human health, since the eggs are required for patients suffering from coronary heart disease. However, this study needs more investigation to clarify these findings.

Changes in nutrients digestibility of Lohmann White laying hens fed diets with different DWM levels with or without EZ are presented in Table 9. It is clear that, digestibility coefficients of different nutrients of the studied experimental diets revealed that incorporating 5% DWM as substitution of yellow corn resulted in an improvement in nutrients digestibility (P≤0.05) as compared to those of the control group. Similar results were observed with increasing the DWM inclusion levels up to 15% with the crude fiber. Improving ether extract digestibility also might be due to different inclusion levels of DWM as a result of the improved fatty acid profile of DWM (Mahmoud, 2005). In regard to the crude protein and fiber digestibility of the experimental diets, it is statistically equal to that of the control for all dietary

Table 9: Effect of different dietary date waste meal levels and enzyme mixture supplementation on nutrients digestibility of Lohmann White layers at 44 weeks of age

Treatments		Digestibility coefficient, %				
EZ[1]	DWM[2], %	OM	CP	EE	CF	NFE
DWM, %	0	74.85^{b}	85.54^{c}	82.54^{d}	28.22^{c}	78.81
	5	78.37^{a}	86.58^{b}	93.43^{a}	30.00^{b}	80.16
	10	77.05^{a}	87.04^{a}	90.95^{b}	31.21^{a}	79.65
	15	75.95^{b}	86.36^{b}	88.37^{c}	31.78^{a}	78.19
EZ	-	76.13^{b}	86.04	87.87^{b}	29.52^{b}	78.49^{b}
	+	77.29^{a}	86.74	90.52^{a}	31.57^{a}	79.91^{a}
DWM x EZ-	0	74.27^{c}	85.47	80.70^{d}	26.78^{d}	78.16^{cd}
	5	77.93^{ab}	86.64	91.24^{b}	28.50^{c}	79.53^{b}
	10	77.04^{ab}	86.38	90.30^{b}	30.13^{b}	78.85^{c}
	15	75.26^{b}	85.66	89.23^{b}	30.70^{ab}	77.40^{d}
+	0	75.43^{b}	85.61	84.37^{c}	29.65^{c}	79.45^{b}
	5	78.81^{a}	86.51	95.61^{a}	31.50^{ab}	80.78^{a}
	10	78.37^{a}	87.80	91.59^{b}	32.29^{a}	80.45^{a}
	15	76.53^{b}	87.06	90.51^{b}	32.85^{a}	78.98^{c}
SEM	1.81	3.33	4.35	1.02	4.41	
robabilities						
DWM	*[3]	*	**	**	NS[4]	
EZ	**	NS	**	*	*	
DWM x EZ		**	NS	**	**	*

$^{a-d}$Means within a column with no common superscripts differ significantly ($P \leq 0.05$). [1]EZ: kemzyme enzyme mixture. [2]DWM: Date waste meal. [3]**: ($P \leq 0.01$); *: ($P \leq 0.05$). [4]NS: not significant ($P > 0.05$).

Table 10: Effect of different dietary date waste meal levels and enzyme mixture supplementation on some microbial groups counts (log[10]) of the gastrointestinal tract of Lohmann White layers at 44 weeks of age

Treatments		Total Bacteria	Salm-onella	*E. coli*	Fungi
DWM[1], %	0	4.24^{a}	1.97	4.00^{a}	3.54^{a}
	5	4.24^{a}	-	4.10^{a}	3.48^{a}
	10	4.18^{b}	-	3.88^{b}	3.48^{b}
	15	4.18^{b}	-	3.88^{b}	2.54^{b}
EZ	-	4.81^{a}	-	4.00^{a}	3.60^{a}
	+	4.15^{b}	-	3.94^{b}	3.32^{b}
DWMxEZ[2]	0	4.18^{c}	1.85	4.00^{a}	3.70^{a}
	5	4.30^{b}	-	4.00^{a}	3.60^{a}
-	10	4.00^{d}	-	4.00^{a}	3.60^{a}
	15	4.00^{d}	-	4.00^{a}	3.48^{b}
	0	5.30^{a}	-	4.00^{a}	3.60^{a}
+	5	4.30^{b}	-	4.00^{a}	3.30^{c}
	10	4.30^{b}	-	3.70^{b}	3.30^{c}
	15	4.18^{c}	-	3.70^{b}	2.30^{e}
Probabilities					
DWM		**[3]		**	*
EZ		**		**	**
DWM x EZ		**		**	**

$^{a-e}$Means within a column with no common superscripts differ significantly ($P \leq 0.05$). [1]DWM: Date waste meal. [2]EZ: kemzyme enzyme mixture. [3]**: ($P \leq 0.01$); *: ($P \leq 0.05$).

DWM levels. While, different trend was noted with the nitrogen free extract digestibility for all experimental diets which was not different than that of the control. On the other hand, EZ supplemented diets numerically increased the digestion coefficients of nutrients and this agree with results of Abou El-wafa (1993). The benefit gained by the EZ addition is largely due to the partial degradation of the soluble β-glucan which reducing the viscosity of intestinal contents and improving nutrients absorption (Hesselman and Aman, 1986 and Rotter *et al.*, 1989).

Results of Table 10 shows the effect of DWM inclusion levels to layers diets on some specific bacterial and fungi counts of the gastrointestinal tract of Lohmann White layers. It is clear that salmonella cells numbers were not recovered from the gastrointestinal tract of hens fed inclusion levels of DWM 5% and up. However, total bacteria, *E. coli* and fungi group counts were significantly different ($P \leq 0.01$) as compared to those of the control group. There is a tendency to decrease total bacteria, *E. coli* and fungi counts with increasing dietary DWM up to 15%. This finding is in line with that of Cowan (1999) who reported that fruits are rich in a wide variety of secondary metabolites, such as terpenoids which was found to have anti-microbial properties. March (1979) and Sissons (1989) stated that gut microflora enzymes are beneficial to improve the nutrient substance of diet by increasing the digestion of nutrients. Concerning enzymes mixture supplementation, total bacteria, *E. coli* and Fungi counts of gastrointestinal were significantly lower than those fed the control diet. The possible effects of enzymes supplementation on digestion have been outlined by Bedford (1966). It was indicated that adding enzymes mixture to diets might improve ingredients digestibility by manipulation of gut micro-flora population.

In general, it can be concluded that DWM replacement of yellow corn should not exceed 10% in laying hens diets to support proper laying performance. Moreover, supplementation of laying hens diets with EZ had a superior effect when compared to the non-EZ supplemented diets, and an enhancing influence on layers performance at 10% DWM inclusion.

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