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EFFECT OF ADDING OF ENZYME COMPLEX (KEMZYME) TO DIET CONTAINING DATE STONE MEAL ON BLOOD PARAMETERS, IMMUNE RESPONSE, MICROBIAL ACTIVITY AND CARCASS CHARACTERISTICS OF GROWING RABBITS.

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ABSTRACT:

This study aimed to evaluate impact of enzymes mixture (EZ) addition to diets containing date stones meal (DSM) on body weight, blood parameters, immune response and carcass characteristics of growing rabbits. Seventy-five New Zealand White (NZW) rabbits,28 days of age with average weight 491±2.77g, were randomly divided into fiveexperimental groups. The 1st group was fed control diet (Diet A) contained 0% DSM and served as a control. The 2nd, 3rd groups, were fed diets contained 10% DSM either without (Diet B) or with EZ (1 g/ Kg diet) diet addition (Diet C), respectively. The 4th and 5th groups, were fed diets contained20% DSM either without (Diet D) or with EZ (1 g/ Kg diet) addition (Diet E), respectively. At the end of the experimental period five rabbits from each groupwere taken to determine carcass characteristics, blood parameters and meat quality.

Percentages of dressing were significantly (P<0.05) higher in rabbits fed 10% DSM+ EZ than the other groups. The protein and lipid percentage of meat were significantly higher in rabbit fed A, C and E diets than those fed Diet D, the levels of glucose, total lipid, triglycerides, HDL, LDL and RBC's were significantly (P<0.05) higher, whereas GPX and SOD percentages were lower (P<0.05) in rabbits fed A or C diets than the other groups. Higher values of α–globulin, BA and LTT were recorded with rabbits fed A, B or C diets compared with other groups. IgA, IgM and IgG were significantly higher in rabbits fed A, C or E diets than the other groups. No significant differences were

recorded in creatinine, AST, ALT, alkaline phosphates, cholesterol, total protein, albumin, TAC, GSH, globulin, globulin–γ, LA, PI and PA, hematological criteria and differential leucocytes counts among the different groups. Total bacterial, Salmonella, E. Coli and Proteus bacterial counts were significantly lower in rabbits fed B, C, D or E diets than the control group.

***In conclusion**, date stones meal can be included in rabbit diets up to 10% with adding enzyme complex (Kemzyme) without any adverse effects on body weights, blood parameters, immune index, carcass characteristics and meat quality.*

Keywords: Rabbits, enzymes, date stone meal, carcass characteristics, blood parameters.

The feed cost represents more than 70% of the total costs for rabbits production (El-Sayaad, 2002). Due to the constantly growing in the prices of main ingredients in poultry and rabbits feed as the results of feed shortage and in port most of the concentrated feed ingredients from abroad with foreign currency especially soybean meal and corn grains. Therefore, to decrease the cost of feeding, it is necessary to search about alternatives such as agricultural and industrial by-products (El-Manelawy and El-Banna, 2013).

Date stonesare produced in date industry in tremendous amounts in Egypt about 1.1 million tons are produced yearly (Gaber *et al*., 2012). The date stones are the inedible part of date, represents about 10 % of the date's weight, and could be utilized as a feed source for animals (Al-Homidan 2003, Aldhaheri *et al*. 2004 and Zanu *et al*., 2012). The nutritive value of date stonesranged from 5.64 % to 8.20 % crude protein (CP), 1.60–9.35 % ether extract(EE), 9.10–22.0 % crude fibre (CF), 58.5–75.4 % nitrogen free extract (NFE), 38.5–73.1 %neutral detergent fiber (NDF), 17.2–35.3 % acid detergent fiber (ADF), 0.19–0.32 % methionine and cystine, and 0.19–0.30 % lysine. ME value ranges from 1,350 to 1,746 kcal/kg diet (Aldhaheri *et al*., 2004). Date stones are good source of antioxidants, mainly carotenoids and phenolics (Al-Farsi and Lee, 2008). There is a propensity to decline total bacteria and *E. coli* with feeding diets contain DSM (El-Deek *et al*., 2008 and Al-Harthi *et al*., 2009). The total bacterial counts in the jejunum contents showed a decrease when the date seeds were used. Date stone meal(DSM)was used in rabbit diets without adverse effect on rabbit performance (Soliman *et al*., 2009) and on protein digestion, liver and renal functions (Attia, and Al-Harthi, 2015).

Some experiments showed positive effect of EZ addition in the rabbits diets. Ronaled (2007), found that rabbits diet supplementation with multienzymes mixture increase the haemoglobin and leukocyte level in the blood of the experimentalanimals. Abd El-Latif *et al.* (2008) revealed that EZ addition to rabbit diets improved the level of serum total protein, albumin, globulin, glucose, ALT, AST and total lipids. Multienzymes mixture supplementation in the diets led to a reduction in pathogens, total anaerobics, Gram-positive cocci, and enterococci in the intestine microbiota (Tabook *et al.*, 2006).

