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Physiological and Reproductive Responses of Domyati Ducks to Different Dietary Levels of Coconut Oil as a Source of Medium-Chain Fatty Acids during Laying Period

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ABSTRACT

The objective of this study was to determine the optimal level of coconut oil (CO) supplementation in the diet to enhance the performance of Domyati ducks. A total number of 300 Domyati ducks (240 females and 60 males) aged 25-week-old were randomly assigned to 4 experimental groups of three replicates, each replicate included 5 males and 20 females of Domyati ducks. The groups received CO at 0, 1.0, 1.5, and 2.0% during the experimental period. The results indicated that egg weight, egg number, and egg mass significantly increased in treated groups, compared to the control group. Furthermore, fertility and hatchability percentages were superior in 1.0, and 1.5% CO groups, compared to other experimental groups. Low-density lipoprotein in ducks that received CO was significantly lower than that of the control group. It is concluded that the inclusion of CO at a 1.5% level could be enough and useful for improving the reproductive and physiological performance of Domyati ducks.

Keywords: Blood, Coconut oil, Ducks, Egg, hatchability, Laying period

INTRODUCTION

Ducks are the second common strain of poultry in the world and can be considered a potential source of dietary protein for humans (Ali et al., 2018). Domyati ducks are a local breed in Egypt and their meat is more favorable to the Egyptian consumers. However, the relatively high content of fat and cholesterol in meat and eggs may limit their consumption (Pagala and Nur, 2010; Aziz et al., 2012). Consumer anxiety may be eliminated by the production of duck eggs with low cholesterol levels by feeding ducks on diets containing medium-chain fatty acids (MCFA) (Li et al., 2018).

Coconut oil (CO) is considered a highly saturated oil, about 60% of its total fatty acid composition is MCFA with a chain length of 6 to 12 carbon atoms (Bhatnagar et al., 2009). CO is composed primarily of short-chain fatty acids and MCFA; lauric acid (12:0) comprises approximately 46.5% of the fatty acid content (Dauqan et al., 2011). The MCFA are directly absorbed into the portal circulation without any re-esterification in intestinal cells (Ferreira et al., 2012). The MCFAs are partly independent of the carnitine transport mechanism into the mitochondria of the liver and are rapidly and are exclusively oxidized for the production of energy (Rubin et al., 2000). However, most diets commonly contain long-chain fatty acids (LCFA) that are incorporated into chylomicrons after absorption in the small intestine where they are re-esterified and then enter the blood-stream (Ferreira et al., 2012). Most LCFA are stored in the adipose tissue (Rego Costa et al., 2012). While, MCFAs are associated with reduced fat deposition and improved serum lipid profiles in humans and rats (Han et al., 2003; Takeuchi et al., 2006).

However, few researches have been conducted to study the effects of MCFA on broiler chickens, excepting that it reduces weight gain (Santos et al., 2008). In laying hens, Lee et al. (2015) found that chickens that received MCFA had greater egg protein quality (larger Haugh units), stronger egg-shells, and higher content of calcium, and reduced *Escherichia coli* count. Several studies showed that most of fatty acids in CO are potential as antibacterial (Bergsson et al., 2001), antiviral (Bartolotta et al., 2001), and immune-stimulant agents (Witcher et al.,

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1996; El-Kholy et al., 2014 and 2018), which are important to fight infection. Also, MCFA inhibits the production of lipases by the bacterium (Dierick et al., 2002). As lipases are needed to allow the bacteria to attach to the intestinal wall, this process will be prohibited and the bacteria will be washed out. The immune system requires antioxidants to produce and maintain the balance of immune cells, to protect cell membranes from reactive oxygen species, and to fight microorganisms causing disease (Tugiyanti1 et al., 2016).

In addition, CO could improve fat digestion and performance values during the coccidiosis infection in broilers chickens (Adams et al., 1996). Few studies are available on the effects of CO as a source of MCFA on the physiological and reproductive parameters of Domyati ducks. Also, no studies have shown whether it is beneficial to other sources of energy for local duck breeds, especially during the laying period. Moreover, appropriate inclusion level of CO in laying duck's diet is not definitely known. Therefore, the current study was conducted to investigate the effect of diets containing different CO levels on some physiological and reproductive parameters and some egg quality traits of Domyati ducks during the laying period.

