Research Article

Supplementary Practices Improving Holstein Cattle Performance During Hot Seasons

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Abstract | Heat stress is a major problem facing dairy producers and negatively influences production, performance, and welfare. The study aims to improve milk production and quality during the hot season, which supports milk safety and dairy chain sustainability. Forty-two Holstein cows in the middle of the first lactation were used and divided into a control group (22 cows) that received control diet and a treatment group (20 cows) that received a control diet supplemented with 200 g of potassium carbonate through 30 days. Milk yield for all cows were recorded, while a total number of 84 milk samples were collected on the tenth day of the trial and at the last day of the trail. In addition, 16 blood samples were collected for both groups. Milk composition, somatic cell count (SCC), and bacterial profile were evaluated. The results indicated that the average milk yield was higher in the treatment group, which increased by 10.3%, while the fat % was decreased by 21-25% compared with the control group. The physiological parameters gave no significant differences between groups, except urea which recorded higher levels in the treatment group (34.6 vs. 30.6 mg/dl). SCC was significantly decreased by 52.5-56.3% compared with the control. Also, a significant enhancement of milk stability against overheating was recorded in the treatment group. The study proved that using potassium carbonate at the farm level during hot season could improve milk production and quality, subsequently, it might extend shelf life, safety, and stability of raw milk supplied through the dairy chain.

Keywords | Climatic changes, Dairy cattle, Potassium carbonate, Milk safety, Milk stability.

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INTRODUCTION

The climatic change was identified as one of the main challenges that facing the dairy chain through all different chain points in the hot climate. Climate change effects started at feed handling, milk production, milk handling, and ended by packaging and handling of products or even conserving milk to reach consumers (Martinsohn & Hansen, 2012). Heat stress is the main problem facing dairy producers and industries that influence cow production, performance, and animal welfare (Atrian & Shahryar, 2012; Hansen, 2013; Bernabucci et al., 2014; Lees et al., 2019) and it harmed the efficiency and profitability of the dairy enterprises (Esposito et al., 2014; Sammad et al., 2020). Moreover, Tao et al. (2018) reported that milk yield is decreased, and the milk somatic cell count (SCC)

is increased during the hot season. Abdelatty et al. (2018) and Conte et al. (2018) observed that the hyperthermia led to increase respiratory rate accompanied by panting that decreased the HCO_3 buffer levels in saliva and blood and contributed to an increase in incidence rate of ruminal acidosis where acetate concentrations and acetate to propionate ratios increased significantly. The threshold of heat stress in dairy cattle was reported to be above 72 temperature-humidity index (THI) (Wang et al., 2020). Recently, many management plans were set to avoid heat stress consequences that are considered one of the main climatic change's manifestations.

Potassium (K) is quantitatively the third most present monovalent cation in the body, and it is a fundamental element for cell functioning (Harrison et al., 2012), where animal's requirement of K is the highest compared with all other cations in the diet. Potassium is also involved in the acid-base balance process, nerve transmission, muscle functions, and maintenance of normal cardiac. Also, it is the most common intracellular electrolyte and renal function (Udensi & Tchounwou, 2017), therefore potassium is a particularly dynamic cation. Potassium deficiency could cause a reduction in dairy cow production. This deficiency may occur through some conditions like K^+ secretion via milk representing about 15 to 40% of total daily K intake, high environmental temperatures that led to an increase in potassium loss via sweating (Atrian & Shahryar, 2012; Wang et al., 2020), and low potassium content in animal feeding.

Generally, water consumption of dairy cows was increased due to the increase of potassium carbonate in diet, which influences total milk production and rumen parameters (Fraley et al., 2015). While there is a shortage of information about the effect of potassium carbonate as an additive to cow diets on udder health in terms of total bacterial count, *Staphylococcus spp.*, and *coliform*, and Ethanol or heat milk stability as milk technological traits.

Therefore, the main objectives of the present investigation were to study the effect of potassium carbonate supplementation on milk yield, milk composition, milk technological traits, general health, as well as udder healthy traits, including SCC and milk microbiological contents of lactating Holstein cows maintained under heat stress.

MATERIALS AND METHODS

Ethical statement and approval

Animal ethical code of ethics, all participated authors followed the international, national, and institutional guidelines (Institutional Animal Care and Use Committee (CU-IACUC), Cairo University with approval no. (CU-II-F-25-20).

