Effect of electric stimulation on histological traits and color of carcasses in old duck and chicken

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Abstract. The effect of electric stimulation on some quantitative character of old duck and chicken carcasses were the main aim of the present study. The device for measuring meat tenderness was designed at department of Food Sciences, College of Agriculture, and University of Basrah. A total of 36 old duck and same number of spent layer chickens aged78.21 week's birds were slaughtered with knife manually. They de-feathered, and all internal organs were removed. Carcasses were divided into three groups, the control group (no electric stimulation), the second treatment group (electric conductivity 3.67 V/cm) with low voltage [110 volt]) and 1% saline solution, the third treatment group (7.33 V/cm) with 220 V voltage and 1% saline solution. Traits were measured at 25 min, 6 hrs and 24 hrs after electric stimulation. The carcasses were stored for 30 and 60 days. The following histological parameters were determined; sarcomere length, muscle fiber length, muscle fibres breaking index and color. The results indicated that length of sarcomere and muscle fibre breaking index were significantly($P \leq 0.05$) affected by electric stimulation, because there was an increase in sarcomere length and muscle fiber breaking index of third treatment in chicken with an increasing interval of time. There was a significant ($P \leq 0.05$) decrease in muscle fiber diameter in electric stimulation treatment three (duck and chicken) with the time progress. Duck differed significantly when compared to chicken. Statistical analysis showed a significant effect of electric stimulation on color lightness (L). Duck appeared to be darker in color than chicken. Treatment group three revealed blue coloration at 25min, 60 days in both duck and chicken. Whereas other treatment groups showed yellowness (b) color. Similarly, stimulation affected color redness (a) where all groups showed greenish except 30 and 60 day intervals, their color was reddish and value of (a) of duck was less than of chicken. Electric stimulation (especially 220 V voltage) improve meat tenderness of both chicken and duck.

1. Introduction

There are about 250 million adult chickens marketed annually worldwide. A total of 85% of this chicken are from layers that are aged 1.5 year-old, while the rest are parent stocks that are used for breeding purpose. Old chicken is characterized by stiff, dry and low-grade meat, and tenderness is one of the most important measure of consumer palatability [1]. One of the methods currently used to reduce the time of ripening is the process of electric stimulation after slaughter to accelerate the development of rigors mortis where it can maturate muscles in two hours instead of 6 hours or more and thus reduce the costs of processing, cooling, stores and the power spent in the factory [2]. The

study of electrical stimulation as a means of reducing the time required for aging to prevent the meat hardness has been newly available for commercial use. Electrical stimulation improves the tenderness of meat by decrease cutting pieces and increases the length of sarcomere and reduced the diameter of muscle fibers. In addition to the possibility of cutting of meat in less than two hours after slaughter with a tenderness similar to that of meat after a period of 4 hours after slaughter. This reduce the storage time to 5% or more, reducing the cost of storage, in addition to reducing the force required for feather removal, and electric stimulation also reduces the microbial load on chicken carcasses [3]. Gezgin and Karakaya [4] used electric water bath stunning (30 V, 30 mA, 220 Hz alternative current for 17 s) to treat broilers and found that the effect of stunnin on the color parameters (L*, a*, and b*) was not significant ($p>0.05$). Pulsed electric field processing in meat improves nutritional and physicochemical changes as well as tenderization, [5]. The current study aimed to evaluate the histological and color characteristics of the quality of meat using electrical stimulation.

2. Materials and Methods

2.1. Experiment treatments

In this experiment, thirty six (36) birds comprising of old duck and the same number of spent layer chickens were used. The birds were 1.5 year-old, they were slaughtered manually and after the complete blood depletion period of 150 seconds. Then the feathers and internal organs were measurement removed manually. Carcasses were divided into three electrical stimulation treatment groups with 6 birds per treatment. The study treatments were as follow: the first treatment (T1) was control, the second treatment (T2) was the use of electrical stimulation with low voltage 110 V with a 1% saline concentration, the third treatment (T3) was the use of electrical stimulation with high voltage 220 volts with 1% saline concentration. Traits were measured at 25 minutes, 6 hours and 24 hours. Carcasses were stored in freezer for 30 and 60 days at -18 ^{o}C and the following tests were performed.