MATERIALS AND METHODS

Ethical approval

The current study protocol used in this study was approved by the Animal Care and Use Committee of Damietta University, Damietta, Egypt.

Study design

The current experimental work was carried out at El–Serw Water Fowl Research Station, Animal Production Research Institute. Agricultural Research Center, Ministry of Agriculture, Egypt; to evaluate the impact of adding different levels of CO on reproductive performance of Domyati duck diets during the first 3 months of egg production season (25-36 weeks of age). A total number of 300 Domyati ducks (240 females and 60 males), 25 weeks old, were weighed and randomly distributed into four experimental groups, each group contained 75 ducks. Each group was also subdivided equally into three replicates of 20 females and 5 males each. Ducks within each group were fed with different diets; G1was fed the basal diet and served as the control group, the other three groups (G2, G3, and G4) were fed the basal diet supplemented with different levels of CO (1.0, 1.5, 2.0%/kg feed, respectively).

Fatty acids composition in CO is presented in Table 1. Diets listed in Table 2 were formulated to be iso-caloric and iso-nitrogenous according to NRC (1994) and were offered in mash form throughout the experimental period. Ducks of each replicate were reared in a house (2.3 ducks/m^2) with windows and received additional artificial light to provide 17 h light and 7 h dark daily. Ducks in all treatments were reared under similar hygienic and management conditions.

Table 1. Fatty acids composition of coconut oil[1]

Common name	Percentage (%)
Caproic acid (C 6:0)	0.4 - 0.6
Caprylic acid (C 8:0)	4.6 – 10
Capric acid (C 10:0)	5.0 - 8.0
Lauric acid (C 12:0)	45.1 - 53.2
Myristic acid (C 14:0)	16.8 - 21.
Palmitic acid (C 16:0)	7.5 – 10
Stearic acid (C 18:0)	2.0 - 4.0
Oleic acid (C 18:1)	5.0 - 10.0
Linoleic acid (C 18:2)	1.0 - 2.5
Other (C 18:3 C 24:1)	< 0.5

[1] According to Rossell (1985).

Table 2. Ingredients and chemical analysis of experimental diets

Ingredients (%)	Treatments (coconut oil levels, %)			
	0.0 (G_1)	1.0 (G_2)	1.5 (G_3)	2.0 (G_4)
Yellow corn	65.05	63.10	62.13	60.93
Soybean meal	23.91	26.31	27.51	27.63
Gluten corn	2.30	0.85	0.12	0.00
Coconut oil	0.00	1.00	1.50	2.00
Wheat bran	0.00	0.00	0.00	0.70
Calcium carbonate	6.15	6.15	6.15	6.15
Dicalcium phosphate	1.75	1.75	1.75	1.75
Lysine	0.00	0.00	0.00	0.00
DL.-Methionine	0.09	0.09	0.09	0.09
Vitamin and mineral premix*	0.30	0.30	0.30	0.30
NaCl	0.35	0.35	0.35	0.35
Sodium Bicarbonate	0.10	0.10	0.10	0.10
Calculated analysis				
Crude protein (%)	17.000	17.000	17.000	17.000
ME (Kcal/Kg)	2,800	2,800	2,800	2,800
Lysine (%)	0.75	0.75	0.75	0.75
Crude fiber (%)	3.15	3.10	3.28	3.33
Methionine (%)	0.35	0.35	0.35	0.35
Methionine +Cystine (%)	0.60	0.60	0.60	0.60
Threonine (%)	0.27	0.27	0.27	0.27
Calcium (%)	2.80	2.80	2.80	2.80
Available Phosphorus (%)	0.45	0.45	0.45	0.45
Chlorine (%)	0.22	0.22	0.22	0.22
Sodium (%)	0.17	0.17	0.17	0.17

*Vit+Min premix: Provided per kilogram of the diet Vit. A: 6000 IU, Vit. E (dl-α-) Tocopherylacetate: 10 IU, menadione: 2.5 mg, Vit. D3: 2000 ICU, riboflavin: 2.5 mg, calcium Pantothenate: 10 mg, nicotinic acid :12 mg, Choline chloride:300 mg, Vit. B12: 4µg, Vit. B 6: 5 mg, thiamine: 3 mg, folic acid: 0.50 mg, and biotin: 0.02 mg. Trace mineral (mg/ kg of diet: Mn: 80 mg, Zn: 60 mg, Fe: 35 mg, Cu: 8 mg and Se: 0.1 mg).