The present study was carried out to investigate impact of enzymes mixture (EZ) addition to the diets containing date stones meal (DSM) on some blood parameters, immune index, carcass characteristics and meat quality of growing rabbits.

MATERIALS AND METHODS

The experimental work of this study was carried out at the Poultry Research Unit, Damanhour University, Egypt from December, 2016 to February, 2017.

Seventy-five New Zealand White rabbits (NZW), 28 days of age and approximately 491±2.77g body weight, were randomly divided into five experimental groups (15 rabbits in each one). All rabbits were fed one of the experimental pelleted diets (Table 1). Rabbits of the 1st group were fed control basal diet contained 0% DSM. The 2nd and 3rd groups, were fed diets contained 10% DSM either without or with EZ (1g/ Kg) addition, respectively. The 4th and 5th groups, were fed diets contained 20% DSM either without or with EZ (1g/ Kg) addition, respectively. Enzyme complex (kemzyme) contained from 300 µ/g Beta-glucanase, 5000 µ/g cellulose, 450 µ/g Alfa amylase and 450 µ/g protase and lipase.

Date stones were obtained from El-Wadi El-Gaded Governorate and processed according to (Al-Harthi *et al.*, 2009) by sun-drying for 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 before mixing to the diets.The diets, DSM, barley and wheat bran samples were chemically analyzed for determination of dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), ash and nitrogen free extract (NFE) according to AOAC (2004) as shown in Table 2.

Table (1): Ingredients and chemical analysis of the experimental diets.

Ingredients	Experimental diets (%)				
	Control (Diet A)	10%DSM (Diet B)	10%DSM (Diet C)	20%DSM (Diet D)	20%DSM (Diet E)
Yellow Corn	11.22	11.22	11.22	11.22	11.22
Barley	15.00	10.00	10.00	10.00	10.00
Wheat bran	23.33	18.33	18.30	8.33	8.30
Alfalfa hay	30.12	30.12	30.10	30.12	30.10
Soya bean meal	17.33	17.33	17.33	17.33	17.33
Data stone meal	0.00	10.00	10.00	20.00	20.00
Vit. + Min. Premix*	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.50	0.50	0.50	0.50	0.50
Dicalcium Phosphate	1.20	1.20	1.20	1.20	1.20
Lime stone	1.00	1.00	1.00	1.00	1.00
Enzyme	0.00	0.00	0.05	0.00	0.05
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Chemical analysis					
Dry matter	87.63	87.18	87.08	87.53	87.53
Ash	6.27	6.54	6.57	6.33	6.30
Crude protein	17.69	17.31	17.36	16.87	16.75
Crude fat	2.91	3.09	3.13	3.55	3.58
Crude fiber	12.78	13.25	13.22	14.07	14.00
NDF	37.02	37.73	37.73	38.29	38.28
ADF	20.67	21.65	21.65	22.43	22.42
Hemicellulose	16.35	16.08	16.08	15.86	15.86
Digestible energy	2542	2508	2508	2480	2480

*__Each 3 Kg contain:__ Vitamin A = 12,000,000 IU, D_3 = 2,200,000 IU, E = 10,000 mg, K_3 = 2,000 mg, B_1 = 1,000 mg, B_2 = 5,000 mg, B_6 = 1,500 mg, B_{12} = 10 mg, Niacin = 30,000 mg, Biotin = 50 mg, Folic acid = 1,000 mg, Pantothenic acid = 10,000 mg, Zinc = 50,000 mg, Manganese = 60,000 mg, Iron = 30,000 mg, Copper = 4,000 mg, Iodine = 1,000 mg, Selenium = 100 mg, Cobalt = 100 mg, Calcium carbonate to 3 Kg.

**According to Feed Composition Tables for rabbits (NRC 1977) and Cheeke (1987).
[NDF% = 28.924 + 0.657 (%CF),
ADF= 9.432+0.912 (CF%).
Hemicellulose = NDF% - ADF%, according to Cheeke (1987)
DE (M Cal /Kg)=(4.36-0.0491*NDF)].
DSM= Date stones meal.

Table (2). The chemical analysis of the date stone, barley and wheat bran.

Composition	Moisture	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen free extract
DSM	8.23	6.38	7.41	17.94	1.42	58.62
Barley	12.20	10.30	1.97	4.60	2.22	68.71
Wheat bran	11.50	14.97	3.40	9.48	4.92	58.40

The rabbits were housed under the same environmental and hygieneconditions in wire cages (60 ×55 × 40 cm). Feed were offered to rabbits *ad libitum*by galvanized feeders and fresh tap water was available all the time by automatic drinkers.

