ENHANCING DIVERSITY OF POULTRY PRODUCTS BY PRODUCING HIGH QUALITY BROWN EGGS

Hanan Al-Khalifa
Gehan Ragheb
Kuwait Institute for Scientific Research, Kuwait

Abstract

There has been interest in increasing egg production and enhancing diversity of poultry products in the state of Kuwait. The main objective of the current study is to investigate the differences between production performance between brown and white strains of laying hens. One day old pullets from Lohmann LSL-Classic and Lohmann Brown-Classic strains were used, 600 each. Food and water were provided *ad libitum*. Body weight, total feed consumption, overall feed efficiency, overall percent egg production, overall egg weight, overall egg mass, shell weight, and Haugh Unit (HU) were measured. Mortality, temperature and humidity were recorded daily. Data were analyzed using ANOVA. Means were separated using Tukey's test where significance was set at $P< 0.05$. The results showed that overall body weight gain and feed efficiency for the brown pullets were significantly ($P<0.05$) better than the white pullets. Mass of eggs and feed efficiency in case of brown hens were significantly ($P<0.05$) higher than that of the white hens for the last period (58-69 wk of age). In general, the production performance of the Lohman brown strain was better than that of the LSL white strain. This could enhance diversity of poultry products in the country and provide consumers with high quality eggs.

Keywords: Brown eggs, Lohmann LSL-Classic, Lohmann Brown-Classic

Introduction

The local poultry industry is one of the most important animal industries in the country. Annual egg consumption in Kuwait is one of the largest in the world (240 eggs/person/year). Improving production efficiency is essential for producers to reduce their costs and be able to improve profitability and compete with the imported products in terms of price and quality. This can be achieved by utilizing strains which produce brown eggs with high quality.

There are several studies in the literature that compared the performances of different strains of laying hens. For example, Altan et al. (2000) studied the effects of short-term starvation and midnight lighting on egg production and egg quality of white and brown strains and found that brown layers were significantly affected. Their egg production and egg quality were significantly better than the white layers by applying short-term starvation and midnight lighting regimes. Using brown laying hens (Shaver 579) and white laying hens (Shaver 2000), Riczu et al. (2004) found that eggs from the brown hens were heavier, had more egg shell, and had a higher specific gravity than the white eggs. In addition, Anderson (2002) provided detailed information on the differences in egg production and quality between different white and brown egg strains. Strains used were Hy-Line-W-36, Hy-line-W-98, Bovans (white), DeKalb (white) and DeKalb (sigma) for white eggs, and Hy-Line (Brown), Bovans (Brown) and DeKalb (Brown) for brown eggs. The author found that the average age at sexual maturity for the brown hens was 132.7 d, which was shorter than that of white hens (137.8 d). Also, the overall average of hen-day egg production for the brown hens was 85.6%, which was higher than that of white hens (83.2%). This has a great economic value as a 1% in egg production of laying hens in Kuwait is translated to a gain of approximately KD 115,000. Anderson (2002) found that egg weight was more for brown hens (61.1 g) than that of white hens (58.3 g). The author also found that feed efficiency was similar in both brown and white hens, and percent mortality was higher in brown hens than in white hens. However, the net income per hen (egg income-feed cost) was more for the brown hen than the white hen. Furthermore, Vits et al. (2005) found that % egg production, egg weight, shell thickness, and shell breaking strength of Lohman brown are better than that of Lohman Selected Leghorn (LSL). In addition, Benyi et al. (2006) found that Hy-line laid more but lighter eggs, utilized feed more efficiently and had a lower mortality than Hy-line W-98 white hens.

Interestingly, The nutritional values of brown eggs are known to be better than that of the white eggs. It was reported by Bell and Weaver (2002) that brown eggs have less lipids (8.49% vs. 10.04%) and more vitamin A (317 IU vs. 260 IU) and vitamin B2 (0.254 mg vs. 0.180) than white eggs. These nutritional values were also confirmed by the American Egg Board.

The objective of the current study was to investigate the differences between the production performance and egg quality between of brown and white strains of laying hens under the local environmental conditions of Kuwait.