Productive performance

The number of eggs laid (EN) was daily recorded and also eggs were weighed from 25 to 36 weeks of age. The EN was calculated per duck for 4 weeks as follows:

EN per duck=Total EN per replicate / Number of ducks at house.

Egg weight (EW) was recorded for each replicate. Egg mass (EM) was calculated by multiplying EN by EW. The EM was expressed per duck throughout the experimental period. Feed consumption (FC) of each replicate was weekly recorded; it was then averaged and expressed in gram/duck/4 weeks. Feed conversion ratio (FCR) for egg production was also calculated during the same periods.

Egg quality

At 33rd weeks of age, a total number of 60 eggs (15 from each treatment) were randomly taken to determine egg quality traits. During two successive days per each week during the 33rd to 36th weeks of age, all eggs laid by ducks of each treatment were collected and individually subjected to the following measurements and estimations. Egg was broken and the yolk was separated from albumen. Egg yolk, albumen, and shell (with its membranes) were separately weighted. Relative weights of each component (to the whole EW) were then calculated. Shell thickness was measured at the broad, narrow and the middle ends, using a micrometer. The average shell thickness for all regions was calculated. The egg shape index was calculated according to the following formula:

Egg shape index = (Egg width/ Egg length) × 100

Egg fertility and hatchability percentages

A total of 300 eggs were collected from each treatment during the 34th - 36th weeks of age to determine fertility and hatchability percentages. They were randomly divided into three equal replicates. Fertility percentage was determined on the 10th day of incubation. Hatchability percentage was determined at the end of the incubation period.

Plasma analysis

At the end of the experimental period (36th week), three ducks from each treatment group were randomly taken for blood sampling through wing vein. Blood samples were collected in heparinized test tubes and centrifuged at 3500 rpm for 15 minutes to obtain blood plasma. Plasma samples were stored at –20 °C until analysis to determine total protein (TP) and albumin (Alb) levels. The TP and Alb were determined using commercial kits supplied by Randox (Randox Laboratories Ltd, Crumlin, Co, Antrim, UK) according to Henry et al. (1974). Globulin (Glb) concentration was estimated by subtracting the values of Alb from the corresponding values of TP. Also, the plasma was assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using standard protocol methods (Vogel and Vogel, 1997). The radioimmunoassay method was used for the determination of triiodothyronine (T_3) and thyroxin hormone (T_4) using commercial RIA kits (Medical Technology, USA). Plasma samples were analyzed for concentrations of aspartate transaminase (AST) and alanine transaminase (ALT), phosphorous, and calcium using commercial kits (Linear Chemicals, Barcelona, Spain) according to the manufacturer procedure.

Statistical analysis

Data were subjected to one-way ANOVA using the general linear model (GLM) procedure of SAS software (SAS, 2004) based on the following model:

$Y_{ij} = \mu + T_i + e_{iJ}$

where:

Y_{ij} = An observation; μ = Overall mean; Ti = Effect of treatments (i = 1, 2, 3 and 4); and e_{ij} = Random error component assumed to be normally distributed. Differences between the treatment groups were considered statistically different at $p \leq 0.05$. The significant differences among treatments were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance

As shown in Table 3, EN significantly ($p \leq 0.05$) increased in the G2 group compared to G1, G3, and G4 at the first experimental period (25-28 weeks of age). While G3 recorded the highest EN during the other experimental periods and at the overall period (25-36 weeks of age). EW significantly ($p \leq 0.05$) decreased in the G2 group compared to G1, G3, and G4 in the first experimental period. On the other hand, ducks in G3 recorded the highest EW at both the second experimental period (29-32 weeks of age) and the overall period (Table 3). The results of the analysis indicated that the use of various dietary CO levels significantly ($p \leq 0.05$) affected the EM at all experimental periods. Also, the dietary CO addition significantly ($p \leq 0.05$) improved FCR at all experimental periods compared to the control group. As shown in Table 3, Domyati ducks that received the diet containing 1.5% CO (G3) had significantly ($p \leq 0.05$) better productive performance parameters, except FC, compared to other

groups (G1, G2, and G4) during the overall laying period. Accordingly, 1.5% CO in the diet could be considered suitable for Domyati laying ducks.

