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Research Article

Effect of Different Levels of *Moringa oleifera* Leaves Meal on Productive Performance, Carcass Characteristics and Some Blood Parameters of Broiler Chicks Reared Under Heat Stress Conditions

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Abstract

A growth experiment was conducted to study productive performance, carcass characteristics and some blood parameters of broiler chicks fed corn-soybean meal diets with 3 levels of *Moringa oleifera* leaves meal (MOLM) under heat stress conditions. Two hundred and eighty one days old chicks were randomly assigned to four treatments. The 1st treatment fed a commercial basal diet as a control, while, the other treatments 2, 3 and 4 were fed the commercial basal diet supplemented with MOLM (0.1, 0.2 and 0.3%, respectively). The results showed that body weight gain was increased significantly ($p < 0.05$) as the level of MOLM increase; also, the feed intake had the same trend. Feed conversion ratio was recorded better values as the level of MOLM increase. The levels of MOLM had no significant effect on carcass relative weight, liver, gizzard, heart, abdominal fat, breast and thigh. Haemoglobin (Hb) was increased with increase the level of MOLM, while, haematocrit (Ht) values did not affect. Heterophil/Lymphocyte (H/L) ratio was decreased by increase the level of MOLM. Plasma total protein increased significantly ($p < 0.05$) with increase the level of MOLM. Albumin did not affect while, globulin increased significantly ($p < 0.05$) with increase the level of MOLM. Aspartate transaminase (AST) decreased significantly ($p < 0.05$) while, Alanine transaminase (ALT) did not affect with adding MOLM levels. Thyroid hormones (T_3 and T_4) were significantly ($p < 0.05$) increased with increase MOLM level while, T_3/T_4 ratio did not affect. It could be concluded that addition of *Moringa oleifera* leaves meal up to 0.3% improved broiler performance, physiological parameters and enhanced the ability to resist heat stress conditions of broilers fed corn-soybean meal diet.

Key words: *Moringa oleifera*, heat stress, broiler performance, blood parameters, carcass characteristics

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INTRODUCTION

A temperature above 30°C represents a heat stress condition in poultry and considered one of the most common stress that affect the production criteria. High mortality, decreased feed intake, lower body weight gain and poor feed efficiency are common adverse effects of heat stress often seen in meat type poultry flocks (Sahin *et al.*, 2002a, b). Heat stress increases lipid oxidant as a consequence of increase free radical generation which enhances the formation of reactive oxygen species and induces oxidative stress in cells (Altan *et al.*, 2003).

Natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds are known to be present in certain plants. *Moringa oleifera* is one of such plant that has been identified to contain natural antioxidants (Siddhuraju and Becker, 2003). Moreover, the antioxidant effect of *Moringa oleifera* leaf was due to the presence of polyphenols, tannins, anthocyanin, glycosides and thiocarbamates, which remove free radicals, activate antioxidant enzymes and inhibit oxidases (Luqmans *et al.*, 2012). *Moringa* leaves can serve as a rich source of β -carotene, vitamins C and E and polyphenolics. The growing popularity of the use of *Moringa oleifera* as a feed additive in poultry nutrition necessitates through investigation into its nutritional value, as well its impact on haematological parameters as a measure of both nutritional and medicinal benefits of the leaves in broiler chicks (Ebenebe *et al.*, 2012). *Moringa oleifera* leaves incorporated into maize meal poultry feed led to better growth performance of the chicks and a significant increase in the serum level of biochemical minerals compared to the maize meal feed alone (Donkor *et al.*, 2013). Although, several studies have reported that the use of *Moringa oleifera* leaves as feed supplements in livestock (Ayssiwede *et al.*, 2011; Nkukwana *et al.*, 2014), the optimal concentration of *Moringa oleifera* leaves as a nutritional supplement has not yet been determined and there are only limited reports on the bioactive constituents of *Moringa oleifera* leaves and their impact on meat antioxidant status.

Therefore, the objective of this study was conducted to examine the effect of various levels of *Moringa oleifera* leaves meal as a new source of antioxidant on productive and physiological parameters of broiler chicks under heat stress condition.

