



Olive Cake and Fibrolytic Enzymes Inclusion in Ewes' Diets and its Effect on Blood Values and Reproductive Performance

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Abstract | Enhancing the productive performance of ruminant by improving nutrients metabolism get much attention. The aim of the current study was to investigate the effect of feeding olive cake either treated or untreated with fibrolytic enzymes on the reproductive performance and some blood parameters in ewes. Forty-eight multiparous lactating Barki ewes at second to third season of lactation (42±3 kg of live body weight) were equally and randomly allotted to four dietary groups. Control group (G1) was fed on 60 % concentrate feed mixture (CFM), 40 % green maize, first group (G2) as control with replacing 30% of CFM with olive cake, second group (G3) as control with replacing 30% of CFM with olive cake silage and third groups (G4) as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme. The results indicated no significant change were noted between control and other groups in blood serum total protein, Albumin, Tri-glycerides, glucose, ALT and urea. G1 significantly increased (p<0.05) T3 as compared with G1, while G4 recorded the highest value for progesterone as compared with other treatments. Amongst the four groups, G2, G3, G4 improved lambing rate, prolificacy rate, fertility%, female ratio, litter weight as compared with G1, while, the highest stillbirth being in G1. It could be concluded that inclusion of olive cake silage either treated or untreated with fibrolytic enzyme could improve the reproductive performance of ewes.

Keywords | Olive cake silage, Ewes, Fibrolytic enzyme, Reproductive performance

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INTRODUCTION

The exploitation of agro by-product is the most important means to challenge the shortage of concentrated feed in Egypt (Hadhoud et al., 2020). The olive cake is one of competing for agro-industrial by-products from olive oil which produced by 98% in the mediterranean countries (FAO, 2010). The type of olive fruit and the method of extraction are the two main factors that influence its composition, also, it is characterized by increasing crude fibre, low protein (Molina-Alcaide and Nefzaoui, 1996;

Abbeddou et al., 2011) and rich in monounsaturated fatty acids, that decrease low-density lipoprotein (LDL) within the body as well as high-density lipoprotein (HDL) (Savarese et al., 2007). Likewise, triglyceride accumulation was decreased in the liver, the level of lipid peroxides was decreased, improved liver damage markers and inhibit liver fibrosis (Karakilcik et al., 2004). On the other hand, there were anti-nutritional factors like tannins, lead to decreased nutrient digestibility and nutritive value of olive cake (Abd El Tawab et al., 2016). Moreover, fibrolytic enzymes can be an effective additive with agro-industrial by-product to

improve the nutritive value of fibre-rich diets (Khattab and Abd El Tawab, 2018; Khattab et al., 2019; Abd El Tawab et al., 2019) and improve productive performance (Khattab et al., 2012; Abd El Tawab et al., 2017; Abd El Tawab et al., 2020).

The reproductive performance in sheep is affected by genetic and environmental factors such as breed and nutrition (Petrovic et al., 2012). The nutritional sources are critical for each stage of the reproductive process of sheep, from puberty to conception (Blache and Martin, 2009). Through two directions, the first direction is directly by equipping the basic nutrients required for the reproductive processes and other direction by indirect effects on the circulating concentrations of the hormones and other nutrient-sensitive metabolites that are important for the success of these processes (Fernando et al., 2011). Olive oil by-product may have a positive effect on fertility and most breeders prefer feed pregnant ewes on it due to its balanced valuable fats that required for intrauterine fetal development (Aguilera et al., 1992). In recent study El-Tarabany et al. (2018) found that olive cake in the diet decreased significantly the level of E2 and increased P4 levels during pregnancy, fertility hormones regulate the reproductive cycle in females and used test for infertility and early or delayed puberty. These are associated with ovulation, ovarian syndrome and premature ovarian failure. Fat in olive cake has been involved in the production and regulation of steroid hormones, which are hydrophobic molecules made from cholesterol in the smooth endoplasmic reticulum, a compartment within a cell in which lipids, hormones, and proteins. Steroid hormones are essential in regulating sexuality, reproduction, development of the sex organs and regulating the water balance in the body.