The FC values were not significantly affected by feeding different levels of CO during 29-32 weeks of age and the whole period from 25-36 weeks of age (Table 3). The group fed diet containing 1.0% CO recorded significantly higher value of FC from 25-28 weeks of age, whereas, ducks receiving 1.5% CO had significantly higher value of FC during 33- 36 weeks of age compared to the other treatments group.

The improvement of EM may be due to the higher EN and EW associated with the diet containing 1.5% CO. These results are in agreement with Wang et al. (2009) and Lee et al. (2015) who reported that dietary supplementation of MCFA improved egg production in the laying hens. In contrary, Klementavičiūtė et al. (2016) reported that laying hens fed the diet supplemented with MCFA showed a low egg production rate. These differences may be due mainly to different levels of CO and differences in bird species. Furthermore, it is proposed that CO can lead to a better condition of digestion and absorption so that the ration becomes more efficient to produce eggs as mentioned by Hanczakowska et al. (2013). These effects may be attributed to a healthier and more stable gut environment created by the MCFA. Also, the antimicrobial activity of MCFA (Ferreira et al., 2012) diminishes intestinal infection pressure and improves intestinal morphology, resulting in better digestive and absorptive capacity (Batovska et al., 2009).

Table 3. Effects of supplementing coconut oil to the basal diet on productive performance of Domyati ducks during laying period.

Periods (weeks of age)		Treatments (Coconut oil levels)				SEM	p-value
		G1 (0%)	G2 (1.0%)	G3 (1.5%)	G4 (2.0%)		
Egg number/ duck	25-28	6.8^{b}	8.5^{a}	7.7 ^{b}	7.1^{b}	0.12	0.0001
	29-32	11.4^{d}	15.9^{b}	17.2^{a}	12.2^{c}	0.11	0.0001
	33-36	15.2^{d}	17.7^{b}	18.2^{a}	16.0^{c}	0.08	0.0001
	25-36	33.5^{d}	41.3^{b}	42.2^{a}	35.3^{c}	0.15	0.0001
Egg weight (g)	25-28	60.4^{a}	57.6^{b}	61.5^{a}	61.1^{a}	0.57	0.0047
	29-32	62.5^{c}	62.3 ^{c}	68.6^{a}	63.2^{c}	0.11	0.0001
	33-36	67.2^{d}	72.5 ^{a}	70.5^{b}	68.3^{c}	0.11	0.0001
	25-36	63.2^{d}	66.2 ^{b}	68.5^{a}	64.5^{c}	0.08	0.0001
Egg mass (g) / duck	25-28	412.7^{c}	487.7^{a}	434.6^{b}	433.7^{b}	6.35	0.0002
	29-32	712.1^{d}	988.5^{b}	1177.6^{a}	773.5^{c}	6.0	0.0001
	33-36	1019.0^{c}	1281.4^{a}	1283.1^{a}	1092.8^{b}	6.0	0.0001
	25-36	2115.1^{d}	2732.7^{b}	2904.4^{a}	2275.9^{c}	11.42	0.0001
FC (g) / duck	25-28	4247.0^{b}	4325.0^{a}	4225.0^{d}	4233.0^{c}	1.73	0.0001
	29-32	5070.0	5166.7	5035.0	5028.0	82.6	0.6341
	33-36	5007.0^{c}	5012.0^{c}	5067.0^{a}	5035.0^{b}	1.67	0.0001
	25-36	14324.0	14503.7	14327.3	14296.0	82.67	0.3337
FCR (g feed/ g egg mass) /duck	25-28	10.3^{a}	8.9^{c}	9.7^{b}	9.7^{b}	0.14	0.0006
	29-32	7.1^{a}	5.2^{c}	4.3^{d}	6.5^{b}	0.09	0.0001
	33-36	4.9^{a}	3.9^{c}	4.0^{c}	4.6^{b}	0.03	0.0001
	25-36	6.8^{a}	5.3^{c}	4.9^{d}	6.3^{b}	0.08	0.0001

$^{a,b,c,\ d}$ means within rows with different superscripts are significantly different ($p \leq 0.05$). SEM: standard error mean.