2.2. Length of the sarcomere

The length of the sarcomere was measured as described by [6]. Homogenized 5 g of meat sample (breast) was mixed with 35 mL of a solution containing 0.25M sucrose for a minute with a mixer at low speed. After completion of the homogenization process, a drop of solution was placed on a glass slide and placed with the Eocene, hematoxylin and a drop of glycerin, then was covered with a coverslip and examined microscopically using a 10X magnification. The length of 10 randomly selected sarcomeres were measured and then averaged

2.3. Fiber Muscle Diameter

The diameter of the fiber was measured according to the method described by [7] with some modifications. Five grams of meat were taken into small pieces and blender was mixed at low speed for 5 minutes with a 5-second rest and mixed with 30 mL of solution containing 0.25M sucrose, Ethylen Tetra-Acetic Acid (1mM EDTA). A drop was placed on the glass slide and added Eocene and glycerin dye to give the color to clarify the diameter of the fiber after covering the specimen with coverslip. The diameter of ten fibers was measured randomly under objective lenses of x10. Average diameter was computed.

2.4. Muscle fiber Index (MFI).

This trait was calculated according to the method described by [7], where frozen cubes were taken and mixed with 50 mL of solution containing 0.25M of cold sucrose and 0.02M of potassium chloride. Leaving the pieces for 5 minutes after melting from freezing and grinding for 40 seconds with high strength and then filtered with filter paper and placed in a drying oven under 40 ° C for 40 minutes.

2.5. Color measurement.
Image processing method was use for the analysis of the color characteristics of the ducks and chickens carcass. Where the images were taken to the plates for each carcass by a high resolution digital camera (8 mega pixel) at suitable lighting and using Adobe Photoshop CS2 program for image analysis according to [8]. L*: Lightness, a*: Redness, b*◦: Yellowness.

2.6. Statistical Analysis
Data was statically analyzed by using Statistical Package of Social Science (SPSS). Factorail experiment with complete randomize design was used. Means were compared by Revised Least Significant Differences (RLSD) at 5% significant level [9].

3. Results and Discussions

3.1. Length of sarcomere
The results showed in table 1 that there was a significant effect of electrically stimulated treatment and time on the sarcomere length of old ducks and chickens. The control treatment showed significant increase ($P<0.05$) in the length of sarcomere when compared to the other treatment groups. There was significantly higher differences in the older chickens ($p<0.05$) than in the other treatments (2.22 ± SEM μm) compared to the control and the second treatment which recorded 1.98 and 1.55 microns respectively. The table also showed a significant ($P<0.05$) effect of the storage time (60 and 30 days) on ducks and chickens carcasses. There was a significant ($p <0.05$) increase in length of sarcomere at different timing intervals. Duck showed a mean of 2.29 ± SEM and 2.28 ± SEM compared to those of chicken 2.19 ± SEM and 2.03 ± SEM microns for both ducks and chicken respectively. The control treatment during the 60 days storage period showed the longest sarcomere in the adult ducks (2.15 ± SEM microns) and the chicken (2.33 ± SEM microns). The table shows that the control treatment at day 60 was significantly higher (2.51± SEM micron). In the chicken, the third treatment exceeded 60 days with a significant gain of 2.33 ± SEM micron. In Table 3 there are no significant differences between ducks and chickens in the length of the sarcomere. The effect of electrical stimulation in the length of the sarcomere may be due to the fact that electrical stimulation activates enzyme systems leading to complete rigors mortis. It also increases the breakdown of sugars and prevents the occurrence of cooling shortage when meat is preserved after slaughtering [10]. Other reasons for increasing the length of sarcomere as a result of electrical stimulation are that electrical stimulation may break down the transverse bridges between actin and myosin. Because the number of transverse bridges is linked to the length of the sarcomere, the increase in sarcomere length may be due to the reduction in the number of transverse bridges. Insufficient energy compounds in electrically stimulated muscles, making the risk of shortening in sarcomere very low due to insufficient muscle energy and allowing it to contract [11]. The results of this study agreed with that of [12] where it was observed that there was an increase in the length of the sarcomere when the voltages increased for older chickens and differed with respect to the ducks. There was no increase in sarcomere length with increased voltages. On the other hand this study was not consistent with the study of [12] as it did not notice an increase in the length of the sarcomere when increasing the time of voltages within a single treatment. This study is in agreement with many studies, noting that the length of the sarcomere is not affected by the voltages and the time difference within the voltages [13,14].