Materials and Methods

Birds

Two strains of layer hens were used: Lohmann LSL-Classic strain which was white egg producers and Lohmann Brown-Classic strain which was brown egg producers. One day old female chicks (pullets) from the two strains were used, 600 chicks from each.

Housing

One pullet house was used for birds grown from one day until 19 weeks of age.and one layer house was used for birds raised from 19 weeks until 70 weeks of age. For each strain, the pullets were distributed between three batteries providing space of 333-364 cm^2 per pullet. At 19 wks of age, pullets were transferred to the layer house in which all hens were raised until 70 wks of age. The hens were housed in cages and each caged contained sex hens providing 500 cm^2 per hen.

All pullets and hens were provided with food and water *ad libitum*. Feed rations were prepared according to pullet and laying hen feed ration specifications at the poultry research farm. Photoperiod regimens that was used for pullets and laying hens followed the recommendations by the strain producing company. Mortality, temperature and humidity were recorded daily.

Diet

The pullets were fed grower ration from day one until 8 wk of age (18.5% protein, 2750 kcal kg), developer ration from 8 wk-16 wk of age (14.5 % protein, 2750 kcal kg), and pre-lay from 16 wk until 24 wk of age (17.5% protein, 2750 kcal kg), and laying ration from 24 wk of age to the end of the laying period (18.0% protein, 2900 kcal kg)

Lighting system

The one-day old pullets received 24 h/day of light in the first 3 d to provide the chicks enough time to locate the feed and water. Thereafter, duration of the lighting period was decreased until reached 8 hours/day of light at 8 weeks of age and remained until 18 weeks of age. Then, duration of lighting period was increased until reached 14 hours/day of light at 24 weeks of age and remained the same till the end of the experiment

Vaccination program

Vaccination program was conducted in accordance with the recommendations of the manufacturing company.

Data Collected

Pullets Period

The pullet production data collected included body weight, total feed consumption and overall feed efficiency was measured till 20 wk of age.

Laying Period

Overall percent egg production, overall egg weight, overall egg mass and total feed consumption, and overall feed efficiency were measured till 70 weeks of age. In addition, components of brown and white eggs, shell weight, and HU were measured. Finally, economic impact study on the return on investment from raising brown or white hens was conducted.

Data analysis

The data were analyzed using ANOVA utilizing the S-Plus statistical program (Crawley, 2002). Means were separated using Tukey's test, and the significance was set at $P< 0.05$.

Results and Discussion

Pullet Production

Body Weight, Feed Consumption and Feed Efficiency: Data for total body weight gain (g/bird), total feed consumption (g/ bird) from 0-20 weeks of age, and feed efficiency (g feed/ g gain) for both Lohman brown and LSL pullets are shown in Table 1.

As shown in the Table 1, body weights of brown and white pullets were similar until 4 wk of age, and then, the body weights of brown pullets were higher than that of white pullets (data are not shown). Also, overall body weight gain for the brown pullets was significantly ($P<0.05$) higher than the white pullets. The Lohmann Tierzucht Company reported in their management's guide similar results. They reported that Lohman brown pullets were heavier than the LSL at the same age. Our results agree with the findings of Renema and Robinson (2001) who found that white egg strains have lower body weight at sexual maturity than brown egg strains. The current data on body weight is important because it implies that the cost of raising Lohman brown pullets is less than that of LSL and that will be reflected in the total cost of production of Lohman brown hens vs. LSL.

Although the cumulative feed consumption until 20 wk of age for the brown pullets was higher than that for the white pullets, the difference was not statistically significant ($P>0.05$), and the overall feed efficiency for brown pullets was significantly ($P<0.05$) better than that for the white pullets. These results indicate that production performance for brown pullets was better than that of white pullets. This result is important because it imply that the cost of raising Lohman brown pullets is less than that of LSL and that will be reflected in the total cost of production of Lohman brown hens vs. LSL. This information is of great significance to the local egg industry.