Enhancing reproductive efficiency in ewes maintained in less than optimal environments is an important goal; therefore, the objectives of this study were to determine the reproductive response and some blood parameters of ewes, fed on ensiling olive cake treated with or without fibrolytic enzymes.

MATERIALS AND METHODS

STUDY AREA AND ANIMAL CARE

The experiment was carried out from May to December 2018 at Animal Production Experimental Farm (30.28547430865962, 31.385319302925275), Nuclear Research Center (Egypt), while chemical analyses were performed at the Laboratory of dairy animal production, national research centre (Egypt). Ewes were managed in accordance with the guide for the care and use of agricultural animals in agricultural research and teaching (Federation of Animal Science Societies; Champaign, IL, USA).

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COLLECTION AND ENSILING OF CRUDE OLIVE CAKE

The Fresh olive cake (*Olea europaea*) collected from a modern olive oil extraction plant at Al salhiya agricultural company, factory-sharkia governorate, Egypt, was used for the preparation of treated olive cakes. The crude olive cake was prepared simply by drying to reach 65-70% moisture before ensiling. Olive cake silage was prepared by spraying fresh olive cake with four-litres of enzyme solution per ton DM of olive cake (treated silage), or without crude enzyme (untreated silage). The olive cake silages were ensiled in plastic bags and kept for two months before feeding to the ewes.

ENZYME SOURCES

Fibrolytic enzymes produced from anaerobic bacteria (*Clostridium butyricum*) at the dairy science department, national research centre, Dokki, Giza, Egypt. Each gram of enzymes powder contain an enzyme mixture of 5179 IU g⁻¹ of cellulase and 866 IU g⁻¹ of tannase (Khattab et al., 2017).

EXPERIMENTAL DESIGN AND ANIMAL WELFARE

Forty-eight lactating Barki ewes at second to the third season of lactation, with average live body weight (42±3 kg), were randomly divided according to previous lactation season productivity into four groups (12 animals each). Control group (G1) was fed on 60 % concentrate feed mixture (CFM), 40 % green maize, first group (G2) was as control with replacing 30% of CFM with olive cake, second group (G3) fed as control with replacing 30% of CFM with olive cake silage and third groups (G4) fed as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme. Animal were fed individually twice daily at 0800 and 1700 according to NRC (1985). The freshwater was always available to the animals. The experiment were extended for 90 days and started one week after parturition. Ingredients used in rations and their chemical compositions are summarized in Table (1 and 2).

BLOOD SAMPLING AND BIOCHEMICAL ANALYSIS

Blood samples were collected in vacuum tubes from the jugular vein biweekly after pregnancy diagnosis. On the day of sampling, blood was taken at 8.0 am into tubes. Blood serum samples were obtained by centrifugating at 3500 rpm for 15 min within an hour of collection and stored at -20°C until analysis. Serum total protein, Albumin and triglycerides were estimated as described by (Azzaz et al., 2020), Serum globulin records were obtained by difference between total protein and albumin, Progesterone hormone levels were estimated by according to (Nulsen and Peluso, 1992). While, serum total triiodothyronine (T3) and thyroxine (T4) concentrations were determined according to

Table 1: Ingredient of the experimental concentrate feed mixture

Ingredient (%)	Experimental groups			
	G1	G2	G3	G4
Yellow corn	22	10	10	10
Sugar beet pulp	31	19	19	19
Wheat bran	27	19	19	19
Soya bean meal	7	9	9	9
Uncorticated cotton seed cake	10	10	10	10
Dried olive cake	-	30	-	-
Silage olive cake	-	-	30	-
Silage olive cake with enzyme	-	-	-	30
NaCl	1	1	1	1
Dicalcium phosphate	1.5	1.5	1.5	1.5
Vitamins (AD ₃ E)	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.1	0.1	0.1	0.1
Mineral mix	0.3	0.3	0.3	0.3

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme. Mineral mix. : 30 mg Zn as Zn SO₄.7H₂O; 20 mg Mn as MnSO₄.H₂O; 0.5 mg I as KI; 0.1 mg Co as CoCl₂; 0.1 mg Se as Na₂SeO₃,1500 IU vitamin A; 250 IU vitamin D and 16 IU vitamin E per Kg DM.