At the end of the experimental period (12 weeks of age), five rabbits were randomly taken from each group and fasted for 12 hours before slaughtering to determine carcass characteristics. Slaughter procedure and carcass analysis were carried out as described by Blasco *et al*. (1993). After complete bleeding, pelt, viscera's and tail were removed then the carcass and its components were weighed as edible parts. The non-edible parts including lung, spleen, stomach, large intestine, small intestine and kidney fat were also weighed as percentage of pre-slaughter weight. Dressing percentage was calculated by dividing the hot dressed carcass weight by pre-slaughter weight and expressed as a percentage according to Steven *et al*. (1981).

Five blood samples were taken from each experimentalgroup at the time of slaughter for haemato-biochemical analysis. Each blood samples were taken into two tubes, the $1^{st}$was supplemented with heparin as anticoagulationwhile, the $2^{nd}$was without heparin. Plasma was separated by centrifugation of the blood at 3000 rpm for 20 minutes and stored at -20°C until performance of biochemical analysis

Blood plasma was analyzed for determination of glucose levels (mg/dl), total protein (g/dl), albumin (g/dl) and globulin (g/dl) whichwere measured according to Trinder (1969),Henry *et al*. (1974), Doumas (1971) and Coles(1974), respectively and different types of globulin (α-, β- and γ-globulins) were determined according to Bossuyt *et al*. (2003). In addition, creatinine, urea-N, total lipids, triglycerides, total cholesterol, HDL and LDL were determined according to (Fabiny and Ertingshausen, 1971), (Sampson *et al.*, 1980), (Chabrol and Charonnat, 1973), (Fossati and Prencipe, 1982), (Stein, 1986), (Lopez-Virella, 1977) and (Friedewald *et al*., 1972), respectively. Thyroid hormones T_3 were analyzed by using radioimmunoassay kits as described by Sharp *et al*. (1987).

The activity of Alkaline phosphates was measuredaccording to Belfield and Goldberg (1971) as well as activities of aspartate amino transferase (AST), and alanine amino transferase (ALT), were estimated according to Reitman and Frankle (1957).

White blood cells, red blood cells and different subclasses of WBC's (lymphocytes, neutrophils, monocytes, eosinophils and basophils percentages) were counted according to Feldman *et al*. (2000). Packed cell volume (PCV; %).

Haemoglobin concentration and red cell indices were determined according to the following equations:

Mean Corpuscular Hemoglobin (MCH; Pg) = Hb x10/ Red blood cell

Mean Corpuscular Hemoglobin Concentration(MCHC,g/dl) = $\frac{\text{Hb x100}}{\text{Packed cell volume}}$

Total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, glutathione peroxidase (GPX) activity and glutathione (GSH) activity were determined according to Koracevic *et al*. (2001), Misra and Fridovich (1972), Paglia and Valentine (1967) and (Ellman, 1959), respectively.

Phagocytic activity (PA) and index (PI) were determined according to Kawahara *et al.* (1991) as following equation:

PA = Percentage of phagocytic cells containing yeast cells.

PI = Number of yeast cell phagocytized / Number of phagocytic cells.

Serum immunoglobulins (IgY, IgM and IgA) were determined using commercial ELISA kits (Kamiya Biomedical Company, USA) according to Bianchi *et al*. (1995).

Lymphocyte transformation test (LTT) was determined following the method described by Balhaa *et al.* (1985).

Serum bactericidal activity (BA) to *Aeromonas hydrophila* strain was determined according to Rainger and Rowley (1993).

Serum lysozyme activity was determined through the turbidimetry described by Hultmark *et al.* (1983) by using lyophilized *Micrococcus lysodekticus* ($OD_{570\ nm}$ = 0.3) as the substrate in phosphate buffer (0.1 M, PH 6.4). Fifty microlitres of fish serum was added to 3 ml of bacterial suspension. The 570 nm absorbance-was-measured-after-mixture (Ao) and incubation for 30 min at 37 ^{o}C(A). The result was expressed by the formula:

Lysozyme activity = (Ao - A) / A

The effect of dietary treatments on the microbial activity of the digestive system include: total bacteria count which was determined according to the method of ICMSF, 1980, as well as the detection of *Salmonella* and

Escherichia coli strains following themethod of ISO-6579: 2002 food microbiology procedure employing the horizontal method of food and animal feeding stuffs (ISO Standards catalogue 07.100.30; WHO, 2010).

Data were statistically examined by analysis of variance (ANOVA) according to Snedecor and Chochran (1982) using SPSS system (2006) as the following model.

$$Yij = \mu + Ti + eij$$

Where: μ= Overall mean of Yij, T = Effect of treatment, i = (1, 2…, etc) and eij= Experimental error.

The differences between means were tested by using Duncan's New Multiple Range Test, (Duncan, 1955).

RESULTSAND DISCUSSION

Carcass characteristics:

The results for Pre-slaughter weight**,** g and carcass characteristics of NZW rabbits as affected by dietary supplementation with EZ are shown in Table 3. The results indicated that the rabbits fed diet containing 10 and 20% DSM without EZ supplementation had lower final body weight. However, the rabbits fed diets containing 10% DSM with EZ supplementation was similar to the rabbits fed basal diet and significantly higher than the other groups.