Egg Production

Data on egg production for both LSL and Lohman brown laying hens from 22 wk of age and until 69 wk of age are shown in Table 2. The data are presented for 4 periods, each consisted of 12 wk. Overall average percent egg production for the 48- wk period reported here for the brown hens

(90.0), was slightly higher than that for the white hens (89.6), but the difference was not significant (P>0.05). Our results are different from that reported in the guideline published by the company from where the present chickens were purchased. They reported that the white hens produced more eggs than the brown hens. However, Lewis et al. (2004) reported similar results as this study did. They indicated that ISA brown hens had higher egg production than Shaver white hens. Silversides et al. (2006) also reported that ISA brown hens had higher egg production than ISA white hens. In addition, Anderson (2002) provided detailed information on the differences in egg production and quality between different white and brown egg strains. The author also found that the overall average of hen-day egg production for the brown hens was higher than that of white hens. All the above results indicated that brown hens lay more eggs than white hens but the difference in production varies between studies.

The results shown in Table 2 showed that percent egg production for the brown hens for the last period measured (58-69 wk of age) was significantly (P<0.1) higher than that of the white hens. This finding is very important since it proved that brown hens are more persistent in production than white hens, indicating that if hens are to be kept for a longer period of egg production, brown hens would produce more eggs than white hens. In addition, results imply that since brown hens are more persistent in production than white hens, if there is a desire to induce molting and utilize the hens for a second cycle, it is expected that brown hens would perform better.

Egg Weight

Data on egg weight for both LSL and brown eggs at different ages are shown in Table 3. The data are presented for 4 periods, each consisted of 12 wk. The results showed no significant difference (P>0.05) between the overall weight of the brown and the white eggs. Results differed from that of Scott and Silversides (2000) who found that eggs from ISA-Brown hens were heavier than those from ISA-White hens. In addition, Riczu et al. (2004) found that eggs from the brown hens were heavier than white eggs. However, it should be mentioned that these results for egg mass (Table 4), shows that the overall egg mass of this study's Lohman brown hens was more, but not significantly more than that of the LSL. These results agree with the findings of Grobas et al. (2001) who compared production performance of ISA-Brown hens with Dekalb Delta, a White Leghorn egg layer strain and found that egg mass from ISA-Brown was more than that from Dekalb Delta. In addition, the results from this study agreed with the guideline published by the company from where the chickens in the current study were purchased. Egg mass for the brown hens for the last period measured (58-69 wk of age) was significantly (P<0.05) higher than that of

the white hens. This finding is very important as it showed that brown hens are more persistent in the total egg mass than white hens, indicating that if hens were to be kept for a longer period of egg production, brown hens would produce more egg mass than white hens. In addition, as mentioned previously, the results of the present study would imply, that since brown hens are more persistent in production of more egg mass than white hens, if there is a desire to induce molting and utilize the hens for a second cycle, it is expected that brown hens would perform better by producing more egg mass.

Feed Consumption and Feed Efficiency

Data for feed consumption and feed efficiency for both LSL and brown eggs at different ages are shown in Tables 5 and 6, respectively. The data are presented for 4 periods, and each consisted of 12 wks. Overall feed consumption for brown and white hens was similar with no significant difference ($P>0.05$); however, feed consumption for the brown hens for the last period measured (58-69 wk of age) was significantly ($P<0.05$) less than that of the white hens. This finding is important, since it could imply that brown hens would eat less as the hens get older, and they would have better feed efficiency at a later age, and therefore, can be kept for a longer period of egg production. Actually, results on feed efficiency (Table 6) showed that even though overall feed efficiency was similar between brown and white hens, feed efficiency for the brown hens for the last period measured (58-69 wk of age) was significantly ($P<0.05$) better than that of the white hens. Grobas et al. (2001) compared production performance of ISA-Brown hens with Dekalb Delta, a White Leghorn egg layer strain and found that feed efficiency from ISA-Brown was better than that from Dekalb Delta. Results indicated that production performance and efficiency for brown laying hens (Lohman brown) was better than that of white laying hens (LSL).

Egg Quality

Data for yolk, albumen, shell, and Haugh Unit for both LSL and Lohman brown eggs at different ages are shown in Tables 7, 8, 9, and 10, respectively. The data are presented for 4 periods.