Table 2: Chemical analysis of feed ingredients (on DM basis).

	OM	CP	NDF	ADF	EE	Ash
CFM	93.98	12.73	35.7	17.6	6.28	6.02
Green maize	86.95	7.45	73.1	40.7	6.25	13.05
crude olive cake	94.71	6.17	67.4	53.1	12.76	5.29
Untreated olive cake silage	97.37	5.16	71.5	53.7	14.79	2.63
Treated olive cake silage with enzymes	96.78	5.38	69.5	50.0	12.36	3.22
Experimental ration						
G1	91.17	10.62	6.27	50.64	26.83	8.83
G2	91.14	11.97	6.52	53.77	31.20	8.86
G3	91.19	10.93	7.42	51.43	30.18	8.81
G4	91.48	12.15	6.85	52.63	31.10	8.52

DM: dry matter; OM: organic matter; CP: crude protein; NDF: natural detergent fiber; ADF: acid detergent fiber; EE: ether extract; CFM: Concentrate feed mixture

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme.

the procedure specified with the RIA coated tubes kits produced by RIAKEYTUBE II®, Republic of Korea (Code No: RT10&RT02).

REPRODUCTIVE PERFORMANCE

During the experimental period ewe’s reproductive data were recorded. The teaser ram was introduced two times daily at 9.00 am and 4.00 pm of each group for an hour to detect the onset of estrus. Ewes being receptive for teaser and standing for mounting by the teaser were considered in estrus. Ewes in estrus were mated by a mature and healthy ram. The pregnancy rate was diagnosed by trans-abdomi-

nal ultrasonography (SonoVet 2000; 4.5 to 6 MHz convex transducer; MedisonCO, Seoul, Korea) on days 35 to 40 after mating when ewes passed two estrous cycles with the absence of heat signs. Litter weight and birth weight were recorded. Reproductive evidence was calculated for each animal in four groups. Fertility %: number of ewes that kidded/number of ewes joined to the rams x 100. Prolificacy rate (Litter size): number of kids born/number of ewes that kidded. Fecundity %: number of kids born/number of ewes joined to the rams x 100. Mortality and survivability rates (stillbirth) were calculated (Kuźnicka and Rant, 2013).

Table 3: Blood serum constituents of ewes as affected by the experimental rations (data are expressed as mean ±SE)

Items	Experimental groups			
	G1	G2	G3	G4
TP (g/dl)	6.91±0.50	7.34 ±0.50	7.92±0.50	7.82±0.50
Albumin (g/dl)	3.46±0.21	3.92±0.23	3.80±0.25	3.35±0.20
Globulin (g/dl)	3.45 ±0.31 b	3.42 ±0.35 ab	4.12 ±0.29 a	4.47 ±0.37 a
A/G ratio	1.00±0.08 ab	1.15±0.10 a	0.92±0.08 b	0.75 ±0.10 b
Tri-Glycerides (mg/dl)	76.26±13.43	84.57±13.43	68.61±13.43	74.84±13.43
Cholesterol (mg/dl)	109.93 ±12.33 a	117.25 ±12.33 a	103.42 ±12.3 ab	97.13 ±12.33 b
glucose (mg/dl)	65.9±7.37	74.68±7.78	74.68±7.37	83.84±7.78
Urea (mg/dl)	40.64±5.32	50.54±5.32	47.23±5.32	42.76±0.15
AST (U/ml)	43.51 ±2.31 a	45.18 ±2.31 a	38.63 ±2.31 ab	33.94 ±2.31 b
ALT (U/ml)	27.96±3.48	24.82±3.48	24.03±3.48	22.2±3.48

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme. SE: standard error

Different letters a, b in the same row differ significantly ($p < 0.05$)