Animals and management

The trial was conducted using 42 first lactation Holstein cows in the middle of lactation. The trial was conducted from August to September in one commercial farm located in Faiyum Governorate at the middle of Egypt (120 km southwest of Cairo, at a latitude 29°18'35.82" N and a longitude 30°50'30.48" E). The cows were fed total mixed ration (TMR) 3 times a day with free access to fresh water, all cows were housed in two shaded freestalls and milked 3 times/ day in a milking parlor. The parasitic control program was applied prior to calving period. Regular spraying against external parasites was conducted using safe pesticide. The average temperature and humidity were 34°C and 50% respectively at farm circumstances, where the temperature-humidity index was 84 that was considered a tough natural heat stress condition for Holstein (Wildridge et al., 2018). Dairy cows were distributed and divided into control (22 cows) and treatment groups (20 cows) according to the milk yield. The control was fed on a total mixed ration (TMR) and the treatment group was fed on a total mixed ration supplement with 200g/head/day of potassium carbonate. Milk yield data were collected through the experimental period.

Milk Sampling and Analysis

Sterile tubes (50 mL) were used to collect forty-two milk samples two times through the trial period (in total 84 samples), where the test was conducted on the 10th day of the trial (first test) and the 30th day (second test). Foremilk samples were collected during the second milking through the day (01: 00 pm) following to stripping step, from all teats (composite milk). Milk samples were stored undercooling (icebox) till reaching the laboratory. Samples were kept at 4°C for 8hrs before analysis.

Milk composition

Lactoscan MCC Combo, Ultrasonic milk analyzer (Stara Zagora, Bulgaria) was used to determine the milk composition for all samples in terms of fat %, lactose %, protein %, solids not fat (SNF)%, salt %, and density.

Udder health parameters

Udder health traits were evaluated by determining SCC, total bacterial count (TBC), *Staphylococcus ssp.*, and *coliforms* groups of milk samples collected from both control and treatment groups. SCC was conducted on all milk samples by Fluorescent Somatic Cell Counter machine (Milkotronic Lactoscan, Bulgaria) using the SCC kits ×4. TBC was determined by the pour plate count technique as described by Markey et al. (2013). C*oliform*: MacConkey agar was used to count the coliform group and the plates were incubated for 24 h at 37°C. *Staphylococcus* medium

No.110 was used to count the pathogenic *staphylococci* following the colony morphology on the culture medium S110 according to Da Silva et al. (2018).

Blood sampling and general health measurements

A total number of 16 **b**lood samples were collected from animals of both groups through milk veins in heparinized Falcone tubes (15 ml) and kept in an icebox during transfer to the laboratory. Centrifugation of blood samples was conducted at 3000 rpm for 30min and the plasma was aspirated into capped Eppendorf tubes then stored at -18°C until analysis. Total protein, alkaline phosphatase, creatinine, urea, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined by Bio-diagnostic® kits using a colorimetric method (STAT LAB SZSL60- SPECTRUM).

Milk stability tests (ethanol and heat treatments)

These tests were based on the addition of alcohol (two vol. of ethanol 78% to one vol. of milk) and the heating of milk at 135°C for 10 min have been studied according to Machado et al. (2017).

Statistical analysis

The statistical analysis was applied using the R language program. The one-way GLM procedure was used to test the significance of effects on response traits. Days in milk at the beginning of the trial (DIMS), average days in milk through the trial (ADIM), last test day milk yield taken through the trial (LMTD), accumulative milk yield through 30 days of the trial (TMY), and average milk yield for each cow through the trial period (AMY) were estimated and analyzed. Data of SCC, *Staphylococcus* ssp., and coliforms group were logarithm transformed to be analyzed. The model that used was as follows:

$$Y_{ij}=\mu+Tr_i+e_{ij}$$

where, Y_{ij}=the measured trait, μ=overall mean, Tr_i=Treatment Effect type (i=1 and 2; where 1=basic diet and 2=basic diet plus 200gm of K_2CO_3) and e_{ij}=Random error. A simple correlation coefficient was estimated between milk yield and milk composition using the Python language program.