These results showed that overall average of yolk weight and yolk percentage of the brown eggs were less than that of the white eggs, even though the difference was not significant ($P>0.1$). Similar trends were observed for most of all the periods studied. Scott and Silversides (2000) found that eggs from ISA-Brown hens had less yolk than those from ISA-White hens. However, Ayerza and Coates (2002) reported that white eggs had less yolk than brown eggs. It should be mentioned that this study also found that overall albumen weight and overall percent albumen (Table 8) were more for the Lohman brown eggs than for the LSL eggs. Scott and Silversides (2000) found similar results. The authors found that eggs from

ISA-Brown eggs had more albumen than ISA-White hen eggs. These results indicated that brown eggs might have less total lipids than white eggs which give brown eggs more advantages over white eggs. In addition, the results of this study also indicated that brown eggs might have more protein than white eggs, which is an important part of the egg and that which gives more advantage to the brown eggs.

Results on shell weight and percent shell shown in Table 9 showed that there were no significant differences between the Lohman brown and the LSL white egg shells ($P>0.05$). These results differ from that reported by Scott and Silversides (2000) and Silversides and Scott (2001), who found that eggs from ISA-Brown hens had more shell than ISA-White hen eggs. In addition, using brown laying hens (Shaver 579) and white laying hens (Shaver 2000), Riczu et al. (2004) found that eggs from the brown hens had more egg shell, and had a higher specific gravity than that of the white eggs.

The results on Haugh Unit (HU), an indicator of internal egg quality, are shown in Table 10. Results showed no significant differences ($P>0.05$) between the Lohman brown and LSL eggs. However, the overall average of the HU of the white eggs was significantly ($P<0.01$) more than that of the brown eggs.

Conclusion

In conclusion, the production performance of the Lohman brown pullets was better than that of the LSL white pullets. Also, the Lohman brown eggs could have less fat and more protein than the LSL white eggs. Using the brown eggs could enhance biodiversity of poultry products that could compete with the imported products in terms of quality and price.

References:

Altan, O., S. Ozkan, A. Altan, Y. Akbas, V. Ayhan, and K. Ozkan. Effects of short-term fasting and midnight lighting on egg production traits of laying hens during summer season. *Archiv-fur-Geflugelkunde* 64: 85-89, 2000.

Al-Nasser, A. Poultry Industry in Kuwait. *World's Poultry Science Journals*., 62:702-708, 2006.

Anderson, K.E. First cycle report. North Carolina layer performance and management test, 34:1-35, 2002.

Ayerza, R., and W. Coates. Dietary levels of chia: influence on hen weight, egg production and sensory quality for two strains of hens. *British Poultry Science*., 43: 283-290, 2002.

Bell, D.D., and W.D. Weaver, Jr. Commercial Chicken Meat and Egg Production. 5th ed. Cambridge, Massachusetts: Kluwer Academic Publisher, 2002.

Benyi, K., D. Norris, and P. M. Tsatsinyane. Effect of stocking density and group size on the performance of white and brown Hyline layers in semi-arid condition. *Tropical Animal Health and Production.*, 38: 619-624, 2006.
Crawley, M. J. Statistical Computing: An Introduction to Data Analysis Using S-Plus. Wiley, New York, NY, 2002.
Grobas, S., J. Mendez, R. Lazaro, C. de Blas, and G.G. Mateos. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poultry Science.*, 80:1171-1179, 2001.
Lewis, P. D., P. J. Sharp, P. W. Wilson, and S. Leeson. Changes in light intensity can influence age at sexual maturity in domestic pullets. *British Poultry Science.*, 45:123-132, 2004.
Razzaque, W. P., and A. Al-Nasser. Status of animal production sub-selector and animal origins food in Kuwait and recommended measures for improvements. Technical Report. Kuwait Institute for Scientific Research. Report No. KISR 6722, 2003.
Renema, R.A., and F.E. Robinson. Effects of light intensity from photostimulation in four strains of commercial egg layers: 1. Ovarian morphology and carcass parameters. *Poultry Scence.,*. 80:1112-1120, 2001.
Riczu, C. M., J. L. Saunders-Blades, A. K. Yngvesson, F. E. Robinson, and D. R. Korver. End-of-cycle bone quality in white-and brown-egg laying hens. *Poultry Science.*, 83: 375-383, 2004.
Scott, T.A., and F.G. Silversides. The effects of storage and strain of hen on egg quality. *Poultry Science.*, 79:1725-1729, 2000.
Silversides, F.G., and T.A. Scott. Effect of storage and layer age on quality of eggs from two lines of hens. *Poult. Sci.* 80:1240-1245, 2001.
Sliversides, F. G., T. A. Scott, D. R. Korver, M. Afsharmanesh, and M. Hruby. A study on the interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens. *Poultry Science.*, 85: 297-305, 2006.
Vits, A., D. Weitzenburger, H. Hamann, and O. Distl. Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes. *Poultry Science.*, 84: 1511-1519, 2005.