MATERIALS AND METHODS

Experimental procedures and design: A total number of 281 days old chicks with average body weight (40 g) were

used. The chicks were individually weighted and distributed randomly into four treatments equal in number and nearly in body weight. Fourteen replicates (5 chicks each) were assigned to each treatment. The first group (T1) was fed on commercial basal diets (starter diet contained 22% CP and 3100 Kcal kg⁻¹, while, the grower diet contained 20% CP and 3000 kcal kg⁻¹) and used as control. Groups 2nd, 3rd and 4th were fed the same basal diet supplemented with *Moringa oleifera* leaves meal at levels 0.1, 0.2 and 0.3%, respectively. All groups were maintained under the same environmental and management conditions. Water and feed were provided *ad-libitum*.

Productive performance parameters: All birds were weighted weekly through the experimental period (35 days) and feed intake was recorded per replicate. Body weight gain and feed conversion ratio were calculated. At the end of the experimental period, seven birds from each group were randomly taken, weighed and slaughtered, de-feathered and eviscerated. Carcass weight and weights of breast, thigh, liver, heart, gizzard and abdominal fat were recorded and calculated as percentage of live body weight.

Physiological and biochemical parameters: Blood samples were collected from the seven slaughtered birds in heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min and plasma obtained was stored at -20°C in eppendorf tubes until analysis. Few drops of fresh blood samples were taken to determine blood haemoglobin (Hb) and packed cell volume (haematocrit, Ht %). Blood smears were also done, stained with Wright's stain procedure and used to calculate the number of Lymphocytes (L) and Heterophils (H) in 100 white blood cells then the H/L ratio was calculated.

The plasma total proteins (g dL⁻¹) were determined according to the method described by Henry (1974). The determination of plasma albumin (g dL⁻¹) based on a colorimetric method described by Doumas *et al.* (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein. The enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by using available commercial kits purchased from Diamond Diagnostics Company. Plasma thyroxin (T₄) and triiodothyronine (T₃) were determined by RIA technique using Gamma-Coat ¹²⁵I RIA Kits, Clinical Assay, Cambridge, Medical Diagnostics, Boston, MA, as reported by Akiba *et al.* (1982).

Statistical analysis: The obtained data was statistically analyzed using the general linear model procedure described

in SAS User's Guide (SAS., 2001). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance: Results concerning Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) for the experimental periods (0-14, 14-35 and 0-35 days) are presented in Table 1. The results showed that BWG increased significantly ($p < 0.05$) with increasing the level of *Moringa oleifera* leaves meal (MOLM) at all ages. At 14 days of age, birds fed MOLM (T2, T3 and T4) had gained more weight compared to the control group (T1). Also, at 35 days of age treated groups (T2, T3 and T4) significantly ($p < 0.05$) gained more weight (1408, 1488 and 1543 g, respectively) compared with the control group (1307 g). The improvement of BWG with MOLM addition in 0.1, 0.2 and 0.3% levels were about 8, 14 and 18% respectively, compared with the control group at 35 days of age.

Feed intake significantly ($p < 0.05$) increased with increasing the level of MOLM at all ages. At 35 days of age FI ranged from 2771-2871 g for T2-T4 respectively, compared with the control group (2681 g). Adding MOLM improved significantly ($p < 0.05$) FCR. However, the best FCR was recorded with birds fed on T3 and T4 at all periods with no significant differences between them. In 0-35 days of age percentage of improvement in FCR were 8.78 and 9.26% for T3 and T4 respectively, compared with the control group.

The results obtained is in agreement with Donkor *et al.* (2013) and Teteh *et al.* (2013), who reported that broilers fed MOLM increased weight gain significantly compared to broilers fed a nutritional diet without MOLM. Also, the enhancement of feed intake of chickens fed MOLM diets is in agreement with the findings of Melesse *et al.* (2011), who

reported a higher FI value for broiler chickens fed on diets containing 2 and 6% MOLM under tropical climate. Melesse *et al.* (2013) showed that chickens reared of tropical regions on diets containing 5% MOLM significantly consumed more feed than those of the control diet.