On the other hand, the group fed 20% DSM with EZ showed significantly ($P<0.05$) greater final body weight than the 20% DSM without EZ supplementation. The negative effects of dietary addition DSM on rabbits growth performance agree with those reported by Masoudi *et al.* (2011) who found that rabbits fed diet containing date stone at levels of 10 and 20 % had lower final body weight than the control group. This may be due to the low levels of protein and energy and high fibre level in DSM. This is agree with the results reported by Soliman *et al.* (2009), Ibrahim *et al.* (2010) and El-Manylawi and El-Banna (2013) who noted that adding EZ to diets contain DSM led to improve the rabbits growth performance. This improvement can be attributed to increase the digestibility and the nutrients availability of the ingested diet.

The rabbits fed 10% DSM with EZ presented significantly ($P<0.05$) higher percentages of dressing than the other groups. Carcass characteristics such as giblets (liver, heart, kidney), lungs, stomach, abdominal fat, spleen, pancreas and cecum were not significantly affected by the treatments. However, the group fed the 20% DSM with EZwas significantly higher percentages of intestinal than the other groups. The present results indicate that dietary DSM addition had no

Table (3): Carcass characteristics and relative weight of internal organs of NZW rabbits fed diets containing different levels of date stones meal (DSM).

	Experimental diets (%)						
Items	**Control (0)**	**10 % DSM**	**10 % DSM + EZ**	**20 % DSM**	**20 % DSM+ EZ**	**SEM**	**Sig.**
Preslaughter weight, g	1885[a]	1731[c]	1898[a]	1656[d]	1790[b]	18.6	0.001
Dressing (%)	59.33[b]	56.36[b c]	64.05[a]	54.91[c]	57.18[bc]	1.034	0.006
Heart (%)	0.44	0.44	0.39	0.41	0.41	0.215	0.506
Liver (%)	2.65	3.48	3.15	3.53	3.33	0.215	0.078
Kidney (%)	0.76	0.82	0.83	0.78	0.74	2.246	0.433
Giblet (%)	3.85	4.64	4.37	4.72	4.48	**??**	**??**
Lungs (%)	0.54	0.700	0.630	0.62	0.660	0.044	0.207
Stomach(%)	2.26	2.380	2.570	2.86	2.590	0.255	0.527
Intestinal(%)	2.94[b]	2.60[b]	2.48[b]	4.33[a]	3.55[ab]	0.41	0.026
Abdominal fat (%)	1.370	1.050	1.440	1.22	1.090	0.107	0.071
Spleen (%)	0.070	0.070	0.080	0.07	0.050	0.008	0.128
Pancreas(%)	0.140	0.150	0.190	0.22	0.130	0.025	0.118
Cecum (%)	1.830	2.460	2.020	2.05	2.340	0.181	0.135

[a,b] Values within a row with different superscripts differ significantly ($P<0.05$).
SEM, Standard error of mean's.

effects on internal organs weights. These results are disagreement with the results reported by Garcia *et al*. (1999) who reported that carcass weight was reduced when adding 6 and 10% palm kernel cake in broiler diets. However, Al-Harthi *et al*. (2009) showed that carcass weight, dressing and intestine length of hens fed dietary date waste meal containing diets had no significant differences. While, abdominal fat percent was decreased ($P\leq0.05$) with increasing date waste meal inclusion level up to 20%. Similarly, Toson *et al*. (1995) reported that inclusion of DSM to replace up to 20% of rabbits basal diet had no adverse effect on carcass traits of growing rabbits. Also, Masoudi *et al*. (2011) revealed that the carcass characteristics of broiler chickens fed diets containing date stone showed the same dressed weight and liver percentage as the control.

Furthermore, Attia, and Al-Harthi (2015) found that inclusion of different concentrations of date stoneshad not significantly effect on dressed carcass weight and total edible parts percentage.

The improvement in dressing percentage in groups fed 10% DSM plus EZ may be attributed to that EZ supplementation increased the digestibility and availability of nutrients in the low density diets containing DSM therefore improved the percentages of dressing. This results agree with those reported by Ibrahim *et al*. (2010) who revealed that kemzyme addition to diets containing 50 or 100% ground date stone increased the dressing percentage compared to the other groups. Also, Attia *et al*. (2014) found that multienzymes addition to broiler diets led to increase in relative dressingweight and total edible parts compared to the control group. Similarly, Attia *et al*. (2003) and Salem *et al*. (2003) concluded that the improvement in carcass yield due to enzyme supplementation is a reflection of increase in nutrient availability for tissue growth. However, El-Manylawi and El-Banna (2013) showed that replacing DSM at levels 10 or 20% without or with Allzyme ® SSF in the diets, led to insignificant differences in dressing and giblets% of rabbits compared to the control.