Table 1: Body Weight Gain (g/bird), Feed Consumption (g/b) and Feed Efficiency (g feed / g gain) for the Lohmann Brown and LSL Pullets (0- 20 weeks of age)

Parameter	Strain	
	Lohman Brown	LSL

Body Weight Gain (g/bird)	1436.5[*] ± 16.1 [a]	1350.6 ± 33.8 [b]
Total Feed Consumption (g /bird)	7522 ± 62.7 [a]	7501 ± 53.6 [a]
Feed Efficiency (g feed/ g gain)	5.233 ± 0.05 [a]	5.550 ± 0.12 [b]

[*]Values are Means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 2. Percent Egg Production for Lohman Brown and LSL Hens at Different Age Periods

Age Period (wks)	Strain	
	Lohman Brown	LSL
1 (22-33)	89.51[*] ± 1.98[a]	90.82 ± 1.84[a]
2 (34-45)	92.07 ± 1.65[a]	92.15 ± 1.51[b]
3 (46-57)	90.44 ± 2.16[a]	90.69 ± 2.34[a]
4 (58-69)	87.97 ± 0.80[a]	84.49 ± 0.61 [b]
Overall	90.00 ± 1.65[a]	89.54 ± 1.58[a]

[*]Values are Means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 3. Egg Weight (g) for the Lohman Brown and LSL Hens at Different Age Periods

Age Period (wks)	Strain	
	Lohman Brown	LSL
1 (22-33)	58.76[*] ± 0.62 [a]	57.99 ± 0.96 [b]
2 (34-45)	65.30 ± 0.53 [a]	65.23 ± 0.52 [b]
3 (46-57)	68.35 ± 0.41 [a]	68.54 ± 0.29 [a]
4 (58-69)	67.72 ± 0.84 [a]	68.69 ± 0.58 [b]
Overall	65.03 ± 0.60 [a]	65.11 ± 0.59[a]

[*]Values are Means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 4. Egg Mass (kg) for the Lohman Brown and LSL Hens at Different Age Periods

Age Period (wks)	Strain	
	Lohman Brown	LSL
1 (22-33)	4.43[*] ± 0.08 [a]	4.36 ± 0.13 [b]
2 (34-45)	5.05 ± 0.11 [a]	5.05 ± 0.08 [b]
3 (46-57)	5.30 ± 0.14 [a]	5.22 ± 0.14 [a]
4 (58-69)	5.04 ± 0.14 [a]	4.87 ± 0.03 [b]
Overall	4.96 ± 0.12 [a]	4.87 ± 0.10 [a]

[*]Values are Means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 5. Feed consumption (kg/bird/period) for the Lohman Brown and LSL Hens at Different Age Periods

Age Period (wks)	Strain	
	Lohman Brown	LSL
1 (22-33)	8.41* ± 0.15 [a]	7.86 ± 0.37 [b]
2 (34-45)	8.15 ± 0.12 [a]	7.71 ± 0.38 [b]
3 (46-57)	8.01 ± 0.05 [a]	8.05 ± 0.08 [a]
4 (58-69)	8.07 ± 0.26 [a]	8.24 ± 0.16 [b]
Overall	8.16 ± 0.15 [a]	7.97 ± 0.28 [b]

*Values are means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 6. Feed Efficiency for the Lohman Brown and LSL Hens at Different Age Periods

Age Period (wks)	Strain	
	Lohman Brown	LSL
1 (22-33)	1.90* ± 0.07 [a]	1.81 ± 0.11 [b]
2 (34-45)	1.62 ± 0.05 [a]	1.53 ± 0.08 [b]
3 (46-57)	1.51 ± 0.04 [a]	1.54 ± 0.05 [a]
4 (58-69)	1.60 ± 0.07 [a]	1.69 ± 0.03 [b]
Overall	1.66 ± 0.06 [a]	1.64 ± 0.07 [b]

*Values are Means ± SD.
[ab] Means within the same row with different letters are significantly different (P<0.05).