The data for FCR showed that the MOLM groups were more efficient in converting feed into meat than the control group. These results are in agreement with the findings of Onu and Aniebo (2011), who showed that birds fed MOLM at 2.5 or 5% gained significantly ($p < 0.05$) higher BWG and superior FCR than birds fed the control diet. Nkukwana *et al.* (2014) found also that BWG and FCR were the best ($p < 0.05$) in birds supplemented with MOLM (0.1-2.5%). This suggests that birds fed MOLM based diets had better utilization potential of the nutrients probably because of the increased bulkiness as the inclusion level increased (Onunkwo and George, 2015). El-Badawi *et al.* (2014) found that supplementation of *Moringa oleifera* dry leaves of growing rabbits diets at 0.15 or 0.30% of the daily ration improved growth performance and carcass traits and promote dietary energy and protein utilization efficiency. El Tazi (2014) found that body weight gain, feed intake and feed conversion ratio were improved significantly ($p < 0.05$) with the inclusion of MOLM at 5% in the broiler diet as compared to other experimental diets.

The improvement of performance with MOLM groups may be due to the high content of vitamin C in *Moringa olifera*, which able to cover the adverse effect of heat stress and enhance the productive responses. El-Moniary *et al.* (2010) reported that supplementing vitamin C to broiler diets under summer stress conditions could improve the productive performance.

In the present study, adding MOLM decreases the bad effect of heat stress which help the birds to consume more feed and gain more weight. The improvement of BWG and FCR may be due to the improvement of CP digestibility and

Table 1: Productive performance of broiler chicks fed various levels of *Moringa oleifera* leaves meal under heat stress condition

Items	Days	Treatments				Significance
		T1	T2	T3	T4	
BWG (g)	0-14	211.00±1.93 ^c	226.00±1.01 ^b	236.00±1.22 ^a	242.00±3.38 ^a	*
	14-35	1096.00±2.38 ^d	1183.00±8.87 ^c	1251.00±3.07 ^b	1301.00±3.71 ^a	*
	0-35	1307.00±1.65 ^d	1408.00±9.13 ^c	1488.00±2.84 ^b	1543.00±2.94 ^a	*
FI (g)	0-14	385.00±1.09 ^d	414.00±0.76 ^c	419.00±0.98 ^b	423.00±0.98 ^a	*
	14-35	2296.00±2.33 ^d	2357.00±1.56 ^c	2369.00±1.95 ^b	2448.00±2.10 ^a	*
	0-35	2681.00±3.40 ^d	2771.00±2.31 ^c	2788.00±2.90 ^b	2871.00±3.06 ^a	*
FCR (g/g)	0-14	1.83±0.02 ^a	1.83±0.01 ^a	1.77±0.01 ^b	1.75±0.02 ^b	*
	14-35	2.09±0.01 ^a	1.99±0.02 ^b	1.89±0.01 ^c	1.88±0.01 ^c	*
	0-35	2.05±0.01 ^a	1.97±0.01 ^b	1.87±0.01 ^c	1.86±0.01 ^c	*

^{a,b,c}Means within rows with no common superscripts differ significantly, * $p < 0.05$, T1: Control, T2: 0.1% MOLM, T3: 0.2% MOLM, T4: 0.3% MOLM, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, MOLM: *Moringa oleifera* leaves meal

Table 2: Carcass characteristics of broiler chicks fed various levels of *Moringa oleifera* leaves meal under heat stress condition

Items (%)	Treatments				Significance
	T1	T2	T3	T4	
Carcass	72.74±0.99	73.31±0.71	72.79±0.57	73.76±0.23	NS
Liver	2.93±0.08	2.56±0.09	2.67±0.17	2.91±0.09	NS
Gizzard	2.46±0.21	2.56±0.08	2.30±0.12	2.25±0.07	NS
Heart	0.59±0.03	0.58±0.01	0.57±0.02	0.57±0.02	NS
Abdominal fat	2.21±0.12	2.34±0.07	2.30±0.03	2.18±0.09	NS
Breast	24.51±0.19	24.64±0.56	24.50±0.45	24.33±0.71	NS
Thigh	18.96±0.38	19.20±0.30	19.08±0.64	19.02±0.15	NS

NS: Non significant, T1: Control, T2: 0.1% MOLM, T3: 0.2% MOLM, T4: 0.3% MOLM, MOLM: *Moringa oleifera* leaves meal

the nutrient utilization as result of the presence of flavonoids which react as antibacterial and antioxidant. Also, this improvement may be due to moringa beneficial effect on the microbial environment in the gut, which might enhance digestion, absorption and utilization of nutrients.