Chemical composition of rabbits meat :

The chemical characteristics of rabbit meat as affected by feeding DSM and EZare shown in Tables 4. Protein and lipid percentage of meat was significantly lower in rabbit fed 10 and 20% DSM with EZ as compared to those fed the control diet. Moreover, the groups fed 10 % DSM with EZ showed significantly higher moisture percentage in rabbits meat than those fed 10 and 20% DSM without EZ. While, Meat ash percentage showed no significant differences between groups.In harmony with the obtained results of meat quality, El-Manylawi and El-Banna (2013) showed that dietary inclusion of DSM at 10 and 20 % of the diet reduced the ether extract of meat compared to the control group, while, moisture of meat was reduced at dietary addition of 20 % DSM compared to the control group. Similarly, Attia *et al*. (2014) found that meat protein percentage was increased while meat moisture decreased by multienzymes addition in diets. This indicate that multienzymes addition to improvement in meat quality traits. However, there was no significant effect of inclusion of whole inedible dates in diets up to 15% on dry matter, crude protein, ether extract and ash of meat (El-Deek *et al*., 2010).

Similarly, Al-Homidan (2003) observed that whole inedible dates did not significantly affect carcass quality of broilers. Also, Abaza and Omara (2011) recorded insignificant differences in the chemical composition of NZW rabbit meat due to feeding diets supplemented with EZ.

Table (4). Meat quality of NZW rabbits fed diets containing different levels of date stone meal (DSM).

Items	Experimental diets (%)					SEM	Sig.
	Control	10 % DSM	10 % DSM + EZ	20 % DSM	20 %DSM+EZ		
Moisture	67.78^{ab}	66.32^{bc}	71.28^{a}	62.84^{c}	69.48^{ab}	1.533	0.010
Protein	22.47^{a}	20.55^{ab}	21.57^{a}	19.43^{b}	21.66^{a}	0.67	0.041
ASH	1.154	1.142	1.164	1.186	1.176	0.018	0.448
Lipid	5.520^{a}	5.120^{ab}	5.380^{a}	4.300^{b}	5.480^{a}	0.303	0.054

^{a,b} Values within a row with different superscripts differ significantly (P<0.05).
SEM, Standard error of mean's.

The blood constituents:

The results of blood biochemical constituents of NZW rabbits fed DSM supplemented with EZ are shown in Table 5. Feeding 10% DSM plus EZ and control diets resulted in significantly higher total lipid, triglycerides, HDL, LDL and glucose than those fed the other groups, however, the difference between rabbits fed 10 and 20% DSM plus EZ were not significant in HDL and LDL. In addition, rabbits fed 20% DSM diets with or without EZ had significantly higher urea-N than other groups. On the other hand, rabbits fed 10 and 20% DSM with EZ had significantly (P<0.05) higher T_3 than those fed20% DSM without EZ supplementation. Moreover, rabbits fed10% DSM with EZand controldiets had significantly lower GPX and SOD than the other groups.

No significant differences were recorded in total protein, albumin, AST, ALT, alkaline phosphates, cholesterol, creatinine, TAC and GSH among the different experimental groups. The present results of biochemical constituents of blood are in agreement with the results reported by Al-Bowait and Al-Sultan (2006) who reported that blood glucose levelwas not significantly affected in birds fed diet containing date stones. Similarly, El-Deek *et al*. (2008) and Al-Harthi *et al*. (2009) found insignificant reduction in plasma total protein levels in rabbits fed diet containing date waste meal. Also, There was no significant effect of addition of date stones to diets on HDL (Masoudi *et al*., 2011). The present results agree with these reported by Mousa (2013) who found that concentrations of serum cholesterol, glucose and activity of SALT and SAST did not significantly differ among the three experimental groups fed date stones. Similarly, Attia and Al-Harthi (2015) reported that broilers can be fed date waste up to 200 g/kg from 21 to 40 days of age without adverse

effects on protein metabolism and liver and renal functions. El-Kelawy and El-Kelawy (2016) showed that glucose was significantly ($P<0.05$) higher in rabbits fed diet supplemented with EZ compared to those fed basal diet (control).

On the other hand, there was no significant effect of EZ supplementations on blood plasma albumin, total lipids, cholesterol, creatinine, AST, ALT and alkaline phosphates. Also, results regarding albumin and globulin are in agreement with those reported by Abaza and Omara (2011) who found no significant changes in the levels of the albumin and globulin in the blood of rabbits fed diets supplemented with EZ. Results regarding blood glucose are in agreement with those reported by Attia *et al*. (2012) who recorded significant increase in blood glucose of rabbits supplemented with kemzyme compared to controls. Also, Saleh *et al*., (2006) found that kemzyme supplementation to the ration of growing turkey resulted in a significant increase level of blood glucose (within normal range). They attributed such finding to the improvement of digestibility and metabolism of different nutrients in the diet by the addition of exogenous enzymes thus, actively affects the blood levels of basic nutrients (Mathlouthi *et al*., 2003). Moreover, dates and date stones are a good source of antioxidants, mainly carotenoids and phenolics (Al-Farsi and Lee, 2008). Masoudi *et al*. (2011) found that blood glucose concentration was increased while, the triglyceride and LDL concentration were not significantly different between control and date stones diets. Furthermore, Mousa (2013) found that serum total protein, albumin and globulin for growing rabbits significantly ($P<0.05$) decreased by 30% date stone inclusion in the diet compared with those fed control or 20% date stones diets.