Table 7. Yolk Weight and Yolk Percentage for the Lohman Brown and LSL at Different Ages

Age Period (wks)	Parameters			
	Yolk Weight (gm)	Yolk Weight (gm)	Yolk (%)	Yolk (%)
	Lohman Brown	LSL	Lohman Brown	LSL
1(23 - 34)	13.7* ± 0.7[a]	13.4 ± 0.7 [a]	24.2 ± 1.8 [a]	24.1 ± 1.8 [a]
2 (35 - 46)	17.5 ± 1.0 [a]	18.2 ± 0.8 [a]	29.6 ± 1.1 [a]	31.0 ± 2.1 [b]
3 (47 - 54)	18.4 ± 1.2 [a]	19.0 ± 1.5 [a]	31.3 ± 2.2 [a]	32.8 ± 4.2 [a]
4 (56 - 66)	16.3 ± 0.9 [a]	17.1 ± 1.1 [a]	28.1 ± 2.1 [a]	28.6 ± 2.9 [a]
Overall average	16.5 ± 1.0 [a]	16.9 ± 1.0 [a]	28.3 ± 1.8 [a]	29.1 ± 2.8 [a]

*Values are Means ± SD.
[ab] Means within the same row and within the same parameter with different letters are significantly different
(P<0.05).

Table 8. Albumen Weight and Albumen Percentage for the Lohman Brown and LSL Hens at Different Ages

Age Period (wks)	Parameters			
	Albumen Weight (g)	Albumen Weight (g)	Albumen (%)	Albumen (%)
	Lohman Brown	LSL	Lohman Brown	LSL
1(23 - 34)	36.1* ± 3.3 [a]	34.9 ± 3.4 [a]	63.2 ± 3.4 [a]	62.5 ± 4.2 [a]
2 (35 - 46)	34.3 ± 1.7 [a]	32.0 ± 2.6 [b]	58.1 ± 3.4 [a]	54.3 ± 2.4 [b]
3 (47 - 54)	33.9 ± 4.5 [a]	30.7 ± 6.0 [a]	57.3 ± 4.6 [a]	52.0 ± 6.1 [b]
4 (56 - 66)	34.6 ± 2.7 [a]	35.3 ± 4.2 [a]	59.1 ± 2.4 [a]	58.6 ± 4.3 [a]
Overall average	34.7 ± 3.0 [a]	33.2 ± 4.0 [b]	59.5 ± 3.4 [a]	56.8 ± 4.3 [b]

*Values are Means ± SD.

[ab] Means within the same row and within the same parameter with different letters are significantly different
($P<0.05$).

Table 9. Shell Weight and Shell Percentage for the Lohman Brown and LSL Hens at Different Ages

Age Period (wks)	Parameters			
	Shell Weight (g)	Shell Weight (g)	Shell (%)	Shell (%)
	Lohman Brown	LSL	Lohman Brown	LSL
1(23 - 34)	7.6* ± 0.8 [a]	7.8 ± 1.3 [a]	13.4 ± 1.4 [a]	14.1 ± 2.3 [a]
2 (35 - 46)	7.7 ± 0.3 [a]	8.2 ± 0.5 [b]	13.0 ± 0.4 [a]	14.0 ± 1.4 [b]
3 (47 - 54)	7.6 ± 0.7 [a]	7.6 ± 4.2 [a]	12.9 ± 1.3 [a]	13.1 ± 1.7 [a]
4 (56 - 66)	7.3 ± 0.6 [a]	7.5 ± 0.7 [a]	12.6 ± 1.1 [a]	12.7 ± 1.7 [a]
Overall average	7.6 ± 0.6 [a]	7.8 ± 1.7 [b]	13.0 ± 1.1 [a]	13.4 ± 1.8 [a]

*Values are Means ± SD.

[ab] Means within the same row and within the same parameter with different letters are significantly different
($P<0.05$).