3.2. Diameter of muscle fibers
Table 2 shows the effect of electrical stimulation on the diameter of muscle fibers of chicken and ducks. The table shows that the treatment had a significant effect ($P <0.05$) on the diameter of muscle fiber.The third treatment (T3) showed a significant ($P<0.05$) decrease in the diameter of the muscular fiber of the duck and the old chicken, which reached 52.27 ± SEM and 55.90 ± SEM

microns compared to the control treatment of 64.62 ± SEM microns in ducks and 63.66 ± SEM micron for chicken.

Table 2 also showed a significant effect (P <0.05) for the time, with 60 days showed a significant decrease in muscle fiber diameter (53.84 ± SEM, 57.11 ± SEM micron for duck and chicken respectively) compared with 25 minutes of 64.71 ± SEM and 66.72 ± SEM microns for duck and chicken, respectively.

The table shows that the third treatment at a 30 day storage period showed a significant (P<0.05) decrease in muscle fiber diameter (51.29 ± SEM) micron compared to 25 minutes for the control treatment of duck. The chicken showed at 60 days reduction (50.81 ± SEM micron) compared with 25 Minute (58.88 ± SEM microns). The table showed a significant effect (P<0.05) on the diameter of muscle fiber, where ducks showed a significant decrease in fiber diameter compared to chicken (60.41 ± SEM, 58.32 ± SEM micron respectively).

These results were not consistent with the results of [15] and [16] They did not observe any significant differences in muscle fiber diameter. However, the present results were consistent with [14] and [16]. Note that there was a significant (P<0.05) decrease in the diameter of the muscle fiber at 330 and 220 volts and decreased the diameter of the muscle fiber after the electrical stimulation at 220 and110 volts compared to the control group. It may be due to the difference in animal species, muscle type and sarcomere length. After the death of the animal, oxygen associate with the myosin in a chemical bond, making the meat solid and inflexible. The electrolysis leads to a decrease in the diameter of the muscle fiber and the stimulation of an enzyme that processes the proteins.

Table 1. Effect of electrical stimulation on the length of sarcomere (μm) of old duck and spent layer chikens (mean ± standard error)

Species	Time	Treatments			Mean of time
		Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	
Duck	0.25H[a]	1.50±0.11	1.42±0.11	1.35±0.08	1.42±0.06
	6H	1.89±0.11	1.60±0.08	1.39±0.09	1.63±0.06
	24H	2.10±0.07	2.00±0.09	2.14±0.15	2.08±0.06
	270(30D)	2.30±0.08	2.23±0.11	2.30±0.11	2.28±0.06
	1440(60D)	2.51±0.10	2.19±0.05	2.18±0.08	2.29±0.05
	Treatment mean	2.06±0.06	1.89±0.06	1.87±0.07	1.94±0.04
Chicken	0.25H	1.21±0.05	1.55±0.11	2.17±0.05	1.64±0.09
	6H	1.38±0.07	1.97±0.15	2.14±0.05	1.83±0.08
	24H	1.48±0.11	1.93±0.23	2.27±0.07	1.89±0.10
	270(30D)	1.71±0.10	2.19±0.17	2.19±0.08	2.03±0.08
	1440(60D)	1.98±0.10	2.26±0.09	2.33±0.07	2.19±0.06
	Treatment mean	1.55±0.05	1.98±0.08	2.22±0.03	1.92±0.04

[a]: H= Hours, D= Days, T2 (V/cm=110 V), T3 (V/cm= 220 V), LSD_{Time}=0.11, $LSD_{treatment}$= 0.09, $LSD_{species}$= 0.07, $LSD_{time\ x\ treatment}$= 0.20