RESULTS

Milk yield and composition traits

The effect of potassium carbonate on milk production as shown in Figure-1, where no significant differences observed until day 3 of the experiment then the differences in production were recorded. The DIMS showed no significant differences between the two groups (Table-1). Aaccumulative milk yield through the 30 days of the trial (TMY) and average milk yield for each cow (AMY) showed significant differences between groups (*P<0.05*).

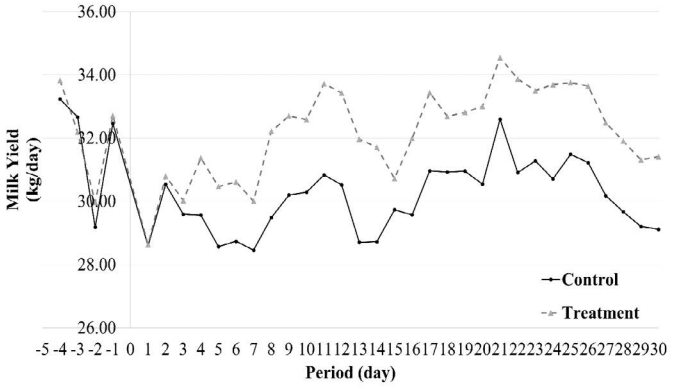

Figure 1: Effect of potassium carbonate on milk yield through trial period for control and treatment groups.

Table 1: Milk yield parameters of control and treatment groups through hot days.

Variable[1]	Control (Mean±SE)	Treatment (Mean±SE)	P vlaue
DIMS (day)	121.6±17.6	119.0±10.6	0. 214
LMTD (kg)	29.1±0.3[b]	31.4±0.3[a]	0.007
TMY (kg)	873±14.2[b]	962±23.0[a]	0.008
AMY (kg)	29.1±0.6[b]	32.1±0.8[a]	0.009

[1]DIMS: days in milk at the beginning of the trial. LMTD: last test day milk yield taken through the trial. TMY: accumulative milk yield through 30 days of the trial. AMY: Average milk yield for each cow through the trial period. Superscript letters (a, b) show significant difference between control and treatment groups (*P 0.05*).

The milk composition in Table (2) revealed that protein, lactose, SNF, Salt %, and density did not show any significant differences between the two groups. While the fat % was significantly (P<0.05) higher in the control group (1.5-1.9 %) compared with the treatment group (0.9-1.2 %).

The correlation between milk yield and milk composition (Figure 2) revealed an inverse relationship between milk yield and fat %. Also, fat % and density of milk revealed a negative correlation. Moreover, SCC had an opposite correlation with protein, lactose, SNF %, and density. While, there was a positive correlation between SNF, protein, lactose, and density.

Blood biochemical parameters

Blood biochemical parameters in Table (3) did not reveal any statistical differences between groups, except blood

Table 2: Milk quality parameters of control and treatment groups through hot days.

Item	First time			Second time		
	Control (Mean±SE)	Treatment (Mean±SE)	P value	Control (Mean±SE)	Treatment (Mean±SE)	P value
Fat (%)	1.9±0.1	1.5±0.1	0.09	1.2±0.1[a]	0.9±0.1[b]	0.012
Protein (%)	3.3±0.03	3.3±0.04	0.214	3.4±0.02	3.3±0.03	0.211
Lactose (%)	4.9±0.04	5.0±0.06	0.210	5.1±0.03	5.0±0.04	0.212
SNF (%)[1]	8.9±0.1	9.1±0.1	0.229	9.2±0.1	9.1±0.1	0.208
Salt (%)	0.72±0.01	0.74±0.01	0.203	0.75±0.01	0.74±0.01	0.186
Density	32.0±0.03	32.9±0.04	0.06	33.6±0.2	33.6±0.3	0.333

[1] SNF: solids not fat. Superscript letters (a, b) show significant difference between control and treatment groups (*P 0.05*).

Table 3: Blood biochemical parameters analyzed of the control and treatment groups.