Blood hematological parameters

Enzyme supplementation to 10% DSM diet resulted in significantly higher RBC's than the control and other groups. Nevertheless, the other blood plasma components, haematological criteria and differential leucocytes counts were not significantly affected by the dietary treatments (Table 6).

The present results of blood hematological parameters are in agreement with the results reported by Afsari *et al*., (2013) who stated that date stones and olive pulp with non-phytate phosphorus and phytase supplementation did not significantly affect white blood cell count (heterophils, eosinophils, basophils, lymphocytes and monocytes). Also, Kurtong (2014) found that rabbits fed diets supplemented with EZ showed no significant difference ($P>0.05$) for, Hb, WBC and PCV among all treatments. Results of El-Kelawy

Table (5): Blood biochemical constituents of NZW rabbits fed diets containing different levels of date stones meal (DSM).

Items	Experimental diets (%)						
	Control	10 % DSM	10 % DSM+ EZ	20 % DSM	20 % DSM + EZ	SEM	Sig.
Total protein (g/dl)	5	4.95	5	4.75	4.95	0.155	0.771
Albumin (g/dl)	2.2	2.28	2.08	2.48	2.2	0.093	0.074
AST(U/L)	20.5	22	22.4	20.1	18	1.029	0.059
ALT (U/L)	62.3	57	59.3	59.3	59	1.228	0.083
Alkaline phosphates(IU/L)	12.8	12.8	12.3	13.5	14.3	0.547	0.452
Urea (mg/dl)	17.2^{b}	17.5^{b}	17.9^{b}	19.7^{a}	20.0^{a}	0.341	0.001
Creatinine (mg/dl)	0.975	0.8	0.9	0.875	1.003	0.049	0.06
Total lipid (mg/dl)	456^{a}	384^{b}	440^{a}	400^{b}	384^{b}	6.314	0.001
Triglycerides(mg/dl)	167^{b}	164^{c}	171^{a}	165^{c}	166^{c}	1.625	0.001
Cholesterol (mg/dl)	185	184	180	184	181	1.666	0.365
HDL(mg/dl)	41.3^{b}	41.5^{b}	43.3^{a}	40.8^{b}	42.5^{ab}	0.55	0.029
LDL(mg/dl)	97.8^{ab}	$93.5c^{d}$	98.3^{a}	92.3^{d}	$95.5b^{c}$	0.885	0.003
Glucose (mg/dl)	83.3^{ab}	78.3^{b}	85.0^{a}	78.5^{b}	80.3^{ab}	1.781	0.054
T3 (ng / ml)	2.21^{ab}	2.21^{ab}	2.25^{a}	2.16^{b}	2.23^{a}	0.019	0.024
TAC (mg/dl)	420	422	420	421	422	1.567	0.76
GPX (mg/dl)	0.475^{b}	0.525^{a}	0.450^{b}	0.523^{a}	0.508^{a}	0.01	0.016
GSH (mg/dl)	989	996	990	990	997	2.072	0.065
SOD (mg/dl)	239^{b}	244^{a}	239^{b}	247^{a}	244^{a}	1.321	0.001

$^{a,b...d}$ Values within a row with different superscripts differ significantly ($P<0.05$).
SEM, Standard error of mean's; AST=Aspartate amino transferase; ALT=Alanine amino transferase; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; T3= Triiodothyronine; T4=Thyroxine; TAC= Total antioxidant capacity; GPX = Glutathione peroxidase; GSH= Glutathione; SOD=Superoxide dismutase.

and El-Kelawy (2016) suggested that Hemoglobin, PCV, MCV, MCH, MCHC, WBC's and differential leukocytes counts were not significantly affected by EZ supplementation to rabbits diets.

Enzyme supplementation to 10% DSM diet resulted in significantly higher β-globulin than the other groups. Moreover, rabbits fed 10% DSM with

Table (6): Blood hematological constituents of NZW rabbits fed diets containing different levels of date stones meal (DSM).