3.3. Muscle fracture index

Table 3 shows the effect of electrical stimulation on the muscle fiber fracture index for ducks and older chickens. It is shown from the table that treatment has an effect on the muscle fiber fracture index of the ducks, the control treatment showed a significant increase (p<0.05) in muscle fiber fracture compared to the third treatment. The electrical conductivity of 7.33V / cm revealed an index of 127.04 ± SEM and 177.67 ± SEM for duck and chicken respectively. While chicken showed a significant (P<0.05) decrease in muscle fiber compared to that of duck at the third treatment (134.67 ± SEM, 95.33 ± SEM respectively). The time period showed an effect on muscle fiber fracture. The 24-hour storage time showed a significant increase (p< 0.05). in the fiber fracture index (158.67 ±

SEM). While the storage period of 60 days, the third treatment showed a significant increase (P<0.05) in chickens it reached 184.78 ± SEM compared to the rest of the time periods. The table showed significant differences between treatment and time, where the standard treatment at (6-24) hours showed a significant increase (P<0.05) in the muscle fiber breakout index, it reached 249.33 ± SEM and 240.00 ± SEM, respectively, compared with the rest of the treatments. The lowest treatment (3.67 conductivity intensity) at 25 minutes was 77.33 ± SEM micron while the third treatment (7.33 conductivity intensity) at 60 days in the chicken showed a significant increase (P<0.05) compared to the other treatments. The lowest treatment was the control treatment at (25) minutes, reaching 55.33 ± SEM. The table shows that the species has an effect on the muscle fiber fracture index. The ducks show a significant increase (P<0.05) on the chickens with an estimates of 116.20 ± SEM and 143.04± SEM respectively. The effect of the electrical stimulation may be due to the muscle fiber fracture guide by its effect on the structural breakdown of the fiber muscle, rupture and fracture in Z-line [15]. This finding did not agree with the study [16], where no significant differences were observed between the control treatment and the second treatment. This study also did not agree with [17]. The study showed a significant effect (P< 0.05) in the muscle fiber breakdown index and for the different periods, noting that the highest value appeared in the stimulation treatment (200) volts and the lowest was from the control group.

Table 2. Effect of electrical stimulation and time on diameter of muscle fibers (μm) of old duck and spent layer chikens (± Standard Error)

species	Time	Treatments Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	Mean of time.
Duck	0.25H[a]	1.23±76.65	63.79±1.72	53.70±1.07	64.71±1.90
	6H	2.27±67.93	59.93±1.718	52.88±2.06	60.25±1.61
	24H	0.88±62.90	58.63±1.89	51.35±1.23	57.63±1.18
	270(30D)	1.99±59.15	55.08±1.65	51.29±1.48	55.17±1.13
	1440(60D)	2.35±56.45	52.95 ±1.08	52.11±1.32	53.84±0.99
	Treatment mean	1.29±64.62	58.08±0.89	52.27±0.65	58.32±0.70
Chicken	0.25H	74.19±1.20	67.08±1.56	58.88±2.64	66.72±1.57
	6H	68.64±2.56	61.86±2.43	61.08±2.63	63.86±1.55
	24H	57.21±2.81	57.07±1.90	56.01±1.78	56.76±1.24
	270(30D)	59.07±2.67	60.95±1.72	52.73±1.20	57.58±1.27
	1440(60D)	59.21±2.60	61.32±2.41	50.81±0.78	57.11±1.44
	Treatment mean	63.66±1.41	61.66±0.98	55.90±0.99	60.41±0.71

[a]: H= Hours, D= Days, T2 (V/cm=110 V), T3 (V/cm= 220 V), LSD_{Time}=2.18, $LSD_{treatment}$= 1.69, $LSD_{species}$= 1.38, $LSD_{time\ x\ treatment}$= 2.00

Table 3. Effect of electrical stimulation on muscle fracture index of old duck and spent layer chicken (± standard error)

Species	Time	Treatments Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	Mean of time.
Duck	0.25H[a]	178.67±1.86	77.33±1.20	93.33±0.88	116.44±15.74
	6H	240.00±2.89	112.33±1.45	101.00±0.58	151.11±22.30
	24H	249.33±3.48	123.00±1.53	103.67±0.88	158.67±22.87
	270(30D[a])	119.00±0.58	124.67±3.53	161.33±0.67	135.00±6.72
	1440(60D)	101.33±0.67	180.33±1.45	180.33±7.69	154.00±13.36
	Treatment mean	177.67±16.19	123.53±8.89	127.93±9.63	143.04±7.73
Chicken	0.25H	55.33±1.45	82.67±2.33	97.67±1.45	78.56±6.26
	6H	63.67±1.33	82.67±2.33	113.33±0.88	86.56±7.28
	24H	86.67±1.45	117.00±3.06	125.00±0.58	109.56±5.92
	270(30D)	103.33±0.88	120.33±0.88	141.00±2.08	121.56±5.49
	1440(60D)	167.67±1.76	190.33±0.88	196.33±3.18	184.78±4.50
	Treatment mean	95.33±10.69	118.60±10.54	134.67±9.10	116.20±6.21