Table 4: Thyroid and progesterone (ng/mL) of ewes as affected by the experimental rations (data are expressed as mean ±SE)

Items	Experimental groups			
	G1	G2	G3	G4
T3	1.25 ±0.07 bc	1.4 ±0.01 a	1.23 ±0.01 c	1.38 ±0.06 ab
T4	87.70 ±1.6 bc	80.50 ±4.0 c	97.20 ±2.7 a	95.00 ±1.7 ab
progesterone p4	9.53 ±0.33 b	9.31 ±0.43 b	9.39 ±0.23 b	10.43 ±0.35 a

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme . SE: standard error

Different letters a, b, c in the same row differ significantly ($p < 0.05$)

STATISTICAL ANALYSIS

The experimental data were statistically analyzed using the general model program (SAS, 2004). Data were subjected to analysis of variance using one way according to the following model. $Y_{ijk} = \mu + T_i + E_{ij}$. Where: Y_{ijk} = any observation. μ =overall mean. T_i = the fixed effect of the estimate. E_{ij} = general error The differences among means were tested using Duncan's multiple range test ($p < 0.05$) (El-Zaher et al., 2020).

RESULTS AND DISCUSSION

THE BLOOD CHEMICAL COMPOSITION

Table 3 shows the blood chemical composition. Total protein and albumin did not differ significantly among the four groups. Whilst, a significant high ($P < 0.05$) proportion of globulin was detected with ewes in G4 (4.47 g/dl), the change in total protein concentrations maybe for the contain of olive cake an anti-nutritional factor-like tannins (Al-Masri and Guenther, 1995) that decreases nutrient digestibility and nutritive value. Ewes in G4 and G3 showed significantly ($p < 0.05$) lower A/G ratio than G2 and G1,

even though, Tri-glycerides of ewes in G3 exhibited lower level than those of ewes in G4, G1 and G2, the increases in triglycerides were due to triglycerides synthesis in the epithelium of the small intestine and liver cells was improved also increasing the absorption of these fats from the small intestine (Chichlowski et al., 2005). Regarding the differences between treatments, cholesterol was significantly ($P < 0.005$) different, being higher in ewes fed on the olive cake than ewes fed on control, olive cake silage and olive cake silage with fibrolytic enzyme, it can be also assumed that decreased cholesterol level in ewes fed on treated olive cake silage reflects the balance between mono- and poly-unsaturated fats in the olive cake, and these balances maintain the balance between HDL and LDL cholesterol (Mensink et al., 1992). Previous study also suggested that diets contain olive cake high mono-unsaturated, omega-3 fatty acids and non-fat components (phenolic compounds) which contributed low cholesterol level (Visioli and Galli, 2002). A higher level of glucose was observed in ewes fed on olive cake silage with fibrolytic enzyme and was similar in G2 and G3, but lower in G1, increased levels of glucose reflects the improvement in diet energy utilization. A high-

Table 5: Effect of different experimental diets on the reproductive performance of ewes (data are expressed as mean ±SE)

ITEMS	Experimental groups			
	G1	G2	G3	G4
No. of ewes	12	12	12	12
DMI (kg/head/day)	1.76	1.78	1.59	1.59
Lambing rate (%)	91.66±4.17	100±4.17	100±4.17	100±4.17
Sex ratio				
Male	58.33±1.44a	50.00±1.44a	58.33±1.44a	41.67±1.44b
Female	50.00±1.57c	66.67±1.57ab	58.33±1.57bc	75.00±1.57 a
Reproductive efficiency:				
Fertility (%)	87.50±6.85	90.28±6.85	91.67±6.85	91.67±6.85
Prolificacy rate (Litter size)	1.08±0.12	1.17±0.12	1.17±0.12	1.17±0.12
Fecundity (%)	104.17±12.52	106.94±12.52	108.33±12.52	100±12.52
Number of service per conception (NS/C)	1.17±0.15	1.42±0.15	1.00±0.15	1.17±0.15
Conception rate from first Insemination	76.92±9.76	69.23±9.76	76.92±9.76	84.61±9.76
Conception rate %	92.00±5.58	81.00±5.58	92.00±5.58	96.00±5.58
Litter traits				
Litter weight (kg)	3.74±0.21	4.24±0.21	4.29±0.21	3.86±0.21
Ewes weight (kg)	43.90±1.46	44.83±1.40	46.50±1.40	46.08±1.40
Stillbirth (%)	25±0.99 a	0.00±0.99 c	16.67±0.99 b	16.67±0.99 b