Carcass characteristics: The effects of dietary treatments on carcass characteristics at 35 days old broilers are shown in Table 2. Addition of MOLM (0.1-0.3%) to broiler diets had no significant effects on carcass percentage and the relative weights of the liver, gizzard, heart, breast, thigh and abdominal fat.

These results are in agreements with Nuhu (2010), who reported that there were no significant differences among treatments in carcass characteristics of weaned rabbits fed MOLM. Ayssiwede *et al.* (2011) reported that inclusion of MOLM had no significant effect on the dressing percentage of indigenous chicken. Nkukwana *et al.* (2014) found that addition of MOLM (0.1-2.5%) to broiler diets have no significant effects on carcass weight, dressing percentage and the relative weights of the liver, gizzard, heart and spleen.

Haematological parameters: Results concerning the haematological parameters of broiler chicks fed various levels of MOLM under heat stress condition are presented in Table 3. The results indicated that all levels of MOLM supplementation significantly ($p < 0.01$) increased haemoglobin concentration (Hb) compared with the control (T1) group ($8.93 \text{ mg } 100 \text{ mL}^{-1}$). Additionally, the highest value was recorded for T4 and T3 (9.46 and 9.33, respectively). This result may be due to the high content of MOLM from iron (Ogbe and Affiku, 2012). In this respect, Olugbemi *et al.* (2010) mentioned that Red Blood Cells (RBC) are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of haemoglobin hence higher values indicate a greater potential for this function and a better state of health.

Moringa oleifera leaves meal had no significant effect on haematocrit values (Ht %) although the values were numerically increased with increase MOLM level. The high level may be attributed to the influence of Moringa on the haematological parameters. Furthermore, Onu and Aniebo (2011) reported that haematocrit is an index of toxicity reduction in the blood usually and suggest the presence of a toxic factor which has an adverse effect on blood formation.

Heterophil/Lymphocyte (H/L) ratio is an indicator to the immune responses of the bird, the immune response enhanced as the H/L ratio decrease. All levels of MOLM supplementation significantly decreased H/L ratio compared with the control (T1) group (0.65). Additionally, the lowest H/L ratio was recorded for the highest level of Moringa in T4 (0.58) followed by T3 (0.59) then T2 (0.60). The antimicrobial and antioxidant properties of Moringa may be responsible for these findings; this indicates that the experimental levels enhanced the birds ability to wade infection. (Ebenebe *et al.*, 2012). Also, the Moringa leaves are highly nutritious containing significant quantities of vitamins especially vitamin C (Onu and Aniebo, 2011; Asante *et al.*, 2014) which play, as an antioxidant and a role in alleviating the negative responses to heat stress condition on birds to become healthy.

Biochemical parameters: Plasma total protein, albumin and globulin of broiler chicks fed various levels of MOLM under heat stress condition are shown in Table 4. Plasma total protein level was significantly increased as MOLM increased in the diet. The highest level was recorded for T4 and T3 (4.44 g dL^{-1} and 4.42 , respectively) with no significant differences between them, followed by T2 (4.11), while the lowest level was recorded for the control group T1 (3.91).