[a]: H= Hours, D= Days, T2 (V/cm=110 V), T3 (V/cm= 220 V), LSD_{Time}=1.44, $LSD_{treatment}$= 1.11, $LSD_{species}$= 0.91, $LSD_{time\ x\ treatment}$= 2.49

3.4. Color

Color is one of the most important qualitative character of food products and has a significant impact on the consumer's desire and the final price of the product [18] The values of L * can be determined if the color is dark (L = low) and light (L * high).

3.4.1. Lightness (L)*

Table 4 shows the effect of electrical stimulation on the value of L for duck and old chickens. For the effect of time after stimulation, the results showed that the highest value of L was 47.20 after 0.25 hours of electrical stimulation of the duck. This indicates that the color of the ducks was light and turned to a darker color with increasing time but unevenly. The lowest value of L reached 39.98 after 6 hours of electrical stimulation as the color was darker than the rest of the treatments and the difference between them and the first treatment (0.25 hours) was significant ($p<0.05$). The results also showed that the difference between the first treatment (0.25 hours) and the fifth treatment (60 days) was insignificant ($p<0.05$). The significant effect of time on the L value might be due to the high pH value which leads to a dark color as shown in Table 4, reaching 6.07. For electrically stimulated chickens, the value of L increased significantly ($P<0.05$) with time after stimulation, reaching 60.79 after 0.25 hours and increased to 71.99 after 30 days after stimulation. The table shows that the third treatment (7.33V / cm) was significantly ($P<0.05$) higher than the control

treatment (41.65 and 47.58 respectively) in ducks. In the chicken, the control treatment was significantly (P<0.05) higher (74.42). The table shows the superiority of the third treatment (electric field intensity of 7.33V / cm) at the time of 60 days at 57.66 in ducks. In the chicken, the control treatment at 24 hours was significantly higher (p<0.05) at 88.85. The table showed a significant superiority of chicken (p<0.05) on ducks (44.47 and 66.12 respectively). The reason for the increase in color in chickens after stimulation and lack of coloration in ducks might be due to high fat in ducks, which cause pale duck meat compared to chicken meat [19].

Table 4. Effect of electrical stimulation on the value of L for duck and aged chicken.

species	Time	Treatments			Mean of time
		Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	
Duck	0.25H[a]	44.78	43.91	52.91	47.20
	6H	37.85	40.18	41.92	39.98
	24H	33.76	45.74	44.62	41.37
	270(30D[a])	54.90	44.69	40.76	46.78
	1440(60D)	36.95	46.41	57.66	47.01
	Treatment mean	41.65	44.19	47.58	44.47
Chicken	0.25H	60.35	62.05	59.97	60.79
	6H	61.62	71.51	56.69	63.27
	24H	88.85	56.03	51.32	65.40
	270(30D)	81.43	69.10	65.44	71.99
	1440(60D)	79.83	66.09	61.49	69.14
	Treatment mean	74.42	64.96	58.98	66.12

3.4.2. Yellowness/ Blueness (b)*

The value of b * yellowish colours when the value of b * is positive (+), but if it has a negative value (-), it means blueish [18]. The value of b * is between -120 and 120 [8,20,21]. When the value of b is zero, this means there is no colour (neutral colour) or grey. Table 5 shows that the predominant colour of the duck and the electrically stimulated chickens is yellowish, except for the treatment after 0.25 hours at 3.67 volts/ cm and after 60 days at 7.33 volts/ cm where the predominant colour is bluish in ducks and chick. The yellowishness of ducks in the electric field of 7.33 V/ cm was higher than the electric field strength of 3.66 V / cm as it increases by increasing the electric field strength in ducks and chickens. The highest value for b * after 60 days was 23.27 for ducks while the highest value for b * after 30 days was 37.23. of third treatment (-84.21) where it was greener compared to other treatments. The table also shows time superiority of the second treatment (electric field strength 3.67V / cm) at 30 and 60 in ducks and chickens where the colour was red and it was 5.18, 16.16 in ducks and 6.12, 3.61 in chickens. The table shows no significant differences between ducks and chickens with reddish colour.