Items	Experimental diets (%)						
	Control	10 % DSM	10 % DSM + EZ	20 % DSM	20 % DSM+ EZ	SEM	Sig.
RBC's (10^6/cmm^3)	1.45b	1.45b	1.68a	1.50b	1.40b	0.057	0.026
Hemoglobin (g/100ml)	9.50	10.00	9.75	10.75	10.00	0.354	0.179
PCV %	29.00	30.30	29.50	30.30	29.80	0.879	0.831
MCH (Ug)	201.0	210.0	198.0	181.0	215.0	2.986	0.098
MCHC (%)	65.80	69.50	65.40	64.30	72.00	0.895	0.36
WBC's (10^3/cmm^3)	32.70	33.10	33.00	35.70	33.60	0.444	0.164
Monocytes (10^3)	4.50	4.50	3.75	5.00	4.25	0.308	0.109
Basophils (10^3)	0.50	0.50	1.00	1.00	0.50	0.224	0.24
Eosinophils (10^3)	9.50	9.50	9.75	8.75	9.75	0.377	0.348
Neutrophils (10^3)	49.50	50.00	50.00	48.80	48.80	0.886	0.735
Lymphocytes (10^3)	36.00	35.50	35.50	36.50	36.80	0.444	0.202

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.
SEM, Standard error of mean's; RBC's= Red blood cell; PCV=Packed cell volume; MCH=Mean corpuscular hemoglobin; WBC's=White blood cell

or without EZ and control groups had significantly higher α–globulin, BA and LTT than those fed diet containing 20% DSM with or without EZ. However, the differences between the 10 and 20%DSM with EZ was not significant in BA and LTT. In addition, EZ supplementation to diet containing 10 and 20% DSM and control diet resulted in significantly higher IgA, IgM and IgG than the other DSM groups. However, the differences between 10% DSM with or without EZ were not significant in IgA.

Immune response

On the other hand, no significant differences were recorded in globulin, γ- globulin, LA, PI and PA among the experimental groups (Table 7). El-Kelawy and El-Kelawy (2016) showed that rabbits fed diet supplemented with EZ had significant higher PA% than those fed basal diet (control).In addition, However, Attia, and Al-Harthi (2015) reported that inclusion of date waste up to 200 g/kg did not affect antibody titers to NDV at 40 days of age.

Table (7): Immune indices in blood of NZW rabbits fed diets containing different levels of date stones meal (DSM).

Items	Experimental diets (%)						
	Control	10 % DSM	10 % DSM + EZ	20 % DSM	20 % DSM+ EZ	SEM	Sig.
Globulin (g/dl)	2.80	2.68	2.93	2.28	2.75	0.171	0.12
α–globulin (g/dl)	1.08^{a}	1.08^{a}	1.13^{a}	0.900^{b}	0.925^{b}	0.046	0.007
B-globulin (g/dl)	0.750^{b}	0.850^{b}	1.000^{a}	0.825^{b}	0.850^{b}	0.044	0.013
Globulin–γ (g/dl)	0.975	0.75	0.8	0.55	0.975	0.175	0.417
LA (IU%)	0.09	0.115	0.103	0.118	0.103	0.007	0.067
BA (%)	37.8^{c}	38.5^{bc}	41.0^{a}	37.3^{c}	40.3^{ab}	0.61	0.001
LTT(%)	30.5^{c}	29.3^{c}	34.5^{a}	31.5^{bc}	33.5^{ab}	0.963	0.006
PI (%)	1.780	1.800	1.800	1.780	1.850	0.760	0.956
PA (%)	16.30	18.00	18.30	16.50	17.80	0.577	0.075
IgA (mg/100 ml)	86.1^{a}	83.5^{ab}	86.3^{a}	83.0^{b}	86.5^{a}	0.979	0.035
IgM (mg/100 ml)	242^{a}	237^{b}	241^{a}	235^{b}	240^{a}	1.49	0.046
IgG (mg/100 ml)	983^{a}	980^{b}	987^{a}	978^{b}	985^{a}	1.49	0.01

^{a,b} Values within a row with different superscripts differ significantly (P<0.05).
SEM, Standard error of mean's; LA= Lysozyme activity; BA=Bactriocide activity ; LTT=Lymphocyte transformation test; PI=Phagocytic index; PA =Phagocytic activity.

Microbial activity of the digestive system

The microbial activity of the digestive system of rabbit as affected by DSM and EZ supplementation are shown in Tables 8. The groups on the 10 and 20% DSM with or without EZ showed significantly lower total bacterial count, *Salmonella, E. Coli and Proteus* than the control groups. This results of microbial activity of the digestive system are in agreement with the results reported by El-Deek *et al*. (2008) who found that total bacteria, E. coli and fungi group counts were decrease with increasing the dietary date waste meal level up to 15% as compared to those of the control group. Also, this finding agree with that of Al-Harthi *et al*. (2009) who found that the total bacteria and *E. coli* were decreased with inclusion date waste meal up to 20% of the diet. Also, Abd El-Megeed (2016) revealed that total bacterial counts on the jejunum contents showed a decrease when used the date stones in broiler chicks diets. The improvement in performance of rabbits with multienzymes

Table (8). Microbial activity in digestive canal of NZW rabbits fed diets containing different levels of date stones meal (DSM).