Item[1]	Control (Mean±SE[2])	Treatment (Mean±SE)	P value
Total protein (g/dl)	5.3±0.2	5.7±0.3	0.300
Urea (mg/dl)	30.6±2.1[b]	34.6±3.3[a]	0.016
Creatinine (mg/dl)	1.2±0.2	1.1±0.2	0.873
Alkaline phosphatase (IU/l)	20.5±1.9	22.5±2.4	0.878
ALT (U/L)	26.0±1.1	25.6±1.5	0.842
AST (U/L)	48.5±3.0	59.1±3.6	0.168

[1]ALT: alanine aminotransferase. AST: the aspartate amino transferase. [2]SE: Standard Error. Superscript letters (a, b) show significant difference between control and treated groups *(P 0.05)*.

Table 4: Milk microbits profile of control and treatment groups.

Item	First time (log cfu/ml)[1]		P value	Second time (log cfu/ml)		P value
	Control (Mean±SE[2])	Treatment (Mean±SE)		Control (Mean±SE)	Treatment (Mean±SE)	
Total Bacterial Count	4.8±0.1	4.6±0.1	0.248	4.1±0.4	3.8±0.4	0.602
Staphylococcus ssp.	2.8±0.1	2.5±0.2	0.139	2.83±0.1	2.4±0.2	0.139
Coliform groups	2.8±0.1	2.8±0.2	0.937	2.8±0.1	2.7±0.2	0.937

[1]cfu: colony forming unit, [2]SE: Standard Error.

urea that recorded significantly higher values in the treatment group (34.6 mg/dl) compared with the control group (30.6 mg/dl) (*P<0.05*).

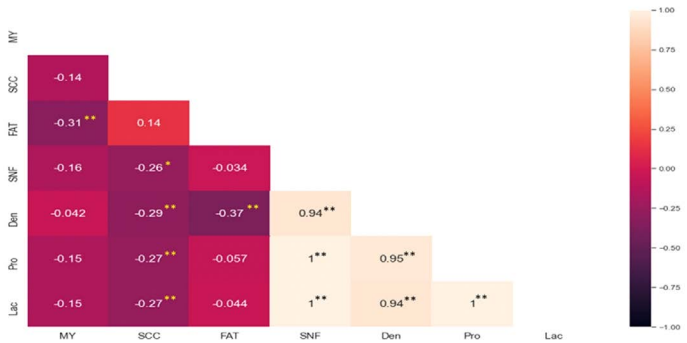

Figure 2: Correlation between milk yield and milk composition through the trial period.
** (P<0.001) and * (P<0.05)

Udder health traits

The SCC revealed in Figure-3, where a significant decrease was observed in animals that received supplemented K_2CO_3 in feed by 53-56% compared to the control group. The total bacterial count, *Staphylococcus ssp.*, and *coliform* groups in Table-4 revealed no significant differences between groups (*P>0.05*), however, the treatment group had a lower proportion of high bacterial content compared with threshold values, 100,000 CFU/ml *TBC* in raw milk,500 CFU/ml *for Staphylococcus* Spp., and less than 100 *CFU/* ml for Coliforms (Hillerton & Berry, 2004) as shown in Figures (4-6).

Milk stability

There was a significant enhancement as described in Figures-4: 7, where milk stability against overheating for samples collected from the treatment group. Moreover, milk

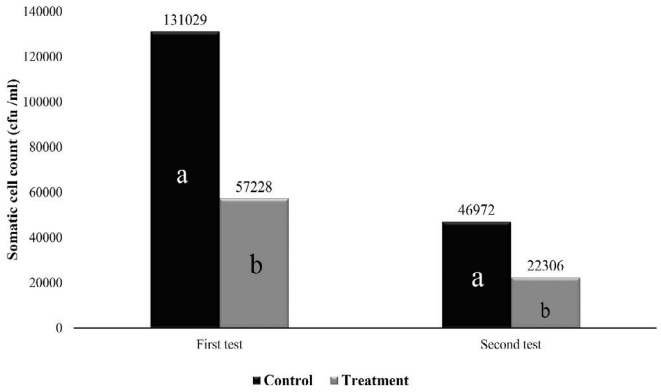

Figure 3: Effect of potassium carbonate on somatic cell count (cfu/ml) in milk for control and treatment groups at two times. Superscript letters (a, b) show significant difference between control and treatment groups (*P* 0.05).