3.4.3. Redness/greenness (a)*

The value of a* indicates reddish colours if the value is positive (+), but if it is negative (-), the colour greenish [20].The value of a * is between -120 and 120 [22]. The results showed in Table (6) that there were significant (P<0.05) differences between the control treatment and the third treatment (the intensity of the electric field 7.33V / cm) where the control treatment exceeded (-40.78) that of third treatment (-84.21) where it was greener compared to other treatments. The table also shows time superiority of the second treatment (electric field strength 3.67V / cm) at 30 and 60 in ducks

and chickens where the colour was red and it was 5.18, 16.16 in ducks and 6.12, 3.61 in chickens. The table shows no significant differences between ducks and chickens with reddish colour.

Table 5. Effect of electrical stimulation of the ducks in the b* values of old duck and spent layer chickens.

Species	Time	Treatments			
		Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	Mean of time
Duck	0.25H[a]	19.66	-50.43	5.96	-8.26
	6H	30.08	2.46	4.39	12.31
	24H	3.45	20.56	7.37	10.46
	270(30D[a])	3.45	3.61	25.90	10.99
	1440(60D)	34.83	37.39	-2.40	23.27
	Treatment mean	18.29	2.72	8.24	9.75
Chicken	0.25H	8.54	0.94	-0.46	3.005
	6H	43.37	11.76	30.11	28.41
	24H	63.81	3.34	15.02	27.39
	270(30D)	66.22	9.02	36.44	37.23
	1440(60D)	26.82	3.78	14.80	15.13
	Treatment mean	41.75	5.77	19.18	22.23

[a]: H= Hours, D= Days, T2 (V/cm=110 V), T3 (V/cm= 220 V), LSD_{Time}=27.22, $LSD_{treatmen\ t}$ = 21.09, $LSD_{species}$= 17.22, $LSD_{time\ x\ treatment}$= 38.50, The value of b * yellowish colours when the value of b * is positive (+), but if it has a negative value (-), it means blueish.

Table 6: Effect of electrical stimulation of the ducks in the a* values of old duck and spent layer chickens.

species	Time	Treatments			
		Control (T1)	3.67 V/cm (T2)	7.33 V/cm (T3)	Mean of time
Duck	0.25H[a]	-22.84	-26.41	4.55	-14.90
	6H	-57.88	-9.47	-17.33	-28.23
	24H	-31.01	-34.14	5.09	-20.02
	270(30D[a])	-59.61	16.16	-8.13	-17.19
	1440(60D)	-32.58	5.18	-16.40	-14.60
	Treatment mean	-40.78	-9.74	-6.44	-18.99
Chicken	0.25H	-73.39	3.76	2.35	-29.71
	6H	-65.80	4.18	5.21	-18.80
	24H	-103.06	-.47	-36.71	-46.75
	270(30D)	-95.22	3.61	-23.24	-38.28
	1440(60D)	-83.61	6.12	-26.96	-34.82
	Treatment mean	-84.21	3.42	-17.17	-33.85

[a]: H= Hours, D= Days, T2 (V/cm=110 V), T3 (V/cm= 220 V), LSD_{Time}=17.22, $LSD_{treatment}$= 21.09, $LSD_{species}$= 29.82, $LSD_{time\ x\ treatment}$= 47.15, The value of a * indicates reddish colours if the value is positive (+), but if it is negative (-), the colour greenish

4. Conclusions

The present assessment obviously indicated that all native biological control agents (*T. harzianum, B. subtilis* and *B. cerus*) examined showed robust antagonistic activity against *R. solani* infection on

cowpea crop. Consequently, they could be used as alternative methods of fungicide application for management of the diseases caused by *R. solani* on cowpea crops or others crops.

5. Acknowledgements
The authors thankful department of Animal Production and department of food science, College of Agriculture, University of Basrah, Iraq for available of laboratories.

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