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme. SE: standard error

Fertility %: number of ewes that kidded/number of ewes joined to the rams x 100. Prolificacy rate (Litter size): number of kids born/number of ewes that kidded. Fecundity %: number of kids born/number of ewes joined to the rams x 100. Stillbirth: defined as death of a lamb that occurs just prior to, during, or within 48 h from parturition.

-her level of glucose in ewes G3 is evidence of the lower energy deficit of the ewes receiving fibrolytic enzymes. In spite of, the insignificance of differences between treatments in other characteristics, there was the tendency of higher concentration of urea and AST for ewes feed on olive cake ration than other groups (Table 3), whereas, ewes fed on olive cake silage with fibrolytic enzyme had a lower liver enzyme (ALT and AST). Urea and AST were higher for ewes feed on olive cake ration than other groups, this can be attributed to changes in the rumen fermentation processes under the influence of olive by-product (Hadhoud et al., 2020). Olive cake by-product characterized by a lower percentage of saturated fatty acids and a higher percentage of unsaturated (Wong et al., 2014). This inhibit oxidative stress and fat deposition. Decreased levels of urea in treated olive silage this agree with Tavafi et al. (2012) and Visioli et al. (2009), who found that treated olive by-product makes kidney normally work. Nekooeian et al. (2011) reported that olive cake by-product inhibits lipid peroxidation, enhancing renal glutathione content and antioxidant enzymes activity, also, this may be due to olive oil cake silage is rich in monounsaturated fatty acids, the well-balanced diet that can decrease low density (LDL) within the body as well as boost high density

(HDL) (Smith et al., 1989). Finally, the current results of serum blood parameters levels are within the physiologically normal range.

THYROID AND PROGESTERONE HORMONE CONCENTRATION

The effect of treatments on thyroid hormone levels of mature female sheep are illustrated in Table 4. Data revealed that dried olive cake increased (P<0.05) T3 concentrations more than control and olive cake silage groups with a mean value of 1.40, 1.25 and 1.23 ng/mL, respectively. On the other hand, feeding on olive cake silage with fibrolytic enzymes increased (P<0.05) T3 more than olive cake silage only with a mean value of (1.38 and 1.23) ng/mL, respectively. On the other hand, olive cake silage increased T4 concentrations (P<0.05) more than control and olive cake groups with the highest mean value of 97.2 ng/mL. Moreover, olive cake silage with fibrolytic enzymes increased T4 concentrations (95 ng/mL) more than olive cake (80.50 ng/mL) only. Ewes in fed olive cake silage with fibrolytic enzyme showed significant (p<0.05) higher progesterone level than other dietary groups, while G2 and G3 were nearly similar (Table 4) and (Figure 1).

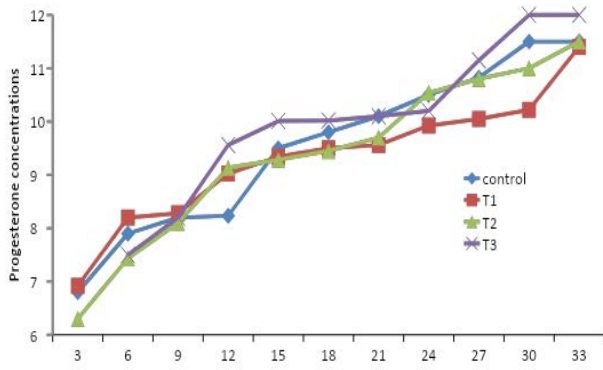


Figure 1: Progesterone concentration (ng/mL) of ewes as affected by the experimental rations.