Plasma albumin level was insignificantly increased with the increase of MOLM in the diet. However, plasma globulin level was also higher in the same manner like total protein with no significant differences between T3 and T4 group, which may indicate better immune response of broilers. It is

Table 3: Haematological parameters of broiler chicks fed various levels of *Moringa oleifera* leaves meal under heat stress condition

Items	Treatment				Significance
	T1	T2	T3	T4	
Hb (mg/100 mL)	8.93±0.15 ^c	9.16±0.06 ^b	9.33±0.06 ^{ab}	9.46±0.06 ^a	**
Ht (%)	34.00±1.00	34.00±1.10	35.00±1.10	37.00±2.80	NS
H/L ratio	0.65±0.01 ^a	0.60±0.01 ^b	0.59±0.01 ^b	0.58±0.02 ^b	**

Hb: Hemoglobin, Ht: Haematocrit, H/L: Heterophil/Lymphocyte, ^{a,b,c}: Means within rows with no common superscripts differ significantly, **p<0.01, NS: Non-significant, T1: Control, T2: 0.1% MOLM, T3: 0.2% MOLM, T4: 0.3% MOLM, MOLM: *Moringa oleifera* leaves meal

Table 4: Plasma protein fractions of broiler chicks fed various levels of *Moringa oleifera* leaves meal under heat stress condition

Items	Treatment				Significance
	T1	T2	T3	T4	
Total protein (g dL ⁻¹)	3.91±0.04 ^b	4.11±0.23 ^{ab}	4.42±0.03 ^a	4.44±0.01 ^a	**
Albumin(g dL ⁻¹)	1.75±0.01	1.76±0.04	1.77±0.01	1.80±0.03	NS
Globulin(g dL ⁻¹)	2.16±0.05 ^b	2.35±0.21 ^{ab}	2.65±0.02 ^a	2.64±0.03 ^a	**

^{a,b,c}: Means within rows with no common superscripts differ significantly, **p<0.01, NS: Non-significant, T1: Control, T2: 0.1% MOLM, T3: 0.2% MOLM, T4: 0.3% MOLM, MOLM: *Moringa oleifera* leaves meal

postulated that plasma protein profile of a given bird is a reflection of the metabolic activities related to protein synthesis and/or degradation. Since, it is well known that stress conditions could stimulate the adrenal gland cortex for corticosterone secretion, which caused a considerable increase in protein catabolism due to its gluconeogenic activity (Tollba and Hassan, 2003). Increased levels of MOLM in the diet significantly elevated the plasma total protein which is consistent with the findings of Teye *et al.* (2013). Increased total proteins in chickens fed the MOLM diet may reflect a more intensive metabolism of the proteins in the chicken's organ as suggested by Melesse *et al.* (2013). Also, this result may be due to high content of *Moringa* from antioxidant (Onu and Aniebo, 2011; El-Wardany *et al.*, 2012; Asante *et al.*, 2014) which increases blood total protein by decreasing corticosterone secretion which could limit protein catabolism under heat stress condition.

The results of plasma aminotransferase activity (AST and ALT) of broiler chicks fed various levels of MOLM under heat stress condition are presented in Table 5. Aspartate aminotransferase enzyme (AST) level was significantly decreased in plasma with supplementation of MOLM in the diet. Additionally, the lowest level was recorded for T4 and T3 with no significant differences between them followed by T2 (139.3, 142.6 and 159.3 U L⁻¹, respectively) while, the highest level was recorded for the control group T1 (197.3). While, alanine aminotransferase enzyme (ALT) level did not significantly affect by MOLM supplementation. The liver is reported to contain enzymes like ALT and AST, it releases these enzymes in the blood when damaged (Sherwin, 2003). Elevation of serum AST and ALT can occur with states of altered hepatocellular membrane permeability either due to

circulatory hypoxia, exposure to toxins and toxemia, inflammation, metabolic disorders or proliferation of the hepatocyte. In the stunting syndrome of broiler chickens, increased activities of AST and ALT were also associated with liver and intestinal damage (Rani *et al.*, 2011). Hence, the decrease of AST level and the absence of significant differences among treatment diets in serum ALT in the present study may collectively reflect the normal liver and intestinal functions of chickens fed diets containing MOLM during heat stress condition. This result is also supported by the works of Olugbemi *et al.* (2010) and Melesse *et al.* (2013), who reported *Moringa oleifera* leaves have a beneficial effect to enhance the immune responses and improve intestinal health of broilers. This could reflect a protective influence of MOLM supplementation on liver tissues.