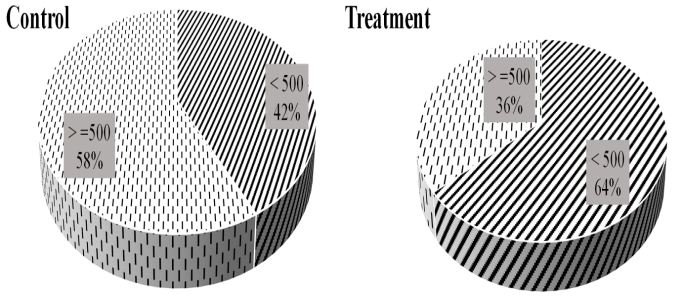

Figure 4: Effect of potassium carbonate on proportions of milk samples exceed 500 *Staphylococcus* pathogen count (cfu/mL).

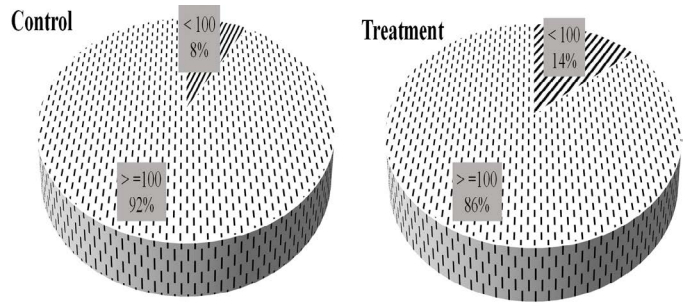

Figure 5: Effect of potassium carbonate on proportions of milk samples exceed 100 *Coliform* groups (cfu/mL).

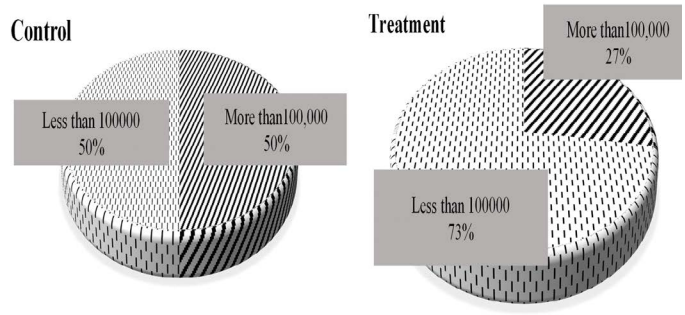

Figure 6: Effect of potassium carbonate on proportions of milk samples exceed 100,000 total bacterial count (cfu/mL).

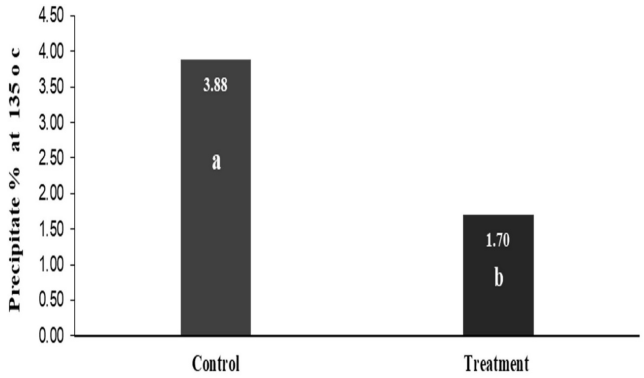

Figure 7: Milk heat precipitation of control and treatment groups. Superscript letters (a, b) show significant differences between control and treatment groups (*P* 0.05).

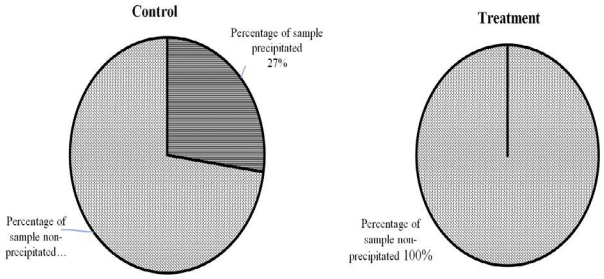

Figure 8: Proportion of samples coagulated in milk ethanol stability test of control and treatment groups.

samples collected from the treatment group that showed better stability against the ethanol test compared with the control group (100 vs. 75% of the samples, respectively) (Figure 8).