G1: Control fed on 60 % concentrate feed mixture (CFM), 40 % green maize, G2 : as control with replacing 30% of CFM with olive cake , G3 :as control with replacing 30% of CFM with olive cake silage, G4 : as control with replacing 30% of CFM with olive cake silage treated with fibrolytic enzyme.

Reproductive performance was recorded in different groups during the treatment period (Table 5). Lambing rate, which reflects differences found in the number of lambs born per ewes, was insignificantly lower in control than other groups but it was similar in other groups. Also, the sex ratio of born lambs was associated with the type of rations. The male ratio was insignificantly ($p < 0.05$) lower in ewes feed on olive cake silage with fibrolytic enzyme compared with ewes in G1, G2, and G3 (Table 5). The number of ewes kidded / the number of ewes which joined to ram (fertility %) was available for analysis from 12 ewes for each treatment. No significant differences were observed in fertility %, prolificacy rate and fecundity. Ewes fed on control ration showed lower fertility % and prolificacy rate. However, data revealed that females had olive cake have a higher number of insemination per conception, lower conception rate from first insemination and percent of conception rate than other groups. While ewes in G4 had better reproductive parameters such as conception rate from the first insemination and percent of conception rate. Also, no significant differences in litter and ewes weight were observed between the treatments, ewes fed on olive cake silage was higher in weight and born weighted lambs more than other groups. Furthermore, litter and ewes weight was lower for G1. However, the percentage of lambing stillbirth differed ($P > 0.05$) 0, 16.67, 16.67 and 25% for G2, G3, G4 and G1, respectively.

The positive effects of feeding untreated or treated olive cake silage enzyme were accordance with Bilik et al. (2009) who demonstrated that cows fed on a diet supplemented with (15 g/day) fibrozyme TM preparation containing a blend of active xylanase and cellulose a higher pregnancy rate after the first insemination and needed a

lower number of inseminations to conceive compared to the control group. It is also possible that several other environmental factors (related to oestrus detection, insemination technique and semen quality) could affect fertility indices of the cows (Stevenson, 2001). Improving reproductive performance may be due to one or more of the following reasons, improving feed digestibility means an increase in energy intake, which has a positive correlation with body condition and reproduction of bovine (Peters and Ball, 1995). Increasing glucose level may improve the reproductive efficiency through coordinating the biological activity of gonadotropin hormones (Hafez, 1993). Increase blood serum glucose causes an increase in serum insulin-like growth factors 1 (IGF1), this may be a possible hormonal mechanism by which nutritional effects might be recognized centrally. Furthermore, IGF1 has an effect on the rate of increase in the bioactivity of LH and to augment FSH-stimulated induction of LH receptors and subsequent progesterone synthesis (Peters and Ball, 1995). However, the females fed treated olive cake silage with fibrolytic enzyme had a lower cholesterol level, in addition, the decreasing level of serum cholesterol may be attributed to the increasing demand of cholesterol in the biosynthesis of the steroid hormones towards the sexual maturity since about 25% of daily-formed cholesterol is used in the synthesis of steroid hormones (Sommer, 1969).

CONCLUSION

In conclusion, the olive cake is a highly fibrous feed, the ensiling process has been shown to be a feasible means of converting and utilizing olive cake into the reasonably nutritious feed. It is important to an adverse effect on digestibility and growth performance of the lactating ewes. Also, the inclusion of olive cake silage either treated with or without fibrolytic enzymes in ewes' diets may increase the reproductive performance of dairy ewes, and has a positive effect on by increasing lambing rate, fertility % and litter weight. The use of the enzyme preparation in practical feeding of ewes will be conditional on reproduction costs, ease of use and efficiency of use under production conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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Khatab MSA, and Abd El Tawab AM designed the study. Shaaban MM, Eid SY, Mostafa MMM, El-Zaher HM conducted the farm experiment, Abd El Tawab AM and Khatab MSA, Hadhou FI, El-Ganainy SMM conducted the chemical analysis for samples, El-Ganainy SMM, and Khatab MSA wrote the draft and all authors revised the manuscript.

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