The results of thyroid hormone levels, triiodothyronine (T₃) and thyroxin (T₄) and the T₃/T₄ ratio of broiler chicks fed various levels of MOLM under heat stress condition are presented in Table 5. The result showed that T₃ level was significantly increased in all groups which supplemented MOLM compared with the control group. The highest level was recorded for T3 and T4 with no significant differences between them (4.68 and 4.35 ng dL⁻¹, respectively) followed by T2 (4.25) while, the lowest level was recorded for T1 (3.55). Moreover, T₄ level was significantly (p<0.05) increased in T3 group (25.51 ng dL⁻¹) followed by T4 (22.70) compared with T2 and T1 (19.48 and 18.0 ng dL⁻¹, respectively). However, T₃/T₄ ratio did not significantly change with MOLM supplementation compared with the control group.

Triiodothyronine (T₃), the metabolically active thyroid hormone, plays an active role in energy metabolism and metabolic rate. Any pronounced alteration in thyroid function

Table 5: Plasma aminotranseferase activity and thyroid hormones levels of broiler chicks fed various levels of *Moringa oleifera* leaves meal under heat stress condition

Items	Treatment				Significance
	T1	T2	T3	T4	
AST (U L ⁻¹)	197.300±1.45 ^a	159.300±4.37 ^b	142.600±1.76 ^c	139.300±1.76 ^c	**
ALT (U L ⁻¹)	11.000±0.57	10.000±0.57	11.000±0.57	10.330±0.66	NS
T ₃ (ng dL ⁻¹)	3.550±0.34 ^b	4.250±0.03 ^{ab}	4.680±0.24 ^a	4.350±0.18 ^a	**
T ₄ (ng dL ⁻¹)	18.000±0.31 ^c	19.480±0.46 ^c	25.510±0.86 ^a	22.700±0.56 ^b	**
T ₃ /T ₄ ratio	0.197±0.02	0.218±0.01	0.184±0.01	0.191±0.01	NS

^{ab,c}Means within rows with no common superscripts differ significantly, **p<0.01, NS: Non-significant, T1: Control, T2: 0.1% MOLM, T3: 0.2% MOLM, T4: 0.3% MOLM, MOLM: *Moringa oleifera* leaves meal, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

(hyperthyroidism or hypothyroidism) is reflected in an altered metabolic rate. Geraert *et al.* (1996) reported that the importance of the thyroid hormone in the physiological adaptation to heat stress is related to their role in regulation of metabolic process in birds, correspondingly the thyroid hormone output is depressed as the ambient temperature increased. Exposure of broiler chickens to heat stress was significantly reduced T₃ level when compared with control reared at 22 °C (Tollba and Hassan, 2003).

The elevation in T3 level of MOLM groups may be due to the high content of antioxidant particularly vitamin C which able to alleviate the adverse effect of heat stress and enhance the physiological responses of birds to overcome heat stress. The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* leaves. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in *Moringa* leaves (Siddhuraju and Becker, 2003). *Moringa* has a host of antioxidant in combinations, which are highly beneficial and easily absorbed such as vitamin C (Onu and Aniebo, 2011; Asante *et al.*, 2014). Hemid *et al.* (2013) found that vitamin C supplementation to broiler diets under heat stress condition resulted in a greater serum T₃ and T₄.

The thyroid hormones play important roles in growth and protein turnover (Hayashi, 1993) therefore, the decrease in T₃ production leads to decrease the protein turnover followed by growth retardation and vice versa is true. The addition of MOLM (0.2%) in the diet led to an increased level of thyroid hormones, which in turn led to increase the proportion of plasma total proteins followed by protein synthesis which reflected positively on the productive performance of broilers (increased live body weight and body weight gain), this explains the results obtained in the present study.

CONCLUSION

It could be concluded that supplementation of MOLM in the diets during heat stress condition with the present

experimental levels (0.1, 0.2 and 0.3%) had positive effects on productive performance, physiological responses and enhance the ability of broilers to resist the heat stress condition. Moreover, the best level of MOLM is 0.2%, which enhanced the productive performance, carcass characteristics, haematological and biochemical parameters in the present study.

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