Items	Experimental diets (%)						
	Control	10 % DSM	10 % DSM + EZ	20 % DSM	20 % DSM+ EZ	SEM	Sig.
TBC	6250^{a}	3850^{b}	4000^{b}	3500^{b}	2750^{b}	456	0.004
E. coli	4000^{a}	1600^{b}	2500^{b}	2950^{b}	1150^{b}	682	0.002
Salmonella	5250^{a}	1375^{b}	2300^{b}	2425^{b}	1250^{b}	589	0.022
Proteus	1800^{a}	625^{b}	1050^{b}	1000^{b}	1000^{b}	175	0.003
Mortality rate	0.0	0.0	0.0	0.0	0.0	--	--

^{a,b} Values within a row with different superscripts differ significantly (P<0.05).
SEM, Standard error of mean's.

addition may be due to a reduction in counts of pathogens, Gram-positive cocci, total anaerobes, and enterococci bacteria in the intestines (Tabook *et al*., 2006).

In conclusion, date stones meal can be included in the rabbit diets up to 10% with adding enzyme complex (Kemzyme) without adverse effects on body weights, blood parameters, immune index, carcass characteristics and meat quality.

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تأثير إضافة مستحضر انزيمات (كيزيم) في العلائق المحتوية على مسحوق نوى البلح على وبعض مكونات بلازما الدم والاستجابة المناعية والنشاط الميكروبى وخصائص الذبيحة في الأرانب النامية.

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أجريت هذه الدراسة لتقييم تأثير إضافة مستحضر انزيمات (كيزيم) لعلائق محتوية على مسحوق نوى البلح على وزن الجسم وبعض مكونات الدم والاستجابة المناعية وخصائص الذبيحة للأرانب النامية عمر 28 يوم ومتوسط مزن 491 ± . تم توزيع عدد خمسة وسبعون أرنب نيوزيلندي أبيض بالتساوي عشوئيا إلى خمس مجموعات تجريبية. المجموعة الاولي غذيت على العليقة الكنترول التي لا تحتوي على مسحوق نوى بلح (مجموعة كنترول) والمجموعات الثانية والثالثة غذيت على عليقة تحتوي على 10% مسحوق نوى بلح بدون أو مع إضافة الإنزيمات على التوالي. والمجموعات الرابعة والخامسة غذيت على عليقة تحتوي على 20% مسحوق نوى بلح بدون أو مع إضافة الإنزيمات على التوالي. في نهاية الفترة التجريبية تم أخذ عدد خمسة أرانب من كل مجموعة لتقدير صفات الذبيحة وبعض مكونات الدم وجودة اللحم.

أوضحت النتائج أن الأرانب المغذاه على 10% مسحوق نوى بلح مع إضافة الإنزيمات كانت أعلى في وزن الجسم ونسبة الذبيحة مقارنة بالمجموعات الأخرى.

كانت نسبة البروتين والدهن في لحم الأرانب المغذاه على 10 و 20 % نوى بلح مع إضافة الانزيمات او بدون أقل من تلك المغذاه على العليقة الكنترول . أدت إضافة الانزيمات للعليقة المحتوية على 10% من نوى البلح إلى زيادة معنويافي مستويات الجلوكوز والدهون الكلية والجلسريدات الثلاثية والكولسترول عالي ومنخفض الكثافة وعدد كرات الدم الحمراء وانخفاض معنويا في نشاط انزيمات الأكسدة GPX وSOD مقارنة بمجموعات نوى البلح الأخرى. كانت الأرانب المغذاه على علائق 10% مع او بدون الانزيمات و مجموعة الكنترولأعلى معنويا في الألفا جلوبيولين ونشاط مقاومة البكتريا ومعامل تحويل الخلايا الليمفاوية (LTT) من المجموعات الأخرى. أدت إضافة الانزيمات للعلائق المحتوية على 10 و 20% من نوى البلح و مجموعة الكنترول إلى زيادة معنوية في الجلوبيولينات المناعية عن مجموعات نوى البلح الأخرى. لم تسجل أي فروق معنوية في نشاط انزيم ناقل الألانين (ALT) ونشاط انزيم ناقل الاسبارتات (AST) والكرياتينين و الكولسترول والبروتين الكلي والألبيومين ونشاط انزيمات الأكسدة TAC وGSH والجلوبيلولين و الجاما جلوبيولين ونشاط الليزوزيم والنشاط البلعمي ودليل النشاط البلعمي وأنواع كرات الدم البيضاء بين المجموعات المختلفة. المجموعات المغذاه على نوى البلح مع أو بدون الإنزيمات أدت إلى خفض العد البكتيرية بالجهاز الهضمي مقارنة مع مجموعة الكنترول.

التوصية: توصي نتائج التجربة بإمكانية إضافة مسحوق نوى البلح في علائق الأرانب حتى 10% مع إضافة مستحضر الانزيمات كيمزيم بدون أي أثار سيئة على وزن الجسم وصفات الذبيحة ومكونات الدم والاستجابة المناعية.