DISCUSSION

In the present study, the milk yield of lactating cows was influenced by supplementing the diet with potassium carbonate which agrees with Harrison et al. (2012) who recorded that the milk production of cows in the treatment group increased by 10.3%. The increase in milk production could be attributed to the role of potassium carbonate (as a DCAD and/or buffer) which increased DMI and water intake (Fraley et al., 2015; Sharma et al., 2018) which have been observed in our study. The treatment group tended to visit drinking troughs more frequently compared with the control group. Therefore, Wang et al. (2020) recommended supplementation of an appropriate electrolyte dose for *Bos Taurus* exposed to heat stress, that enhanced milk production in the treatment group, while the fat % was decreased logically.

The current results showed that most blood biochemical parameters such as total protein, urea, and creatinine contents were similar between the control and treatment groups. Serum total protein concentrations were not affected by the treatment, while urea was higher in the treat-

ment group which explained the potentiality of K_2CO_3 to increase the protein absorption and subsequent increase on serum urea level.

SCC of milk is affected by animal productivity, health, lactation stage, and breed (Alhussien & Dang, 2018). Furthermore, any stressors on dairy cattle are led to increase the SCC. Nasr and El-Tarabany (2017), reported that there is a positive relationship between SCC and heat stress. Where Yadav et al. (2016) found that heat stress is affecting on the immune system and inflammatory response. The current study showed lower SCC in animals fed diets supplemented with K_2CO_3.In the contrast, Fraley *et al.* (2015) found that the lowest SCC was observed in cows receiving 0.75% K compared with those fed on a diet with 1.5 or 1.67% K. Warken et al. (2018) found that cows that take minerals such as potassium had lower SCC compared with control, this could be related to enhance the immunity of cows that received minerals. The SCC had an inverse relationship with protein %, lactose %, SNF %, and density which agrees with Alhussien & Dang, (2018) and Kull et al. (2019) who reported that K_2CO_3 in cow diet could lead to an increase in the immunity system which had a positive effect on animal health particularly udder health.

In general, the bacterial load was increased in the hot season particularly pathogenic bacterial (Gao et al., 2017). Moreover, heat stress demonstrates a negative impact on udder health causing an inhibition of the immune system of cows in particular increasing intramammary infections. Despite the microbiological profiles (*Staphylococcus, Coliform,* and TBC) showing no significant differences between groups, but they recorded a lower proportion in animals' milk that received potassium supplementation (treatment group) compared to the control group. These results could be attributed to the influence of feed additives on enhancing the immune system of treatment cows, where the finding of this study agreed with results concluded by Armstrong et al. (2018). Steele M (2016) reported that the natural immune function might be suppressed in lactating cows during heat stress which led to an increase in the risk of clinical diseases like mastitis and metritis. Moreover, the author mentioned that the immunity indicators were all decreased due to heat stress. So, K_2CO_3 supplementation could enhance the internal environment of cows that led to stress reduction and led to an increase in the immunity system which had a positive effect on animal health particularly udder health.

There was an increase in milk stability (heat and ethanol) in response to potassium carbonate supplementation, while a destabilization occurred for milk samples collected from the control group. This result is agreed with Rose and Tessier (1959), where sodium and potassium chlorides exert the stabilizing action of skimmed milk by reducing the concentration of calcium and phosphate in the portion of milk. The improvement of milk stability could be attributed to improving the mineral balance that happened through heat stress relevant in the treatment group.

CONCLUSIONS

In the present experiment, milk yield was improved significantly due to adding K_2CO_3. Also, udder health was enhanced in terms of decreasing somatic cell counts and microbial contents (TBC, *Staphylococcus ssp*., and *coliform* groups). Moreover, the milk stability was improved. These results suggest that mineral supplementation as one of the feeding management techniques could alternate the composition, quality, and milk stability that might benefit dairy supply chain performance through the hot season.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Sherif Abdelghany: Set the work plan, participated in data analysis, and participated in writing and reviewing the manuscript. Lynda Allouche: Participated in writing the Manuscript. Ahmed Ali Abd El-Maksoud: Participate in collecting samples, participated in conducted lab work, and participated in writing. Ehab N. Daoud: Conducted whole field work and conduct sampling process. Saleh A. Kandeal: Reviewing the scientific content. Mohamed A. Radwan: Participate in collecting samples, participated in conducted lab work, participate in data analysis, and participate in writing.

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