Egg quality traits

The results of feeding with different levels of CO and their effects on egg quality traits are shown in Table 4. The group fed the diet containing 2.0% CO (G4) recorded higher values of shell thickness. It was found that with increasing CO levels in the diet, shell thickness increased. However, there were insignificant differences in shell weight (%) for ducks that received 2.0% CO (G4) compared to the control group (G1).

These results are in agreement with Świątkiewicz et al. (2010) and Klementavičiūtė et al. (2016) who found that the addition of MCFA had a positive influence on eggshell characteristics including egg-shell weight as percentage, density, and breaking strength. This influence can probably be attributed to the increased availability of Ca and P, due to a decrease in pH in the upper part of the intestinal tract and the stimulating effect of fatty acids on the villus height, which was observed in broilers by Hanczakowska et al. (2013).

Hence, maintaining a good quality shell throughout the production cycle is of importance for the egg consumers and producers in terms of health and economics (Hughes et al., 1986). Also, eggshell porosity is of concern during embryonic development in the breeding

industry (Reynard and Savory, 1999). The most commonly used indicators of Ca metabolism in laying hens are shell quality assessment parameters (Gordon and Roland, 1998). Data presented in Table 4 also revealed that different levels of CO in the diet had no significant effects on EW, absolute and percentage weight of yolk and albumin, absolute shell weight, and egg shape index. These results were in agreement with the findings of Klementavičiūtė et al. (2016) who indicated that the inclusion of MCFA in birds' diet reduced egg and yolk weight, but the difference with the control group was not significant. Also, Danicke and Halle (2002) demonstrated that the yolk and albumen weights were not significantly affected by different sources or inclusion levels of lipids.

Table 4. Effects of supplementing coconut oil to the basal diet on egg quality traits of Domyati layer ducks

Items	Treatments (Coconut oil levels)				SEM	p-value
	G1 (0%)	G2 (1.0%)	G3 (1.5%)	G4 (2.0%)		
Egg weight (g)	70.00	71.80	71.60	69.80	2.18	0.8743
Shell weight (g)	9.20	8.80	9.60	9.80	0.31	0.1446
Shell weight (%)	13.14^{ab}	12.26^{b}	13.41^{a}	14.08^{a}	0.01	0.0076
Shell thickness (mm)	0.29^{c}	0.30^{bc}	0.31^{ab}	0.32^{a}	0.01	0.0133
Yolk weight (g)	23.20	24.60	24.00	24.60	0.90	0.6618
Yolk weight (%)	33.25	34.26	33.54	35.18	0.01	0.4953
Albumin weight (g)	37.60	38.40	38.00	35.40	1.47	0.4977
Albumin weight (%)	53.61	53.48	53.05	50.74	0.01	0.1834
Egg shape index	0.78	0.78	0.79	0.79	0.02	0.9367

$^{a,b,c,\ d}$ means within rows with different superscripts are significantly different ($p \leq 0.05$). SEM: standard error mean.

Reproductive traits

There were significant differences in fertility percentages among the treatment group as shown in Table 5. Also, two CO levels (1.0 and 1.5%) significantly increased ($p \leq 0.05$) hatchability percentage of set and fertile eggs as compared to both control and group fed 2.0% CO in the diet. This supports the previous findings that the incorporation of CO-derived MCFA into hen's diet is readily utilized by the embryos (Ding and Lilburn, 1997). It is clear from the results that, inclusion levels of 1.0 and 1.5% CO in the diet could be considered suitable for hatching eggs, where, the groups fed diet contained 1.0 and 1.5% CO showed a good quality of shell thickness and high percentages of hatchability as compared to the other treatment groups. It was found that the hatchability of thick-shelled eggs is higher than that of thin-shelled eggs (Narushin's and Romanov, 2002). It was reported that reduction in egg-shell quality decreases hatchability and is associated with the weakening of the embryos (Peebles et al., 1987). In contrary, Yamak et al. (2016) and Ergun and Yamak (2017) indicated that differences in hatching rates of eggs with different shell thicknesses were not statistically significant.

Blood plasma parameters

Some blood plasma parameters of Domyati layer ducks were influenced by diets supplemented with different levels of CO (Table 6). However, no significant differences in plasma T_3, T_4, P, Ca, ALT, AST, TP, Alb, and Glb values were observed among the treatment groups. The ALT and AST values were 20.2-23.9 and 75.7-87.0 U/l throughout the whole experimental period, respectively. These results are in agreement with those of Ali et al. (2018) who found that ALT and AST values varied between 20.2 to 24.3 U/l and 83.3 to 88.1 U/l, respectively. Regarding the effect of dietary CO on lipid profile, no significant differences were observed in plasma cholesterol and triglyceride (Table 6). However, the groups fed with the diet containing 2.0% CO recorded significantly ($p \leq 0.05$) higher and lower values of HDL and LDL, respectively, as compared to 1.0% CO and control group. Moreover, no significant differences were observed between G1 and G2 in HDL and LDL values.

Wang et al. (2015) showed that with increasing CO levels, serum levels of total cholesterol, LDL, and LDL/HLDL linearly decreased. In general, usage of CO at some levels is a popular concept that is believed to increase the productive and reproductive performance of birds. Similarly, the administration of some levels for long periods is believed to be more effective without considering the other adverse effects on the birds. Previous studies have shown some benefits such as immune modulation and anti-inflammatory effects. Besides efficacy, these supplements also have to be safe for the animals, consumers of products, and the environment.

Table 5. Effects of coconut oil supplemented to basal diet on fertility and hatchability traits of Domyati duck eggs.

Items	Treatments (Coconut oil levels %)				SEM	p-value
	G1 (0%)	G2 (1.0%)	G3 (1.5%)	G4 (2.0%)		
Fertility (%)	93.0^{b}	96.0^{a}	96.0^{a}	94.0^{b}	0.58	0.0139
Hatchability of set eggs (%)	71.0^{c}	76.0^{a}	77.0^{a}	73.0^{b}	0.58	0.0003
Hatchability of fertile eggs (%)	75.4^{b}	79.2^{a}	80.2^{a}	76.7^{b}	0.61	0.0018

^{a,b,c,d} means within rows with different superscripts are significantly different (p≤0.05). SEM: standard error mean.

Table 6. Effects of supplementing coconut oil to the basal diet on some blood plasma parameters of Domyati ducks during laying period

Items	Treatments (Coconut oil levels %)				SEM	p-value
	G1 (0%)	G2 (1.0%)	G3 (1.5%)	G4 (2.0%)		
T_3 (ng/ml)	3.30	3.20	3.30	3.20	0.22	0.9804
T_4 (ng/ml)	20.20	18.70	18.40	19.10	1.17	0.7132
Pi	5.70	5.80	6.00	6.20	0.25	0.4348
Ca	21.60	20.60	22.60	21.80	1.18	0.6065
ALT (U/l)	23.60	22.20	21.50	21.90	1.99	0.8930
AST (U/l)	84.20	87.00^{c}	82.80	75.70	4.83	0.4395
Triglyceride (mg/dl)	117.5	123.2	133.0	129.4	6.73	0.4302
Cholesterol (mg/dl)	175.6	175.9	178.1	170.1	6.33	0.8305
HDL (mg/dl)	60.60^{b}	60.80^{b}	67.70^{a}	67.90^{a}	1.87	0.0336
LDL (mg/dl)	91.50^{a}	90.40^{a}	83.80^{ab}	76.30^{b}	3.49	0.0507
Total protein (TP, g/dl)	5.14	5.73	5.80	5.77	0.25	0.3618
Albumin (Alb, g/dl)	3.06	3.50	3.50	3.60	0.25	0.1095
Globulin (Glb, g/dl)	2.13	2.28	2.21	2.13	0.13	0.8307

^{a,b,c,d} means within rows with different superscripts are significantly different (p≤0.05). SEM: standard error mean.

CONCLUSION

Inclusion of coconut oil at a level of 1.5%/kg feed to diet can improve the physiological and reproductive performance of local Domyati ducks during the laying period.

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

Khaled H. El-Kholy designed the proposal of this study. Aymen I. Ghonim; Mahmoud A. Atef.; Hoda A. Gad.; Mervat N. Ghazal; Mosad A. El-Aik and Reham A. Ali developed the concept for the manuscript. Khaled H. El-Kholy and Ayman I. Ghonim wrote the manuscript.

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